NEUROPHYSIOLOGICAL CORRELATES OF ECSTASY/MDMA USE ON EXECUTIVE FUNCTIONING

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A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree Doctor of Philosophy.

November 2013

Acknowledgements

I would like to thank the School of Natural Sciences and Psychology at Liverpool John Moores University for their support and funding over the last three years. I would like to thank all of the members of my supervisory team; Professor Stephen Fairclough, Professor Francis McGlone and in particular Dr. Cathy Montgomery. Dr. Montgomery has provided invaluable help and mentoring over the course of this PhD, her suggestions on drafts of papers and this thesis have been highly valued at every point during the last three years. I would also like to thank my Mum and Dad, as well as Laura, Julian and Evan Neale for their emotional support.

Abstract

The purpose of this thesis was to assess the integrity of the serotonin system, by measuring the neurophysiological response to tasks that measure executive functions, and neuroendocrine function in ecstasy users and non-users. Each of the proposed executive functions outlined in Miyake *et al.*'s (2000) conceptual framework (inhibition, switching and updating) as well as the addition of access to semantic/long term memory made by Fisk and Sharp (2004), was assessed using behavioural tasks in combination with EEG and fNIRS.

Behavioural performance between ecstasy users and various controls (polydrug and drug naïve) was equivalent throughout the thesis. However ERP analysis revealed ecstasy-related atypicalities in cognitive processing during inhibitory control, switching and access. Ecstasy users displayed increases in P2 and N2 components during these tasks that reflect recruitment of additional resources. A diminished P3 response during the switching task was evident for ecstasy users and polydrug users relative to controls. Regression analyses suggest that lifetime cannabis use may be an important factor for this function. Results from fNIRS suggest that ecstasy users show an increased haemodynamic response during all four executive functions relative to non-users, which suggests that ecstasy users are engaged in more effortful cognition than controls. Increases in neuronal activation whilst performing at a similar level behaviourally are understood as recruitment of additional resources. Again during switching cannabis use may have been an important factor.

Another aim of this thesis was to assess neuroendocrine function. Ecstasy users displayed elevated basal cortisol levels relative to polydrug controls and drug naïve controls. The results suggest that ecstasy is detrimental to the integrity of the HPA-axis.

This thesis provides support for ecstasy-related damage to the serotonergic system and should be used in educating prospective ecstasy users of relative harms.

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Chapter 1: Overview of thesis

Chapter 1 provides a brief overview of each chapter that this thesis comprises. Chapter 2 provides a brief introduction to the study of working memory and the fractionation of the central executive. It is these theoretical models of executive functioning that form the basis for behavioural assessment in this thesis. This chapter briefly introduces the reader to the study of ecstasy use and executive function and provides a rationale for studying each function separately.

Chapter 3 reviews studies into cognition in ecstasy users, briefly starting with intelligence and then focusing more in depth on each of the executive functions that are later investigated. This chapter reviews the current understanding of how ecstasy affects executive functioning and provides a rationale for further clarification in this research area.

Chapter 4 defines the theoretical background of the neuroimaging techniques that are used in this thesis, including how they work, what the data that they generate may tell us and advantages and limitations of each technique. Furthermore this chapter provides a rationale for using the two techniques employed in this thesis in a complimentary fashion.

Ecstasy is proposed to damage the serotonergic system and is a proposed neurotoxin. It is understood that damage to the serotonin system may underlie any observed cognitive deficits. As such Chapter 5 reviews the literature on objective measures of serotonergic neurotoxicity in human ecstasy users from various functional and structural neuroimaging methods.

Chapters 6, 7, 8 and 9 are the empirical chapters of this thesis. The first of these assesses each of the four executive functions (using function specific tasks) and their electrophysiological correlates from ERPs in ecstasy users, polydrug controls and drug naïve

controls. Chapter 7 assesses the haemodynamic response to memory updating using fNIRS and two updating tasks (letter updating and spatial updating) in ecstasy users, polydrug controls and drug naïve controls. Chapter 8 assesses the haemodynamic response to inhibition (using a random letter generation task), switching (using the number-letter task) and access to semantic memory (using the Chicago Word Fluency Task) in ecstasy users and controls.

The results from these three chapters indicate that ecstasy users perform at a similar level to controls in the executive functioning tasks employed in each chapter. However they show neurophysiological responses that reflect compensatory mechanisms/recruitment of additional resources to enable equivalent performance.

Chapter 9 investigates the haemodynamic response to multitasking in ecstasy users, polydrug controls and drug naïve controls. Importantly, this chapter also investigates the integrity of the HPA-axis and the neuroendocrine response to stress, through salivary cortisol sampling.

Finally Chapter 10 provides a general discussion of the results and places them in the context of the existing literature on ecstasy use, executive function and serotonin system degradation. This chapter discusses these results in terms of implications for drug users and suggestions for future research.

Chapter 2: Working memory and the central executive

2.1 Chapter overview

This chapter briefly describes Baddeley's working memory model and more recent work that has built upon this model exploring the central executive, executive processing and the fractionation of the central executive. This gives the theoretical basis for further exploration of executive functions that are discussed in Chapter 3.

Theory of working memory, executive functioning and ecstasy use.

Baddeley's (1986) multi-component model of working memory is a key construct in cognitive psychology. Initially proposed as a three component model, this comprises a modality free control system, called the central executive, with limited storage capacity that is subserved by two "slave" storage systems. The two slave systems are: the phonological loop, which is involved in processing sound and language, and the visuospatial sketchpad, which processes visuospatial information. A fourth component- the episodic buffer was later added (Baddeley, 2000) to bridge the gap between the limited capacity of the initial three components and long term memory. This was added after observing an amnestic patient with severe damage to long term memory who was able to recall passages of prose that were beyond the capacity of the phonological loop or the visuospatial sketch pad. The episodic buffer is regarded as the storage component of the central executive (Baddely, 2003), and is thought to be involved in transfer of episodic information to and from long term memory stores.

The central executive is an integral component of working memory and is responsible for coordinating the processing of information from the subsidiary components. Initially understood to operate as a single unit, studies on individual differences (Miyake *et al.,* 2000) and patients with frontal lobe damage (Shallice & Burgess, 1991) started to suggest that

perhaps the central executive was not a unified construct. Lehto (1996) explored the relationship between working memory capacity and a variety of executive functioning tasks in a normal 15-16 year old student population. It was observed that performance on complex span measures (working memory) had high inter-correlations with memory updating. However, although performance on the Wisconsin Card Sorting Task (WCST) correlated with working memory measures, performance on two further executive measures (Tower of Hanoi and Global Search Test) did not. Perhaps more interesting, is that none of the executive measures correlated significantly with one another, which led Lehto to conclude that the central executive was not unified.

Miyake *et al*. (2000) explored the separability of executive functions further, by examining three possible discrete executive functions: mental set shifting ("shifting"), information updating and monitoring ("updating") and inhibition of prepotent responses ("inhibition") and their contributions to the complex neuropsychological tasks used to assess executive function. In this study, performance on three tasks, each proposed to target a proposed executive function (WCST, Tower of Hanoi –ToH, and random number generation - RNG), as well as two other commonly used executive tasks (operation span and dual tasking) were correlated. It was observed, from confirmatory factor analysis, that the three target executive functions were moderately correlated with one another, but were distinctly separable. Furthermore structural equation modelling revealed that each function contributed separately to each task, with performance on the WCST relating to the executive function of shifting, ToH pertaining to inhibition, operation span to updating and RNG loaded on both inhibition and updating.

Fisk and Sharp (2004) investigated the separability of executive functions in their research on age related cognitive impairment and observed findings largely consistent with

Miyake *et al.*'s fractionated components of the central executive. However an additional component termed "access" that involves access to long term memory was proposed. This addition was proposed due to word fluency tasks often being used as a measure of executive function, and apparent impairment of word fluency after damage to frontal brain regions (Stuss *et al.,* 1998). Word fluency involves temporary access of long term memory stores and does not seem to fit as well with the three initial proposed components of executive function. Furthermore Baddeley (1996) postulated that temporary activation of long term memory was a key executive process. In this study a battery of executive tests were administered to an elderly cohort, including the WCST, Random Letter Generation (RLG), Brooks spatial sequences, reading and computation span, word fluency and a measure of dual task performance. All of the tasks loaded on at least one of Miyake's executive processes apart from word fluency and the redundancy measure of RLG (the extent to which a letter is produced with the same overall frequency), which loaded on their proposed fourth executive function of access.

The study of executive functions is complicated by task impurity, for example the WCST, a commonly used task to assess mental set shifting, requires sorting cards based on a particular theme (e.g. colour, shape, number) then switching to another theme at the experimenter's request. This not only involves shifting of the mental set, but also perceptual and motor cognitive abilities necessary for sorting cards and monitoring verbal feedback (Friedman *et al.,* 2008). As such purer tasks of executive function are required. Further work into the separability of executive functions has been conducted by Friedman *et al.* (2006) who suggest that the three executive functions identified in Miyake's model are differentially related to intelligence, with updating showing close relations with crystallised and fluid intelligence, but shifting and inhibition showing no such relationship. Furthermore Friedman *et al.* (2008) also suggest that the executive functions are correlated by hereditary factors that

go beyond speed of processing or intelligence, but are separable due to other genetic factors unique to each function.

This fractionation of the central executive into four discrete components has helped the progression of research into the effects of ecstasy/MDMA on cognition. Many earlier studies into ecstasy use and working memory refer to the central executive as a single entity, and have yielded equivocal results, whereby users show deficits in some tasks but not others. For example Halpern *et al.* (2004) administered a large battery of neuropsychological tests to a relatively pure MDMA user group and found that heavy users were impaired on performance of a Stroop task (supposed to be related to inhibition), but did not find significant performance deficits on many other tasks (including the WCST, Controlled Oral Word Association, WAIS-R digit span subtest, WAIS-R digit symbol subtest, the Rey-Osterrieth complex figure text and the California verbal word learning test). Fox *et al.* (2001) also observed ecstasy users to be unimpaired on the WCST, supposedly pertaining to the executive function of shifting, whereas Fox *et al.* (2002) show evidence of ecstasy-related impairment in shifting as well as verbal fluency, and spatial working memory. Morgan *et al.* (2002) conversely report little performance deficits in the Stroop task or word fluency. Due to equivocal findings there was no definitive consensus on whether executive functions were impaired in ecstasy users or not. As such, Montgomery, Fisk, Newcombe and Murphy (2005), applied Miyake *et al.*'s (2000) and Fisk & Sharp's (2004) framework to the research on ecstasy users, suggesting that ecstasy users display differential impairments in executive function. It is argued that a systematic approach is necessary, using "pure" tasks that tap one function only, to observe how MDMA affects each component of the central executive. Ecstasy-related impairments were observed in the updating and access components of executive function, but not in the switching and inhibition components.

In light of these findings, this thesis focuses on the separable executive functions and uses function-specific tasks to assess each component of executive function in ecstasy users. Furthermore neurophysiological measures such as electroencephalography (EEG) and functional Near Infrared Spectroscopy (fNIRS) are employed as more sensitive measures of cognitive impairment. The literature on the research pertaining to ecstasy-related deficits on executive function briefly touched upon here will be reviewed in greater detail in Chapter 3 whereby each component of the central executive will be reviewed separately.

Chapter 3: Review of the literature on cognitive deficits in ecstasy users

The recreational drug ecstasy/MDMA (3,4-methylenedioxymethamphetamine) is a potent indirect monoaminergic agonist, that is structurally similar to amphetamine and mescaline (Morgan, 2000). The acute psychological and physiological effects include feelings of euphoria and empathy, increased energy, dilated pupils and tight jaw (trismus) (Davison & Parrott, 1997) and are thought to result primarily from serotonin and dopamine agonsim (McDowell & Kleber, 1994). However ecstasy has been classed under the novel pharmacological category of entactogens (from Greek and Latin roots, meaning to produce a "touching within" Nichols, 1986) owing to its unique psychoactive profile that can be differentiated from classic hallucinogens and stimulants (Morgan, 2000). MDMA increases emotional sensitivity and empathy, but does not produce hallucinations, as such it cannot be classified as an hallucinogen or psychostimulant (Cole & Sumnall, 2003). Working memory deficits, and those particularly associated with higher level executive functioning tasks appear to be most prominent. This is particularly salient given the continued prevalence of ecstasy/MDMA use; for example the British Crime Survey (2012) states that 3.3% of 16-24 year olds report use of MDMA in the last year, and the negative psychological consequences could have real world functional significance (Montgomery *et al.,* 2010). MDMA related changes in cognition are believed to be related to the drug's effects on the serotonin system (Gouzoulis-Mayfrank *et al.,* 2000) and have been shown to be long lasting (Gerra *et al.,* 2000). Specifically serotonin, understood to be implicated in supporting working memory processes, is densely innervated in the prefrontal cortex (Pazos *et al*., 1987), and as such it is integral in executive processing.

There have been a number of investigations into working memory deficits in human ecstasy users compared to drug naïve controls. This chapter reviews the literature of several

aspects of cognition that have been investigated including intelligence, and the four executive functions outlined in Chapter 2: shifting, inhibition, updating and access. Although many publications cover several executive functions, this chapter has been subdivided into sections for each component of the central executive, and thus papers may have been cited multiple times.

3.1 Intelligence:

As mentioned in Chapter 2, the executive functions are differentially correlated with intelligence so this thesis controls for intelligence throughout. Studies on ecstasy and cognition do attempt to control for intelligence, often using Raven's Standard Progressive Matrices (SPM), the Weschler Abbreviated Scale of Intelligence (WASI) or a test of crystallised intelligence (e.g. The National Adult Reading Test - NART).

Gouzoulis-Mayfrank *et al.* (2000) measured both crystallised and fluid intelligence in ecstasy users, cannabis users and non-drug users. Fluid intelligence was assessed using a German version of the Weschler Adult Intelligence Scale – Revised (WAIS-R), Mosaic test, in which, participants must reproduce complex visual patterns using cubes (this task assesses visuomotor performance, planning and problem solving) and the LPS-4 (a problem solving test assessing abstract thinking). Crystallised intelligence was assessed with the German WAIS-R general knowledge test. Ecstasy users performed significantly worse than both non-users and cannabis users on all three intelligence measures. Due to the ecstasy user group having lower IQ measures, the researchers had to control for intelligence in their subsequent analyses. Gouzoulis-Mayfrank *et al.* (2003) also observed deficits in crystallised intelligence in heavy ecstasy users compared to moderate users and non-users, using the WAIS-R General Knowledge test.

However, not all studies find differences in intelligence. For example using the NART, which involves participants reading 50 words of decreasing fluency in the English language, with atypical phonology, so that words should not be guessed correctly using standard grammatical pronunciation (a measure of crystallised intelligence), Fox *et al.* (2002) observed no significant differences between ecstasy users and non-users. Furthermore Morgan *et al.* (2006) used the NART to assess premorbid intelligence in ecstasy polydrug users, non-ecstasy polydrug users and drug naïve controls and observed no significant between group differences in their estimated IQ (ecstasy users: 111.8, polydrug controls : 112.1, drug naïve controls: 111.2). Similarly, using a Dutch variant of the NART (the DART) Renememan *et al*. (2006) transformed the number of correctly read words into an estimate of verbal IQ and observed that former ecstasy users (male: 105.9, female: 102.0), heavy current users (male: 106.0, female: 104.5), moderate users (male: 111.2, female: 112.2) and non-ecstasy polydrug users (male: 104.7, female: 106.9) had comparable premorbid IQ regardless of gender. Moreover Dafters *et al*. (1999) observed that level use of ecstasy was not correlated with performance on the NART.

Montgomery, Fisk and Newcombe (2005) used the NART in ecstasy polydrug users compared to drug naïve controls and observed no significant between group differences in this measure. The same study also found no significant differences between users and non-users on Raven's SPM, whereby participants are required to study a series of problems presented as a symbolic sequence and select an appropriate response to complete the sequence from a choice of 6/8 options. Montgomery and Fisk (2008) again observed no differences between ecstasy users and controls on the NART and Raven's SPM. Raven's SPM has been used frequently in the literature to assess fluid intelligence, and has repeatedly yielded no observable significant differences between ecstasy users and non-users (Fisk *et al.*, 2004; Montgomery, Fisk & Newcombe, 2005). However, Montgomery *et al.* (2010) found

ecstasy users to score significantly lower than non-users on this measure. Furthermore Halpern *et al.* (2011) found performance on Raven's SPM and WAIS-R digit symbol subtest to be significantly reduced in moderate users, but not heavy users in comparison to drug naïve controls. However in an earlier study by the same research group (Halpern *et al.*, 2004) on a similarly pure ecstasy using cohort, no such differences were observed.

Verbal intelligence (using the WAIS III vocabulary subtest) and performance intelligence (using the WAIS III block design subtest) in ecstasy users was explored in a longitudinal study by Zakzanis and Young (2001) to observe whether these measures were robust to continuing ecstasy use over time. The tests were administered twice, one year apart, with continued ecstasy use in between testing dates. It was observed that there were no significant differences in WAIS vocabulary score between the first testing session (mean = 53.0) and the one year follow up score (mean = 52.1), nor was there significant decline in performance in WAIS block design performance between time one (mean = 49.0) and time two (mean= 48.4). However there was a significant correlation between frequency of MDMA use and performance change (time one – time two score) on the vocabulary subtest, suggesting that verbal intelligence is adversely affected by frequency of ecstasy use. Thomasius *et al*. (2003) also explored premorbid intelligence using the German multiple choice test of vocabulary knowledge (Mehrfachwahl-Wortschatztest – MWT-B), in their initial study (Thomasius *et al.,* 2003) they report that ecstasy users (IQ score – 102.5), former ecstasy users (IQ – 106.48), polydrug controls (IQ -104.28) and drug naïve controls (IQ - 104.97) showed no significant differences in IQ, and this remained non-significant in their follow up study (Thomasius *et al.,* 2006) (ecstasy users – 101.36, former users – 106.48, polydrug controls – 107.91, drug naïve controls – 105.20). Currently abstinent ecstasy users were assessed on verbal intelligence using the WAIS-III vocabulary subtest and the NART in a study by McCann *et al.* (2007) and again it was observed that differences between abstinent

ecstasy users and drug naïve controls on their estimated baseline intelligence were non-significant (WAIS- III; MDMA users 44.44, controls 40.39, NART; MDMA users 102.74, controls 99.38); it was suggested that these tests provide estimates of verbal intelligence that are insensitive to MDMA related neurotoxicity.

In summary, it would appear that most studies in this area attempt to control for IQ differences, and in the majority of cases there is little difference in IQ between ecstasy using populations and controls. In studies that do show between group differences in IQ measures, IQ is used as a covariate and statistically controlled for in subsequent analysis. Although one longitudinal study (Zakzanis & Young, 2001) observed a negative correlation between frequency of ecstasy use and performance on verbal intelligence measures, suggesting that ecstasy use could affect intelligence over time, other longitudinal studies have found little effect of use on measures of intelligence (Thomasius *et al.,* 2006). However in line with most other research in this area, the studies presented in this thesis all have at least one control intelligence measure.

3.2.1 Mental set switching

Mental set "switching" or "shifting" is the ability to switch attention between task types, whereby a switch between tasks is associated with a performance cost, either in accuracy or time, compared to completing two tasks in succession (Jersild, 1927). Switching reflects cognitive flexibility and is one of the core executive functions outlined in Miyake *et al.*'s (2000) framework. Several tasks have been used in the literature to assess this function in ecstasy users, including the WCST, the number-letter task, plus-minus task, and a switching variant of the Stroop task. Findings of performance of this executive function in ecstasy users are equivocal.

Fox *et al.* (2001) used a computerised version of the WCST whereby participants have to learn a rule in order to sort a pack of 128 cards, along three possible 'dimensions' (colour, shape or number). After 10 consecutive successful card placements, the rule by which the cards were being sorted was changed (switch). There were six switch trials, where the rule changed (from colour to shape to number then repeated). In this task participants are scored for number of correctly completed trials, number of trials to complete the first category, percentages of perseverative (the amount by which they fixated on a rule after it had changed) and non-perseverative errors and failure to maintain set. No significant performance differences on the task were reported between controls and 'problem' or 'non-problem users' (defined as problems attributed to use of ecstasy), furthermore lifetime dose of ecstasy (low = < 100 tablets, medium = 100-500 tablets, high = > 500 tablets) had no significant effect on performance. The WCST was administered in a neurocognitive test battery to current ecstasy users [15 male, mean age = 24.5, mean lifetime dose (MLD) for males = 1033.77, females = 600.42 tablets], former users (16 male, mean age = 24.13, MLD for males = 987.31, females = 533.80 tablets), polydrug controls (15 male, mean age = 24.41) and drug naïve controls (15 male, mean age = 23.13) by Thomasius *et al*. (2003), planned comparisons revealed that the polydrug user group produced a significantly higher amount of perseverative errors than both ecstasy using groups. Reneman *et al*. (2006) compared 15 moderate MDMA users (9 male, mean age = male 25.6, female 22.7, MLD = male 29.5, female, 27.3), 23 heavy MDMA users (12 male, mean age = male 27.1, female 25.0, MLD = male 831.8, female 200.9 tablets), 16 former users (8 male, mean age = male 26.4, female 24.1, MLD = male 126.9, female 409.3 tablets) and 13 ecstasy naïve, but drug taking controls (7 male, mean age = male 29.3, female 23.3) on performance on the WCST and observed little difference on any of the performance measures of the task. Similarly Back-Madruga *et al*. (2003) observed no differences in performance on the WCST between recreational ecstasy

users (n=22, 14 male, mean age = 37.0, mean lifetime occasions = 74.6) and controls (n=28, 23 male, mean age = 39.9). Halpern *et al*. (2004) also observed no significant differences in performance on the WCST between 23 MDMA users with minimal exposure to other drugs (8 male, median age = 20, median lifetime MDMA episodes = 60) and 16 ecstasy naïve comparison individuals involved in rave subculture (9 male, median age = 22). However, when the ecstasy user group was further subdivided into light (less than 50 occasions of use) and heavy (more than 50 occasions of use) users, heavy users performed worse compared to non-users after adjusting for age, sex and family of origin on the "total categories" score of the task. However this score does not relate to the executive function of switching in the same way as total perseverations does and may not reflect switching deficits. In a follow up study (Halpern *et al.*, 2011) with similarly ecstasy pure participants (n=52, 30 male, median age = 22, median lifetime episodes of MDMA use = 43.5) versus rave subculture matched controls (n=59, 38 male, median age = 24) it was again observed that there were no significant between group differences in performance on the WCST. However this changed when the ecstasy user group was subdivided into heavy and moderate users. This time WCST total category score was significantly reduced among moderate, but not heavy users. Results from studies administering the WCST as a measure of switching seem to suggest that this function is relatively robust to ecstasy use. However as discussed in Chapter 2, this task has been criticised for not necessarily being a pure measure of mental set switching. Therefore, it is important to consider other tasks that have assessed this function.

Fox *et al*. (2002) compared 20 ecstasy polydrug users (10 male, mean age = 27.3, MLD = 172.0 tablets) and 20 ecstasy naïve polydrug users (8 male, mean age = 27.5) on their ability to effectively "switch" attention using the 3D IDED (intra-dimensional/extra dimensional) attention shift task as well as a switching version of the Go/NoGo task. Based on a task in the CANTAB neuropsychological battery, the 3D IDED comprised of eight

stages related to forming, maintaining and shifting attentional set. Participants are required to learn two alternative forced choice discriminations and their reversals. The stimuli used, varied on three possible dimensions (one dimension is relevant and the other two are not). In the first and simplest stage (visual discrimination) two stimuli are presented and these vary on one of the three dimensions (e.g. colour) and in the reversal stage the previously incorrect item becomes the correct item. Following this, there is the compound visual stage in which the two stimuli are different on all three possible dimensions. In the intra dimensional shift stage, the "relevant" dimension (e.g. colour) remained the same despite the introduction of two novel stimuli. Finally in the extra-dimensional shift stage participants are required to shift their response set to a previously irrelevant dimension (e.g. shape). Each stage has a reversal stage and participants progress to the next stage by achieving six successive discriminations. Although increases in errors and reaction time were observed within groups as difficulty increased, there were no significant between group differences in performance at any stage on non-reversal trials. However on reversal trials the difficulty by group interaction was approaching significance, with ecstasy users making more errors on simple and compound reversal trials but fewer errors on the extra dimensional reversal condition than controls. Furthermore ecstasy users were significantly slower than controls at all levels of reversal, indicating performance deficits. In the same study, switching was also assessed with a switching variant of the Go/NoGo task with 10 blocks, each containing 18 symbols appearing rapidly on the centre of a screen. Half of the symbols were "targets" and half were "non-targets" comprising of letters (from A-G) and numbers (2-9). Participants had to press the space bar when a target appeared on the screen and were to withhold a response to non-targets. The targets (either letters or numbers) switched every two blocks. Mean errors were calculated (failure to press space bar) as well as mean distractors (pressing space bar when it

should not have been pressed) and mean reaction time for correct responses. No between group differences were observed on any of the measures analysed on the task.

The number-letter task is considered to load on the executive function of switching only, and was used by Montgomery, Fisk, Newcombe and Murphy (2005), to assess switching performance in 51 ecstasy polydrug users (27 male, mean age 21.96, MLD = 345.96 tablets) compared to 42 non-user controls (8 male, mean age 20.83). In this task (adapted from Rogers & Monsell, 1995), participants are presented with a number-letter pair (e.g. "D4") in one of four quadrants on a computer screen. If the number-letter pair appears in one of the top two quadrants of the screen, participants must indicate whether the letter is a vowel or a consonant. If the pair appears on the bottom half of the screen participants should indicate whether the number is odd or even. In the main three blocks of the task, number-letter pairs appear 64 times, in the first block they alternate between the two quadrants on the top half of the screen. In block two, pairs alternate between the bottom two quadrants. However on the third block the pairs rotate clockwise around all four quadrants of the screen. As such every second trial in block three requires a switch in categorisation. A switch cost is calculated by subtracting the average time taken to complete trials on the first two blocks (where no switching is required) from the mean trial reaction time in block three. There were no differences between users and non-users in this task, and groups had equivalent age, premorbid intelligence, fluid intelligence and years in education. In the same study, the plus-minus task also measured set switching, and involves three blocks of mental arithmetic; in block one participants are given a list of 30 two-digit numbers (10-99) and are required to add three to each number, in block two participants are given another 30 two-digit number list and are required to subtract three from each. In the final block participants are given a third 30 two-digit list and participants are to alternate between adding three and subtracting three from each number on the list. This final block involves a shift/switch and the switch cost is

calculated by subtracting mean completion times for lists one and two from the time taken to complete list three. No between-group differences were observed on this task, and the main effect of ecstasy use on switching was non-significant.

Using a modified version of the Stroop task, to assess inhibition and task switching, Dafters (2006) compared performance of 18 ecstasy users (12 male, mean age = 23.24, MLD = 522.33), 15 ecstasy users who did the task in reversed order (9 male, mean age = 22.93, MLD = 475.87), 17 cannabis users (13 male, mean age = 23.19) and 18 controls (10 male, mean age = 22.67) whom had never used either drug. The switching component came in the fourth phase of the task whereby colour words appeared on a screen and participants were required to name the ink colour rather than read the word. On half of the trials the word was underlined in black and participants were required to select the colour name rather than the ink colour, hence a switch in rule. Reaction times were analysed for the switch trials and it was reported that ecstasy users performed worse than both cannabis users and non-drug user groups on the switching component of the task, after covarying for other drug use. However, the mapping of tasks onto individual executive functions is difficult, and as this task is usually implemented to assess inhibition, perhaps this manipulation does not necessarily tap switching exclusively. It could be that these results still reflect inhibition deficits (Murphy *et al.,* 2009**).** Dafters *et al.* (1999) had previously observed dose related impairment in switching using a derivative of the WCST called the Behavioural Assessment of the Dysexecutive Syndrome (BADS) rule shift cards test, whereby MDMA use was negatively correlated with performance on the task. There was, however, no control group in this experiment, and when heavy ecstasy (at least 50 MDMA tablets over lifetime)/ cannabis (1680.7 mean lifetime joints) users, were compared to light ecstasy (below 50 tablets)/cannabis users (1252.9 mean lifetime joints), cannabis only users (1023.1 lifetime mean lifetime joints) and non-drug

controls, this task yielded no significant differences between groups after covarying for alcohol, amphetamine cocaine and LSD (Dafters *et al.,* 2004).

von Geusau *et al*. (2004) assessed cognitive flexibility in 26 ecstasy using first year university students (17 male, mean age = 21.4 MLD = 53.82 tablets, 9 female, mean age = 21.7, MLD = 38.78) and 33 non-user controls (12 male, mean age = 22.0, 21 female, mean age = 21.4) using the Dots-Triangles task and the Local-Global task. In the Dots-Triangles task participants are presented with a 4x4 grid on a computer screen in which varying numbers of dots or triangles appear. When dots appear, participants have to decide whether there are more dots in the left half of the screen or the right half, and when triangles appear a decision has to be made as to whether there are more triangles in the top half of the screen or the bottom half. In blocks one and two, all trials are dots or triangles (randomised), whereas in block three it alternates between dots and triangles being presented every four trials. The Local-Global task involves participants responding to rectangles and squares. Larger (global) rectangles or squares consist of smaller (local) rectangles or squares. Participants respond to either the local or global figures only, in the first two blocks of the task. In the third block, the rule alternates between local and global every fourth trial, initiating a switch. Male users displayed a significantly higher switch cost reaction time than non-users in the dots triangles task, although they were also shown to be significantly more accurate. Female users and non-users were equivalent in performance on this task. Moreover on the Local-Global task male users were significantly slower than controls and had a higher switch cost. However there was no significant difference in accuracy on this task.

In summary, it appears from the literature that the majority of studies suggest that this executive function is relatively stable after ecstasy use, although there may be issues with the purity of some of the tasks. However there is some evidence to suggest that this executive

function warrants further investigation. Although von Geusau *et al*. (2004) suggests that this function is more affected in males, this could be an effect of dose, as the males in this sample had a higher mean lifetime dose than females and the range was much larger for males. This is interesting to consider given that Dafters *et al*. (1999) showed evidence of a relationship between dose and performance on set switching. Halpern *et al.*'s (2004) study also showed an effect in heavy users compared to light users. However this was contradicted in a follow up study (Halpern *et al.*, 2011) with moderate users performing worse than heavy users. Furthermore Dafters' 2006 study showed evidence for deficits in this area. Perhaps the addition of neuroimaging techniques in combination with performance on these tasks can help to address equivocal findings. As such the effect of ecstasy use on mental set switching will be investigated in this thesis both behaviourally and with EEG and fNIRS, using the number-letter task in Chapters 6 and 8.

3.2.2 Inhibitory control

Inhibitory control, or response inhibition is one of the executive processes outlined in Miyake *et al.*'s (2000) framework and involves the inhibition of prepotent, or dominant responses when they are not necessary. This function has been assessed in ecstasy using populations with several tasks, including: the traditional Stroop task, RLG, RNG, ToH, Stop Signal and Go/NoGo tasks.

The most frequently used task to assess this function in the literature is the Stroop task (Stroop, 1935). Conventional Stroop measures involve comparing reaction times of participants to name the ink colour of a colour named word (e.g. the word "yellow" written in red ink), to naming the ink colour when the stimulus and colour match (e.g. "red" written in red ink) or the stimulus is not a word (e.g. an asterisk) (Murphy *et al.*, 2009). Morgan *et al.* (2002) examined performance on the Stroop task in 18 current heavy ecstasy users (9 male,

mean age = 23.4, MLD = males 513 tablets, females 93 tablets), 15 former heavy ecstasy users (4 male, mean age = 24.7, MLD = males 336 tablets, females 577 tablets, abstinent for at least 6 months), 16 ecstasy naïve polydrug controls (8 male, mean age = 22.1) and 15 drug naïve controls (6 male, mean age = 22.4). No significant between group differences were observed for number of errors made or reaction time. Dafters (2006) used a modified version of the Stroop task, in which standard colour-word interference trials were interspersed with trials where the target colour was the same as the distractor word from the previous trial. Performance was compared between ecstasy users, cannabis users and controls (as described in Chapter 3.2.1). After covarying for cocaine, amphetamines, alcohol and tobacco, ANOVA revealed no significant between group differences on the magnitude of Stroop interference reaction times (pre potent response inhibition). However a difference was observed on the magnitude of negative priming, whereby ecstasy users showed a reduced priming effect (reduced short term residual inhibition) compared to both other groups. It was suggested that these two inhibition types are regulated by separable processes and future work should investigate the microstructure of cognitive subcomponents. Back-Madruga *et al*. (2003) failed to observe behavioural differences in the Stroop task between ecstasy users and controls matched for age, IQ and education (described in Chapter 3.2.1). Similarly Gouzoulis-Mayfrank *et al.* (2000) observed no significant differences on Stroop performance between ecstasy users, cannabis users and non-drug users. However Halpern *et al*. (2004) did observe ecstasy-related performance deficits on the Stroop task, after subdividing the ecstasy using population into heavy and light users (as described in chapter 3.2.1). Heavy users showed significantly longer reaction times and more Stroop errors on interference trials. However, these findings were not replicated in a follow up study (Halpern *et al.,* 2011). A longitudinal test on 149 new ecstasy users (<5 MDMA use occasions before participating in the study) was conducted by Wagner *et al*. (2012) to examine whether abnormalities in executive

function existed prior to drug using. This was followed up one year later with 109 remaining participants; Of these, 43 did not use illicit substances other than cannabis over the 1 year (classed as non-users for analysis) and 23 took more than 10 MDMA tablets over the one year period (mean = 33.6). The remaining participants used MDMA more than once, but had taken less than 10 tablets, and so were excluded from follow up analysis. Using a German variant of the Stroop task, no significant differences were found in performance between groups, or between baseline and follow up sessions. This suggests that performance on this task does not decline following continued use in new users over a one year period.

Wareing *et al.* (2000) assessed performance of 10 ecstasy users (mean age = 22.2, mean duration of use = 4.1 years), 10 former users (mean age = 22.6, mean duration of use = 3.9 years, abstinence of at least 6 months) and 10 non-users (mean age = 22.6) on RLG. Participants were instructed to speak aloud consonants in random order and to avoid repeating letter sequences, producing alphabetical sequences and to try and produce each letter with the same overall frequency. Participants were required to produce three sets of 100 letters, at a different rate (every 4 seconds, 2 seconds or 1 second – presentation randomised). This task yields three performance measures; redundancy - the extent to which each letter appears with the same overall frequency, number of letters produced at each rate (often due to more accelerated rates participants will lapse and produce fewer letters) and number of vowel intrusions. A low score on redundancy and vowel intrusions is desirable for good performance on this task, whereas a high score on the number of letters produced is indicative of good performance. Ecstasy users (both groups) performed worse on the task compared to controls, with more vowel intrusions at all three rates, and higher redundancy and lower number of letters produced relative to controls at the 1s rate. It is suggested that this function is impaired in ecstasy users and this persists after six months, furthermore ecstasy users perform worse when greater demand is placed on them. However the sample size here is

relatively small and these results were not replicated in a follow up study with a larger sample (Fisk *et al.*, 2004) where it was observed that ecstasy users (n=44, mean age = 21.52, MLD = 343.38 tablets) were unimpaired on all measures of RLG performance relative to controls (n=59, mean age = 21.37). Moreover, Montgomery, Fisk, Newcombe and Murphy (2005) observed ecstasy users (described in Chapter 3.2.1) to perform better at RLG than non-users with users producing significantly more letters than controls. Other measures (alphabetic sequences, repeat letters and redundancy) were non-significant.

RLG was also administered to 15 ecstasy users (3 male, 1 transsexual, mean age = 24.5, MLD = 364.8 tablets), 12 cannabis only users (6 male, mean age = 21.9) and controls (6 male, mean age = 19.6) who had never used either drug in a study by Murphy *et al.* (2011). No between group differences were observed in measures of alphabetic sequences or repeat sequences (measures of impulsivity) and ecstasy use did not predict performance on these measures. However there were between group differences in 'redundancy' which the authors suggest pertains more to access to long term memory and as such will be discussed in Chapter 3.2.4.

More recently, Clark *et al.* (2009), suggested that disrupted 'reflection' impulsivity may be more related to cannabis use than MDMA use. In this study 46 current ecstasy users (33 male, mean age = 24.2, MLD = 609.1 tablets), 14 former ecstasy users (6 male, mean age = 27.9, MLD = 1000.8 tablets, abstinence of at least 1 year), 15 cannabis users (5 male, mean age = 22.3) and 19 drug naïve controls (12 male, mean age = 24.0) were compared on performance of a novel information sampling task (IST). The IST comprised of two conditions; the fixed reward (FR) condition and the reward conflict (RC) condition. Participants had to make judgements on which colour (out of a choice of two) was most frequently contained inside 25 boxes. Participants could open as many boxes as they desired

before making the judgement. In the FR condition participants were awarded 100 points for a correct response regardless of how many boxes were opened before reaching the decision. In the RC condition 250 points were available to win at the start of the trial, which decreased by 10 points with every box that was opened, creating conflict between reward and certainty level. Moreover, 100 points were deducted for incorrect responses. Performance was indexed by average number of boxes opened, as well as calculating the probability of a correct response at the point of decision [P(correct)]. Post-hoc analysis revealed that cannabis users opened significantly less boxes than current ecstasy users, and this difference was approaching significance with former users and drug naïve controls. There was also a group by gender interaction, and subsequent analysis revealed that male cannabis users had significantly reduced information sampling compared to males in all three other groups. This difference was not the case in females, despite equivalent cannabis use in males and females. However, the results from this study are difficult to interpret given the higher use of cannabis (although not statistically significant) in both ecstasy groups compared to cannabis users. The authors suggest this study shows evidence against a simplistic pathway from ecstasy consumption to elevated impulsivity via serotonin neurotoxicity.

The Go/NoGo task, is believed to have specificity for the executive function of response inhibition. The literature suggests that ecstasy users are relatively unimpaired on this task also. Gouzoulis-Mayfrank *et al.* (2003) used this response inhibition task in which participants are presented with two visual stimuli (e.g. an X and an O) independently, one of these is defined as the critical target and every time this stimulus appears on the screen participants are to respond to it. Whereas the other stimulus is a non-critical target and responses are to be inhibited. Performance is measured by the amount of responses to the non-critical target (errors). In this study no significant differences in performance on the task were observed between 30 heavy ecstasy users (21 male, mean age = 21.5, MLD = 503.2

tablets), 30 moderate users (21 male, mean age = 24, MLD = 39.5 tablets) and non-drug controls (21 male, mean age = 25.37). Similarly Roberts & Garavan (2010) assessed 20 ecstasy users (10 male, mean age = 22.4, MLD = 406.5 tablets) and 20 drug naïve controls (10 male, mean age = 22.5) on the Go/NoGo task in an fMRI study. No significant between group differences were observed on any of the performance measures (% of successful response inhibitions, error of commission reaction times and GO reaction times). However between group differences were observed on neurophysiological data which will be discussed further in Chapter 5.5. Hanson and Luciana (2010) also observed no MDMA related performance deficits in a Go/NoGo task. On the contrary Hoshi *et al*. (2007) observed impaired response inhibition on a Go/NoGo task in current ecstasy users (n=25, mean age = 28.64, mean lifetime uses = 288.00) compared to former users (n=28, mean age = 29.50, mean lifetime uses = 264.86) and drug naïve controls (n=27, mean age = 32.04). However, polydrug controls (n=29, mean age = 31.93) were also impaired in this task compared to former users and drug naïve controls. The authors concluded that recency of use may play a role in response inhibition given that former users do not appear impaired on this function. Moreover recent use of cannabis and cocaine may also play a role in inhibition given that polydrug controls showed impairments compared to former users and drug naïve controls.

The majority of published studies investigating this function using the Stroop task have yielded no ecstasy-related effects in terms of performance. Although Halpern *et al.* (2004) did observe differences with this task after subdividing their ecstasy user group to heavy and light users. This was, however, not replicated in a follow up study, using similarly pure ecstasy users. As such the initial findings should be treated with caution. Wareing *et al.* (2000) observed deficits in ecstasy users (both current and former) compared to non-users with RLG, however again this was not replicated. Of the Go/NoGo tasks reviewed, only one showed drug related deficits behaviourally, and recent cannabis and cocaine use were

implicated here; as such it would appear that this function is relatively robust to MDMA use. Nevertheless this thesis intends to provide a complete analysis of MDMA's effects on executive functioning. Therefore performance in this function will be assessed with the addition of neuroimaging to provide a more complete understanding of the mechanisms that subserve this function. This function will be investigated using the Go/NoGo task and the RLG task using EEG and fNIRS in chapters 6 and 8 respectively.

3.2.3 Updating

The updating function of working memory involves the monitoring and coding of incoming information for task relevance, and updating the items held in working memory by replacing irrelevant information with new incoming relevant information (Miyake *et al*. 2000), and appears to be more consistently affected by MDMA use.

Montgomery, Fisk, Newcombe and Murphy (2005) assessed this function in 27 ecstasy users (14 male, mean age 21.70, MLD = 345.96 tablets) versus 34 non-user controls (10 male, mean age 21.59) using a letter updating task and a computation span task. In the letter updating task participants are presented with a random sequence of between six and 12 consonants. There are 24 trials and participants are unaware of the list length each time. Participants complete six trials at each list length (6, 8, 10 and 12) and in each case need to recall the last six consonants. A composite score for updating can then be calculated. In this study a second measure of updating was also completed (computation span). Participants are given a number of arithmetic problems to solve (e.g. 4 + 7 = ?) and have to circle the correct answer from a choice of three possible answers, as well as simultaneously remembering the second digit of each presented problem. Following each set of problems, the second digits are to be recalled in the order they were presented. The number of arithmetic problems, to be solved whilst remembering second digits, increases as the task progresses. For the first three

trials, one problem is presented, increasing to two for the next three trials and then increased by one every three trials until the participant gives two incorrect answers in a set. Computation span is defined as the maximum number of second digits in serial order correctly remembered, accompanied by correct arithmetic responses. In this study the authors observed no between group differences in background variables such as age, premorbid intelligence, number of hours slept per evening, years of education or fluid intelligence (as measured by Raven's SPM) – although this was approaching significance. Ecstasy users did however score significantly higher than non-users for subjective daytime sleepiness (as measured by the Epworth Sleepiness Scale). It was observed that ecstasy users performed significantly worse than non-users on both the letter updating and computation span task measures. Letter updating was subsequently investigated again by Montgomery and Fisk (2008), with 73 ecstasy users (39 male, mean age = 21.77, MLD = 309.86 tablets) and 73 non-ecstasy user controls (16 male, mean age = 20.73). Separate analyses were conducted according to span length (span = 4, 5 or 6), and it was observed that ecstasy users with simple spans of five and six performed worse than non-users. Those with a span of four were not significantly different. It is suggested that this may be due to small numbers of participants with this span length, thus reducing the statistical power of the analysis. Correlational analysis revealed that higher levels of ecstasy use were associated with poorer performance on the task, whereas indicators of cocaine and cannabis use were not correlated with updating performance.

Fisk *et al.* (2004) had previously observed deficits in updating, using the computation span task, in ecstasy users (described in Chapter 3.2.2) compared to non-users. Performance on this task was significantly worse in the ecstasy using cohort after covarying for cannabis, amphetamine and cocaine use, as well as cigarettes smoked per day and units of alcohol consumed per week. Similarly Wareing *et al.* (2004) observed deficits in both current ecstasy

users (n=42, 22 male, mean age = 24.69, MLD = 553 tablets) and former users (n=17, 9 male, mean age = 26.06, abstinent for at least 6 months, MLD = 385 tablets) compared to non-user controls (n=31, 12 male, mean age = 23.39) in computation span. This remained significant after statistically controlling for cannabis and other drugs and is suggestive of long lasting impairments as the deficits persist after six months abstinence.

Wareing *et al.* (2005) measured computation span in 36 current users (mean age = 21.81, MLD = 591.33 tablets), 12 former users (mean age = 26.83, MLD = 433.36) and 31 controls (mean age = 23.39). The updating component of spatial working memory was also investigated with a maintenance plus type visuo-spatial working memory task. This task involved participants being presented with a 4x4 matrix on a computer screen, in which five cells would be highlighted for three seconds. The task commenced with one matrix being presented three times. On the next three trials two matrices were presented sequentially and this kept on increasing by one matrix every three trials up until a maximum of six matrices per trial. In each matrix, one of the highlighted cells was filled with 0's and participants had to remember the position of this cell, whist simultaneously indicating whether there were more highlighted cells at the top or the bottom of the matrix. After all of the matrices for each trial had been presented, participants had to indicate on a blank grid all of the 0 labelled cells that had appeared in the trial, in the order that they had appeared. This had to be correctly achieved on two out of three trials at each level for acceptance of performance at that level. This task is analogous to computation span, as it requires concurrent processing and storage of incoming information but without the phonological component. It was observed that ecstasy users and former users both performed significantly worse in the computation span task. Moreover both groups of ecstasy users performed significantly worse than non-users in the spatial working memory task. These differences remained after controlling for spatial span and age. Spatial updating was investigated by Montgomery and Fisk (2008) using a task

that is analogous to the letter updating task, in which participants are presented with blocks in a Corsi type arrangement and a random sequence of spatial locations are highlighted. Twenty-four trials (6 at span sequence length, 6 at span+2, 6 at span+4 and 6 at span+6) are undertaken whereby the participant is unaware of the number of locations to be highlighted. The participant is to indicate the last X amount of blocks highlighted in the order they were presented (where X is the participant's span that had been calculated prior to undertaking the updating task). Seventy-three ecstasy users were compared to 73 non-users on task performance (as described above) and separate analyses were conducted for each span length. It was observed that for those participants with a spatial span of five, ecstasy users performed significantly worse than controls. Furthermore heavy ecstasy use was correlated with poorer performance. Spatial span and spatial working memory was assessed in 52 polydrug users (MDMA use 0-150 tablets) and non-drug controls (< 10 occasions of cannabis use and no use of other drugs) by Hanson and Luciana (2010). The Spatial Delayed Response Task (SDRT) was used in which participants had to correctly recall a spatial location that had been highlighted on the screen after various delay intervals (500ms, 4000ms or 8000ms). Polydrug users had poorer spatial memory spans, and were more negatively impacted by increasing delay intervals than controls (as measured by the SDRT). However the polydrug user group was a mixture of ecstasy polydrug users and ecstasy naïve polydrug users. Exploratory correlations showed that the spatial working memory summary score was negatively correlated with average number of ecstasy tablets consumed per session, as well as maximum number of tablets ever taken in one session. Total lifetime dose (tablets) was not correlated with performance suggesting that impairment is associated with the size of an average/maximal dose.

Backward digit span is another measure of updating that has been employed in the research on ecstasy use. Participants are required to repeat sequences of digits in the opposite

order (backwards) to which they were presented. Sequence lengths increase with successful performance and points are gained for sequences that are correctly repeated in order. Reay *et al*. (2006) compared performance in this task between 15 ecstasy polydrug users (9 male, mean age = 25, mean ecstasy use = 11.5 tablets per month for the last 4.3 years) and 15 non-ecstasy polydrug users (7 male, mean age = 21.3, defined as having never used ecstasy); it was observed that performance was not significantly different between the two groups after controlling for cannabis, cocaine, alcohol and tobacco. Similarly, Gouzoulis-Mayfrank *et al.* (2003) observed no performance differences on this task between heavy ecstasy users, moderate users and non-users (as described in Chapter 2.3.2). However in a previous study by the same group (Gouzoulis-Mayfrank *et al.* 2000), it was reported that ecstasy users (at least two tablets per month over the previous two years/at least 25 occasions; no heavy alcohol use/no other illicit drug use except cannabis) performed significantly worse than non-drug users and cannabis matched controls, and this remained significant after covarying for general knowledge. Backward digit span (and forwards digit span) was also administered to 11 MDMA users (4 male, mean age = 22.9, MLD = 32.5 tablets), 13 polydrug users (4 male, mean age = 23.2) and 13 non-users (4 male, mean age = 23.2), in an EEG study by Nulsen *et al.* (2011), who observed no main effect of group on performance. Moreover, MDMA variables did not predict performance in their regression analyses. Croft *et al.* (2001) also used backward and forward digit span as a measure of updating performance to assess the relative contributions of ecstasy and cannabis to cognitive impairment (31 drug naïve controls, 11 MDMA/cannabis users and 18 cannabis users). It was observed from ANCOVA that there were no differences between MDMA/cannabis users and cannabis only users on these measures. However there were significant differences between drug naïve controls and a combined (both MDMA/cannabis users and cannabis only users) drug user group. It was suggested that cannabis use was more closely related to performance deficits, as both drug

user groups had used cannabis and did not differ on performance. Moreover, covarying for MDMA consumption had little effect on results. This highlights the complication of concomitant use of other drugs in this research area, and suggests that cognitive deficits observed in ecstasy users may be related to cannabis use.

Halpern *et al.* (2011) measured backwards digit span in ecstasy users, who report little use of other drugs, and non-users (as described in Chapter 3.2.1). Modest differences between users and non-users were observed, with ecstasy users scoring lower for correct repeated sequences. However it was concluded that differences were sufficiently limited to reject a large effect of ecstasy. As such residual cognitive deficits in ecstasy users were not assumed. However in a reinterpretation of results, Parrott (2011) suggested that the initial interpretation was incorrect and that, these results were in-line with other studies suggesting serotonergic neurotoxicity and cognitive impairment in ecstasy users. It was argued that the participants in Halpern *et al.*'s (2011) study were careful drug users, with lifetime rates that were not particularly high (especially for the heavy user group) and that usage was not intense. The bioenergetic stress model (Parrott, 2006) suggests MDMA damage is greater when taken intensely and cumulatively. Parrott (2011) suggests that even under the neuroprotective circumstances that users in this sample experienced, cognitive damage is still apparent.

Bedi and Redman (2008) assessed backwards digit span in 45 ecstasy polydrug users (ecstasy and cannabis use ≥ 10 times), 48 cannabis polydrug users (cannabis use ≥ 10 times, with variable other drug use) and 40 legal drug users (namely alcohol, > 5 times cannabis use). No differences were observed here on a group level, however hierarchical regression suggested that weak negative semi-partial correlations were apparent with lifetime ecstasy dose and LSD dose with attention/working memory scores (digit span forward and digit span backwards). Other studies that report no ecstasy-related deficits on backwards digit span

include Thomasius *et al.* (2003) and Bhattachary and Powell (2001). Suggesting this task has produced more varying results than other tasks that assess updating. Nevertheless a meta-analysis by Rogers *et al*. (2009) suggested that generally ecstasy users performed worse than controls on common measures of digit span.

The N-back task has been used to assess updating in ecstasy users, often in association with neuroimaging methods (which will be reviewed in a separate chapter). In this task, participants are usually presented with strings of digits (or letters) sequentially, and upon instruction are required to recall the "nth" character back in the sequence (where n=0 is the last character to be displayed in the sequence). This task can be varied for difficulty depending on how far back in the sequence the participant is required to recall. Gouzoulis-Mayfrank *et al.* (2003) observed heavy users, moderate users and non-users (as described in Chapter 2.3.2) performance on a 2-back task (whereby participants must respond to a stimulus if it is the same as one presented two trials earlier), no significant between group differences were observed on measures of performance on this task. Daumann, Fimm *et al*. (2003) subjected 11 heavy ecstasy users (8 male, mean age = 27, MLD = 258.18 tablets), 11 moderate ecstasy users (8 male, mean age = 23.27, MLD = 27.36 tablets) and 11 healthy non-user controls (8 male, mean age = 25.64) to three n-back tasks consisting of sequential presentation of single letters. In the 0-back condition, participants had to respond when a stimulus in the sequence matched a target stimulus. In the 1-back condition, participants were to respond to stimuli that matched the stimuli immediately preceding it, and in the 2-back condition participants had to respond if the stimuli matched a stimulus presented 2 letters earlier. Analysis of behavioural data revealed that although there was a main effect of difficulty, there were no between group differences in performance on the task. In an 18 month longitudinal study (Daumann, Fischermann, Heekeren *et al.*, 2004), 30 ecstasy users (at time 1, whom had consumed ecstasy regularly twice a month over a six month period, or

at least 20 occasions, excluded if they regularly use legal or illegal psychotropic drugs such as opiates and benzodiazepines, or regular heavy use of alcohol), which due to various exclusion reasons reduced to 21 users (16 male, mean age = 24.93) at time 2, completed three n-back tasks (0,1 and 2 back) at time 1 and 2, to observe whether performance deteriorates over time with continued/discontinued use. The 21 returning participants were subdivided into those who had not consumed any MDMA or amphetamine in the 18 month period (n=8) and those who reported continued ecstasy and amphetamine use of at least 20 tablets (n=9). The remaining four participants were excluded from analysis due to sporadic MDMA and amphetamine use between time 1 and 2. The two groups did not differ in performance at baseline or follow up. However both groups tended to respond quicker at time 2 (significantly at 2 back for abstinent users and 0-back for continuing users). The results suggest task performance was not correlated with drug use patterns.

In summary it appears that ecstasy use has a more consistent effect on memory updating. Of the studies reviewed here, ecstasy users performed consistently worse than non-drug controls on letter updating (Montgomery & Fisk, 2008; Montgomery, Fisk, Newcombe & Murphy, 2005). Although there was no polydrug control group employed in either study, greater use of MDMA was associated with poorer performance. The findings are however, more diverse for backwards digit span. No differences between ecstasy users and controls are observed in several of the studies reviewed (Bedi & Redman, 2008; Bhattachary & Powell, 2001; Croft *et al.*, 2001; Gouzoulis-Mayfrank *et al*., 2003; Nulsen *et al*., 2011; Reay *et al.*, 2006; Thomasius *et al.*, 2006). However one of these (Nulsen, *et al.*, 2011) was an EEG study with low n numbers, which may have lacked the statistical power to detect behavioural differences (this study did show between group differences in electrophysiological measures will be discussed in Chapter 5). Another (Reay *et al.*, 2006) failed to detect differences between ecstasy users and cannabis users. However, as discussed by Croft *et al*. (2001),

cannabis may play a role in ecstasy-related impairments. Conversely Gouzoulis-Mayfrank *et al.* (2000) showed deficits in ecstasy users compared to cannabis matched controls, so this warrants further exploration. Halpern *et al.*'s (2011) study could be useful in deciphering the ecstasy/cannabis contribution to this task due to this ecstasy using population being relatively pure. However there is debate about the interpretation of the findings, with Parrott (2011) suggesting that the results here show evidence that intensity of dose is related to cognitive impairment. Finally Bedi and Redman (2008) observed an MDMA related effect on performance on this task with regression analysis, and meta-analysis on this task suggests that overall ecstasy use is associated with poor performance.

Spatial working memory performance was shown to be associated with intensity of MDMA dose (Hanson & Luciana, 2010), and MDMA use was correlated with poor performance in this measure by Montgomery and Fisk (2008). Current and former users were observed to be worse than non-users (Montgomery & Fisk, 2008), suggesting that this deficit is persistent after cessation of use. Moreover the control group here were not drug naïve and these results remained after controlling for other drug use. As such it is unlikely that the deficit is due to continued cannabis use. Computation span regularly yields observable deficits in ecstasy users relative to controls after covarying for other drug use (Fisk *et al.*, 2004; Wareing *et al.*, 2004) and this has also been observed to be persistent after cessation of use (Wareing *et al.*, 2004; Wareing *et al.*, 2005). The N-back task in this review appears to have yielded few ecstasy-related deficits. However this may in part be due these neuroimaging studies recruiting low numbers of participants (Daumann, Fischermann, Heekeren *et al.*, 2004). Moreover, in these studies, the n-back task has not gone beyond a 3-back difficulty; perhaps deficits may become more apparent with increasing difficulty. Further to this, many of these studies have found differences in their neurophysiological

response (Daumann Fimm *et al*., 2003; Daumann, Fischermann, Heekeren *et al.*, 2004) and will be discussed further in Chapter 5.

Chapters 6 and 7 will investigate ecstasy-related differences in memory updating using neuroimaging techniques (EEG and fNIRS). Pure memory tasks that tap this function will be used in combination with the neuroimaging measures. For example computation span relies heavily on ability to conduct mental arithmetic, which should be avoided. As such the n-back task will be used in combination with EEG. Furthermore the difficulty of the task will be increased beyond the level that has been used previously in the research. Letter updating and spatial updating tasks will be used in combination with fNIRS in Chapter 7, due to these tasks consistently yielding behavioural differences. Using these tasks in combination with fNIRS will allow the neurophysiological mechanisms underlying performance to be observed.

3.2.4 Access to semantic/long term memory

The fourth component of the central executive - access to semantic/long term memory (referred to as 'access' from this point on) was added by Fisk and Sharp (2004). This function involves word fluency and efficiency of lexical access. Retrieval of words and semantics involves the ability to access the long term memory store. The efficiency with which this can occur is dependent on areas of the DLPFC (Stuss *et al*., 1998), amongst other subcortical networks. Tasks that have classically been used to study this function in the ecstasy literature include RLG and the Chicago Word Fluency Task (CWFT). RLG is understood to load on two executive processes, with alphabetic sequences and letter repeats loading on inhibition and redundancy loading on access.

Murphy *et al*. (2011) observed that ecstasy users (described in Chapter 3.2.2) performed significantly worse than drug naïve controls, but not compared to cannabis only users on the redundancy measure of RLG, which is suggested to load on access. The cannabis

only group did not have a significantly lower redundancy score compared to drug naïve controls. However regression analysis yielded a significant relationship between cannabis use and redundancy, whereas MDMA use did not. Furthermore members of the ecstasy user group had very high cannabis consumption totals that perhaps contributed to the significant divergence from drug naïve controls in the analysis. Wareing *et al*. (2000) observed that current and former ecstasy users performed worse than controls on the redundancy measure of RLG, however this result was not replicated with a larger sample by Fisk *et al.* (2004) or by Montgomery, Fisk, Newcombe and Murphy (2005).

The CWFT is a measure of word fluency that requires access to semantic long term memory. In this pencil and paper task, there are usually two blocks that increase in difficulty. In the first block participants are required to write down as many words beginning with the letter S, as possible in five minutes. The following block requires participants to write down words beginning with C, with the added restriction that they must only use four letter words. Furthermore in block two the time in which participants are given to produce words is reduced to four minutes. Participants are instructed to avoid place names, people's names and plurals. This task is usually coupled with a semantic fluency task which requires participants to name as many animals (including breeds within species) as possible in a four minute period. The number of appropriate words is given as the total score for each fluency measure. Using these fluency measures Montgomery, Fisk, Newcombe and Murphy (2005) observed a main effect of ecstasy use on word fluency. Ecstasy users produced significantly less S letter and C letter words than non-user controls. This difference was greatest in the C letter category, which places more constraints upon participants and is therefore more demanding. Group differences on the semantic fluency measure were not significant. Due to the small amounts of illicit drug use in the non-user sample in this study, correlations of performance with different measures of ecstasy, amphetamine, cocaine and cannabis use were performed

to investigate the influence of other drug use on word fluency. Although measures of ecstasy were correlated with word fluency performance, measures of cocaine use were similarly correlated and as such it is difficult to attribute the observed effects solely to MDMA use in this study. Montgomery *et al*. (2007) again observed significant performance deficits in ecstasy users compared to non-users on a composite measure of word fluency (letter fluency – C letter words and S letter words combined) after covarying for semantic fluency and controlling for sleep. However, this study was an analysis of participants from several studies from this research lab, and so included the data from the 2005 study. Heffernan *et al*. (2001) measured word fluency/semantic fluency in a similar task with 30 ecstasy users (17 male, mean age 23.9, mean use = 5.6 tablets per month) and 37 non-user controls (10 male, mean age 25.5). In this study, verbal fluency was measured by participants writing down as many C letter words as possible in one minute. Semantic fluency was measured by naming as many animals as possible in a one minute time frame, and a combined verbal/semantic fluency measure was obtained whereby participants had to recall as many household objects beginning with T as possible in one minute. Amounts of cannabis, cocaine and alcohol use were incorporated into an Analysis of Covariance and it was observed that the ecstasy user group performed significantly worse on verbal fluency, semantic fluency and verbal/semantic fluency. Fisk and Montgomery (2009) compared performance on the CWFT (S letter words and 4 letter C words) in ecstasy users (n=117, 64 male, mean age = 21.68, MLD = 328.55 tablets), cannabis only users (n=53, 17 male, mean age = 20.96) and non-users of illicit drugs (57, 14 male, mean age = 20.91) and observed that after controlling for sleep measures, ecstasy users still showed deficits in performance on the CWFT.

An oral variant of the CWFT is also frequently used as a measure of word fluency, known as the Controlled Oral Word Association task (COWA) or the FAS task, this usually involves participants orally producing words beginning with F, followed by words beginning

with A and then S in one minute periods. Results using this variant of the task have provided less consistent findings. Halpern *et al*. (2004) observed no performance differences on this task between heavy users, moderate users and nonusers (sample described in Chapter 3.2.1). Bedi and Redman (2008) found no differences at the group level (45 ecstasy users, 48 cannabis only users, 40 legal drug users described in Chapter 3.2.3) for COWA, and MDMA use variables did not predict word fluency performance in a regression analysis. Morgan *et al.* (2002) used the COWA task with 18 current heavy ecstasy users who also used other illicit drugs, 15 abstinent (at least 6 months) heavy ecstasy users, who continued to use other illicit drugs, 16 polydrug controls who had never taken ecstasy but had similar pattern of other drug use and 15 drug naïve controls (sample described in Chapter 3.2.2). Statistical analysis revealed no significant between group differences on this measure of verbal fluency, although there was a trend for ecstasy user groups to perform worse on a category fluency task whereby participants had 90 seconds to name as many fruits as possible followed by 90 seconds to name as many vegetables as possible.

Bhattachary and Powell (2001) observed ecstasy-related deficits in word fluency using the COWA. In this sample, participants were divided into groups of nonusers (n = 20, mean age = 22.1), novice users (n = 18, mean age = 23.6, 1-5 lifetime doses) regular users (n = 26, mean age = 23.8, modal lifetime doses $\geq$ 51) and currently abstinent users (n = 16, mean age = 24.6, modal lifetime doses $\geq$ 51). All three MDMA user groups performed significantly worse than non-users on this task. Hanson and Luciana (2004) also compared ecstasy users (n=26, 14 male, mean age = 21.3, mean occasions of use = 64.3) and individuals with no history of MDMA use (n=26, 14 male, mean age = 20.7); Although users and non-users had equivalent performance in number of words generated, it was observed that ecstasy users produced more rule-breaking errors. Furthermore in an exploratory analysis of MDMA users with MDMA abuse/dependence (as described by the DSM-IV) verses non-

problem MDMA users, it was observed that those with a clinical diagnosis produced significantly fewer words than those without. Hanson and Luciana (2010) again assessed this function using the COWA in polydrug users and controls observing no between group differences on verbal working memory. Due to the polydrug user group in this study also containing participants who had never used ecstasy, regression analyses were conducted to observe contributions of individual drugs on the neuropsychological measures. It was concluded that MDMA did not correlate with performance on verbal working memory. Croft *et al.* (2001) assessed COWA along with word fluency of 'animals' in their study on 31 drug naïve controls (mean age = 23.5), 11 ecstasy/cannabis users (mean age = 25.7, MLD = 41.9 tablets) and 18 cannabis only users (mean age = 26.6). No differences were observed between drug user groups on this task, however when drug user groups were combined and compared to nonusers it was observed that controls performed better on the 'animals' component of word fluency. After covarying for total cannabis use, total MDMA use, frequency of cannabis use and frequency of MDMA use the authors concluded that the effects observed may be more related to cannabis use than MDMA use.

More recently Raj *et al.* (2010) assessed 16 polysubstance users (10 male, mean age = 23.6, 12 with a history of MDMA use, mean episodes of use = 43.33) on a task that probed semantic verbal memory in a preliminary fMRI study. This task consisted of a word and pseudo-word encoding period and a word and pseudo-word recognition period. During each encoding phase participants were required to memorise a group of five English words and or a group of five pronounceable pseudo-words. After this they were presented with homophone pairs (e.g. prey/pray), one of which was novel, the other of which would have appeared in the encoding period. Participants had to identify which of the homophones was previously presented. Pseudo-words were Dutch words, and pseudo-word homophone counterparts were synthesised English words based on pronunciation. English words in the encoding phase

should have a greater semantic association for participants and as such the difference between words and pseudo-words should isolate semantic memory. Correlations between drug use and performance on task measures (correct responses, omission errors, commission errors and reaction time for correct responses) revealed that MDMA was not associated with recognition task measures. However total lifetime cannabis joints was significantly negatively correlated with accuracy (number of commission errors) on the pseudo word part of the task. Moreover, lifetime exposure to cannabis (both episodes and total joints) was significantly negatively correlated with reaction time on the word part of the task. However due to the nature of this preliminary study and the small sample used, the results need to be treated with caution. The results from fMRI will be discussed in Chapter 5.

In summary it appears that there is evidence for ecstasy-related deficits in word fluency using the written variant of the CWFT. However one of these studies (Montgomery *et al.* 2007) includes data from their 2005 study that observed deficits in this task. The results are less consistent when the task takes on an oral format. However using the FAS one minute version, does not provide a variant of difficulty like the CWFT does, and as was observed by Montgomery, Fisk, Newcombe and Murphy (2005), ecstasy users tend to perform worse in more difficult circumstances when greater load is placed on the central executive. Further to this, it may be that over a short period of time (1 minute) ecstasy users perform comparatively to controls. Perhaps longer periods (such as 4 or 5 minutes in the CWFT) of sustained load, on the central executive, may uncover effects of difficulty and load. Using the short variant COWA, Hanson and Luciana (2004) observed performance deficits in ecstasy users. However this was only with number of rule-breaking errors rather than performance on fluency measures. This may reflect ecstasy users' difficulty in following instructions rather than deficits in access. Furthermore these results were not replicated in a follow up study (Hanson & Luciana, 2010). The RLG studies reviewed show mixed results, with two

suggesting performance deficits in ecstasy users (Murphy *et al.*, 2011; Wareing *et al.*, 2000) and others suggesting no such observable differences (Fisk *et al.*, 2004; Montgomery, Fisk, Newcombe & Murphy, 2005). Moreover, Murphy *et al*. (2011) suggest that performance is more correlated with cannabis use. Cannabis use was also put forward as a greater predictor of performance on the COWA by Croft *et al.* (2001) and in a novel semantic task by Raj *et al.* (2011), so it would seem that the relationship between this function, MDMA and cannabis use needs further exploration.

This thesis will fully investigate the role of ecstasy in access to semantic long term memory. The addition of neuroimaging techniques may provide valuable insight into the nature of processing mechanisms involved in this executive function and untangle the inconsistent evidence in the neuropsychological literature. This function is assessed behaviourally and with EEG in Chapter 6 and with fNIRS in Chapter 8.

Table 3.1: Summary of studies involving behavioural assessment of executive function in ecstasy users

Function/Authors	Sample Details	Task	Main Findings
Switching			
Fox *et al.*(2001)	20 'problem' ecstasy users, 20 'non-problem ecstasy users', 20 polydrug controls	WCST	No performance differences between groups, and no effect of lifetime dose on performance.
Thomasius *et al.* (2003)	30 current ecstasy users, 31 former users (abstinence of at least 5 months), 29 polydrug controls, 30 drug naïve controls	WCST	Polydrug controls produced significantly higher amount of perseverative errors than both ecstasy groups. No other differences observed.
Back-Madruga *et al*. (2003)	22 ecstasy users, 28 controls	WCST	No performance differences observed on WCST
Halpern *et al*. (2004)	23 ecstasy users with minimal exposure to other drugs, 16 ecstasy naïve controls involved in rave subculture	WCST	No significant performance differences on WCST. However after subdividing ecstasy users into heavy and moderate users, heavy users performed worse than non-users on "total categories".
Halpern *et al*. (2011)	52 ecstasy users with minimal exposure to other drugs, 59 ecstasy naïve controls involved in rave subculture	WCST	No significant between groups differences. However after subdividing ecstasy users into heavy and moderate users, moderate users showed significantly reduced "total categories" score compared to controls.
Fox *et al.* (2002)	20 ecstasy polydrug users, 20 polydrug controls	3D IDED task	No between groups differences on non-reversal trials. However ecstasy users slower than controls at all levels of reversal condition indicating performance deficits.
		Switching Go/NoGo	No between groups differences observed on any of the measures analysed on the task
Montgomery, Fisk, Newcombe and Murphy (2005)	51 ecstasy polydrug users, 42 non-user controls	Number-Letter task	No differences between users and non-users on this task
		Plus-Minus task	No between-groups differences observed on this task.

Dafters (2006)	18 ecstasy users, 15 ecstasy users who completed task in reverse order, 17 cannabis only users, 18 drug naïve controls.	Stroop task	Ecstasy users performed worse than both cannabis users and non-drug user groups on the switching component of the task, after covarying for other drug use
Dafters *et al.* (1999)	24 recreational drug users	BADS rule shift cards test	MDMA use was negatively correlated with performance on the task
Dafters *et al.* (2004)	16 heavy ecstasy (+50 tablets) and cannabis users, 19 light ecstasy (-50 tablets) and cannabis users, 15 cannabis only users, 19 drug naïve controls.	BADS rule shift cards test	No significant differences between groups after covarying for alcohol, amphetamine cocaine and LSD
Von Geusau *et al.* (2004)	26 ecstasy users, 33 non-user controls	Dots-Triangles and Local-Global tasks	Male users significantly higher switch cost reaction time than non-users in the dots triangles task, although significantly more accurate. On the Local-Global task male users were significantly slower than controls and had a higher switch cost. However there was no significant difference in accuracy on this task.
Inhibitory Control			
Morgan *et al.* (2002)	18 current ecstasy users, 15 former ecstasy users (abstinent for at least 6 months), 16 polydrug controls, 15 drug naïve controls.	Stroop task	No between groups differences (error or reaction time).
Dafters (2006)	18 ecstasy users, 15 ecstasy users who completed task in reverse order, 17 cannabis only users, 18 drug naïve controls.	Modified Stroop task	No significant between groups differences on the magnitude of Stroop interference reaction times (pre potent response inhibition). However ecstasy users showed a reduced priming effect (reduced short term residual inhibition) compared to both other groups.
Back-Madruga *et al*. (2003)	22 ecstasy users, 28 controls (not specified)	Stroop task	No between groups differences observed.
Gouzoulis-Mayfrank *et al*. (2000)	28 ecstasy users, 28 cannabis users, 28 drug naïve controls	Stroop task	No between groups differences observed.

Halpern *et al.* (2004)	23 ecstasy users with minimal exposure to other drugs, 16 ecstasy naïve controls involved in rave subculture	Stroop task	Ecstasy related performance deficits observed after dividing users into heavy and moderate users, whereby heavy users showed significantly longer reaction times and more Stroop errors on interference trials compared to controls.
Halpern *et al.* (2011)	52 ecstasy users with minimal exposure to other drugs, 59 ecstasy naïve controls involved in rave subculture	Stroop task	No ecstasy related performance deficits on the Stroop task.
Wagner *et al.* (2012)	Longitudinal study on 149 new ecstasy users (<5 MDMA use occasions before participating in the study). One year follow up yielded 43 interim non-users, and 23 regular ecstasy users. Remaining participants excluded from follow up analysis	Stroop task (German variant)	No significant performance differences between groups, or between baseline and follow up sessions
Wareing *et al.* (2000)	10 ecstasy, users, 10 former users, 10 non-users	RLG	Ecstasy users (both groups) performed worse than controls (more vowel intrusions at all 3 rates, and higher redundancy and lower number of letters produced at the 1s rate). Ecstasy users perform worse when greater demand is placed on them
Fisk *et al.* (2004)	44 ecstasy users, 59 non-users controls	RLG	Ecstasy users unimpaired on all measures of performance relative to controls.
Montgomery, Fisk, Newcombe and Murphy (2005)	51 ecstasy polydrug users, 42 non-user controls	RLG	Ecstasy users show improved performance, producing significantly more letters than controls. Differences on other performance measures on this task were non-significant.
Murphy *et al.* (2011)	15 ecstasy users, 12 cannabis only users, 12 drug naïve controls	RLG	No between group differences observed for alphabetic sequences or repeat sequences (measures of impulsivity). Ecstasy use did not predict performance on these measures. However differences were observed on "redundancy" which relates more to access.

Clark *et al.* (2009)	46 ecstasy users, 14 former users (abstinence of at least 1 year), 15 cannabis users, 19 drug naïve controls	IST	Cannabis users opened significantly less boxes than current ecstasy users, and this difference was approaching significance with former users and drug naïve controls
Gouzoulis-Mayfrank *et al*. (2003)	60 ecstasy users (30 heavy users, 30 moderate users), 30 non-user controls	Go/NoGo	Ecstasy users split into heavy and moderate users. No performance differences observed between groups on this task
Roberts and Garavan (2010)	20 ecstasy users, 20 drug naïve controls	Go/NoGo	No between groups performance differences observed
Hanson and Luciana	52 polydrug users, 29 non-drug controls	Go/NoGo	No MDMA related deficits observed for Go/NoGo
Hoshi *et al.* (2007)	25 ecstasy users, 28 former users, 29 polydrug controls, 27 drug naive controls	Go/NoGo	Ecstasy users and polydrug users impaired on this task compared to former ecstasy users and drug naïve controls.
Updating			
Montgomery, Fisk, Newcombe and Murphy (2005)	27 ecstasy users, 34 non-users	Letter updating	Ecstasy users performed significantly worse than controls
		Computation span	Ecstasy users performed significantly worse than controls
Montgomery and Fisk (2008)	73 ecstasy users, 73 non-user controls	Letter updating	Ecstasy users with simple spans of five and six performed worse than non-users. Higher levels of ecstasy use were associated with poorer performance on the task, whereas indicators of cocaine and cannabis use were not correlated with updating performance
		Spatial updating	Ecstasy users with spatial span of five, performed significantly worse than controls. Heavy ecstasy use was correlated with poorer performance
Fisk *et al.* (2004)	44 ecstasy users, 59 non-users controls	Computation span	Ecstasy users performed significantly worse than controls after covarying for cannabis, amphetamine, cocaine, alcohol use and daily cigarette use.

Wareing *et al.* (2004)	42 ecstasy users, 17 former users (abstinent for at least 6 months), 31 non-user controls	Computation span	Performance worse for both ecstasy groups compared to controls
Wareing *et al.* (2005)	36 ecstasy users, 12 former users, 31 non-user controls	Computation span	Ecstasy users and former users both performed significantly worse in computation span task performance.
		Spatial updating	Both groups of ecstasy users performed significantly worse than non-users.
Hanson and Luciana (2010)	52 polydrug users, 29 non-drug controls	SDRT	Polydrug users had poorer spatial memory spans, and were more negatively impacted by increasing delay intervals than controls. Working memory summary score was negatively correlated with average number of ecstasy tablets consumed per session, as well as maximum number of tablets ever taken in one session
Reay *et al.* (2006)	15 ecstasy users, 15 polydrug controls	Backwards digit span	No performance differences observed after controlling for cannabis, cocaine, alcohol and tobacco
Gouzoulis-Mayfrank *et al.* (2003)	60 ecstasy users (30 heavy users, 30 moderate users), 30 non-user controls	Backwards digit span	No performance differences between heavy ecstasy users, moderate users and non-users
		N-back	No between group differences observed
Gouzoulis-Mayfrank *et al.* (2000)	28 ecstasy users, 28 cannabis users, 28 drug naïve controls	Backwards digit span	Ecstasy users performed significantly worse than drug naïve and cannabis matched controls
Nulsen *et al.* (2011)	11 ecstasy users, 13 polydrug controls, 13 non-drug controls	Backwards (and forwards) digit span	No main effect of group on performance
Croft *et al.* (2001)	11 ecstasy/cannabis users, 18 cannabis only users, 31 drug naïve controls	Backwards (and forwards) digit span	Significant differences between drug naïve controls and a combined (both MDMA/cannabis users and cannabis only users) drug user group were observed. No difference between ecstasy/cannabis users and cannabis only users. Covarying for MDMA consumption had little effect on results

Halpern *et al*. (2011)	52 ecstasy users with minimal exposure to other drugs, 59 ecstasy naïve controls involved in rave subculture	Backwards digit span	Ecstasy users showed modest performance deficits, scoring lower for correct repeated sequences.
Bedi and Redman (2008)	45 ecstasy users, 48 cannabis users, 40 legal drug use controls	Backwards digit span	No between groups differences observed. Lifetime ecstasy use showed weak negative semi-partial correlation with performance
Thomasius *et al.* (2003)	30 current ecstasy users, 31 former users (abstinence of at least 5 months), 29 polydrug controls, 30 drug naïve controls	Backwards digit span	No ecstasy related performance deficits
Bhattacharay and Powell (2001)	26 regular ecstasy users, 18 novice ecstasy users, 16 currently abstinent ecstasy users (abstinence of at least 30 days) and 20 non-user controls	Backwards digit span	No between groups performance differences
Daumann, Fimm *et al*. (2003)	11 heavy ecstasy users, 11 moderate ecstasy users, 11 non-user controls	*n*-back	No between groups performance differences
Daumann, Fischermann, Heekeren *et al.* (2003)	18 month longitudinal study with 30 ecstasy users at time 1, which reduced to 21 users at time 2. These were subdivided into those who had not consumed MDMA or amphetamine in the 18 month period (n=8) and those who continued to use ecstasy and amphetamine (n=9). The remaining four participants were excluded from analysis due to sporadic MDMA and amphetamine use between time 1 and 2	*n*-back	No performance differences between groups at baseline or follow up.

Access			
Montgomery, Fisk, Newcombe and Murphy (2005)	27 ecstasy users, 34 non-users	CWFT	Ecstasy users produced significantly less S letter and C letter words than non-user controls.
Montgomery *et al*. (2007)	36 ecstasy users, 63 non-ecstasy users	CWFT	Significant performance deficits observed in ecstasy users compared to non-users on a composite measure of word fluency (letter fluency – C letter words and S letter words combined). However, this study included data from the 2005 study
Heffernan *et al.* (2001)	30 ecstasy users, 37 non-user controls	CWFT and verbal/semantic fluency	Ecstasy users significantly impaired in verbal fluency, semantic fluency and verbal/semantic fluency
Fisk and Montgomery (2009)	117 ecstasy users, 53 cannabis only users, 57 non-drug controls	CWFT	Ecstasy users performed worse than both control groups in on the CWFT after controlling for sleep measures.
Halpern *et al*. (2004)	23 ecstasy users with minimal exposure to other drugs, 16 ecstasy naïve controls involved in rave subculture	COWA	No performance differences observed between heavy users, moderate users and non-users
Bedi and Redman (2008)	45 ecstasy users, 48 cannabis users, 40 legal drug use controls	COWA	No performance differences observed between groups. MDMA use also did not predict performance in regression analyses.
Morgan *et al.* (2002)	18 current ecstasy users, 15 former ecstasy users (abstinent for at least 6 months), 16 polydrug controls, 15 drug naïve controls.	COWA	No performance differences observed on the COWA. However a trend for ecstasy users to perform worse on a category fluency task.
Bhattacharay and Powell (2001)	26 regular ecstasy users, 18 novice ecstasy users, 16 currently abstinent ecstasy users (abstinence of at least 30 days) and 20 non-user controls	COWA	All 3 ecstasy user groups performed significantly worse than non-user controls

Hanson and Luciana (2004)	26 ecstasy users, 26 non-user controls	COWA	Equivalent performance between groups. However ecstasy users produced more rule breaking errors. Subsequent analysis between problem and non-problem MDMA users showed that problem users produced fewer words than non-problem users
Hanson and Luciana (2010)	52 polydrug users, 29 non-drug controls	COWA	MDMA use did not correlate with performance deficits
Croft *et al.* (2001)	11 ecstasy/cannabis users, 18 cannabis only users, 31 drug naïve controls	COWA	No differences were observed between drug user groups. However a combined drug user group (ecstasy user and cannabis users) performed worse than non-user controls on the 'animals' component of word fluency. After covarying for MDMA and cannabis use indices the authors concluded that the effects observed may be more related to cannabis use than MDMA use
Raj *et al*. (2010)	16 polysubstance users	Semantic verbal memory task	MDMA was not correlated with recognition task measures. However total lifetime cannabis joints was significantly negatively correlated with accuracy and lifetime exposure to cannabis (both episodes and total joints) was significantly negatively correlated with reaction time

Chapter 4: Neurophysiology and neuroimaging as indicators of cognition.

4.1 EEG

Physiological Basis of the EEG Signal

Neurons communicate via action potentials which are discrete voltage spikes generated in the cell body that travel down the axon to axon terminals where neurotransmitters are then released. Theoretically, two neurons with action potentials that are sent simultaneously via parallel axons that end in simultaneous firing would summate for a voltage recording at an electrode. However, this rarely happens. Instead, slight differences (at the microsecond level) in neuronal firing typically cancel out action potentials in different axons and are therefore not detectable from electrodes at the scalp. As such the potentials usually observed with an EEG reflect post synaptic potentials (Luck, 2005).

Post synaptic potentials are voltages that occur either after an action potential has travelled along the axon fibre to an excitatory synapse causing and excitatory postsynaptic potential, or an action potential travels along a fibre ending in an inhibitory synapse where hyperpolarization occurs, culminating in an inhibitory post synaptic potential (Speckman & Elger, 2005). Neurotransmitters bind to the membrane on the post synaptic cell which causes the opening or closing of an ion channel, resulting in a change in potential at the cell membrane (Luck, 2005). Unlike action potentials, whose durations are only milliseconds in length, postsynaptic potentials are longer, potentially lasting hundreds of milliseconds. If there is coherence between neurons (i.e. many receiving excitatory neurotransmitter and in a similar orientation), then post synaptic potentials may summate and their voltages will be measurable at the scalp (Schaul, 1998).

EEG recording and processing

EEG recording involves the measurement, amplification and recording of differences between fluctuating electrical field potentials over time (Kamp *et al.,* 2005). EEG recordings should adequately represent the spatial distribution of potentials over the scalp. As such, recording from several electrodes simultaneously is imperative. It is routine for a large number of electrodes to be placed over the scalp of a participant.

During a conventional EEG recording session an electrode cap is fitted to a participant's head and electrodes are placed into standardised positions in the electrode cap. A suitable conducting gel is used to form an electrolyte bridge between the participants head and each electrode. The placement of the electrodes usually corresponds to the Standardised International 10-20 system. This system is based upon internationally recognised anatomical landmarks on the skull and allows for consistency of electrode names and locations across research laboratories. The electrodes themselves have an input amplifier and measurement of electrical field potentials occurs here. The necessity of input amplification is due to relatively low signal voltage amplitudes at the scalp. Once the electrodes have acquired the EEG signal and it has been amplified it is necessary to convert the signal from a continuous analogue voltage to a discrete digital one that is compatible with a computer for display, analysis and storage of data (Luck, 2005).

As voltage is the potential for a current to move between two points, the signal at an electrode represents the difference between the voltage at an electrode site and a predefined reference electrode site (Luck, 2005). There are several ways in which a reference can be provided, including a common average reference representing the mean of all scalp electrodes, linked earlobes or mastoids or the vertex (Hagemann *et al.,* 2001), and Laplacian

calculations based on differences between an electrode and it's 'nearest neighbour' electrodes (Nunez *et al.*, 1997).

Event Related Potentials and components

If EEG as a general method is used to investigate brain reactions to a variety of stimuli, then Event Related Potentials (ERPs) can be globally understood as EEG changes that are time-locked to a stimulus (Lopes da Silva, 2005). They are defined in the time domain as electrical activity from the brain that is caused by a particular event or stimulus. As such ERPs are used to quantify the neurophysiological response to a stimulus and differences between groups and conditions can be observed (Duncan *et al.*, 2009).

ERPs have distinct advantages over measurements of speed and accuracy of motor responses, the first being that they provide a continuous measure of cognitive processing giving rise to the possibility of determining the stage at which processing is affected by experimental manipulation (Luck, 2005). The second is that they can provide a measure of processing of stimuli in cases where no behavioural response is required. For example attended versus ignored stimuli (Luck, 2005) such as that used in a Go/NoGo task. A disadvantage however is that functional significance of an ERP is not as clear as that of a behavioural response, which is perhaps why it is necessary to observe both.

Due to ERPs being small, a relatively large number of trials are necessary to measure them adequately. However due to the fact that ERPs are time-locked to an event, single ERP waveforms can be averaged together to create a grand average (mean) waveform. It is these grand-averaged waveforms that are usually presented in research papers, and they reflect more clearly defined positive and negative deflections that are understood as components (Luck, 2005).

ERP components are usually labelled in such a way that refers to their polarity (P = positive, N= negative) and their position in the waveform or time (for example P1 would refer to the first positive component). Some of these components have been described by Luck (2005) for example P1 is a very early positive going component, typically largest at lateral occipital electrodes, that usually peaks between 100 and 130ms; this appears sensitive to spatial attention and arousal and is not considered to be affected by top down processes. The N1 component follows the P1 wave and has two posterior components that peak usually around 150-200ms and again reflect spatial attention, although there is suggestion that this component is involved in discriminative processing. The second positive component P2 follows the N1 at anterior and central electrodes. This component is usually larger in trials that contain target features which are fairly simple, so this is an early processing stage. The P2 wave can be observed at anterior and central sites, and elicits a larger response to simple target features that are relatively infrequent (Luck & Hillyard, 1994). This component precedes the N2 and is suggested to be involved in the initial inhibition from further processing in target stimuli (Hansen & Hillyard, 1988).

The N2 and P3 components are more widely studied and as such will be described in more detail here. The N2 family has several studied components; a basic N2 can be observed with a repetitive non-target stimulus. However if other deviant stimuli are presented within a repetitive sequence then a larger amplitude of this basic N2 is observed. Task relevant deviants evoke a later N2 effect (sometimes referred to as N2b) which appears largest over central (for auditory stimuli) and parietal (for visual stimuli) sites; this component is assumed to reflect stimulus categorisation processes (Luck, 2005). The N2 component is observed to be involved in inhibition as this component has been suggested to reflect stimulus discrimination (Ritter *et al.*, 1982) and is therefore an important measure of response inhibition. Kok *et al.* (2004) suggest the N2 component shows greater amplitude in trials

where inhibition of response is required (NoGo) than no inhibition (Go) trials. Moreover amplitude of N2 is more prominent in unsuccessful inhibition trials. The N2 component is associated with errors (i.e. "error negativity" or Ne), and is sensitive to monitoring errors. This has been suggested to be a product of activity in medial frontal regions such as the anterior cingulate (Bekker *et al.*, 2005).

The P3 wave follows the N2 wave, and can be subdivided into a frontally maximum P3a component that will increase with unexpected task irrelevant stimuli and P3b component that is largest over parietal sites. Usually when studies refer to the P3 component, it is actually the P3b that they are referring to. Typically cognitive impairment is associated with alterations to the P3 amplitude or latency due to the P3 being involved in higher level processing of stimuli. This component encompasses frontal-parieto network activation (Gaspar *et al.*, 2011) and in normal populations decreases in the amplitude potential reflects increased cognitive load, and diminished P3 reflects cognitive dysfunction. Longer latencies and smaller amplitude of the P3 response are indicative of cognitive impairment. The P3 component is also understood to be associated with the allocation of attentional resources necessary for information processing and also memory function (de Sola *et al.*, 2008).

Strengths and Limitations of EEG

Due to EEG employing a high sampling rate (usually 512Hz) it has excellent temporal resolution that allows tracking of neurophysiological processes at the neuronal rate (milliseconds) (Liu & He, 2010). As described earlier this affords a continuous measure of cognitive processing and direct understanding of how stimuli are processed and which stages of processing are affected by experimental manipulation. Furthermore the EEG signal is directly coupled to neuronal electrical activity (Debener *et al.*, 2006), as opposed to inferred indirect measurements of neuronal activity in neuroimaging methods that rely on

haemodynamic responses such as fMRI and fNIRS. EEG is considered to be much less expensive than other techniques (for example fMRI) and is non-invasive (Luck, 2005). Furthermore systems such as the one used for data collection in this thesis (Biosemi Ag-AgCl active-two electrode system - Biosemi B.V, Amsterdam, Netherlands) contain their own preamplifier, minimising electrode impedance, and this system is also portable and does not require electrical shielding.

A major limitation of EEG is that it has poor spatial resolution in comparison to haemodynamic measurement counterparts. This is due to the electrodes being separated from the source of the activity in the brain by cerebrospinal fluid, the skull and scalp (Nunez, 1981). The ambiguity of the location of active neurons is known as the inverse problem (Michel *et al.,* 2004). Many complex mathematical algorithms have been developed to attempt to solve the so called inverse problem, however many of these are constrained by a priori assumptions on the generation of EEG signals (Michel *et al.,* 2004). It is suggested that the measurements contain inadequate information about the generators of the activity and as such a perfect localization tomography does not exist (Pascual-Marqui, 1999).

4.2 Functional Near-Infrared Spectroscopy (fNIRS).

Introduction and Physiological Basis of the Technique

fNIRS is a novel non-invasive optical neuroimaging technique, that is portable and is used to measure the haemodynamic response to brain activation (Leff *et al*., 2011). This is an indirect neuroimaging measurement based on the assumption that neuronal activity and cerebral blood flow are tightly coupled (Holper *et al,* 2009; Villringer & Dirnagl, 1995). More specifically fNIRS can be used to measure oxygenation changes to oxygenated haemoglobin (oxy-Hb) and deoxygenated haemoglobin (deoxy-Hb) (Jobsis, 1977), by shining light in the near infrared range (700 – 900nm) directly onto the tissue of a

participant's forehead. Oxygenated and deoxygenated haemoglobin have characteristic optical properties in this light range (Izzetoglu *et al.,* 2004) and chromophores can change in concentration with oxy and deoxygenated haemoglobin. Thus, light reflected back to a detector will be attenuated by an increase in chromophores. The differences in light attenuation can be attributed to the oxygenation changes in the haemoglobin. Typically this type of neuroimaging will penetrate to structures around 2-3 mm of the cortex underlying the skull (Firbank *et al*., 1998). Therefore forebrain structures such as the dorsolateral prefrontal cortex (DLPFC) can be easily accessed and observed. Due to the DLPFC being prominent in higher level processing, and due to these structures being easy to access with this type of imaging, it has been used in several studies observing motor control and learning (Leff *et al*., 2011), as well as more complex tasks that involve working memory and category discrimination (Izzetoglu *et al.*, 2004).

The Modified Beer-Lambert Law

fNIRS raw signals are measurements of light intensity (Ayaz *et al*., 2011) and optical density is measured at two wavelengths (one for oxy-Hb and one for deoxy-Hb). These are chosen (within the 700-900nm range) based on intensities whereby oxy-Hb and deoxy-Hb are the dominant chromophores (absorb the majority of light) compared to other tissue chromophores (Ayaz *et al.,* 2011). The isosbestic point (around 805nm) is the point at which oxy-Hb and deoxy-Hb absorption spectrums cross. Hence a wavelength above this is used to assess oxy-Hb changes (850nm in this case) and a wavelength below the isosbestic point is used to maximally assess deoxy-Hb changes (730nm). The Modified Beer-Lambert Law is used to calculate relative changes in oxy-Hb and deoxy-Hb from baseline. It is worth noting that levels of oxy-Hb and deoxy-Hb (given as μmolar) are calculations that are relative to baseline only, and it is not possible to derive absolute values of concentration with fNIRS.

Optical density at a specific wavelength is calculated using the following formula (Modified Beer-Lambert Law):

$$OD_\lambda = \log\left(\frac{I_{in}}{I_{out}}\right) \approx \varepsilon_\lambda . c . d + G$$ (from Ayaz *et al.*, 2011).

Whereby wavelength (λ) is equal to the logarithmic ratio of input light intensity (Iin) and output (detected) light intensity (Iout). This (optical density – OD) is also related to the concentration (c) and extinction coefficient (e) of chromophores, as well as the corrected distance between light source and detector (d) and a constant attenuation factor (G) (Ayaz *et al.*, 2011).

Changes in chromophore concentration lead to changes in optical density, thus by calculating optical density changes at two wavelengths (850 and 730nm), and by applying known extinction coefficients of oxy-Hb and deoxy-Hb at each of those wavelengths, concentration changes in oxy-Hb and deoxy-Hb can be determined (Boas *et al.*, 2001).

Data Collection and Sensor Placement:

Conventional fNIRS recording requires a fNIRS headband that has several light emitting diodes and sensors embedded in it. This is placed over the participant's forehead, ensuring that the sensors make contact with the skin (any hair preventing contact between sensor and skin should be moved) and that there is no ambient light leakage (to this end a further headband may be placed over the fNIRS headband to limit light leakage). The locations of sensors (voxels) can be observed in Figure 4.1.

Figure 4.1. Voxel placement for fNIRS

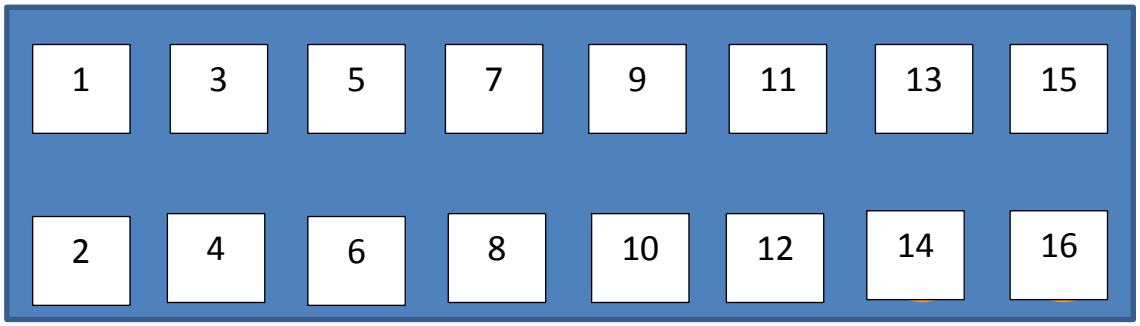

Fig 4.1: Depicts the sensor placement for the fNIRS headband. Odd numbers correspond to superior parts of the PFC, even numbers correspond to inferior parts. Placement at 1 starts over the left side of the brain, with voxels 1-4 referring to the left DLPFC, voxels 5-8 refer to left medial PFC, voxels 8-12 refer to the right medial PFC and voxels 13-16 refer to the right DLPFC.

Advantages and Limitations

There are a number of distinct advantages for studying the cortical response to behavioural tasks using optical neuroimaging methods such as fNIRS. Firstly, as described earlier the prefrontal cortex (PFC) which is understood to subserve executive functions and is densely innervated with serotonergic axons, is particularly accessible using fNIRS (Leff *et al.*, 2011) due to these areas directly underlying the fNIRS sensors (optically accessible). The technology is also portable (only a headband is necessary to attach sensors to the forehead) and robust to movement artefacts, affording measurement of realistic everyday tasks (Leff *et al*., 2011). Movement artefacts are apparent if there is a sustained dip of the head due to gravitational effects on blood flow. However, unlike EEG the data is not affected by blinks, body movements or vocalisation. This allows for verbal and written responses to tasks to be measured. It is also not as restrictive as other haemodynamic measures (fMRI) that require participants to be confined to a set space, and is far less susceptible to movement artefacts compared to fMRI. Another important advantage in comparison to fMRI is that fNIRS

obtains information about oxygenated haemoglobin as well as deoxygenated haemoglobin, as opposed to just deoxy-Hb, as is the case with fMRI (Schecklemann *et al.,* 2010). Although deoxy-Hb is more closely linked to the BOLD response, oxy-Hb is understood to be the best indicator of activation in fNIRS. Furthermore total haemoglobin (Hbt) can be calculated and as such fNIRS gives a multi-dynamic haemoglobin response (Leff *et al*., 2011). A further advantage of fNIRS is that it is relatively low cost, flexible, portable and user friendly (Holper *et al.,* 2009).

fNIRS does also have several disadvantages, primarily that in comparison to fMRI it has relatively poor spatial resolution. Although placement is usually in line with the standardised 10-20 system of anatomical landmarks, it measures cortical activation but does not provide anatomical information about the brain like fMRI (Leff *et al.,* 2011). Furthermore the measurements are confined to relatively superficial cortical areas, as such positioning of the device determines the regions that are examined (Ehlis *et al*., 2008). Moreover fNIRS has relatively poor temporal resolution in comparison to EEG (Ehlis et al., 2008).

Summary

In summary, EEG and fNIRS can provide robust data which details neural and haemodynamic responses to activation. Each method has considerable strengths which are important for the current investigation. The excellent temporal resolution and direct measurement of neuronal activity that EEG affords provides much needed information about the stages of processing that may be affected. Moreover, haemodynamic response to cortical activation in areas of the PFC (which is hypothesised to be damaged with ecstasy use) can be explored with fNIRS. Taken together, these techniques will provide a wealth of information about how cognitive processing may be affected by use of ecstasy. EEG and fNIRS data are implemented in this thesis to investigate functional atypicalities of cognitive processes in

ecstasy users relative to controls. ERP analysis is relatively underused for assessing executive function in ecstasy users, and as such is implemented to achieve a holistic understanding of the contribution of processing deficits in each executive function. fNIRS, to the author's knowledge, has not been used to assess cognitive deficits in ecstasy users, as such this is a novel approach to the investigation of executive functions in ecstasy users as well as the haemodynamic response to multi-tasking.

Chapter 5: Review of Neuroimaging Studies in Ecstasy Users

Executive function and other cognitive deficits were reviewed in Chapter 3. Given the dense innervation of serotonin neurons in prefrontal areas, it is understood that such deficits may be mediated by MDMA related damage to the serotonin system. It is believed that repeated use of MDMA will lead to serotonergic neurotoxicity, or down-regulation of serotonin receptors. Indeed, in the animal literature MDMA has been observed to be toxic to serotonin neurons (see Ricaurte *et al.*, 2000 for a review). In humans direct investigation of serotonergic neurotoxicity is difficult. However, various neuroimaging methodologies have been employed to investigate the integrity of the serotonin system. This chapter reviews the literature from neuroimaging studies in ecstasy users. It is important for this thesis to better understand how MDMA use may affect neuronal activity, as atypicalities in neuronal function are a more sensitive indicator of potential MDMA related neurotoxicity than residual cognitive performance indicators. Each indicator of neuronal activity will be described at the outset of each subchapter and the relationship between MDMA use and neuronal integrity will be discussed.

5.1 Single-Photon Emission Computed Tomography (SPECT)

Single-Photon Emission Computed Tomography (SPECT) is a neuroimaging method that utilises radioligands for the labelling of serotonin transporters (SERT) in the brain, so that they can be tracked and densities of these receptors can be assessed. Owing to the 5-HT transporter being understood as a structural element of the 5-HT neuron, it is a putative reliable marker of the integrity of 5-HT neurons (Reneman, Booij, Majoie *et al.*, 2001). Moreover, lower densities of 5-HT receptors observed with SPECT may reflect damage to the serotonin system, via MDMA use.

The effect of MDMA use on cortical 5-HT$_{2A}$ receptor densities was assessed using SPECT by Reneman, Habraken *et al.* (2000). In this preliminary study, the radioligand [123I]R91150 was administered intravenously to 10 MDMA users (7 male, mean age = 27, MLD = 139 tablets, abstinent for at least 2 months), 5 former users (4 male, mean age = 24, MLD = 218 tablets), and 10 healthy drug naive control subjects (4 male, mean age = 23). Mean cortical 5-HT$_{2A}$ receptor binding ratios were calculated (average of left and right frontal, parietal and occipital binding of [123I]R91150) and it was observed that the current MDMA user group had significantly lower binding ratios than controls and former users. The observed low cortical 5-HT$_{2A}$ receptor densities in the current user group are suggested to be due to downregulation of receptors owing to MDMA induced 5-HT release. However the increase in cortical 5-HT$_{2A}$ receptor densities (approaching significance) in the former user group compared to controls is suggested to be due to upregulation of post synaptic 5-HT$_{2A}$ receptors as a result of low synaptic 5-HT caused by MDMA induced serotonergic neurotoxicity.

The same research group (Reneman, Booij *et al.*, 2000) investigated whether MDMA use produced alterations to post-synaptic 5-HT$_{2A}$ receptors and memory function, by administering the same radioligand [123I]R91150, as well as a verbal memory test (Rey Auditory Verbal Learning Test – RAVLT) to 5 MDMA users (4 male, mean age = 23.6, MLD = 218, mean time since last dose = 4.6 months) and 9 age and education matched healthy controls (4 male, mean age = 22.8). SPECT imaging results revealed that overall binding ratios were higher in the MDMA user group. However this only approached significance in the occipital cortex. Again it is suggested that the higher density of 5-HT$_{2A}$ receptors, reflects upregulation of postsynaptic 5-HT$_{2A}$ receptors as a result of 5-HT depletion. Furthermore, performance on the memory task was significantly reduced in MDMA users relative to controls and this was correlated with 5-HT$_{2A}$ receptor binding in the MDMA group.

The authors suggest that these results reflect memory deficits that are attributable to MDMA induced 5-HT deficits.

Serotonin transporter densities were examined in 22 current MDMA users (11 male, mean age = 26.2, MLD = 485 tablets, mean time since last use = 2.4 months), 13 former users (8 male, mean age = 25.3, MLD = 268 tablets, mean time since last use = 29 months) and 13 controls (7 male, mean age = 25) by Reneman, Lavalaye *et al.*, (2001). This study used the radioligand: iodine 123-labeled 2β-carbomethoxy-3β-(4-iodophenyl) tropane ([^{123} I]β-CIT). SERT and memory function (using the RAVLT) were assessed to observe if there were correlations between the two and whether prolonged abstinence could lead to recovery of any observed deficits. Current MDMA users displayed lower cortical [^{123} I]β-CIT binding than controls, however no significant differences in binding were observed between former users and controls. Immediate and delayed recall performance on the RAVLT was poorer for both ecstasy user groups relative to controls. However this was not correlated with [^{123} I]β-CIT binding. It was concluded that the lower SERT densities in current MDMA users reflects neurotoxic effects, which may be reversible after cessation of use, whereas the effects on memory function may be long lasting. The same research group (Reneman, Booij, de Bruin *et al.*, 2001) used the same radioligand in a SPECT study to investigate the effects of sex, dose and long term abstention from use of MDMA on serotonin neurons. Fifteen moderate ecstasy users (9 male, mean age = males 25.6, females 22.7, MLD = 28.6 tablets, mean time since last use = 3.6 months), 23 heavy users (12 male, mean age = males 27.1, females 25, MLD = 530 tablets, mean time since last use = 2.3months), 16 former users (8 male, mean age = males 26.4, females 24.1, MLD = 268.1, mean time since last use = 29 months) and 15 controls (7 male, mean age = males 29.3, females 23.3) participated in this study. There were no between group differences in age, verbal intelligence or use of alcohol, tobacco and cannabis, although MDMA users reported more use of amphetamines and cocaine than

controls. [^{123} I]β-CIT binding ratios were significantly lower in female, but not male heavy MDMA users compared to controls and this was true for all brain regions analysed. The moderate user group showed equivalent binding ratios for males and females compared to controls. Females showed significantly lower binding ratios than males in the former user group, though this did not differ significantly to controls. Overall SERT binding and log transformed previous MDMA use were significantly correlated in females but not in males. These results suggest that MDMA use can lead to reductions in serotonin transporters that are dependent on gender, and level of use. The study also suggests that MDMA induced reductions in SERTs may be reversible after abstention in females.

Reneman, Booij *et al*. (2002) investigated the densities of nigrostriatal dopamine neurons in 29 ecstasy users (15 male, mean age = 26.1, MLD = 324 tablets), 9 ecstasy and amphetamine users (6 male, mean age = 22.1, MLD = 358 tablets) and 15 non-user controls (7 male, mean age = 26.1) using the radioligand [^{123} I]β-CIT. It is understood that MDMA may affect the dopaminergic system, and tablets sold as ecstasy may also contain known compounds that cause dopaminergic neurotoxicity, such as (meth) amphetamine. Between group comparisons revealed that [^{123} I]β-CIT binding ratios were significantly higher in the ecstasy user group compared to controls, whereas [^{123} I]β-CIT binding ratios were significantly reduced in the ecstasy + amphetamine group relative to ecstasy only users (but not compared to controls). Level use of ecstasy and amphetamine did not correlate with [^{123} I]β-CIT binding ratios. The authors suggest combined use of MDMA and amphetamine may lead to reduced dopamine transporter densities. However this is most likely to result from the amphetamine use, given that ecstasy alone does not appear to have an effect on dopamine neurons, whereas amphetamine has been observed to be neurotoxic to dopamine neurons in animal studies (e.g. Ricaurte *et al.*, 1984).

Chang *et al.* (2000) used SPECT to assess cerebral blood flow (CBF) in 21 ecstasy users (17 male, mean age = 43.4, MLD = 211 tablets) and 21 age, gender and socioeconomically matched controls (17 male, mean age = 43.7). MDMA users showed only slightly lowered global CBF (2.3%) and mild but not significant reductions in regional CBF. Differences in individual regions were not significantly different, and drug use variables (frequency, duration or recency) did not correlate significantly with global or regional CBF. This study also investigated acute effects of MDMA administration. Eight participants were administered MDMA on two separate occasions within one week and were scanned again 2-3 weeks later. Global and regional CBF SPECT scans post MDMA administration showed decreases in CBF in most brain regions compared to baseline and to controls. Decreased regional CBF was greatest in the caudate and superior parietal cortices, and right DLPFC. These decreases were more pronounced in participants who received larger doses of MDMA and most recently. However the data suggest that these effects may be transient.

More recently Klomp *et al*. (2012) suggested that age of first exposure may affect serotonin transporter densities. SPECT analysis of [^{123} I]β-CIT was conducted in 33 ecstasy users stratified for early exposed users (first use at between 14 and 18 years) and later exposed users (first exposure between 18 and 36 years). ANOVA revealed a significant effect of age at first use in midbrain [^{123} I]β-CIT binding ratios (age at scan, gender, duration of use and lifetime dose had no effect). There was also a strong significant negative correlation between age of first use and midbrain [^{123} I]β-CIT binding ratios in the early exposed group, but not the late exposed group. These findings suggest that MDMA affects the developing brain differently to the mature brain and MDMA's neurotoxic effect is dependent on the developmental stage of SERT and maturity of serotonin transmitter function.

The sustained effect of MDMA on SERT densities in novel ecstasy users was assessed by de Win *et al*. (2008a), using the radioligand [^{123} I]β-CIT. In this prospective study 188 ecstasy naïve participants with a high probability of ecstasy use were recruited, based on participants indicating intention to use in the near future. Participants underwent SPECT imaging at baseline and again in a follow up imaging session 12-36 months after baseline. By the follow up testing sessions, 59 participants that were still engaged with the study had started to use ecstasy. This incidental ecstasy user group had a mean lifetime dose of six tablets (range of 0.5-80) with a mean time since last use of 18.7 weeks. From the initial cohort of participants, 56 that were still ecstasy naïve were selected as controls, matched with the ecstasy group for gender, age and cannabis use. At baseline the two groups did not differ significantly in their age, gender, verbal IQ, SERT polymorphism, smoking, alcohol use, or use of cannabis, cocaine and amphetamines. However at follow up the novel ecstasy users showed significantly increased consumption of alcohol, cannabis, cocaine and amphetamine compared to the persistent ecstasy naïve control group. No significant effects of MDMA on [^{123} I]β-CIT binding were observed, with no between group differences at baseline, or follow up, and no significant dose response effects of cumulative doses of MDMA on follow up outcomes.

In summary, the majority of the literature of SPECT imaging in ecstasy users suggests changes in SERT binding ratios. The evidence suggests that heavy users are likely to develop reductions in subcortical serotonin transporter densities (Reneman, Lavalaye *et al.*, 2001) and that there appears to be dose dependent transient reductions in SERT whereby females are more vulnerable than males (Reneman, Booij, de Bruin *et al*., 2001). Reneman, Booij *et al.* (2000) suggest increases in [123I]R91150 binding in ecstasy users, however this study was conducted in a small sample of users (n=5) and the results only approached significance in one area. Recency of last dose may also be of significance given that Reneman, Habraken *et*

al. (2000), observed lower [123I]R91150 binding ratios in recent ecstasy users compared to former users and controls. Moreover, in the subacute effects observed by Chang *et al.* (2000) decreases in CBF after ecstasy use were more pronounced in larger and more recent doses. Klomp *et al.*'s (2012) study suggests that age at first exposure may play a key role in the extent of serotonin transporter density reduction and Reneman, Booij *et al.*'s (2002) results suggest that MDMA may be a selective serotonin neurotoxin (with amphetamine more likely to be culpable for striatal dopamine receptor reduction). The majority of studies show reduced serotonin transporter densities following ecstasy use, which is a putative marker of 5-HT neurotoxicity. The only study that observed no alteration in SERT binding between ecstasy users and non-users (de Win, Jager *et al.*, 2008a) was conducted on novel ecstasy users who had received relatively low doses of MDMA in comparison to other studies.

Evidence from SPECT studies suggests a reduction in SERT densities as a result of serotonergic neurotoxicity. If this is the case and neurotoxicity has occurred in the present sample, differences in behavioural performance as well as changes in processing and haemodynamic response to task may be observable in this thesis.

5.2 Electroencephalography (EEG)

EEG involves the measurement of electrical activity at the scalp, using electrodes that are placed all over a participant's head. Using this technique several methods can be used to investigate various neuronal responses. For example Event Related Potentials (ERPs) are an aggregate of post synaptic action potentials that are time locked to an event, whereas Event Related Synchronisation (ERS) or Event Related Desynchronisation (ERD) reflect increases or decreases in amplitudes of specific frequency bands in relation to an event.

Dafters *et al.* (1999) investigated the relationship between EEG variables (spectral power and coherence) and cognitive/mood variables with level of MDMA use with a 128

electrode EEG apparatus on 23 recreational MDMA users (mean age = 24 years). In this study, six resting (eyes closed) epochs of 60 seconds were recorded from each participant (with 20s re-arousal periods between each recording) for spectral and coherence analysis. Spectral analysis was conducted on one relatively artefact free 60s epoch after visual inspection. The results from spectral analysis revealed that level of MDMA use is positively correlated with an increase in alpha power across left frontal, left posterior and right posterior areas of the brain. Use is also positively correlated with beta power in the left posterior region and is negatively correlated with relative delta power over the whole scalp. Coherence analysis revealed weak but significant negative correlations between MDMA use and sites located over visual tracts (O1-T3, O2-T4). The authors suggest that these results (the desynchronisation of EEG activity – increased high frequency bands alpha and beta coupled with decrease in low frequency band delta) in MDMA users mimic results observed in ageing populations.

Gamma, *et al.* (2005) assessed the ERP P3 component in response to an inhibition task in 16 ecstasy polydrug users (8 male, mean age = 22.6, MLD = 270.2 tablets) and 17 controls (10 males, mean age = 26.0, less extensive drug use). ERPs were evoked by a Continuous Performance Test (CPT A-X), this is analogous to a Go/NoGo task whereby a participant in shown letters sequentially on a screen (A, B, C,D, E, F, G, H, J, L, X), and if an 'A' appears followed directly by an 'X' participants are to respond ("Go"). The presentation of 'A' acts as a cue inducing preparation of a motor response. However the response is to be inhibited if any letter other than 'X' follows. ERPs to the letter following 'A' were used for analysis and the size of the P3 response was calculated as the mean amplitude between 250 and 450ms. Midline electrodes Fz, Cz and Pz were used for analysis, as these had been shown previously to produce maximal P3 amplitude. The averaged P3 amplitude for the 4 quadrants of scalp potential field were also compared (using electrodes Fp1, F3, F7, FC5 and

FT9 for the anterior left quadrant, the corresponding electrodes on the right side of the scalp were used for anterior right quadrant, P3, O1, T5, CP5, TP9 and PO9 were used for the posterior left quadrant and the corresponding electrodes on the right side were used for the anterior right quadrant). The results showed that ecstasy users displayed a significantly reduced P3 in relation to NoGo trials at midline electrodes Fz and Cz. However after controlling for age, education and cannabis use, Fz became non-significant (p=.08). Quadrant analysis revealed one significant difference; in the posterior right quadrant ecstasy users displayed greater P3 positivity than controls – however this again became non-significant after controlling for confounders. There were no between group differences in latencies of P3. Moreover lifetime use of MDMA and cannabis did not correlate with ERP characteristics or performance on the task. Task performance was also equivalent between groups. The authors suggest that although lower P3 amplitudes in ecstasy users are consistent with higher levels of neuronal disinhibition, other results do not reflect disturbed inhibitory brain mechanisms.

Cognitive processing of ecstasy users was investigated using EEG by Mejias *et al.* (2005) using a visual oddball task. In this particular "oddball" paradigm, faces (2 women, 2 men) were presented to participants. These were either, neutral or emotional (happy or fearful). The neutral faces were the frequent stimuli (presented 84 times in a block of 100 trials), and the emotional faces were the infrequent/"oddball" stimuli that were presented 16 times in a block of 100 trials (8 x fearful, 8 x happy). This enables separation of attentional (preparation to process) and response related (preparation to respond) components of an ERP. Thus the N2 component (peaking around 250ms at occipital electrodes) indicates a switch of attention (to prepare) and the P3(b) component (occurring around 450ms at parietal sites) relates functionally to later stages of processing that are conscious, such as decision making and premotor responses. ERPs were recorded from 14 MDMA users (mean age = 24.64, MLD = 143.07 tablets) and 14 controls (mean age = 25.57, matched for scores for depression

and anxiety and cannabis use), whilst performing 16 blocks of the visual oddball task. MDMA users were slower than controls to respond to rare stimuli. Furthermore ecstasy users who had consumed upwards of 100 tablets were significantly slower to respond to rare stimuli than those who had consumed fewer than 100 tablets. A mixed ANOVA revealed that ecstasy users showed a greater latency of the P3b component compared to controls for rare stimuli. There were no differences in latency in the N2 or P3a components, or amplitudes at any measured component. When ERPs for happy vs. fearful faces were compared, there were no between group differences (amplitudes or latencies) for happy faces, whereas ecstasy users showed a greater P3b latency for fearful faces compared to controls. The authors suggest that these results reflect serotonergic neurotoxicity in MDMA users which manifests in attentional deficits. These are indexed neurophysiologically by a postponement of information processing at the attentional level to the decision level (P3b) in MDMA users.

Casco *et al.* (2005) investigated Visually Evoked Potentials (VEPs) to a simple discrimination task in eight heavy MDMA users (7 male, mean age = 28, MLD = 1054 tablets), eight moderate MDMA users (7 male, mean age = 25, MLD = 52.4 years) and 18 drug free controls (limited drug use, split into 2 sub-groups of 19-23 years n=9, 3 male and 24-32 years n=9, 5 male). This two-alternative forced-choice task comprised of digits being presented on a screen randomly (either 1 or 2); when the sequence is interrupted participants indicate whether the last digit that appeared on the screen was a 1 or a 2. There were no between group differences on performance on the task, although pairwise comparisons showed that heavy MDMA users made significantly more errors compared to drug free controls. Amplitudes and latencies of the following components; P100, N150, P200, N250, P300 and N400 were measured at electrodes Oz and Fz. Heavy MDMA users showed significantly reduced P200 and P300 amplitudes at Oz compared to controls. Moderate users also showed significantly reduced P300 amplitude relative to controls. No such differences

were found in latencies at this site. At Fz the P300 was significantly reduced in both heavy and moderate users compared to controls. Moreover the N250 component was significantly reduced in heavy users relative to controls (this was also approaching significance in moderate users). Latency was not significantly different at any component for this electrode. Since the groups do not differ in latencies of components it is suggested that processing speed is not affected in these ecstasy users. However reductions in amplitude at middle (exogenous) and late (endogenous) stages of processing are suggested to be evidence of altered cortical activity associated with low level cognitive processing. It was concluded that recreational use of MDMA is sufficient to cause neurotoxicity that is associated with subtle low-level cognitive deficits in humans.

Auditory ERPs and cognitive performance were assessed in a longitudinal study by de Sola *et al*. (2008). Fourteen ecstasy polydrug users (6 male, mean age = 25.2, mean total lifetime use = 207.4 at baseline) 13 cannabis users (5 male, mean age = 25.1, daily cannabis use or at least 25 times in lifetime) and 22 drug naïve controls (7 male, mean age = 24.3) were recruited. Three ecstasy users, four cannabis users and one drug naïve control had dropped out by the follow up experiment. ERPs were evoked by an auditory oddball paradigm and participants were required to count the infrequent stimuli. Grand averaged P3 amplitudes and P3 latencies were obtained at time 1 and 12 months later at time 2. There were no significant between group differences for P3 amplitude or P3 latency at time 1 or time 2. Correlations between MDMA use and P3 response were not significant at time 1 or time 2. However a significant correlation was observed between lifetime cannabis use and P3 latency at time 1, which was approaching significance at time 2 whereby greater cannabis use improved neuronal processing speed. Conversely a marginally significant correlation between cannabis use and P3 amplitude suggested increased lifetime dose is associated with lower P3 amplitude. Although reduced P3 amplitude and increased P3 latency in ecstasy users

compared to controls was consistent across time 1 and time 2, the results were marginal and non-significant and fail to provide evidence for neurotoxicity associated with MDMA use, though it is suggested that executive functioning tasks are more likely to be impaired at the cognitive level than simple attentional orientation tasks.

More recently Burgess *et al.* (2011) investigated verbal episodic memory ERPs in 15 ecstasy polydrug users (7 male, mean age = 24.1 mean lifetime MDMA uses = 138), 14 cannabis users (7 male, mean age = 21.9) and 13 non-illicit drug users (6 male, mean age = 22.3). Participants undertook two recognition memory tasks, which were identical apart from the type of stimuli; words (verbal) and faces (non-verbal). Over 90 trials participants had to indicate whether the presented stimulus was new (presented first time in the sequence) or old (had been repeated). The tests consisted of 40 stimuli that were repeated and 10 items that were shown only once. No between groups differences were observed on performance on the task. However for the word recognition memory task, Partial Least Squares (PLS) analysis on the ERP data identified a single latent variable that discriminated between correct new responses and correct old responses. A significant repetition effect was observed, with a more positive-going ERP for repeated words centred on the left parietal area maximal in the 500-700ms interval. There was a significant difference between groups in latent variable scores with ecstasy/polydrug users showing significantly reduced amplitude compared to non-drug controls, and this difference was approaching significance with the cannabis user group. This latent variable consisted of two ERP components that were extracted using singular value decomposition. These were identified as a left parietal recollection effect and a midline frontal familiarity effect. Component scores were compared between groups, the recollection component revealed a significantly reduced late positive ERP over left parietal sites in ecstasy users compared to both other groups. No between group differences were observed for the familiarity component. Latent component analysis on the face recognition task did not

provide any between group differences. It is suggested that ecstasy/polydrug users showed an attenuation of a neuronal response that is associated with the 'pure' cognitive process of verbal recollection.

Nulsen *et al*. (2011), observed differences in EEG activity in a cohort of 11 MDMA users (4 male, mean age = 22.9, MLD = 32.5 tablets), 13 polydrug users (4 male, mean age = 23.2) and non-users (4 male, mean age = 23.2), whereby during forward and backwards digit span tasks (described in Chapter 3.2.3), both control groups displayed a significantly reduced P3b in the digit backwards task (more difficult aspect of the task) than the digit forwards task. However the MDMA user group did not show this difference, yet this group displayed the greatest discrepancy between digit backward span and digit forward span. These results suggest that the ecstasy user group found this part of the task more demanding. The authors suggest that ecstasy users' performance was suppressed more by concurrent processing demands of the working memory task than controls, and the ERP data reflect this, showing a reduction in the cognitive resources allocated to processing.

On the whole, the majority of the studies reviewed reflect differences in electrophysiological data that are suggestive of alterations to cognitive processing associated with MDMA use. However there are several studies that fail to show ecstasy-related ERP atypicalities (de Sola *et al.,* 2008; Gamma *et al.,* 2005). Some studies have observed differences in latencies of a P3 component (Mejias *et al*., 2005). Others have observed reductions in P3 amplitude associated with ecstasy use, despite equivalent behavioural performance (Casco *et al*., 2005). Conversely, Nulsen *et al*. (2011) failed to observe reductions in P3 amplitude despite observing performance deficits. Other studies have shown alterations to late ERP components reflective of altered cognitive processing (Burgess *et al.,* 2011). The full extent of MDMA's effect on electrophysiological indices requires

clarification and there is a paucity of EEG data relating specifically to executive processes. As such this thesis aims to observe differences between MDMA users, polydrug controls and drug naïve controls on each executive function, on three well defined components of an ERP; the P2, N2 and the P3(b).

5.3 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is a non-invasive neuroimaging method that relies on a large cylindrical magnet to create a magnetic field around a subject's head. When a magnetic field is created, protons (mainly from H+ hydrogen ions that are abundant in living tissue) align with the same direction as this field (Weishaupt *et al.*, 2008). Radio frequency pulses are then sent to target nuclei (H+ ions) causing them to fall out of alignment (resonance). Following this, protons immediately begin to realign with the magnetic field. The realignment causes a radio frequency which can be received by the scanner. These are known as T1 scans and parameters can be adapted to obtain a high contrast between grey and white matter. As such these scans can provide valuable structural information. MRI is unable to measure neuronal activity, however functional MRI (fMRI) involves parameter manipulation to allow for brain function to be imaged whilst an action is being performed (Kennedy *et al*., 2002) (this will be discussed in more detail in chapter 5.5).

Chang *et al.* (1999) conducted MRI scans in a 1.5 T scanner on 21 subjects with a history of MDMA use (15 male, median age = 43, mean duration since last use = 4 months, average lifetime dose of 13.1 g) and 37 'normal' controls (22 men, median age = 38), and observed all images for MDMA users and controls to be normal with no significant brain atrophy or white matter lesions. Similarly Chang *et al.* (2000) observed normal MRI scans for 21 MDMA using subjects and 21 controls (as described 5.1) in a 1.5 T scanner.

Structural MRI scans of 31 MDMA polydrug users (5+ uses of MDMA and abstinent for at least 3 weeks) and 29 non-MDMA users (age and sex matched that had used a variety of drugs but not MDMA) were compared using voxel based morphometry (VBM) in a study by Cowan *et al.* (2003) to investigate regional brain grey and white matter concentration. It was hypothesised that ecstasy users may show reduced neocortical grey matter as a result of loss of serotonergic trophic effects on cortical cells. Using a 1.5 T scanner T1 weighted scans revealed multiple areas of reduced grey matter concentration in MDMA users relative to controls. The neocortical regions displaying significantly reduced grey matter concentrations included bilateral Brodmann Area (BA) 18 in the occipital lobe, BA 21 in the temporal lobe, and left BA 45 in the frontal lobe, as well as a midline region of the brain stem and bilateral areas of the cerebellum. These findings are interesting to observe if we consider the proposed functionality of these areas. Both BA 45 and BA21 in the neocortex are suggested to play an important role in semantic memory retrieval. This therefore has direct relevance to the current thesis, as this function will be examined with further neuroimaging techniques.

Reneman, Majoie *et al.* (2001) assessed eight ecstasy users (7 male, mean age = 27.6, MLD = 154 tablets, mean duration since last use = 14.6 weeks) and six non-users (3 male, mean age = 22.3 years) on conventional T1 and T2 weighted scans as well as diffusion and perfusion imaging (the results from which will be discussed in chapter 5.6). Perfusion MRI is conducted to assess the vasculature of the brain by calculation of cerebral blood volume (rCBV). Intravenous bolus injections of gadopentetate dimeglumine were administered to participants prior to T2 weighted echo planar scans to provide an endogenous tracer of arterial blood entering the brain (Keston *et al.*, 2003). No oedematous changes in the brains of ecstasy users compared to controls were observed. However the perfusion MRI data provide more interesting results, with ecstasy users having overall higher mean rCBV values than controls. This difference approached statistical significance in the globus pallidus. The

rCBV ratio in the globus pallidus also correlated significantly with extent of previous drug use, and did not correlate with age, sex or IQ values. The authors suggest that increased rCBV in the globus pallidus is a result of vasodilation that occurs in the absence of serotonin controlled vasoconstriction due to serotonergic depletion.

Schouw *et al*. (2012) conducted Pharmacological MRI to assess 5-HT dysfunction in ecstasy users (10 male; 50+ lifetime tablets; 7 healthy controls) by examining the haemodynamic response to injection of a selective serotonin reuptake inhibitor (SSRI – citalopram). MRI was conducted using a 3.0T scanner with pre-infusion anatomical 3DT1 weighted scans conducted for registration with pharmacologically labelled (post infusion) scans. Subtraction of post infusion/citalopram labelled arterial spin labelling (ASL) images from pre infusion/control ASL images yielded whole brain perfusion weighted images. MDMA users displayed a significant cerebral blood flow reduction in response to the 5-HT challenge, most prominent in the left thalamus. A significant decrease in CBF was also observed in the right occipital cortex and the right frontal cortex. Significant CBF increases were observed in the left globus pallidus and left frontal cortex. Controls showed minimal differences between the two scans. Mean whole brain CBF was significantly increased in ecstasy users compared to controls, and CBF was significantly decreased in the left thalamus and bilateral occipital lobe compared to controls after citalopram infusion. It is suggested that ecstasy users showed citalopram evoked haemodynamic changes in cortical regions and subcortical grey matter areas that contain high densities of serotonin receptors in normal populations, as a result of possible neurotoxicity.

Overall it appears that structural MRI scans have yielded little evidence for MDMA related changes to white matter areas (Chang *et al*., 1999; Chang *et al.,* 2000; Cowan *et al.,* 2003) perhaps suggesting that this measure is not sensitive enough to observe a physical

alteration to brain composition after MDMA use. However, reductions in neocortical grey matter have been observed in ecstasy users compared to controls in structural scans, typically in areas that are important for semantic retrieval (BA 21 and 45). Perfusion MRI was successful in identifying increases in cerebral blood volume in the globus pallidus in ecstasy users (Reneman, Majoie *et al.*, 2001). This is a substructure of the basal ganglia, which has been highlighted for its role in access to semantic memory (Copeland, 2003). Furthermore alterations of cerebral blood flow were also observed in this area in ecstasy users from pharmacological MRI (Schouw *et al.*, 2012), as well as CBF reductions in cortical regions and subcortical grey matter areas.

5.4 Magnetic Resonance Spectroscopy (MRS)

Proton Magnetic Resonance Spectroscopy (^{1}H MRS) is another non-invasive technique, that is similar to MRI in that it measures signals from hydrogen protons. However rather than structural information, MRS provides information about relative concentrations of CNS metabolites associated with structural brain integrity (Cowan *et al.*, 2007). These include the neuronal marker *N*-acetylaspartate (NAA) and the putative glial marker myoinositol (MI).

Chang *et al*. (1999) used MRS to evaluate neurochemical abnormalities in 21 MDMA users and 37 controls (as described in Chapter 5.3). Using a 1.5 T scanner, MRS was performed in mid-occipital (grey matter), mid frontal (grey matter) and right parietal (white matter) brain regions. The results showed that metabolite concentrations of NAA, creatine (CR) and choline compounds (CHO) were comparable in all three brain areas measured between ecstasy users and non-users. However ecstasy users showed elevated MI and MI to CR ratios in parietal white matter as well as MI and MI/CR in parietal white matter. Furthermore occipital grey matter positively correlated with MDMA use. The authors suggest

that the lack of NAA change (which is a marker considered sensitive to death or damage to neurons) could reflect down regulation of 5-HT neurons rather than damage, or potentially neuronal recovery. Similarly Liu *et al.* (2011) observed no significant increases in NAA between MDMA users (n = 25, 20 male, mean age = 25.04, MLD = 158.12 tablets) and drug naïve controls (n = 27, 17 male, mean age = 27.04) in the basal ganglia, whereas MDMA users displayed increases in MI concentrations. Correlations between CR concentration and MDMA dose were observed in the right basal ganglia.

Reneman, Majoie *et al*. (2002) investigated NAA/Cr, NAA/CHO and MI/Cr ratios in midfrontal grey matter, mid occipital grey matter and right parietal matter using (^{1}H MRS) in 15 male MDMA users (MLD = 723 tablets, mean time since last use – 12 weeks) and 12 age matched controls. Ecstasy users showed significantly reduced NAA/Cr and NAA/CHO ratios in frontal grey matter compared to controls. Frontal grey matter binding ratios were negatively correlated with MDMA use. No differences were observed in mid occipital grey matter or right parietal white matter between the two groups. These findings are difficult to reconcile with those reported by Chang *et al.* (1999) and suggest that reduced NAA/Cr and NAA/CHO ratios in the frontal cortex reflect neuronal abnormality. As the ecstasy users in Reneman, Majoie *et al.,* (2002) had a higher lifetime dose than Chang *et al.*, (1999), this could be an effect of dosage.

Obergreisser *et al.* (2001) investigated MDMA use on the hippocampus using (^{1}H MRS) in 6 ecstasy users (having at least 100 doses from 3-6 years, range = 120-350) and 5 age matched (26.6 years) controls. It was observed that there were no differences in hippocampal NAA or choline compounds between ecstasy users and non-users, consistent with Chang *et al*.'s (1999) findings. Daumann, Fischermann, Pilatus *et al.* (2004) conducted ^{1}H MRS in the left hippocampus, midfrontal and midoccipital cortex of 13 regular ecstasy

users (10 male, MLD = 324.54 tablets, mean time elapsed since last use = 47.38 days) and a non-MDMA control group (n=13, 10 male) that were matched for age, sex, level of education and cannabis use. No significant between group differences were observed in NAA/Cr ratios in any brain region observed. Furthermore there were no meaningful correlations between NAA/Cr ratios and drug use, or memory performance. It was concluded that ^{1}H MRS was a less sensitive measure of neurotoxicity in ecstasy users than cognitive measures. Similarly, de Win *et al.* (2008a) observed no significant effects of MDMA on brain metabolites (cohort and experiment explained in more detail in Chapter 5.1) in a longitudinal study, nor in a prospective cohort study on sustained effects of low dose ecstasy use on the brain in new ecstasy users (de Win *et al.*, 2007) (this study will be described in more depth in chapter 5.6)

Cowan *et al*. (2007) investigated absolute concentrations of NAA and MI in the occipital lobe in 9 MDMA users (at least 5 occasions of use, aged 18-35) and 7 non-MDMA controls matched for age and sex and had used a variety of other drugs. In this study a higher field proton strength MRS of 4T was used in an attempt to gain a more sensitive measure of neuronal disturbance. There were no statistical differences in absolute NAA or absolute MI levels in the occipital cortex between MDMA users and non-users. The authors concluded that these findings are not supportive of MDMA induced alterations to neurons or glia in the occipital cortex of this small sample of moderate MDMA users.

In summary, MRS studies suggest MDMA has little effect on NAA, which is an indirect measure of neuronal damage. However the samples in these studies are relatively small, furthermore the one study which did yield ecstasy-related differences had a cohort of MDMA users with far greater MDMA exposure than the other studies (Reneman, Majoie *et al.*, 2002). MRS has also been criticised for perhaps not being sensitive enough to detect small changes in NAA that are associated with low level recreational doses of MDMA, or

detect changes to 5-HT terminals only (Chang *et al*., 1999). Although increasing the field power made little difference in the study by Cowan *et al.* (2007) perhaps more work is necessary with larger samples of heavier MDMA users.

5.5 Functional Magnetic Resonance Imaging (fMRI)

Similar to MRI this functional technique relies on magnetic fields being created around a participant's head. However rather than T1 weighted scans (that have a high spatial resolution to give clear structural information) this technique relies on T2 weighted scans at a lower resolution to assess Blood Oxygen Level Dependent (BOLD) signal changes (Kennedy *et al.,* 2002). In basic terms radio frequencies that are delivered to cause transverse magnetisation (falling out of alignment of protons) also cause the spin axes of nuclei to tilt (precess) in phase. When the pulse is stopped, this phase precess will relax, and the time taken for all ions to fall out of phase is called T2. Increases in duration of time to repeat (time between radio frequency pulses and T2) and time to echo (time between radio frequency pulses and signal reception) give T2 weighted scans. T2 weighted scans favour imaging of water, as such by using paramagnetic contrast agents in the blood (deoxy-haemoglobin) blood flow and blood volume changes can be assessed (Huettel *et al.,* 2004).

Daumann, Fimm *et al*. (2003) investigated the cerebral activation during a working memory task using fMRI with 11 moderate ecstasy users (8 male, MLD = 27.3 tablets, mean time since last dose =330.09 days), 11 heavy users (8 male, MLD = 258.18 tablets, mean time since last use = 89.27 days) and 11 healthy controls, all matched for age, sex and level of education. Participants conducted an n-back task (behavioural results discussed in Chapter 3.2.3) in combination with fMRI. The fMRI results showed that all three groups showed significant and localised haemodynamic changes in prefrontal, parietal, occipital and cingulate brain regions. However there were no group differences in activation at any level of

the task at a conservative significance level ($p<0.05$ corrected). Whereas using a more liberal significance level ($p<0.01$, and $p<0.001$ uncorrected) heavy users showed weaker BOLD responses in left frontal and temporal regions on the most difficult level of the task (2-back) relative to the other two groups. Also, both user groups showed increased activation in the right parietal cortex with 1 and 2 back tasks. However extent of previous drug use did not correlate with BOLD signal changes. It is suggested that these results may reflect subtle brain functioning alterations associated with MDMA use, but to treat these results with caution. In a similar fMRI/n-back study, Daumann, Schnitker *et al.* (2003) studied BOLD activation in 8 pure ecstasy users (no regular use of any other drugs, mean age 25.30, MLD = 74.50 tablets), 8 polyvalent ecstasy users (concomitant use of ecstasy and amphetamines and cannabis, mean age = 26.41, MLD = 56.25 tablets) and 8 healthy controls (mean age = 25.55) in an attempt to control for concomitant use of other drugs. Performance on the n-back task was equivalent between the three groups and all groups showed typical cortical activation patterns during the task. However pure MDMA users showed reduced BOLD activation in the temporal gyrus and angular gyrus in the 1-back task compared to controls (polyvalent users did not differ significantly from controls). Moreover pure MDMA users had lower signal changes compared to polyvalent users in the striate cortex and higher BOLD response in the premotor cortex. At the more difficult 2-back level of the task, pure MDMA users showed lower activation than both other groups in the angular gyrus. It is concluded from these results that MDMA is associated with neuronal alterations that may reflect MDMA-induced neurotoxicity and that altered fMRI patterns are not associated with concomitant use of other drugs.

The same research group (Daumann, Fischermann, Heekeren *et al.*, 2004) conducted an 18 month longitudinal fMRI study, again using the n-back task in 30 ecstasy users (at time 1, this reduced to 21 users by time two). This ecstasy using cohort was then subdivided into

two groups based on their drug use in the interval between testing at time 1 and time 2; a group that were abstinent during the interval period (n=8, 6 male, mean age =24.50 MLD = 31.50 tablets, mean time since last dose = 487.5 days) and a group that continued to use ecstasy during the interval period (n=9, 5 male, mean age = 25.67, MLD = 149.44 tablets, mean time since last dose = 15.67 days). The results of the fMRI scan at time 1 suggest no differences in cortical activation between the two groups. At time 2 cortical activation patterns did not alter significantly for any level of the n-back task from baseline in the interim abstention group, whereas the continuing users showed increased activation from baseline in two clusters in the parietal cortex during the most difficult level of the task (2-back). Furthermore correlational analysis revealed that the increase in haemodynamic activation between time 1 and time 2 in the two clusters in the parietal cortex for the continuing users was associated with higher one night dose of MDMA. These results suggest that higher nightly doses may result in higher risk of neuronal damage. The authors also offer that neuronal damage in ecstasy users is long lasting, as the interim abstinent group did not differ (or improve) in their activation at time 2 compared to time 1, assuming that activation at time 1 was atypical.

Moeller *et al*. (2004) used fMRI to study activation during a working memory task in 15 MDMA users (12 male, mean age = 24.7, mean lifetime uses = 193.5, mean time since last use = 37 days) and 19 controls (11 male, mean age = 25.4). Participants undertook an fMRI scan whilst completing an immediate and delayed memory task. SPM99 random effects analysis showed that ecstasy users displayed significantly greater BOLD activation in three clusters of brain regions compared to controls in the delayed memory task. These clusters were; 1- left medial and superior frontal gyri with extending activation to the right medial superior frontal gyri, bilateral anterior cingulate gyri, and right middle frontal gyrus. 2- left thalamus extending to left caudate and putamen, left parahippocampal gyrus, left

hippocampus and left insula. 3- right hippocampal gyrus extending to the right hippocampus, right thalamus, right lentiform nucleus, right putamen, right insula, and right temporal cortex. Most of these effects remained after controlling for other drugs. However after controlling for cannabis, the effect was no longer significant in the prefrontal cortex. The authors suggest that the observed increase in activation of the BOLD signal could be due to MDMA users being less "efficient" at the working memory task, resulting in an increase in neuronal activity to perform at a similar level as controls. They also argue that increased BOLD fMRI activation in the hippocampus may be MDMA specific. Valdes *et al.* (2006) used the same subjects as those used by Moeller *et al.* (2004) and compared fMRI data with scores on the Barrat Impulsivity Scale. There was a significant correlation between activation in two clusters of the DLPFC and scores on the BIS. However there was no group by BIS interaction with DLPFC activation, suggesting that this activation is related to impulsivity independently of MDMA use. In line with Moeller *et al.* (2004), hippocampal dysfunction was observed in adolescent MDMA users, in an fMRI study by Jacobsen *et al.* (2004). Selective and divided attention and verbal working memory was assessed concurrently with fMRI in 6 adolescent MDMA users (average of 10 episodes of MDMA use, mean age at first use = 15.8, little use of other drugs other than cannabis and alcohol, mean age = 17.3) and 6 adolescents with no history of MDMA use (matched for age and gender). The two groups did not differ with regards to consumption of cigarettes, cannabis, years of education, estimated intelligence or self-reported depression or anxiety. Performance on tasks was equivalent, although MDMA users had significantly longer reaction times. MDMA users displayed significantly lowered hippocampal activity relative to controls during the working memory task. Correlational analysis revealed that time since last use was negatively correlated with left hippocampal activity. The authors suggest that abnormal hippocampal function in ecstasy users could be the result of damage to serotonin neurons that normally modulate inhibitory circuits.

Moreover the negative relationship between hippocampal activity and time since last use suggests that function of inhibitory circuits in the hippocampus may recover after long periods of abstinence.

Jager *et al.* (2008) assessed the concomitant use of other drugs in 71 participants recruited on the basis of variations in the amount and type of drugs that they used. Thirty-three heavy MDMA users (MLD = 322 tablets) and 38 non users (both groups showing considerable variation in the type and amounts of drugs they were using) completed tasks of working memory, attention and associative memory tasks in association with fMRI procedures. Analysis of fMRI revealed no significant effects of ecstasy or any other drugs on brain activity relating to working memory (modified Sternberg task) and attention (SAT task). However in the associative learning task ecstasy use predicted lower activity in the left DLPFC and higher activation in the right middle occipital gyrus. Moreover these effects appeared to be independent of cannabis and alcohol use, as well as amphetamine cocaine and tobacco use. The authors suggest these results reflect sustained – possibly even long term adaptation or compensatory reorganisation of a fronto-visual network.

Cowan *et al.* (2006) investigated the BOLD fMRI response to visual cortex activation in ecstasy users. In this study 20 MDMA users (who reported MDMA use on at least 4 occasions, mean age = 20.8, 8 male) and 23 non-users (13 male, mean age = 25.3) were administered photic stimulation using specially constructed fibre optic goggles, whilst an fMRI scan was undertaken. MDMA users had reported significantly greater lifetime use of alcohol, amphetamine, cannabis, cocaine, hallucinogens, opiates, sedatives and phencyclidine than non-users. However there were no differences in visual cortical activation between the two groups. Nevertheless a within subjects analysis in the MDMA user group revealed that degree of prior MDMA exposure was correlated with number of activated pixels for photic

stimulation. Conversely MDMA exposure was not correlated with BOLD signal change, whereas lifetime alcohol, hallucinogens, sedatives and cannabis were all inversely correlated with % BOLD signal change, but not with number of activated pixels. As such the results are inconclusive with regard to the neurophysiological response to visual cortex activation in ecstasy users.

More recently Raj *et al.* (2010) observed reduced BOLD signal change during a semantic recognition task in ecstasy polydrug users. In a cohort and task described in Chapter 3.2.4 it was observed that there were statistically significant correlations of MDMA use and BOLD signal change in left BA 9, 18 and 21/22, but not BA 45 during a semantic recognition task. Lifetime episodes of MDMA use and lifetime dose were both inversely correlated with %BOLD signal change at BA 9. Lifetime episodes of use was inversely correlated with BOLD signal change in BA 18 and 21/22, though no such correlations were observed for the encoding phase of the task, suggesting that MDMA affects verbal recognition but not encoding. These results were complicated by inverse correlations between lifetime cocaine use and BOLD signal activation in left BA 9 and 18 as well as a statistically significant inverse correlation between cannabis use and activation in left BA 9. Nonetheless, after controlling for lifetime cocaine and cannabis use, the association between MDMA use and BA 9 activation remained statistically significant. The findings in this study are consistent with findings of other neuroimaging and behavioural studies suggesting that access to semantic memory may be adversely affected by MDMA use.

Neurophysiological correlates of impulse inhibition were explored in 20 ecstasy users and 20 drug naïve controls (as described in Chapter 3.2.2) by Roberts and Garavan (2010). fMRI data showed that ecstasy users displayed greater activity in right middle and inferior frontal gyri, right middle frontal gyrus and right inferior parietal lobule, during successful

response inhibitions (STOPS) on a Go/NoGo task, compared to controls. Ecstasy users also displayed greater error activation in the right middle and inferior temporal gyri. Deactivation in the left medial frontal gyrus and left posterior cingulate was significantly greater for controls on error trials. It is suggested that the increased activation displayed by ecstasy users despite behaviourally silent differences in performance, shows increased neuronal recruitment to inhibit in this group. Recruitment of additional resources to maintain performance, suggests a subtle functional impairment that would have not been exposed with behavioural measures alone.

The results from fMRI warrant further exploration, although it appears that ecstasy use is generally associated with reductions in the BOLD response in frontal and temporal regions (Daumann, Fimm *et al.,* 2003; Daumann, Schnitker *et al.,* 2003) the DLPFC (Jager *et al*., 2008), hippocampus (Jacobsen *et al.,* 2004) and BA 9 (Raj *et al*., 2010). These reductions may reflect neuronal loss or damage. Increases in BOLD have also been observed in several areas including the thalamus and hippocampus (Moeller *et al.,* 2004) and right middle occipital gyrus (Jager *et al.,* 2008). However, the authors of these studies suggest that this may reflect compensatory mechanisms due to task inefficiency. Furthermore some increases in BOLD have been observed in MDMA users in the parietal cortex and are also correlated with higher nightly doses. These effects may be more pronounced in younger users who have yet to complete neurodevelopment upon initiation of use (Jacobsen *et al.,* 2004). There is also evidence to suggest that ecstasy-related alteration of neuronal activity is long lasting (Daumann, Fischermann, Heekeren *et al.,* 2004). However further exploration of use on executive function is warranted.

5.6 Diffusion Tensor Imaging (DTI)

Diffusion Tensor Imaging (DTI) is an MRI technique that enables diffusional motion of water molecules to be assessed (de Win *et al.*, 2007), and allows imaging of tissue structures at the microscopic level, providing information about neural tissues and changes associated with damage and acute brain ischemia (Le Bihan, 2003). Diffusion in the brain white matter is anisotropic, and motion is restricted by cellular structures (for example axons) (de Win *et al.*, 2007). Damage to axons may cause cytotoxic oedema, causing the cells to swell and hence restrict diffusion motion further, resulting in decreased apparent diffusion coefficient (ADC). Conversely, chronic damage to axons, may lead to increases in extracellular water concentrations, subsequently leading to decreases in fractional anisotropy (FA) and therefore increased ADC (de Win *et al.*, 2007). This neuroimaging technique is used to visualise anatomical functional connectivity between different areas of the brain by mapping the orientation of white matter tracts (Le Bihan, 2003).

In a prospective study (de Win *et al.*, 2007), DTI scans were performed on 188 ecstasy naïve participants who were selected on the basis that there was a high probability of them using ecstasy in the near future. Thirty participants (12 males, mean age 22.5 years, cumulative dose of ecstasy was 1.8 tablets, with an average of 7.7 weeks since last use) were scanned soon after their first ecstasy use. These scans revealed a 0.9% significant increase in FA of white matter in the centrum semiovale as well as a significant decrease (3.4%) of ADC in the thalamus post ecstasy use. However after correction for multiple comparisons and exclusion of participants with continued cocaine use, the increase in FA was no longer significant. The authors suggest that this does not provide evidence of structural neuronal damage. However the sustained decreases in ADC may indicate prolonged vasoconstriction in certain areas even after low doses of ecstasy, although it is not known whether these

effects are permanent. This study was followed up by the same research group (de Win *et al.*, 2008a), using the same initial sample. At the time of this study a total 59 participants had used ecstasy (MLD = 6 tablets). These were compared against 56 controls matched for age, gender and IQ, although the ecstasy users had now used significantly more cannabis, amphetamine, cocaine and alcohol than controls. Ecstasy users showed a significant decrease in FA in the thalamus and fronto-parietal white matter. They also showed an increase in FA in the globus pallidus and ADC in the thalamus relative to controls. It is suggested that decreased FA and increased ADC in the thalamus reflects axonal damage, given that axonal cells are understood to be the main cause for restriction of water diffusion and axonal damage leads to the observed changes in FA and ADC.

The globus pallidus was also indicated as an area of interest in a diffusion MRI study by Reneman, Majoie *et al.* (2001). In this preliminary study 8 MDMA users (described in Chapter 5.3) and 6 non-ecstasy controls undertook diffusion MRI scans. It was observed that ecstasy users displayed a significant increase in ADC in the globus pallidus relative to controls. However, no significant correlations were observed between extent of previous ecstasy use and ADC values. This increase in ADC is attributed to possible axonal injury or loss and not due to an increase in water content in the extracellular space, as local brain oedema was not detected on T2 weighted scans.

Moeller *et al.* (2007) compared FA, mean diffusivity (D_{av}), and longitudinal (diffusion along the direction of fibres) and transverse (perpendicular to the fibre tract axis) diffusivities between 12 MDMA users (10 male, mean age = 27.3, 181 mean occasions of use) and 20 healthy controls (13 male, mean age = 25.5), in six regions of the corpus callosum (Genu, Rostral body, Anterior Midbody, Posterior Midbody, Isthmus and Splenium). Results from DTI showed that MDMA users had significantly reduced longitudinal diffusivities in the

rostral body of the corpus callosum relative to controls, consistent with axonal damage in MDMA users. No significant differences in FA, D_{av} or transverse diffusivity were observed.

Conversely to de Win *et al.* (2008a), Liu *et al.* (2011) observed significant increases in FA in the bilateral thalami in 25 ecstasy users relative to 27 drug naïve controls (as described in Chapter 5.4). In this whole brain DTI study; MDMA users showed clusters with significantly increased FA in posterior parts of bilateral thalami and the retrolenticular parts of internal capsules. Decreased FA was observed in MDMA users in the genu of the corpus callosum which is consistent with findings from Moeller *et al*. (2007). Furthermore MDMA users showed significant decreases in ADCs in the bilateral thalami, posterior internal capsule and corona radiata along the bilateral corticospinal tracts, as well as significantly increased ADC in the bilateral anterior internal capsule, the bilateral superior longitudinal fasiculus and the splenium and genu of the corpus callosum. Decreased ADCs in bilateral thalami and increased FA is consistent with de Win *et al*.'s (2007) initial findings suggesting that integrity of axons in the basal ganglia-thalamocortical circuit may be compromised by MDMA use.

Neurotoxic effects of ecstasy on the thalamus were explored further using DTI by de Win *et al*. (2008b) using a sample of 71 polydrug users (33 of which were defined as heavy MDMA users, MLD = 322 tablets) correlations were conducted between drug use variables and DTI values. Extent of MDMA use was significantly correlated with decreased FA in the thalamus although no significant effect of MDMA on ADC in the basal ganglia was observed.

5.7 Positron Emission Tomography (PET)

Positron Emission Tomography (PET) is another haemodynamic functional neuroimaging technique that involves participants receiving injections of a radioactive tracer (radioligand). PET scanners are then able to monitor the distribution of the tracer in the brain,

which indexes cerebral blood flow, and thus is an indirect measure of neural activity (Cabeza & Nyberg, 1997).

PET may also be used to measure SERT densities, similar to SPECT by using radio ligands that selectively label the 5-HT transporter. McCann *et al*. (1998) used the radioligand carbon-11-labelled McN-5652 to observe differences in SERT binding between 14 MDMA users (9 male, mean age = 26.6, mean use = 228 occasions, mean usual dose = 386mg, mean duration since last use = 19 weeks) and 15 controls (9 male, mean age = 28.3). The distribution volumes ratios (DVR) for binding of the radioligand were significantly globally decreased in MDMA users relative to controls, suggesting that users had lower densities of SERT sites than controls. Moreover, decreases in SERT binding correlated significantly with extent of previous drug use, suggesting MDMA exposure may lead to loss of 5-HT terminals.

The same radioligand was used in a much larger sample by Buchert *et al.* (2003). Thirty current ecstasy users (15 male, mean age = 24.5, MLD = 831 tablets, mean duration since last use = 25 days), 29 former users (15 male, mean age = 24.4, MLD = 793 tablets, mean duration since last use = 520 days), 29 ecstasy naïve drug users (15 male, mean age = 24.4) and 29 drug naïve controls (14 male, mean age = 23.2) were compared for SERT availability in SERT rich areas of the brain including: mesencephalon, putamen, caudate and thalamus. The results showed that ecstasy users had significantly lower DVR in the mesencephalon than all other groups. Ecstasy users had significantly reduced DVRs in the caudate relative to polydrug users and in the thalamus, ecstasy users' DVRs were significantly reduced compared to polydrug users and drug naïve controls. The mean DVRs over all areas were lowest in current users. However, DVRs for former users and drug naïve controls were similar across all areas, suggesting possible recovery. There were no differences between groups in DVRs in the white matter, where there are no SERTs, suggesting that the effects are serotonin specific.

Using the same sample, Buchert *et al.*, (2004) observed that current MDMA users had significantly reduced DVRs in the posterior cingulate gyrus, left caudate, thalamus, occipital cortex, medial temporal lobes including the hippocampus and parahippocampal regions and brainstem with mesencephalon and pons compared to all three control groups. These differences were more pronounced in females than males, suggesting that females may be more susceptible to MDMA related serotonergic changes. Interestingly, there were no significant differences in SERT availability between former users and the two other control groups, suggesting that these effects may be reversible after long periods of abstention. This is also supported by the finding that DVRs and MDMA abstention periods were positively correlated in the brainstem with mesencephalon and pons and the basal forebrain. SERT appeared to normalise in this follow up study, with no significant differences between groups in the mesencephalon. The authors suggest that there were reductions in ecstasy use, which may account for normalising SERT levels. These findings coupled with normal SERT levels in former users suggest that ecstasy-related effects of SERT availability may be reversible.

The effects of MDMA use on cortical serotonin function in females was explored further in a PET study by Di lorio *et al*. (2012) using the 5-HT_{2A} receptor specific radioligand [^{18}F]setoperone. Fourteen female MDMA users (mean age = 21.64, mean lifetime MDMA consumption = 14000mg) were compared with 10 female controls (mean age = 21.60). MDMA users had significantly increased 5-HT_{2A} receptor binding in occipital-parietal, temporal, occipito-temporal-parietal, frontal and fronto-parietal regions. Lifetime use was also significantly correlated with binding increases in fronto-parietal, occipito-temporal, fronto-limbic, and frontal regions. There were no significant effects of duration of abstinence on binding here, suggesting chronic 5-HT neurotoxicity in females.

Thomasius *et al.* (2003) used [^{11}C]McN5652 to assess serotonin transporter density in 30 current ecstasy users (15 male, mean age = 24.5, MLD = 1033.77 tablets, time since last

use = male - 21.6 days, female – 24.73 days), 31 former users (16 male, mean age = 24.13, MLD = 600 tablets, time since last use = male – 485.40, female – 545.13 days) 29 polydrug controls (15 male, mean age = 24.41) and 30 drug naïve participants (15 male, mean age = 23.13). Current MDMA users showed significantly reduced DVRs in the mesencephalon in relation to all other groups. Furthermore typical number of ecstasy exposures was the best predictor of DVRs in the thalamus and caudate nucleus, and number of ecstasy tablets taken in the year leading up to testing was the best predictor of DVRs in the mesencephalon. The authors conclude that these results are in line with the hypothesis that MDMA use may lead to reductions in SERT availability in the central serotonergic system. However these alterations may be reversible after abstention.

McCann *et al*. (2005) used first and second generation SERT ligands [^{11}C]McN5652 and [^{11}C]DASB to investigate MDMA induced brain serotonin neurotoxicity in 23 MDMA users (13 male, mean age = 22.04, mean number of exposures = 96.96, mean usual dose = 1.79 tablets, time since last dose = 4.71 months) and 19 controls (8 male, mean age = 26). Consistent with previous findings, global reductions in DVRs were observed in MDMA users compared to controls with both radio ligands. Correlational analysis also revealed that with both radioligands, global DVRs correlated with duration of abstinence, suggesting that abstention may lead to partial recovery. Global SERT binding DVR was also inversely correlated with typical monthly MDMA dose (for both radioligands) suggesting that loss of SERT is associated with MDMA use intensity. The same research group (McCann *et al.*, 2008) conducted PET using [^{11}C]DASB to investigate SERT binding, alongside [11C]WIN 35,428 to investigate dopamine transporter (DAT) binding. The MDMA users in this study (n=16, 8 male, mean age = 23.5, mean number of uses = 195.3) had all reported having sequential doses of MDMA (2 or more doses over a 3-12 hour period). Subjects also underwent formal neuropsychiatric testing (tests of memory, attention and executive

function). The results indicated that SERT binding was significantly reduced in multiple brain regions for MDMA users relative to controls (occipital cortex, parietal cortex, temporal cortex, anterior cingulate cortex, posterior cingulate cortex, DLPFC and hippocampus). The reductions were greatest in cortical regions (especially the occipital cortex) and there were no significant differences in SERT binding in subcortical regions. However, no differences were observed between users and controls in DAT binding in the caudate or putamen, and no relationship was found between measures of MDMA use and DAT binding. There was, however, a significant negative correlation between SERT availability in the hippocampus and duration of MDMA use. These results reflect the specificity of MDMA as a selective serotonin neurotoxin and suggest that sequential dosing is associated with lasting decreases in SERT. Memory performance was also correlated with SERT binding in the DLPFC, orbitofrontal cortex and parietal cortex, across groups. However this was not significant in MDMA users alone, suggesting that MDMA use disrupts this relationship.

More recently Sudhakar *et al*. (2009) investigated SERT binding using [^{11}C]DASB in 12 former MDMA users (all male, mean age = 28.2, mean lifetime occasions = 243.75, typical session dose = 2.75, time since last use = 2.74 years), 9 polydrug user controls (all male, mean age = 35.6) and 19 drug naïve controls (mean age = 30.5). No significant differences were observed in cerebellar DVR between the three groups and there were no correlations between variables of MDMA use and SERT binding suggesting no long lasting serotonin neuron damage in recreational users.

Presynaptic (5-HT transporter, SERT) and postsynaptic (5-HT$_{2A}$ receptor) markers of serotonin transmission in neocortical areas were investigated in a PET study using the SERT ligand [^{11}C]DASB and the 5-HT$_{2A}$ receptor ligand [^{11}C]MDL by Urban *et al.* (2012). Thirteen current users (8 male, mean age = 30.8, 5.7 weeks mean abstinence, 142 mean MDMA sessions) and 13 matched healthy controls were compared. Presynaptic SERT availability

was reduced overall in ecstasy users compared to controls for cortical but not subcortical regions. The most pronounced differences were observed in the medial prefrontal cortex, occipital cortex and temporal cortex. As predicted, decreased SERT was regionally associated with upregulated 5- HT_{2A} receptor binding. It is suggested that these results reflect MDMA induced damage to 5-HT neuron terminals innervating the cortex. Kish *et al*., (2010) observed SERT binding of [^{11}C]DASB in cortical and subcortical areas using voxel based analysis with 49 chronic MDMA users (28 male, mean age = 25.9, range of cumulative lifetime tablets = 2 – 922, variable use of other drugs) and 50 controls (25 male, mean age = 26, low use of other drugs). ANOVA revealed highly regional-specific decreases in [^{11}C]DASB binding in ecstasy users compared to controls that was restricted to the entire cerebral cortices and hippocampus with the most marked reduction (-46%) in the occipital cortex. No changes were observed in the SERT rich striatum (Caudate, putamen and ventral striatum), thalamus, global pallidus or midbrain. These findings suggest that SERT binding reduction is regionally specific and is unlikely to be explained by recent use of other stimulant drugs, hormonal levels, SERT promoter gene polymorphisms or structural brain changes (as observed from regression analysis).

[H_2 ^{15}O]-PET was used to assess cerebral blood flow after a single dose of MDMA (1.7mg/kg) or placebo in 16 MDMA naïve participants by Gamma *et al*. (2000). It was observed that MDMA produced acute increases in regional cerebral blood flow in the ventromedial, frontal and occipital cortex, inferior temporal lobe and cerebellum, as well as decreases in the motor and somatosensory cortices, left amygdala, insula, cingulate cortex and thalamus.

To sum up, the literature on SERT binding in MDMA users as assessed by PET seems to consistently suggest that use is associated with lower SERT availability (McCann *et al.*, 1998; McCann *et al.*, 2005) globally. However this is usually more pronounced in SERT rich

areas such as the mesencephalon, caudate and thalamus (Buchert *et al*., 2003; Buchert *et al.,* 2004; Thomasius *et al.,* 2003). Findings of decreased DVRs in SERT rich areas coupled with no such differences in white matter (Buchert *et al.,* 2003) reflect that potential neural damage is serotonin specific. This is also reflected by lack of observed differences in dopamine transporter binding between MDMA users and non-users (McCann *et al.,* 2008). There is evidence to suggest that MDMA's effects on serotonin terminals are more pronounced in female users (Buchert *et al.,* 2004; Di lorio *et al*., 2012). Furthermore the majority of studies reviewed suggest that the effects observed may be reversible. Sudhakar *et al*. (2009) observed no differences in SERT between former users and controls and other studies have shown correlations between period of abstention and SERT availability (Buchert *et al.,* 2004; McCann *et al.,* 2005).

Chapter Summary

The evidence from neuroimaging studies suggests that ecstasy does adversely affect the serotonin system. Although it is clear that some methods are not as sensitive as others at detecting perhaps mild cognitive aberrations (MRI, MRS) associated with low recreational doses. Other methods (SPECT, PET, fMRI) consistently show alterations to neuronal activation/ SERT binding that reflect degradation of the serotonin system that is associated with MDMA use. Abstention also appears to play an important role with regards neuronal changes, as many studies suggest that SERT availability returns to normal levels after periods of abstention. However, some of the behavioural deficits noted in chapter 3 have been shown to be long lasting. All participants in this thesis will be required to be abstinent from MDMA use for at least 7 days prior to testing, to observe long lasting effects of drug rather than an acute residual intoxication effect. Other neuroimaging studies that have been coupled with behavioural tasks have observed differences in their neurophysiological performance despite having undetectable behavioural deficits, suggesting neuroimaging techniques are more

sensitive to cognitive impairment than behavioural measures alone. As such all of the studies in this thesis will combine behavioural assessments with assessments of neurophysiological indices. Furthermore after conducting a literature search for fNIRS studies with MDMA users, it has been concluded that this neuroimaging technique has never been used for assessment of cognitive performance in MDMA users previously. As such the application of this technique to this research area is novel, and will provide valuable information about haemodynamics in the PFC of MDMA users.

Table 5.1: Summary of studies assessing objective measures of neurotoxicity in ecstasy users

Measure/Authors	Methodology	Findings
SPECT		
Reneman, Habraken *et al*. (2 000)	Structural analysis, using radioligand [123I]R91150 to assess cortical 5-HT_{2A} receptor binding in 10 MDMA users, 5 former users and 10 drug naïve controls	Current MDMA users had significantly lower binding ratios than controls and former users over an average of left and right frontal, parietal and occipital areas.
Reneman, Booij *et al*. (2000)	Structural analysis using radioligand [123I]R91150 to assess post-synaptic 5-HT_{2A} receptor binding in 5 MDMA users and 9 non-user controls	Overall binding ratios higher for MDMA users. However this only approached significance in the occipital cortex.
Reneman, Lavalaye *et al.* (2001)	Structural analysis using radioligand [^{123} I]β-CIT to assess cortical SERT binding in 22 current MDMA users, 13 former users and 13 controls	Current MDMA users displayed lower cortical SERT binding than controls. No significant differences in binding observed between former users and controls.
Reneman, Booij, de Bruin *et al.* (2001)	Structural analysis using radioligand [^{123} I]β-CIT to assess cortical SERT binding in 15 moderate ecstasy users, 23 heavy users, 16 former users and 15 controls	Binding ratios significantly lower in female heavy users compared to controls for all brain regions analysed. Overall SERT binding and log transformed previous MDMA use were significantly correlated in females but not in males.
Reneman, Booij *et al.* (2002)	Structural analysis using radioligand [^{123} I]β-CIT to assess nigrostriatal dopamine neuron densities in 29 ecstasy users, 9 ecstasy and amphetamine users and 15 controls.	[^{123} I]β-CIT binding ratios significantly higher in the ecstasy user group compared to controls. [^{123} I]β-CIT binding ratios significantly reduced in the ecstasy + amphetamine group relative to ecstasy only users.
Chang *et al*. (2000)	Functional analysis, assessing cerebral blood flow in 21 ecstasy users and 21 controls	Ecstasy users showed slightly lowered global CBF (2.3%) and mild but not significant reductions in regional CBF compared to controls.
Klomp *et al*. (2012)	Structural analysis using radioligand [^{123} I]β-CIT to assess SERT binding ratios in 33 ecstasy users stratified for early (14-18 years) and late (18-36 years) exposure	Significant effect of age at first use in midbrain [^{123} I]β-CIT binding ratios. Also a strong significant negative correlation between age of first use and midbrain [^{123} I]β-CIT binding ratios in the early exposed group, but not the late exposed group.

de Win *et al.* (2008a)	Structural analysis using radioligand [^{123} I]β-CIT to assess SERT binding ratios in prospective ecstasy users in a longitudinal study: 188 ecstasy naïve participants scanned at time 1, with 59 participants subsequently using ecstasy by time 2 (12-36 months follow up). 59 incidental ecstasy users compared to 56 still drug naïve controls	No significant ecstasy related effects on [^{123} I]β-CIT binding observed.
EEG		
Dafters *et al.* (1999)	Functional analysis. Spectral and coherence analysis of 60s resting (eyes closed) epochs and cognitive/mood variables in 23 ecstasy users.	Level of MDMA use positively correlated with an increase in alpha power across left frontal, left posterior and right posterior areas. Use also positively correlated with beta power in the left posterior region and negatively correlated with relative delta power over the whole scalp. Coherence analysis revealed weak but significant negative correlations between MDMA use and sites located over visual tracts (O1-T3, O2-T4).
Gamma *et al.* (2005)	Functional analysis. ERP P3 assessed whilst conducting inhibition task (CPT A-X) in 16 ecstasy users and 17 controls	Ecstasy users show significantly reduced P3 in relation to NoGo trials at midline electrodes Fz and Cz. After controlling for age, education and cannabis use, Fz became non-significant. No between group differences in P3 latencies. No correlation between P3 amplitude or latency and lifetime MDMA use.
Mejias *et al.* (2005)	Functional analysis. ERP components assessed while conducting visual oddball task: 14 ecstasy users, 14 controls.	Ecstasy users showed a greater latency of the P3b component compared to controls for rare stimuli.
Casco *et al.* (2005)	Functional analysis. ERP components of VEPs assessed during a simple discrimination task: 8 heavy ecstasy users, 8 moderate users and 18 drug free controls	Heavy users showed significantly reduced P2 and P3 amplitudes at Oz compared to controls. Moderate users showed significantly reduced P3 amplitude relative to controls. P3 was significantly reduced in both heavy and moderate users compared to controls at Fz. N250 significantly reduced in heavy users relative to controls.

de Sola *et al.* (2008)	Functional analysis. ERP components assessed in relation to an auditory oddball paradigm in 14 ecstasy users, 13 cannabis users and 22 drug naïve controls. Longitudinal study.	No significant between group differences for P3 amplitude or latency at time 1 or time 2. Correlations between MDMA use and P3 response not significant at time 1 or time 2. However lifetime cannabis use and P3 latency significantly correlated at time 1, with greater cannabis use associated with increased neuronal processing speed.
Burgess *et al*. (2011)	Functional analysis. ERPs analysed during two recognition memory tasks in 15 ecstasy users, 14 cannabis users and 13non-drug users	Significantly reduced late positive ERP over left parietal sites in ecstasy users compared to both other groups for the recollection component of the task.
Nulsen *et al*. (2011)	Functional analysis. ERPs analysed during forward and backwards digit span task: 11 ecstasy users, 13 polydrug controls, 13 non-drug controls	Both control groups show significantly reduced P3 in the digit backwards task than the digit forwards task. This difference is not evident in ecstasy users, despite showing greatest discrepancy in performance between the two tasks.
MRI		
Chang *et al*. (1999)	Structural analysis: 21 ecstasy users, 37 non-users	Ecstasy users and controls showed normal scans with no significant brain atrophy or white matter lesions.
Chang *et al*. (2000)	Structural analysis: 21 ecstasy users, 21 non-users	Normal MRI scans for ecstasy users and controls.
Cowan *et al.* (2003)	Structural analysis: 31 ecstasy users, 29 non-users	Ecstasy users showed reduced grey matter concentrations relative to controls in bilateral BA 18 in the occipital lobe, BA 21 in the temporal lobe, and left BA 45 in the frontal lobe, as well as a midline region of the brain stem and bilateral areas of the cerebellum.
Reneman, Majoie *et al.* (2001)	Structural analysis with conventional T1 weighted scans as well as diffusion and perfusion MRI (Intravenous bolus injections of gadopentetate dimeglumine administered prior to T2 weighted echo planar scans) in 8 ecstasy users and 6 non-user controls	Perfusion MRI showed ecstasy users had overall higher mean rCBV values than controls. This approached significance in the globus pallidus. The rCBV ratio in the globus pallidus also correlated significantly with extent of previous drug use. No differences were observed on other MRI measures.

Schouw *et al.* (2012)	Structural analysis. Pharmacological MRI, 3.0T T1 weighted scans performed pre and post infusion with SSRI Citalopram. Haemodynamic response to SSRI investigated in 10 male ecstasy users and 7 non-user controls	Ecstasy users displayed a significant cerebral blood flow reduction in response to 5-HT challenge, most prominent in the left thalamus.
MRS		
Chang *et al.* (1999)	Structural analysis from relative concentrations of CNS metabolites associated with structural brain integrity: 21 ecstasy users, 37 non-user controls	Concentrations of NAA, CR and CHO comparable in all 3 brain areas measured (mid occipital, mid frontal and mid parietal brain regions) between ecstasy users and non-users. However ecstasy users showed elevated MI and MI to CR ratios in parietal white matter as well as MI and MI/CR in parietal white matter. Occipital grey matter positively correlated with MDMA use.
Liu *et al.* (2011)	Structural analysis from relative concentrations of CNS metabolites associated with structural brain integrity: 25 ecstasy users, 27 drug naïve controls	No significant differences in NAA between groups in the basal ganglia. However ecstasy users displayed increases in MI concentrations. Correlations between CR concentration and MDMA dose observed in the right basal ganglia.
Reneman, Majoie *et al.* (2002)	Structural analysis from relative concentrations of CNS metabolites associated with structural brain integrity: 15 male ecstasy users, 12 non-user controls	Ecstasy users show significantly reduced NAA/Cr and NAA/CHO ratios in frontal grey matter compared to controls. Frontal grey matter binding ratios were negatively correlated with MDMA use. No differences observed in mid occipital grey matter or right parietal white matter between groups.
Obergreisser *et al.* (2001)	Structural analysis from relative concentrations of CNS metabolites associated with structural brain integrity. Assessment of MDMAs effects on the hippocampus in 6 ecstasy users and 5 non-user controls.	No differences in hippocampal NAA or choline compounds between ecstasy users and non-users.
Daumann, Fischermann, Pilatus *et al*. (2004)	Structural analysis. ^{1}H MRS in the left hippocampus, midfrontal and midoccipital cortex of 13 ecstasy users and 13 non-user controls	No significant between group differences in NAA/Cr ratios in any brain region observed. No meaningful correlations between NAA/Cr ratios and drug use.

de Win *et al.* (2008a)	Structural analysis from relative concentrations of CNS metabolites associated with structural brain integrity: 188 ecstasy naïve participants scanned at time 1, with 59 participants subsequently using ecstasy by time 2 (12-36 months follow up). 59 incidental ecstasy users compared to 56 still drug naïve controls	No significant effects of MDMA on brain metabolites.
Cowan *et al.* (2007)	Structural analysis, MRS 4T was used to investigate absolute concentrations of NAA and MI in the occipital lobe in 9 ecstasy users and 7 non-user controls	No statistical differences in absolute NAA or absolute MI levels in the occipital cortex between MDMA users and non-users.
fMRI		
Daumann, Fimm *et al*. (2003)	Functional analysis during n-back task: 11 heavy ecstasy users, 11 moderate ecstasy users, 11 non-user controls	Heavy users showed weaker BOLD responses in left frontal and temporal regions on the most difficult level of the task (2-back) relative to the other two groups (at liberal significance level $p<0.01$, and $p<0.001$ uncorrected). Both user groups showed increased activation in the right parietal cortex with 1 and 2 back tasks. Extent of previous drug use did not correlate with BOLD signal changes.
Daumann, Schnitker *et al.* (2003)	Functional analysis during n-back task: 8 pure ecstasy users, 8 polyvalent ecstasy users, 8 non-user controls	Pure MDMA users showed reduced BOLD activation in the temporal gyrus and angular gyrus in the 1-back task compared to controls. Pure MDMA users had lower signal changes compared to polyvalent users in the striate cortex and higher BOLD response in the premotor cortex. Pure MDMA users showed lower activation than both other groups in the angular gyrus during 2-back (more difficult) level of the task.
Daumann, Fischermann, Heekeren *et al.* (2004)	Functional analysis during n-back task, in an 18 month longitudinal study: 30 ecstasy users (at time 1, reducing to 21 users by time two)	Continuing users showed increased activation from baseline in two clusters in the parietal cortex during the most difficult level of the task (2-back) at time 2 compared to time 1. Increase in haemodynamic activation between time 1 and time 2 associated with higher one night dose of MDMA.

Moeller *et al.* (2004)	Functional analysis during immediate and delayed memory task: 15 ecstasy users, 19 non-user controls	Ecstasy users displayed significantly greater BOLD activation compared to controls in three clusters: 1-the left medial and superior frontal gyri with extending activation to the right medial superior frontal gyri, bilateral anterior cingulate gyri, and right middle frontal gyrus. 2- left thalamus extending to left caudate and putamen, left parahippocampal gyrus, left hippocampus and left insula. 3- right hippocampal gyrus extending to the right hippocampus, right thalamus, right lentiform nucleus, right putamen, right insula, and right temporal cortex. Effects remained after controlling for other drugs except in the prefrontal cortex after controlling for cannabis use. Increased activation due to MDMA users being less "efficient" at task, resulting in an increase in neuronal activity to perform at a similar level as controls.
Jacobsen *et al*. (2004)	Functional analysis during selective and divided attention and verbal working memory: 6 adolescent ecstasy users, 6 adolescent ecstasy naïve controls	Ecstasy users displayed significantly lowered hippocampal activity relative to controls during the working memory task. Time since last use was negatively correlated with left hippocampal activity.
Jager *et al*. (2008)	Functional analysis during working memory, attention and associative memory tasks: 33 ecstasy users, 38 non-user controls	No significant effects of ecstasy or any other drugs on brain activity relating to working memory (modified Sternberg task) and attention (SAT task). However in the associative learning task ecstasy use predicted lower activity in the left DLPFC and higher activation in the right middle occipital gyrus, reflecting compensatory mechanisms.
Cowan *et al*. (2006)	Functional analysis during photic stimulation using specially constructed fibre optic goggles: 20 ecstasy users, 23 non-user controls	No differences in visual cortical activation between the two groups. No correlation between MDMA exposure and BOLD signal change.

Raj *et al*. (2010)	Functional analysis during a semantic recognition task in 16 ecstasy polydrug users	During semantic recognition, but not encoding- lifetime episodes of MDMA use and lifetime dose were both inversely correlated with %BOLD signal change at BA 9. Lifetime episodes of use was inversely correlated with BOLD signal change in BA 18 and 21/22. After controlling for other drugs the correlation at BA 9 remained significant.
Roberts and Garavan (2010)	Functional analysis during Go/NoGo task in 20 ecstasy users and 20 drug naïve controls.	Ecstasy users displayed greater activity in right middle and inferior frontal gyri, right middle frontal gyrus and right inferior parietal lobule, during successful response inhibitions, compared to controls. Ecstasy users also displayed greater error activation in the right middle and inferior temporal gyri. It is suggested that increased activation despite equivalent performance, shows increased neuronal recruitment to inhibit in ecstasy users.
DTI		
de Win *et al.* (2007)	Structural analysis: 30 participants scanned before ecstasy use and soon after first reported ecstasy use	0.9% significant increase in FA of white matter in the centrum semiovale as well as a significant decrease (3.4%) of ADC in the thalamus observed post ecstasy use. However increases in FA did not remain after correction for multiple comparisons and exclusion of participants with continued cocaine use.
de Win *et al.* (2008a)	Structural analysis: 59 novice MDMA users, 56 non-user controls	Ecstasy users showed a significant decrease in FA in the thalamus and fronto-parietal white matter. Ecstasy users also showed an increase in FA in the globus pallidus and ADC in the thalamus relative to controls.
Reneman, Majoie *et al.* (2001)	Structural analysis: 8 ecstasy users, 6 non-user controls	Ecstasy users displayed a significant increase in ADC in the globus pallidus relative to controls. No significant correlations were observed between extent of previous ecstasy use and ADC values.

Moeller *et al*. (2007)	Structural analysis of 6 regions of the corpus callosum (Genu, Rostral body, Anterior Midbody, Posterior Midbody, Isthmus and Splenium): 12 ecstasy users, 20 non-user controls	MDMA users had significantly reduced longitudinal diffusivities in the rostral body of the corpus callosum relative to controls, consistent with axonal damage in MDMA users. No significant differences in FA, D_{av} or transverse diffusivity were observed.
Liu *et al.* (2011)	Structural analysis. Whole brain DTI on 25 ecstasy users and 27 non-user controls.	MDMA users showed clusters with significantly increased FA in posterior parts of bilateral thalami and the retrolenticular parts of internal capsules compared to controls. Decreased FA was also observed in MDMA users in the genu of the corpus callosum. Furthermore MDMA users showed significant decreases in ADCs in the bilateral thalami, posterior internal capsule and corona radiata along the bilateral corticospinal tracts, as well as significantly increased ADC in the bilateral anterior internal capsule, the bilateral superior longitudinal fasiculus and the splenium and genu of the corpus callosum.
de Win *et al*. (2008b)	Structural analysis. DTI of the thalamus in 71 polydrug users.	Extent of MDMA use was significantly correlated with decreased FA in the thalamus. No significant effect of MDMA on ADC in the basal ganglia.
PET		
McCann *et al*. (1998)	Structural analysis using radioligand carbon-11-labelled McN-5652 to observe differences in SERT binding between 14 ecstasy users and 15 non-user controls	DVRs for binding of the radioligand were significantly globally decreased in MDMA users relative to controls. Decreases in SERT binding correlated significantly with extent of previous ecstasy use.

Buchert *et al*. (2003)	Structural analysis using radioligand carbon-11-labelled McN-5652 to assess SERT binding in the mesencephalon, putamen, caudate and thalamus of 30 ecstasy users, 29 former users, 29 polydrug controls and 29 drug naïve controls	Ecstasy users had significantly lower DVRs in the mesencephalon than all other groups. Ecstasy users had significantly reduced DVRs in the caudate relative to polydrug users and in the thalamus, ecstasy users' DVRs were significantly reduced compared to polydrug users and drug naïve controls. Mean DVRs over all areas were lowest in current users. However, DVRs for former users and drug naïve controls were similar across all areas. There were no differences between groups in DVRs in the white matter.
Buchert *et al*. (2004)	Structural analysis using radioligand carbon-11-labelled McN-5652: 30 ecstasy users, 29 former users, 29 polydrug controls and 29 drug naïve controls. Follow up from the 2003 study	Ecstasy users had significantly reduced DVRs in the posterior cingulate gyrus, left caudate, thalamus, occipital cortex, medial temporal lobes including the hippocampus and parahippocampal regions and brainstem with mesencephalon and pons compared to all 3 control groups. More pronounced in females than males. No significant differences in SERT availability between former users and the two other control groups. DVRs and MDMA abstention periods were positively correlated in the brainstem with mesencephalon and pons and the basal forebrain. SERT appeared to normalise in this follow up study.
Di Iorio *et al*. (2012)	Structural analysis using radioligand [^{18}F]setoperone in 14 female ecstasy users and 10 female non-user controls	Ecstasy users had significantly increased 5-HT$_{2A}$ receptor binding in occipital-parietal, temporal, occipito-temporal-parietal, frontal and fronto-parietal regions. Lifetime use was significantly correlated with binding increases in fronto-parietal, occipito-temporal, fronto-limbic, and frontal regions. No significant effects of duration of abstinence on binding.

Thomasius *et al.* (2003)	Structural analysis using the radioligand [^{11}C]McN5652 to assess SERT density in 30 ecstasy users, 31 former users, 29 polydrug controls and 30 drug naïve controls	Ecstasy users showed significantly reduced DVRs in the mesencephalon in relation to all other groups. Number of ecstasy exposures was the best predictor of DVRs in the thalamus and caudate nucleus, and number of ecstasy tablets taken in the year leading up to testing was the best predictor of DVRs in the mesencephalon.
McCann *et al.* (2005)	Structural analysis using radioligands [^{11}C]McN5652 and [^{11}C]DASB: 23 ecstasy users, 19 non-user controls	Global reductions in DVRs observed in MDMA users compared to controls with both radio ligands. Global DVRs correlated with duration of abstinence. Global SERT binding DVR was also inversely correlated with typical monthly MDMA dose for both radioligands.
McCann *et al.* (2008)	Structural analysis using radioligands [^{11}C]DASB to investigate SERT binding, alongside [11C]WIN 35,428 to investigate dopamine transporter binding: 16 ecstasy users, 16 non-user controls	SERT significantly reduced in occipital cortex, parietal cortex, temporal cortex, anterior cingulate cortex, posterior cingulate cortex, DLPFC and hippocampus for ecstasy users. The reductions were greatest in cortical regions and there were no significant differences in SERT binding in subcortical regions. No differences observed between users and controls in DAT binding in the caudate or putamen, and no relationship between MDMA use and DAT binding.
Sudhakar *et al.* (2009)	Structural analysis using [^{11}C]DASB in 12 former ecstasy users, 9 polydrug controls and 19 drug naïve controls	No significant differences observed in cerebellar DVRs and no correlations between MDMA use and SERT binding.
Urban *et al.* (2012)	Structural analysis using SERT ligand [^{11}C]DASB and the 5-HT2A receptor ligand [^{11}C]MDL: 13 ecstasy users, 13 non-user controls	Presynaptic SERT availability reduced overall in ecstasy users compared to controls for cortical but not subcortical regions. Most pronounced differences in the medial prefrontal cortex, occipital cortex and temporal cortex. Decreased SERT regionally associated with upregulated 5- HT_{2A} receptor binding.

Kish *et al*. (2010)	Structural analysis using [^{11}C]DASB in cortical and subcortical areas: 49 ecstasy users, 50 non-user controls	Regional-specific decreases in [^{11}C]DASB binding in ecstasy users compared to controls was restricted to the entire cerebral cortices and hippocampus with the most marked reduction (-46%) in the occipital cortex. No changes were observed in the SERT rich striatum (Caudate, putamen and ventral striatum), thalamus, global pallidus or midbrain.

Chapter 6: Electrophysiological indices of executive function

6.1 Chapter overview

Chapter 3 reviewed the literature on executive function deficits in relation to MDMA use. Furthermore Chapter 5 reviewed the evidence of structural and functional neural alterations in relation to MDMA use. It was observed that there was a paucity of neurophysiological data systematically assessing Miyake *et al.*'s (2000) conceptual framework of executive function. One of the aims of this thesis is to fully characterise the nature of MDMA's effects upon the central executive of working memory. The following chapter investigates each of the four previously defined executive functions with behavioural tasks assumed to tap one function and their electrophysiological correlates. Twenty ecstasy polydrug users, 20 ecstasy naïve polydrug controls and 20 drug naïve controls were recruited and Go/NoGo, number-letter, n-back and semantic association tasks were undertaken. ANOVA revealed no significant between group differences on performance measures for the Go/NoGo, number-letter and semantic association tasks. There were no differences between groups in terms of errors on the n-back task, however reaction time data revealed that drug naïve controls were significantly slower to respond than polydrug controls on all levels of the task. The ERP data showed drug related atypicalities in the P2 component during, the Go/NoGo task. There were also drug related differences in the N2 component in the semantic association task, as well as drug effects on positive components (P2 and P3) during the number-letter task. There were no between groups differences on the ERP data during the n-back task. The results from this chapter reflect ecstasy/polydrug related atypicalities of processing during tasks that tap inhibitory control, switching and access that may reflect compensatory mechanisms/cognitive reallocation of resources to attenuate behavioural differences. The results from the data on inhibition, switching and access have been

published in three separate journal articles (Roberts *et al.*, 2013a, b and c – in press) and copies of these publications can be observed in the appendices of this thesis.

6.2 Introduction

Areas that are involved in working memory such as the DLPFC are richly innervated with 5-HT receptors; therefore degradation to the serotonergic system via ecstasy use could lead to deficits in cognitive processes associated with these forebrain structures. Significant deficits have been observed in ecstasy users compared to non-users in components of working memory such as spatial working memory (Wareing *et al.*, 2005), access to semantic memory, and memory updating (Fisk *et al.*, 2004; Montgomery, Fisk, Newcombe & Murphy,2005). Furthermore, ecstasy users perform poorly in information processing tasks when cognitive demand is high **(**Wareing *et al.,* 2000). It has been suggested (Cole *et al.,* 2002), that a lack of sleep (among other possible lifestyle variables), may exacerbate or indeed cause the observed cognitive deficits in ecstasy using populations. Furthermore, several characteristics of sleep such as sleep quality, length of sleep (hours) and related changes in alertness have been reported to be altered in ecstasy users relative to controls (Allen *et al.*, 1993). However such deficits appear to have little mediating effect on ecstasy-related cognitive deficits (e.g. Montgomery *et al.*, 2010).

When looking at executive functioning in ecstasy users, some functions appear to be more affected than others (See Chapter 3 for a review). There is a differential pattern of impairment based on previous drug use history and type of function, with the updating function of the executive being particularly susceptible to ecstasy use (Montgomery & Fisk, 2008; Montgomery, Fisk, Newcombe & Murphy, 2005) along with access to long term memory (Montgomery, Fisk, Newcombe & Murphy., 2005). Inhibitory control and set switching appear to be more robust to ecstasy-related deficits; however, recent research

suggests that even in the absence of behavioural differences, ecstasy users may show electrophysiological differences related to task demands (Burgess *et al.* 2011). Consequently, participants in previous studies displaying no impairments in the behavioural tasks may not necessarily be exhibiting "normal" functioning. The present study therefore sought to assess all aspects of executive functioning (in relation to Miyake *et al*.'s 2000, and Fisk & Sharp's 2004 frameworks) in ecstasy users through behavioural and electrophysiological assessments of performance.

Inhibitory control (see Chapter 3.2.2 for review) requires effortful control over instinctive (predominant) responses. Although the DLPFC, ACC and Inferior Frontal Cortex are commonly activated during working memory performance (Duncan & Owen, 2000), neuroimaging and lesion data indicates that the inferior frontal cortex (IFC) may have a particularly important role in inhibitory control (Aron *et al*., 2004). Furthermore this area may also play a role in mental set switching, given that task switching may require inhibition of responses to a now inappropriate task (Aron *et al.*, 2004). The Stroop task has been used in several studies to test whether ecstasy use impairs inhibitory control (Back-Madruga *et al.*, 2004; Gouzoulis-Mayfrank *et al.*, 2000; Morgan *et al.*, 2002), with all studies reporting no ecstasy-related impairment. Wareing *et al.* (2000) employed the random letter generation measure of inhibitory control and observed performance deficits in ecstasy users compared to non-users, however there have been failures to replicate this (Fisk *et al.*, 2004). A review by Murphy *et al.* (2009) found that the literature on inhibition in ecstasy users was unclear, although there is little evidence to suggest ecstasy-related impairments. Furthermore any perceived impairment can be obscured by confounding variables such as polydrug use and although the use of ANCOVA and regression are usually employed to statistically control for this, the majority of findings in the literature need to be interpreted with some degree of caution.

A commonly used task in the inhibition literature is the Go/NoGo task. This task requires participants to only respond to certain stimuli and therefore inhibit prepotent or dominant responses. Previous studies using this task with ecstasy users (e.g. Gouzoulis-Mayfrank *et al.* 2003), have observed little difference in performance on the task between non-users, moderate users and heavy users. However it has been suggested that 5-HT depletion, as well as impaired executive functions may play a role in inhibitory control (Morgan *et al.*, 2006). One study conducted on ecstasy users with minimal exposure to other drugs reported that heavy use of MDMA led to notable impairments in inhibition and impulsivity (Halpern *et al.*, 2004).

Although much of the research on behavioural tasks assessing inhibitory control in ecstasy users has provided inconclusive evidence, perhaps such cases where no differences have been observed can be attributed to compensatory mechanisms. This was proposed in an fMRI study by Roberts and Garavan (2010), where increased activation was seen in right middle and inferior frontal gyri, right middle frontal gyrus and right inferior parietal lobule, in ecstasy users relative to controls in a Go/NoGo task, despite equivalent task performance (see Chapter 5.5).

Mental set switching (or shifting; as defined in Chapter 3.2.1) is the ability to alternate attention as required between two tasks, or between different components of a task. This executive function reflects cognitive flexibility and deficits here may have implications for real world situations; for example in the work environment where reallocation of attention (or switching between tasks) is required continually. The neural basis of this executive function is proposed to be localised to the lateral prefrontal cortex (Dove *et al.*, 2000) and left DLPFC, parietal and temporal regions (Smith, Taylor *et al*, 2004). In ecstasy users, research in switching is equivocal (Fox *et al.*, 2001; Fox *et al*., 2002). However tasks used do not always

solely assess switching (Fisk & Sharp, 2004). The WCST has been employed frequently to assess switching (Reneman *et al.,* 2006**;** Thomasius *et al*., 2003) yielding no ecstasy-related deficits. The number-letter task (Rogers & Monsell, 1995) has also been used (Montgomery, Fisk, Newcombe & Murphy, 2005), showing no clear ecstasy-related deficits. Conversely Halpern *et al.* (2004) observed deficits in switching using the WCST. The cohort in this sample had minimal exposure to other drugs and as such potential confounds from polydrug use were reduced. However, in a follow up study (Halpern *et al*., 2011) with a larger sample and similar controls for concomitant drug use and other lifestyle variables, no behavioural deficits in switching were observed. However, Dafters (2006) did observe deficits in ecstasy users relative to cannabis users and controls, in a task switching version of the Stroop task. As such the impact of MDMA exposure on this executive function remains unclear.

The updating component of the central executive involves monitoring and updating incoming information and replacing no longer relevant information with salient information. This requires active manipulations of incoming information rather than simply acting as a short term memory store (Morris & Jones, 1990). The updating memory paradigm (Morris & Jones, 1990; Pollack *et al.,* 1959) has been used to investigate the neural basis of the central executive and to distinguish between this executive function and slave (storage) systems. Neuroimaging studies have confirmed the dissociation between passive storage of information and active manipulation of incoming information by localising the two processes to separate areas (parietal lobes and frontal lobes respectively) (Smith & Jonides, 1997). More recent neuroimaging studies have often used the n-back task to study this executive function, finding activation in the left frontopolar cortex, bilateral dorsolateral prefrontal and premotor cortex, bilateral intraparietal sulcus, right inferior parietal lobule and the cerebellum (Collette *et al*., 2007). Updating as reviewed in Chapter 3 appears to be more reliably affected by ecstasy use (Montgomery & Fisk, 2008: Montgomery, Fisk, Newcombe & Murphy, 2005).

However there is still a plethora of studies that observe no ecstasy-related deficits in this area (Bhattachary & Powell 2001; Thomasius *et al.,* 2003). The n-back task can be varied for difficulty and is ideal for using during EEG as it is computerised with button responses. Results from the n-back task in ecstasy using populations have not been as consistent as results from consonant updating or spatial updating. Several studies have reported no significant differences between users and controls (Daumann, Fimm *et al.,*2003; Daumann, Fischermann, Heekeren *et al.,* 2004; Gouzoulis-Mayfrank *et al.,* 2003). However, the samples in these studies were smaller than the present study. Moreover task difficulty has rarely been varied to extend further than 2-back and there is evidence to suggest that ecstasy-related deficits may be more pronounced with increased cognitive load (Wareing *et al.,* 2000). As such this study will include a 4-back condition. Further to this, some of the studies on the n-back task that yielded no between group differences behaviourally, were combined with neurophysiological measures and showed subtle brain functioning alterations in ecstasy users (Daumann, Fimm *et al.,* 2003), highlighting the sensitivity of neurophysiological measurements for assessment of cognitive impairment.

Access requires activation of long term memory networks. Although not included in the initial conceptualisation of Miyake *et al*.'s (2000) framework of executive function, Baddeley (1996) suggested that temporary activation of long term memory stores was an important function of the central executive. Indeed in Fisk and Sharp's (2004) work on cognitive ageing, the factor structure obtained was consistent with Miyake *et al*. (2000), though an additional factor was obtained that reflected the efficiency of access to long term memory, as measured by word fluency. Significantly, word fluency has been observed to have neurological correlates in the left prefrontal cortex – left inferior frontal gyrus, anterior cingulate and superior frontal sulcus (Phelps *et al*., 1997). For access to semantic memory some studies using the COWA task have yielded deficits in ecstasy users compared to

controls (Bhattachary & Powell, 2001**;** Fox *et al*. 2002**)**, whereas others report no such deficits (e.g. Halpern *et al.,* 2004). However, as a written variant of the COWA task, the Chicago Word Fluency Test appears to yield more consistent observable deficits in ecstasy users (Montgomery, Fisk, Newcombe & Murphy, 2005; Montgomery *et al.*, 2007). It remains a possibility that a verbal one minute retrieval task, with no restrictions upon word type or length is too simple to require the involvement of the central executive and as such ecstasy users may not show any impairment on the COWA. It has been noted that ecstasy users have shown impairments on difficult aspects of tasks, yet appear unaffected on simple tasks that require relatively automatic processing (Fox *et al*., 2002). Consequently further investigation of ecstasy-related deficits in access to semantic memory is required.

Whitney *et al.,* (2011) investigated the neuronal network involved in semantic retrieval and processing, manipulating strength of semantic association with the cue word (low vs. high). Transcranial Magnetic Stimulation (TMS) was employed to disrupt processing in the Inferior Frontal Gyrus (IFG) and the posterior middle temporal cortex. Disruption to both of these sites produced attenuation of effective processing of executively demanding processes. However processing of cue-target stimuli with strong semantic association (that are relatively automatic) was unaffected by the disruption. It was concluded that there is a network of prefrontal and posterior temporal regions that underlie semantic control, and may provide an explanation of why ecstasy users may be unaffected in relatively simple semantic retrieval tasks, such as the COWA. As such in this experiment a similar semantic association task will be used that has semantic strength manipulation.

Neuroimaging techniques such as EEG are useful in providing a clearer indication of alterations of normal cognitive functioning; for example in patients with Alzheimer's disease, who exhibit increases in prefrontal activity in comparison to controls during executive

functioning tasks. Saykin *et al*. (1998) observed that Alzheimer's patients displayed additional activation in frontal regions which they postulated reflects recruitment of additional resources from local and remote regions when conducting a semantic memory task (see also Grady *et al*. 2003; Woodard *et al*. 1998 for other examples of compensatory mechanisms). Similarly, fMRI research in ecstasy users, has revealed increased BOLD response, during working memory tasks, despite equivalent performance, which has been suggested to reflect compensatory mechanisms due to task inefficiency (Jager *et al.*, 2008; Moeller *et al*., 2004)

ERP research has demonstrated that cognitive impairment is associated with alterations to the P3 amplitude or latency, as the P3 is involved in stimulus processing. Such alterations in P3 activity have been reported in ecstasy users, for example, Casco *et al.* (2005) observed a reduction in P3 amplitude in both heavy and moderate ecstasy user groups compared to controls in Visually Evoked Potentials (VEP) pertaining to a simple discrimination task, though no differences in latency were observed. Furthermore Mejias *et al.* (2005) report longer P3 latencies for detection of target stimuli in a visual oddball task, suggesting reduced cognitive processing. de Sola *et al.* (2008) observed a reduced auditory ERP P3 amplitude in ecstasy users compared to non-drug controls and cannabis users, although this was non-significant.

The Go/NoGo task requires continuous attention to the stimuli to effectively make (Go) or inhibit (NoGo) responses, and is useful for measuring processing and attentional capacity in ERPs (Smith *et al*., 2004). The P3 component, although a significant component in many cognitive tasks due to its involvement in attentional processing, does not appear to have a consistent role in response inhibition. This is possibly due to this component occurring relatively late and therefore not in the initial early inhibition processes.

The N2 component, understood to be important in inhibition, due to this component reflecting stimulus discrimination (Ritter *et al.,* 1982), has been observed to be larger in inhibition trials (NoGo) than non-inhibition (Go) trials (Kok *et al.,* 2004). This component also reflects neuronal processes involved in conflict monitoring, and is determined by the processing of distracting information. Therefore the N2 is often increased in high conflict trials (Yeung & Cohen, 2006). As such it may be expected that switch trials in the number-letter task cause an increase in N2 amplitude.

Low association trials of the semantic association task in the present study possess increased conflict compared to high association trials. This task requires participants to link a cue word with a target word based on their semantic association, whist ignoring irrelevant distractor words. In high association trials the association between the cue and the target is very strong, whereas this is much weaker in the low association trials hence producing conflict. Related tasks that produce conflict, for example the Stroop task have yielded increases in negativity in waveforms of incongruent Stroop trials (West & Alain, 1999) and this has been suggested to reflect increases in attention resources (Potter *et al.,* 2002). Indeed, studies on participants with mild head injuries have observed equivalent performance to controls on cognitive tasks, coupled with increased N2 components that reflect recruitment of additional resources (Rugg, *et al.,* 1993). It is suggested by Rugg *et al.,* (1993) that greater negativity observed in head injury patients ERPs reflect allocation of attention resources necessary to cope with task demands and to achieve similar performance output to controls. Furthermore Suwazono *et al.* (2000) suggest that posterior N2 reflects the degree of attention required for processing stimuli. Increases in attentional demand may reflect allocation of additional resources.

The P2 wave can be observed at anterior and central sites, and elicits a larger response to simple target features that are relatively infrequent (Luck & Hillyard, 1994). This component precedes the N2 and is thought to be involved in the initial inhibition from further processing in target stimuli (Hansen & Hillyard, 1988). The P2 component is an early component in an ERP waveform, and thus is associated with early orienting and stimulus evaluation. Furthermore this component has been observed to increase with age (Amendo & Diaz, 1999; Ford & Pfefferbaum, 1991). Garcia-Larrea *et al*. (1992) suggest that in ageing populations a growing deficit in ability to withdraw attention from stimuli becomes apparent. Increases in the P2 component may reflect early orienting increases in cognitive allocation, or fixation as a function of cognitive ageing, or may even reflect increased impulsivity (Fritzsche *et al.*, 2011).

Recently, Burgess *et al.* (2011) looked at ERPs as evidence for selective impairment of verbal recollection in currently abstinent recreational MDMA/polydrug users. Interestingly, there appeared to be no significant differences between ecstasy users, polydrug controls and drug naïve controls on the behavioural tasks (memory tasks which involved recognition of words and faces). However the ecstasy user group showed attenuation of late positivity over left parietal scalp sites, which is a component associated with the memory process of recollection. Ecstasy users showing a durable abnormality in this ERP component exemplifies how EEG is a much more sensitive measure of cognitive impairment than behavioural measures alone. This point is further elucidated by Nulsen *et al*. (2011) where ecstasy users displayed alternative patterns of activity in ERPs compared to drug naïve and polydrug controls in short term and working memory tasks, despite no significant behavioural differences.

The aim of the current study was to observe whether there are any behavioural or electrophysiological differences between ecstasy users and controls in tasks measuring inhibitory control (Go/NoGo), mental set switching (number-letter task), updating (n-back task) and access (semantic association task). It is predicted that group differences in performance on the Go/NoGo task and the number-letter task will be negligible. However it is expected that ecstasy users may show impaired performance on the n-back task and the semantic association task. Regardless of behavioural performance observable differences in components of the elicited ERPs are predicted. It is envisaged that ecstasy polydrug users will show a diminished P3 response, in line with cognitive impairment. Furthermore if behavioural differences are silent, ERP responses in line with compensatory mechanisms/cognitive impairment are expected. More specifically, increases in N2 and P2 amplitudes that reflect compensatory mechanisms and recruitment of additional resources.

6.3 Method

Design:

In all analyses, a between groups factor of drug user group with three levels (ecstasy user, non-ecstasy polydrug controls and drug naive controls) was employed. Univariate ANOVA was conducted on the behavioural data for the Go/NoGo (inhibition) and the number-letter task (switching) with scores on the Go/NoGo (NoGo errors) and composite scores on the number-letter task (switch cost) as the dependent variables respectively. Mixed ANOVA was conducted on the behavioural data for the semantic association (access) and n-back (updating) tasks, with difficulty level as the within subjects factor (2 levels for semantic association – high association vs. low association, and 3 levels for n-back – n=0, n=2 and n=4).

ERP data on all tasks was analysed using mixed ANOVA, with drug user group as the between subjects factor and electrode site as within subjects factors for the three ERP components. The n-back task and the semantic association task had an extra within subjects factor of difficulty. Mean amplitudes (μvolts) at the selected electrodes for the various components were the dependent variables. Where appropriate significant main effects were further investigated using ANOVA and Tukey HSD tests.

Participants:

Twenty ecstasy users (mean age = 23.95, SD = 0.57, 10 male), 20 non-drug user controls (mean age = 23.1, SD = 0.66, 7 male) and 20 non-ecstasy drug user controls (mean age = 22.58, SD = 0.79, 9 male) were recruited via direct approach to university students and club goers. In terms of statistical power, with 20 participants in each of the three groups, the sample is sufficient to detect a difference between pairs of means of at least 1 standard deviation at alpha = .05 and beta =.20 (Hinkle *et al*., 1994).

Inclusion in the ecstasy user group required participants to have taken ecstasy/MDMA on 5 or more occasions over their lifetime (actual minimum = 5 ecstasy tablets). Indices of ecstasy use were as follows: total lifetime dose 177.65 tablets ± 301.73; mean amount used in last 30 days 0.6 tablets ± 2.26, and frequency of use 0.24 times/week ± 0.42. Furthermore for inclusion in both control groups participants must have never used ecstasy/MDMA, however all other illicit substances were permitted for the poly drug user control group.

All participants were asked to abstain from consuming ecstasy for a minimum of 7 days prior to testing and urine samples were collected upon arrival to the lab to confirm abstinence (after ingestion, MDMA is generally accepted to be detectable in urine for 1-3 days, this is the same for cocaine and amphetamines, with cannabis being detectable for anything up to 95 days Verstraete, 2004). Participants were also requested to abstain from use

of other illicit drugs for a minimum of 24 hours prior to participating and ideally for 7 days. Tobacco smoking was permitted on the day of testing. All participants reported no current or last year diagnosis of psychological disorders.

Materials

Several questionnaires were issued to participants upon entering the lab, these included: A background drug use questionnaire which provides the researcher with indices of drug use patterns and other lifestyle variables. In this questionnaire comprehensive details of ecstasy use as well as other illicit drug use are requested, such as first and last drug use, patterns of drug use, frequencies and doses over time. Using a method employed by (Montgomery, Fisk, Newcombe and Murphy, 2005), estimates of total lifetime drug use of each drug were calculated. Totals for last 30 days drug use as well as weekly drug use estimates were also calculated. This questionnaire also sought information about health, age, years of education and changes to mood and cognition amongst other lifestyle variables.

Measures of sleep quality

Several questionnaires assessing sleep quality and alertness were employed to investigate any possible relationship between sleep quality and cognition. These include a sleep quality questionnaire, exploring typical quantities of sleep (how many hours slept typically, how many hours over the last 3 nights) and level of quality of sleep. The Epworth Sleepiness Scale (ESS, Johns, 1991), explores the chances of dozing or falling asleep in various situations. A high total score here is indicative of increased subjective daytime sleepiness. The Morningness-Eveningness Questionnaire (MEQ, Terman *et al.*, 2001) is a self-assessment of morningness-eveningness in human circadian rhythms (originally developed by Horne & Östberg, 1976). A high score on this questionnaire is indicative of a morning type person and a low score is indicative of an evening type person. Finally the

Karolinska Sleepiness Scale (KSS) (Akerstedt & Gillberg, 1990), is a self-assessment of sleepiness at the current moment in time, therefore this can be administered at different time points of the experiment to assess sleepiness,

State mood.

State Anxiety, Arousal and Depression were measured using scales devised by Fisk & Warr (1996) (The UWIST mood adjective checklist – UMACL). Participants are required to rate on a 5 point likert scale from 1 = not at all, to 5 = extremely, how they are feeling at the time of testing. A high score on each subscale indicates increased hedonic tone/anxiety/arousal.

Raven's SPM (Raven *et al*., 1998)

Raven's standard progressive matrices (SPM) were used an indicator of fluid intelligence. This involves a series of problems (5 sets of 12, 60 in total), presented as a symbolic sequence. Participants are required to select an appropriate response to complete the sequence from a choice of six options. Successful completion of the task requires an understanding of the parts of the sequence and their interaction with one another. Each block of 12 problems begins with an intuitively simple problem and the problems become progressively more difficult as the task continues.

NASA-TLX (Hart & Staveland, 1988)

This is a multi-dimensional scale, consisting of six sub-scales (mental demand, physical demand, temporal demand, personal performance rating, effort and frustration). Participants are required to place a mark on a 100ml VAS, indicating where they perceive their demand to be on the scale. These are administered to observe whether there are any differences between ecstasy users and non-users in demand perceived by the participant as it

has been suggested that ecstasy users may be more susceptible to stress than non-users (Wetherell *et al.*, 2012).

Tasks

All behavioural tasks were programmed in Inquisit version 3.0.6.0 (Millisecond software, 2011).

Inhibitory Control: The Go/NoGo task is frequently used in combination with EEG to assess inhibitory control (Gamma *et al*, 2005; Kok, 1986; Oddy & Barry, 2009). Participants are required to "Go" (press the space bar) when an X appears on the screen, however they are to inhibit their response "NoGo", when any other letter appears (W, Y or Z). The task is designed such that "X" appears 75% of the time and the "NoGo" letters appear only 25% of the time. Thus, the task builds up a pre-potent response to "Go". Furthermore, the first block of the task has "X" appearing 100% of the time, again to build up a pre-potent/dominant response which participants are required to inhibit. The task therefore comprises of two blocks; a practise block with 60 "Go" trials, followed by an interval and then a larger main block whereby participants are required to attend to 240 trials (180 Go/ 60 NoGo) lasting a total of approximately 15 minutes. The task has an inter-trial interval of 1.5 seconds and participants had an epoch of 2.5 seconds from stimulus onset to respond. Participants were instructed to respond as quickly and as accurately as possible.

Mental set switching: This executive function was investigated using the number-letter task as per Rogers and Monsell (1995). During this task, number-letter pairs e.g. "B6" are displayed in one of four quadrants on a screen. If the number-letter pair appears in one of the top two quadrants, participants attend to the letter and respond to whether it is a vowel or a consonant. If the pair appears in the bottom two quadrants, participants are required to attend to the number and respond to whether it is odd or even. In the first block of trials the

number-letter pairs alternate between the top two quadrants; in the second block the pairs alternate between the bottom two quadrants. In the final block, the pairs are presented in anti-clockwise rotation, therefore every two responses requires a switch in the mental set between letters and numbers. The latency difference between the trials with the switch and those not requiring a switch is the "switch cost". The task is comprised of six blocks, the first two of which are practise blocks consisting to 62 trials in each. This is followed by four main blocks, each consisting of 64 trials (31 "switch" trials). There were 124 "switch" trials in total. There was an inter-trial interval of 1.5 seconds and participants were allocated an epoch of 5 seconds to respond. Participants were instructed to respond as quickly and as accurately as possible, and overall the task took around 20 minutes to complete.

Access to Semantic Memory: This was assessed using a semantic association task based on those used by Whitney *et al.* (2011) and Badre *et al.* (2005); two types of semantic judgement which differed in their level of difficulty (high association/low association) were used. In both difficulty levels participants were presented with a cue word in the centre of a computer monitor followed by three target words, one which had a semantic association with the cue, and two distracters. Participants had to decide which of the three target words had the strongest semantic association with the cue word. Participants selected their answer by pressing one of three buttons on a response box which corresponded to their position on screen. They were either high association between cue and target words (e.g. candle - flame) or low association (e.g. detective - search). The low association judgement is deemed to be more difficult and require more processing than the relatively automatic high association semantic judgements. As such the low association between cue and targets leads to a less obvious dissociation from distracters requiring recruitment of additional executive resources in the semantic network (Whitney *et al.*, 2011). The stimuli used were matched for word length, frequency and cue-target association strength (Badre *et al*., 2005; Whitney *et al.*, 2011)

and were kindly provided by Whitney *et al*. The task consisted of a practise round followed by 4 blocks of 30 trials, with both high and low association trial types appearing in each block pseudo-randomly (15 of each in each block). The cue word was presented for 1-second in the centre of a computer screen. After this the three target words appeared below aligned to the left, centre and right of the monitor. Participants were instructed to respond by pressing a button on the response box corresponding to the position of the target on the screen (left, centre, right). The targets remained on screen until a response was made or until the trial timed-out (time out set to 8.5 seconds). An inter trial interval of 2 seconds was employed. The task took around 20 minutes to complete. Participants were instructed to respond as quickly and as accurately as possible.

Updating: This function was assessed using an n-back task. A variant of the task first implemented by Kirchner (1958) was designed, whereby participants were shown a series of digits presented singularly (either 7 or 8 digits in a series) followed by a probe requiring participants to recall the "nth" digit back in the sequence, if n=0 was the last digit presented. A version of the task was used in which after the series of digits were displayed participants were required to recall n = 0, n = 2 or n = 4. Three blocks of 65 trials were completed, with trial types appearing in each block pseudo randomly. Participants responded by selecting the desired number (0-9), via scrolling through the numbers using the arrow keys on a likert scale. The scale stayed on the screen until a response was made or until the trial timed-out (time out set to 12 seconds). There was an inter trial interval of 3.5 seconds. The task took around 60 minutes to complete and participants were instructed to respond as quickly and as accurately as possible.

Equipment

Electroencephalography (EEG) was recorded using a 64 channel Biosemi Ag-AgCl active-two electrode system (Biosemi B.V, Amsterdam, Netherlands) with pin type electrodes mounted in a stretch-lycra headcap (Biosemi). Electrodes were positioned according to the international 10-20 system. Electrical activity was recorded from the following sites: frontal (FPz, FP1, FP2), anterior-frontal (AFz, AF3, AF4, AF7, AF8), frontal (Fz, F1, F2, F3, F4, F5, F6, F7, F8), frontocentral (FCz, FC1, FC2, FC3, FC4, FC5, FC6), central (Cz, C1, C2, C3, C4, C5, C6), temporal (FT7, FT8, T7, T8, TP7, TP8), parietocentral (CPz, CP1, CP2, CP3, CP4, CP5, CP6), parietal (Pz, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10), occipitoparietal (POz, PO3, PO4, PO7, PO8) and occipital (Oz, O1, O2, Iz). Sigma electrolyte gel was used to ensure contact between scalp and electrodes. Vertical and horizontal electro-occulograms were recorded using bipolar, flat Ag-ACl electrodes positioned above and below the left eye as well as to the outer side of each eye. Data was digitized at a sampling rate of 512Hz and no filters were applied online so that the data could be visually inspected for noise and offline filtering could be performed.

Procedure

Testing sessions commenced at 9.30am or 1.30pm, and equal amounts of participants from each condition were tested in the morning as were in the afternoon. Upon entering the lab, participants were given a brief description of the experiment and written consent was obtained. Following this, participants were required to give a urine sample. The urine sample was frozen at -25 Celsius and later transported to the clinical laboratories for analysis. First, participants were required to fill out the battery of questionnaires whilst their head circumference and other details were measured, and an electrode cap and electrodes were fitted. The questionnaires were administered in the following order: Background drug use

questionnaire, Morningness-Eveningness questionnaire, sleep quality questionnaire, mood scale, Epworth Sleepiness Scale, Karolinska Sleepiness Scale (pre-test) and fluid intelligence was assessed using Raven's SPM. Following completion of these questionnaires, providing the EEG setup was correct and actiview running, the computerised tasks was completed on a desktop computer running inquisit version 3.0.6.0 (Millisecond software, 2011). The NASA-TLX questionnaire was completed after each task. Upon completion of the tasks a final Karolinska Sleepiness Scale (after) was administered. Finally participants were fully debriefed and paid £20 in store vouchers. The study was approved by the Liverpool John Moores University Research Ethics Committee, and was administered in accordance with the ethical guidelines of the British Psychological Society.

EEG Analysis – Go/NoGo

The EEG data was analysed using BESA 5.3 (MEGIS software GmbH, Gräfelfing, Germany)**.** All recordings were visually analysed offline, using high and low pass filters of 0.1Hz and 40 Hz respectively. Any channels judged to be bad were replaced by interpolation and all data were EOG-corrected using BESAs PCA based algorithm. All trials judged to be bad after this point were discarded.

Go/NoGo: EEG was segmented into epochs from -500 to 1000ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (e.g. correct "go" responses, correct "NoGo" responses and incorrect "NoGo" responses in the Go/NoGo task) could be generated for each individual. Only ERPs for correct responses on the "NoGo" condition were included in the subsequent analysis. There were 240 trials in the main block of the task, 60 of which were "NoGo" trials. The mean number of good "NoGo" trials retained for grand averaging per subject was 51.92 (average of 13.5% rejected trials), after rejecting incorrect

trials (5%) and those containing artefacts (8.5%). Grand averages were made for each group (ecstasy user, polydrug user and drug naïve) on each task condition (correct "Go" responses, correct "NoGo" responses). The overall P3 response was defined as the mean amplitude between 352 and 452ms. This time window was centred on the positive peak latency and the duration was chosen due to this epoch containing the majority of positive activity for all conditions by observing topographic maps (See Figure 6.1). Midline electrode activity was obtained in this epoch from electrodes Fz, FCz, Cz, CPz and Pz, as much of the activity could be observed in these sites as well as these midline electrodes being commonly used for this task in the literature (Jonkman 2006; Kato *et al.,* 2009). In addition further components were analysed for between group differences, including the N2 and P2 components. The N2 of subjects in response to the inhibitory condition, was defined as the mean amplitude between 260 and 330ms, this epoch was based around the mean local negative peak at midline sites and encompassed the majority of negative activity for all conditions. The P2 epoch was obtained from using a small, 50ms epoch (200-250ms) based around the positive peak from the grand averages of all conditions, directly preceding the N2.

Figure 6.1. Topographies at midpoints for each component (P2, N2 and P3) for the Go/NoGo task.

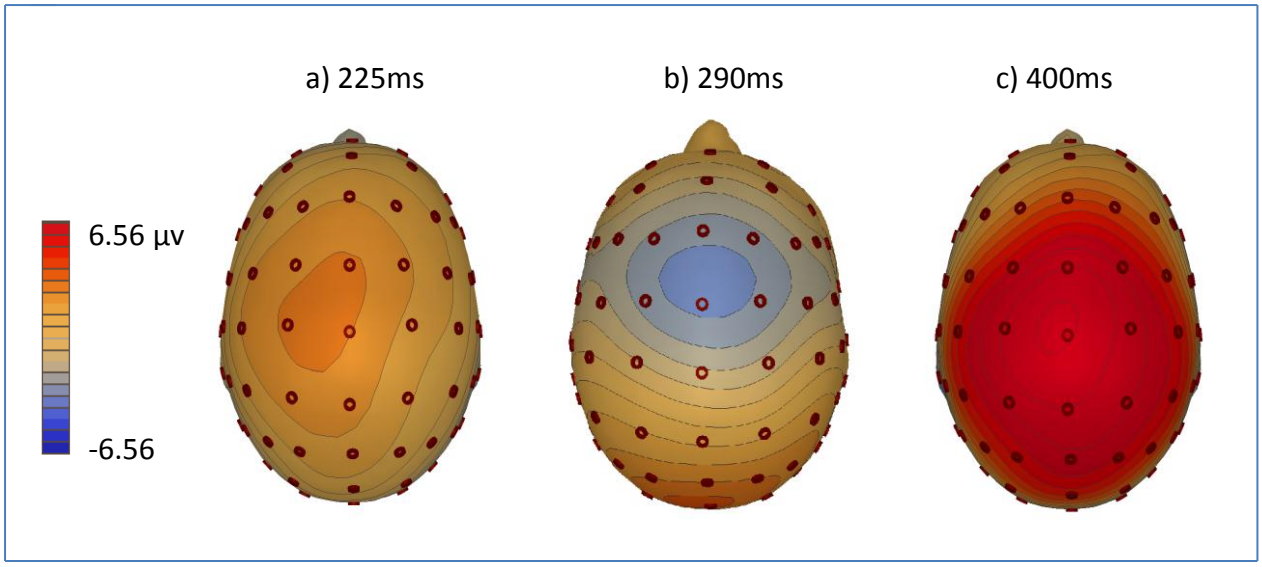

Fig. 6.1: Depicts grand average topographies for the central point of each component. Note that this is from grand averages of each group combined. P2 positivity (red) is clustered around the midline electrodes (a). N2 negativity (blue) is greatest in anterior midline electrodes (b). P3 positivity has a wide spread of activity peaking at central electrodes (c).

Number-Letter: EEG was segmented into epochs from -500 to 1000ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (e.g. correct "switches", correct "non-switches" and incorrect "switches" and "non-switches") could be generated for each individual. Only ERPs for correct responses on the "switch" condition were included in the subsequent analysis. There were 124 "switch" trials in the entirety of the task. The mean number of good "switch" trials retained for grand averaging per subject was 96.37 (average 22.28% rejected trials), after rejecting incorrect trials (4.48%) and those containing artefacts (17.8%). Grand averages were made for each grouping condition (ecstasy user, polydrug user and drug naïve) on each task condition (correct "switches", correct "non-switches"). The overall P3 response was defined as the mean amplitude between 290 and 400ms (the window was centred on the positive peak latency and the duration was chosen as this epoch contained

the majority of positive activity for all conditions. See Figure 6.2). Electrode activity was analysed in this epoch from parieto-occipital and occipital electrodes POz, PO3, PO4, PO7, PO8, Oz, O1 and O2, as the greatest amount of activity in the P3 component could be observed at these sites. Further components were also analysed for between group differences, including the N2 and P2 components. The N2 component appeared to be largest over occipital and parieto-occipital sites P7, P8, POz, PO3, PO4, PO7, PO8, Oz, O1, and O2, between 170-220ms, this epoch was based around the mean local negative peak at these sites and encompassed the majority of negative activity over all 3 conditions. The P2 epoch was most visible as a positive peak between 200-250ms at frontal, fronto-central and central sites Fz, FCZ, FC1, FC2, FC3, FC4 and Cz. The mean amplitudes at these sites from the epoch based around the positive peak from the grand averages of all conditions were analysed.

Figure 6.2. Number-letter task topographies at midpoints for each component (P2, N2 and P3).

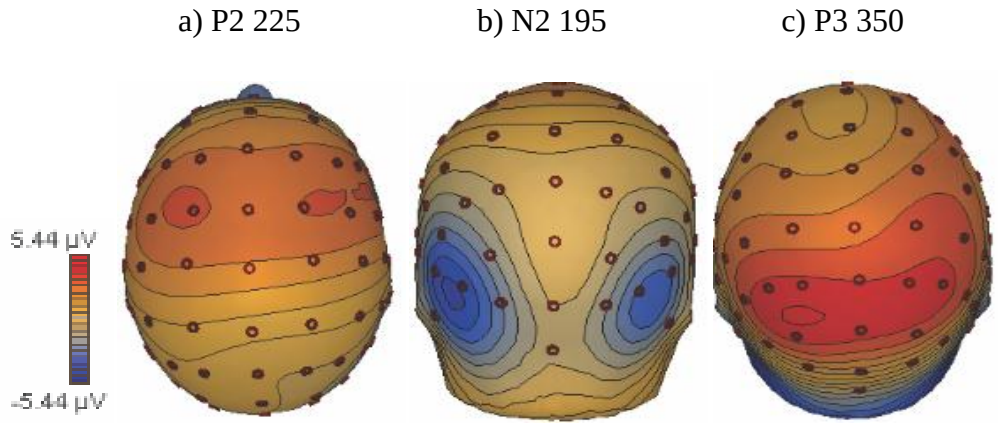

Fig. 6.2: Grand averaged topographies from the central point of each component during the number-letter task. Positivity (red) in the P2 component in fronto-central electrodes can be observed (a). N2 Negativity (in blue) can be observed over occipital and parieto-occipital sites (b). P3 positivity is greatest over occipital and parieto-occipital sites (c).

Semantic Association: EEG was segmented into epochs from -500 to 1000ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (i.e. correct "high associations" and incorrect "high associations" and correct "low associations" and incorrect "low associations") could be generated for each individual. Only ERPs for correct responses were included in the subsequent analysis. There were 120 trials in total, the mean number of good trials retained for grand averaging per subject was 109.66 (average of 8.6% rejected trials), after rejecting incorrect trials (6.1%) and those containing artefacts (2.5%). Grand averages were made for each group on each condition (correct "high associations", correct "low associations"). The overall P3 response was defined as the mean amplitude between 280 and 350ms, for the low association condition and 250-350ms for the high association condition. These time windows were centred on the positive peak latency and the duration was chosen due to this epoch containing the majority of positive activity for all conditions by observing topographic maps (See Figures 6.3 & 6.4). Electrode activity was analysed in this epoch from parieto-occipital and occipital electrodes PO7, PO3, O1, OZ, POZ, PO4, PO8 and O2, as the greatest amount of activity in the P3 component could be observed at these sites. Further components were also analysed for between group differences, including the N2 and P2 components. The N2 component was also largest over parieto-occipital and occipital electrodes (PO7, PO3, O1, OZ, POZ, , PO4, PO8 and O2) , between 120-190ms in the low association condition and 120-200ms in the high association condition, again epochs were based around the mean local negative peak at these sites and encompassed the majority of negative activity over all 3 groups. The P2 component was most visible as a positive peak between 170 and 230ms (for both low and high association) at anterior and midline sites (FZ, FCZ, FC1, FC2, CZ, C1 and C2) the mean amplitudes at these sites from the epochs based around the peaks from the grand averages of all conditions were analysed.

Figure 6.3. Semantic association task topographies at midpoints for each component (P2, N2 and P3) in the high association condition.

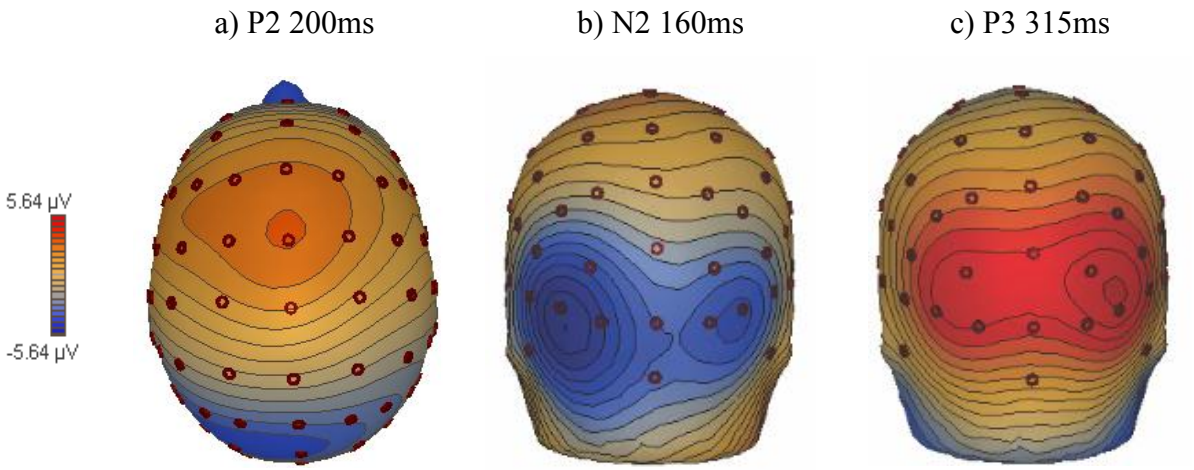

Fig. 6.3: Grand averaged topographies for central points of each component during high association trials. P2 positivity is greatest at midline/anterior electrodes (a). N2 negativity is greatest in occipital and parieto-occipital electrodes (b). Positivity in the P3 component is greatest around occipital and parieto-occipital electrodes (c).

Figure 6.4. Semantic association topographies at midpoints for each component (P2, N2 and P3) in the low association condition.

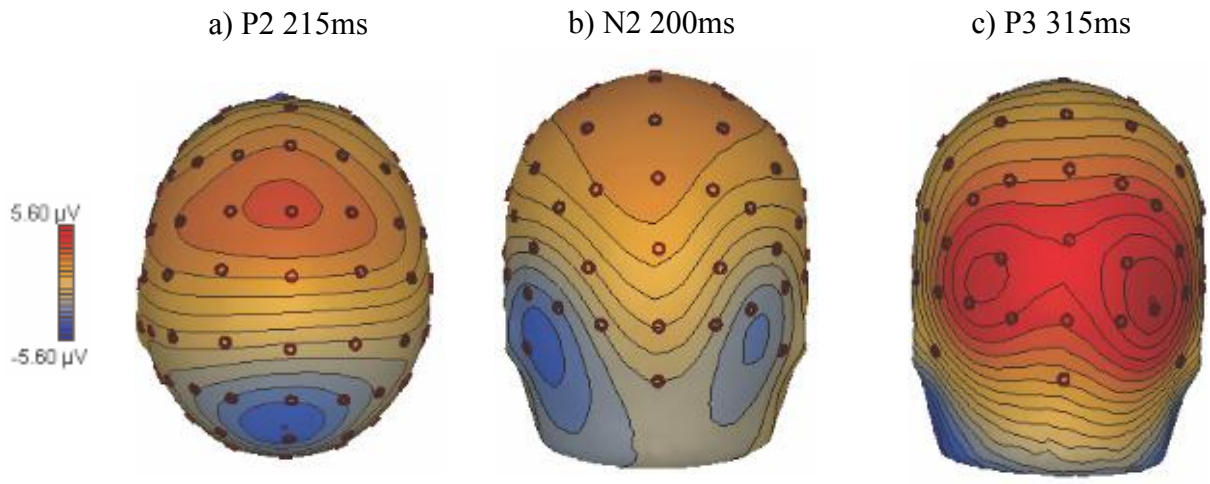

Fig. 6.4: Grand averaged topographies for central points of each component during low association trials. P2 is greatest at midline/anterior electrodes (a). N2 negativity is greatest around occipital and parieto-occipital electrodes (b). Positivity in the P3 component that is greatest over occipital and parieto-occipital electrodes can be observed (c).

N-back: EEG was segmented into epochs from -500 to 1000ms from time of probe onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each (e.g. correct "N = 0" responses, correct "N = 2" and correct "N = 4" responses and incorrect "N = 0", "N = 2" and "N = 4" responses) could be generated for each individual. Only ERPs for correct responses were included in the subsequent analysis. There were 195 trials in total, the mean number of good trials retained for grand averaging per subject was 114.97 (average of 41.03% rejected trials), after rejecting incorrect trials (35.15%) and those containing artefacts (5.88%). Grand averages were made for each group on each task condition (correct "N = 0", "N = 2" and "N = 4"responses). The overall P3 response was defined as the mean amplitude between 280 and 400ms. This time window was centred on the positive peak latency and the duration was chosen due to this

epoch containing the majority of positive activity for all conditions by observing topographic maps (See Figure 6.5). Posterior electrode activity was obtained in this epoch from electrodes P7, P5, PO7, PO3, O1, OZ, POZ, PO4, O2, PO8, P8, P6, as much of the activity could be observed in these sites. The N2 was defined as the mean amplitude between 140 and 230ms, this epoch was based around the mean local negative peak at posterior sites (P6, P8 & PO8) and encompassed the majority of negative activity over all 3 conditions. The P2 epoch was obtained from using a small, 50ms epoch (200-250ms) based around the positive peak from the grand averages of all conditions, here midline and anterior electrodes were used for analysis (F1, F3, FC1, Fz, F2, F4, FC2, FCz & C2).

Figure 6.5: N-back topographies at midpoints for each component (P2, N2 and P3) in the n=2 condition.

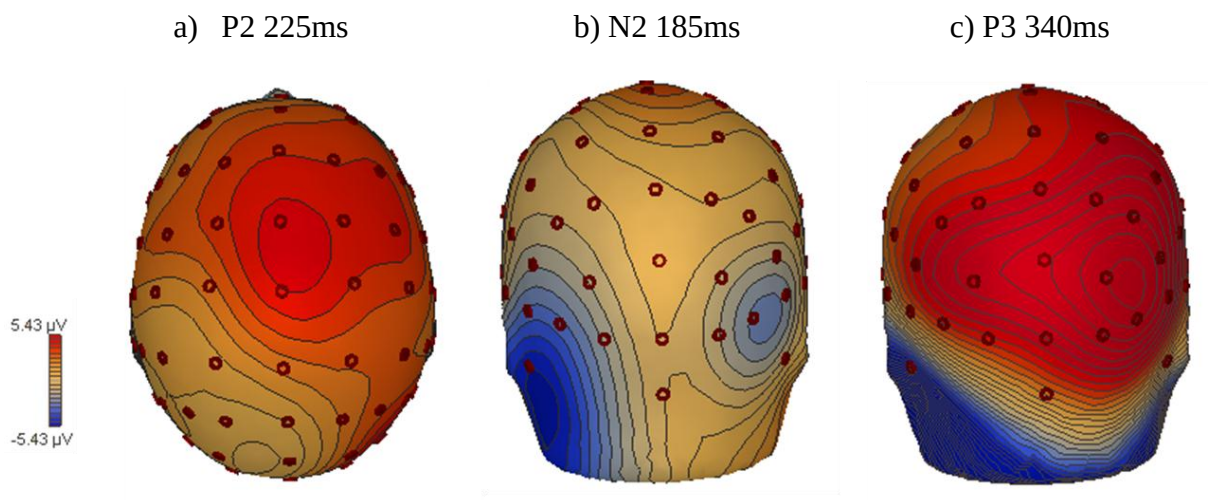

Fig. 6.5: Grand averaged topographies for central points of each component during n=2 trials. P2 is greatest at anterior electrodes (a). N2 negativity is greatest around occipital and parieto-occipital electrodes (b). Positivity in the P3 component is greatest over occipital and parieto-occipital electrodes (c).

Urinary Analysis

Frozen urine samples were delivered to University Hospital Aintree (NHS) and were analysed using Solid Phase Extraction (Mixed Mode Phase) followed by Reverse Phase HPLC MS/MS detection using BOTH Positive & Negative Ion Multiple Reaction Monitoring (MRM). Urine Specimens were been tested for the Synthetic Cannabinoids (JWH-018, JWH-073, JWH-250, JWH-398, JWH-122, JWH-019, AM-694, WIN 48098 & WIN-55212-2), as well as the 'designer' drugs 'Mephedrone', bk-MDMA or 'Methylone', bk-MBDB or 'Butylone', bk-PMMA or 'Methedrone', 1-benzylpiperazine, TFMPP, mCPP and MDPV. In addition they were tested for were a series of 12 Piperazine compounds, 4 β-Keto Amphetamines, a series of 11 Methcathinone compounds, 4-Fluoroamphetamine, Bupropion & the Hallucinogenic Amphetamines: D.O.B. ('bromo-STP' or 'Brolamphetamine'), D.O.C. and D.O.I. and 'Traditional' Drugs of Abuse: Amphetamine(s) including M.D.M.A., M.D.A. & M.D.E.A., Barbiturates, Benzodiazepines, THC & Cannabinoids, Buprenorphine, Cocaine & metabolites, Methadone & metabolites, Opiates & Opioids (Morphine, Codeine, Dihydrocodeine, Tramadol, d-Propoxyphene, Oxymorphone & Oxycodone), LSD, G.H.B. (and the Lactone Precursor), Psilocybin, Ketamine and Methaqualone.

6.4 Results

Socio-demographic information about the participants, anxiety, depression and arousal scores from the mood scale and sleep measures are shown in Table 6.1. Indices of other drug and alcohol use are displayed in Table 6.2.

Table 6.1 – Indices sleep quality, fluid intelligence and socio-demographic variables

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
Males: n (%)	10 (50)	9 (45)	7 (35)
Age (SD)	23.94 (2.50)	22.58 (3.45)	23.10 (2.94)
University degree: n (%)	14 (70)	12 (60)	11 (55)
Employment status			
Student; n, (%)	12 (60)	14 (70)	17 (85)
Employed; n (%)	4 (20)	4 (20)	3 (15)
Unemployed; n (%)	4 (20)	2 (10)	0 (0)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Ravens Progressive Matrices (maximum 60)	48.68 (5.96)	48.35 (5.83)	51.35 (5.01)
Sleep – Hours/night	7.13 (1.91)	7.8 (1.39)	7.05 (1.16)
ESS Score (maximum 24)	6.5 (3.3)	6.7 (3.15)	6.5 (3.32)
KSS before	5.05 (1.93)*	3.75 (1.48)	4.79 (1.23)
KSS after	6.53 (2.03)	5.85 (1.53)	6.56 (1.46)
MEQ total	42.10 (10.15)	45.70 (9.40)	47.90 (8.30)
UMACL anxiety	11.4 (4.08)	12.44 (2.18)	11.75 (2.12)
UMACL depression	13.1 (3.91)	12.61 (2.40)	12.1(3.14)
UMACL arousal	19.7 (4.54)	20.5 (3.68)	20.1 (3.02)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Table 6.2: Indices of other drug use

	Ecstasy users		Polydrug controls		Drug naive controls	
	Mean (SD)	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**n**
Cannabis						
Frequency (times/wk)	2.67 (3.24)	12	0.95 (1.9)	13	-	-
Last 30 days (joints)	32.77 (53.75)	15	6.09 (15.34)	17	-	-
Total use (joints)	5057.88 (7504.30)	16	1091.71 (2531.65)	19	-	-
Cocaine						
Frequency (times/wk)	0.15 (0.14)	11	0.27 (0.34)	2	-	-
Last 30 days (lines)	0.4 (1.12)	15	1.60 (3.58)	5	-	-
Total use (lines)	813.97 (1940.19)	16	107.30 (208.43)	5	-	-
Ketamine						
Frequency (times/wk)	0.26 (0.42)	5	0.02 (-)	1	-	-
Last 30 days use (grams)	1 (2.65)	9	-	-	-	-
Total use (grams)	31.26 (70.61)	11	1.13 (1.62)	3	-	-
Alcohol units p/w	15.33 (15.29)	20	10.53 (8.37)	20	9.93 (11.58)	20

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;

† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

One way ANOVAs revealed that there were no significant between group differences on measures such as age, average hours sleep per night, total score on the Epworth Sleepiness Scale, Morningness-Eveningness questionnaire total score, post-test Karolinska Sleepiness Scale, levels of arousal, depression and anxiety, total score on Ravens SPM or average weekly alcohol consumption. However there were between group differences in the pre-testing Karolinska Sleepiness Scale (i.e. how sleepy the participants felt before the test battery) $F(2,58)=3.78$, $p<.05$, planned comparison t-tests revealed that the ecstasy user group felt significantly more sleepy prior to testing than the polydrug control group $t(38)=2.39$, $p<.05$, but not the drug naïve control group $t(37)=0.50$, $p>.05$.

t-tests between the ecstasy user group and polydrug controls revealed that the ecstasy user group had a larger lifetime total of cannabis joints smoked (5057.88 ± 7504.30) than the non-ecstasy drug users (1091.71 ± 2531.65), that is approaching significance $t(17.88)=2.02$, $p=.06$ (Levene's test was significant so degrees of freedom have been adjusted accordingly). The ecstasy users had also smoked more joints within the last 30 days (32.77 ± 53.75 compared to 6.09 ± 15.34) and this difference was approaching significance $t(16.01)=1.86$, $p=.08$. There were however no differences between these two groups on other drug intake variables. However as can be seen from table 6.2, the ecstasy user group can be described as polydrug users.

Urinary analysis

The following metabolites were found in participants' urine.

Table 6.3: Amounts of various drug metabolites found in urine samples (mg/L)

		THC	**Δ^9 THC**	**11-hydroxy-Δ^9-THC**	**1-Benzylpiperazine**	**TFMPP**
Ecstasy users	N	3	3	3	1	1
	Mean	0.0083	0.16	0.003	0.84	0.18
	SD	0.01185	0.18286	0.00346	-	-
Polydrug controls	N	1	1	1	-	-
	Mean	0.001	0.41	0.0020	-	-
	SD	-	-	-	-	-

As participants were asked to remain abstinent before attending the lab, relatively low levels of drug metabolites were found. As such, we re-ran all main analyses excluding the participants who had metabolites in their urine. This did not affect the significant and non-significant results so the analyses reported below contain all participants.

Behavioural Data Analysis

All behavioural data was analysed using SPSS (17). Incorrect answers in each case were given a score of 0. Therefore an error count could be performed. Furthermore these trials were not included in reaction time analysis. Mean reaction times were calculated for correct responses only. Reaction time data reduction involved excluding reaction times less than 200ms and greater than 5000ms as these reaction times represent pre-emptive responding and a loss of concentration respectively. Individual trial reaction times that were more than 3 standard deviations above the individual mean were discarded.

The Go/NoGo task: Reaction time was not an appropriate measure for correct "NoGo" responses. Univariate Analysis of Variance (ANOVA) revealed that there was no significant difference between groups in performance on this task $F(2,57)=1.15$, $p>.05$. The mean "NoGo" errors (i.e. responding to a letter other than an X that required no response/an inhibition of response) were used as the measure of performance in this case (ecstasy users: 2.7± 1.95, polydrug controls: 3.4 ± 2.80, drug naïve: 4.35 ± 4.92).

Post task NASA TLX scores were analysed using a MANOVA. This revealed no overall between group differences in task load $F(12,102)=0.52$, $p>.05$, nor any between group differences on the individual sub-scales (Mental demand; $F(2,55)=0.15$, $p>.05$, Physical demand; $F(2,55)=0.71$, $p>.05$, Temporal demand; $F(2,55)=1.11$, $p>.05$, Effort; $F(2,55)=0.09$, $p>.05$, Performance; $F(2,55)=0.45$, $p>.05$, Frustration; $F(2,55)= .01$, $p>.05$).

Number-Letter task: Mean reaction times were calculated for correct switch trials as well as correct non-switch trials so that a switch cost could be calculated. The mean percentage of outliers that were discarded from each group were: ecstasy users 1.27 (±0.73) (rank = 24.58), polydrug controls 1.64 (± 0.77) (rank = 33.75), drug naïve 6.56 (±22.0) (rank = 33.18), Levene's test was violated so an independent samples Kruskal-Wallis test was conducted, there were no between group differences in amount of outliers ($H(2) = 3.53$, $p>.05$). Switch cost was calculated by subtracting the mean reaction time from two preliminary blocks with no switching (all letters, followed by all numbers) from the mean reaction time from the switch trials (from letters to numbers) in the main blocks of the task. One participant in the drug naïve group had an incomplete dataset for this task and was excluded from analysis. ANOVA revealed that there was no significant difference between groups on switch cost $F(2,56)=0.41$, $p>.05$ (ecstasy users: 303.56 ± 194.15, polydrug controls: 331.44 ± 229.47, drug naïve controls: 274.09 ± 158.27).

Post task NASA TLX scores were analysed using a Multivariate Analysis of Variance MANOVA. This revealed no overall between group differences in task load $F(12,100)=1.62$, $p>.05$ for Pillai's trace, nor any between group differences on the individual sub-scales (Mental demand; $F(2,54)=2.21$, $p>.05$, Physical demand; $F(2,54)=2.07$, $p>.05$, Temporal demand; $F(2,54)=2.41$, $p>.05$, Effort; $F(2,54)=1.58$, $p>.05$, Frustration; $F(2,54)=0.37$, $p>.05$ with the exception of performance $F(2,54)=2.99$, $p=.06$ (approaching significance), multiple comparisons revealed that polydrug controls thought they had performed significantly better than ecstasy users $p<.05$.

Semantic Association Task: The mean percentage of outliers that were discarded from each group were; ecstasy users 1.46 (±0.66), polydrug controls 1.42 (± 1.05), drug naïve controls 1.71 (±0.92), there were no between group differences in amount of outliers $F(2,57)=0.63$, $p>.05$. Performance on the semantic retrieval task was measured both in terms of number of errors made (incorrect responses) and reaction time. Mixed ANOVA on error count revealed no significant effect of difficulty $F(1,57)=0.04$, $p>.05$, no main effect of group $F(2,57)=1.56$, $p>.05$ and no group by difficulty interaction $F(2,57)=0.01$, $p>.05$. Similarly using reaction time as the dependent variable no significant between group differences were observed $F(2,57)=0.07$ $p>.05$. Difficulty and group by difficulty interactions were non-significant $p>.05$ in both cases (Table 6.4).

Table 6.4: Performance data (means and SDs of error count and reaction times) for all participants in both conditions of the semantic association task.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
High association errors	4.00 (2.34)	4.60 (2.78)	5.25 (2.77)
Low association errors	4.10 (2.57)	4.60 (2.09)	5.35 (2.92)
High association RT (ms)	1282.26 (255.91)	1294.43 (354.77)	1209.39 (230.89)
Low association RT (ms)	1265.14 (250.85)	1294.21(308.44)	1180.39 (198.60)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Post task NASA TLX scores were analysed using a multivariate analysis of variance MANOVA. This revealed no overall between group differences in perceived demand $F(12,104)=0.94$, $p>.05$ for Pillai's trace, nor any between group differences on the individual sub-scales of their perception of subjective workload (Mental demand; $F(2,56)=1.06$, $p>.05$, Physical demand; $F(2,56)=0.10$, $p>.05$, Temporal demand; $F(2,56)=1.56$, $p>.05$, Effort; $F(2,56)=0.48$, $p>.05$, Performance; $F(2,56)=2.62$, $p>.05$, Frustration; $F(2,56)=0.77$, $p>.05$).

N-back task: The mean percentage of outliers that were discarded from each group were; ecstasy users 1.18 (±0.71), polydrug users 6.54 (±22.01), drug naïve controls 6.12 (±22.11), there were no between group differences in amount of outliers $F(2,57)=0.55$, $p>.05$.

A mixed analysis of variance (ANOVA) was conducted on both the mean reaction times and number of errors on the *n*-back task, with between subjects factor of user group (3 levels) and within subject factor of difficulty (ranging from low difficulty; n=0, to medium difficulty; n=2 and high difficulty; n=4). The error count yielded a significant effect of difficulty (3 levels) $F(2,110)=3.27$, $p<0.05$, but no significant main effect of group $F(2,55)=1.35$, $p>0.05$ and no group by difficulty interaction $F(4,110)=0.30$, $p>.05$.

Mixed ANOVA on the mean reaction times revealed a significant effect of difficulty $F(2,110)=16.92$, $p<0.001$, and also a main effect of group $F(2,55)=3.80$, $p<0.05$. There was, however no group by difficulty interaction $F(4,110)=0.22$, $p>.05$.

To explore the main effect of group further, univariate ANOVAs were conducted on each difficulty level for mean reaction time. Significant between group differences in reaction time were observed at n=0 $F(2,55)=3.18$, $p<0.05$, n=2 $F(2,55)=4.50$, $p<0.05$ and n=4 $F(2,55)=4.50$, $p<0.05$. Planned comparisons revealed that drug naïve participants took significantly longer to respond than polydrug users at each level ($p<.05$ in each case), there were no significant differences between ecstasy users and the two control groups in reaction time at any level of the task (table 6.5).

Table 6.5: Performance data (means and SDs of error count and reaction times) on the n-back task.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
N=0 errors	21.90 (15.21)	15.68 (8.21)	17.11 (10.75)
N=2 errors	21.80 (16.11)	15.37 (9.26)	17.89 (12.15)
N=4 errors	22.65 (14.67)	16.95 (8.87)	18.63 (10.54)
N=0 RT (ms)	2895.12 (699.09)	2894.72 (678.82)†	3387.54 (698.82)
N=2 RT (ms)	2755.59 (607.23)	2670.61 (2670.61)†	3205.60 (543.65)
N=4 RT (ms)	2754.19 (651.71)	2674.15 (606.48)†	3162.48 (666.97)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Post task NASA TLX scores were analysed using a multivariate analysis of variance MANOVA. This revealed no overall between group differences in perceived demand F (12, 104) = 0.94, p>.05 for Pillai's trace, nor any between group differences on the individual sub-scales of their subjective perception of subjective workload (Mental demand; F(2, 56) = 1.06, p>.05, Physical demand; F(2, 56) = 0.10, p>.05, Temporal demand; F(2, 56) = 1.56, p>.05, Effort; F(2, 56) = 0.48, p>.05, Performance; F(2, 56) = 2.62, p>.05, Frustration; F(2, 56) = 0.77, p>.05).

ERP analysis

Go/NoGo: Mean amplitudes for each condition and electrode are given in Table 6.6. Due to some unusable EEG data, 1 participant is excluded from statistical analysis on the EEG data, from the drug naïve group (n=19).

Table 6.6: Mean amplitudes (μvolts) across components, for each electrode measured (Go/NoGo).

User group	CPz	Cz	FCz	Fz	Pz
	P2				
Ecstasy users	2.17 (1.82)	1.94 (2.69)	2.08 (2.15) *†	1.43 (2.13)*	1.45 (1.84)
Polydrug controls	1.3 (1.28)	1.16 (1.9)	0.29 (2.22)	0.40 (1.94)	1.43 (1.92)
Drug naïve controls	1.49 (3.24)	0.84 (2.1)	-0.14 (2.12)	-.30 (1.79)	1.64 (2.51)
	N2				
Ecstasy users	1.38 (2.43)	-0.58 (3.60	-1.92 (3.27)	-2.00 (2.14	2.66 (1.72)
Polydrug controls	0.78 (2.67)	-0.82 (2.95)	-3.21 (3.33)	-2.87 (2.96)	2.16 (2.61)
Drug naïve controls	0.41 (3.50)	-1.42 (4.37)	-3.44 (4.33)	-3.12 (3.20)	2.16 (2.43)
	P3				
Ecstasy users	4.94 (2.15)	5.04 (2.82)	4.06 (2.22)	1.05 (1.74)	4.29 (1.95)
Polydrug controls	4.07 (2.84)	4.56 (4.20)	2.91 (3.93)	0.49 (3.06)	3.79 (2.50)
Drug naïve controls	4.76 (2.65)	5.12 (2.77)	3.59 (3.23)	0.93 (3.12)	4.35 (2.10)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Mixed ANOVA[1] of mean amplitudes at component P3 (352-452ms) revealed a significant main effect of electrode site $F(2.55, 143.03)=38.01$, $p<.01$. However, the electrode by user group interaction was non-significant $F(5.11, 143.03)=0.11$, $p>.05$. There was no main effect of group $F(2,56)=0.61$, $p>.05$. As such this component is not discussed further.

At the N2 component (260–330ms), mixed ANOVA revealed a significant main effect of electrode site $F(2.28, 127.64)=59.92$, $p<.01$. However there was no significant electrode by group interaction $F(4.56, 127.64)=0.25$, $p>.05$. There was no main effect of group in this component $F(2,56)=0.86$, $p>.05$.

[1] In all mixed ANOVAs (for electrode data in Chapter 6), Mauchley's test was significant so adjusted degrees of freedom are reported in line with the Greenhouse Geisser statistic.

Mixed ANOVA on the mean amplitudes measured for the P2 component (200–250ms) revealed a significant main effect of electrode site $F(2.24, 125.50)=3.56$, $p<.05$. The electrode by group interaction was non-significant $F(4.48, 125.50)=1.41$, $p>.05$. However there was a significant main effect of group $F(2,56)=3.27$, $p<.05$. To explore this effect further, a series of univariate ANOVAs were conducted at each electrode site. Significant group differences were observed at electrode FCz $F(2,56)=5.81$, $p<0.01$ and also electrode Fz $F(2,56)=3.84$, $p<0.05$. Post-hoc Tukey's test revealed that the ecstasy users had significantly greater mean amplitudes than drug naïve controls at electrode site Fz ($p<0.05$). Furthermore the ecstasy users showed significantly greater amplitude than polydrug controls and drug naïve controls at electrode FCz ($p<.05$ in both cases). The grand average waveforms for each group (users, polydrug nonusers and drug naïve controls) for the electrodes showing significant differences can be observed in Figure 6.6.

Figure 6.6. Grand average waveforms for the 3 groups across electrodes: FCz and Fz.(correct trials on Go/NoGo task)

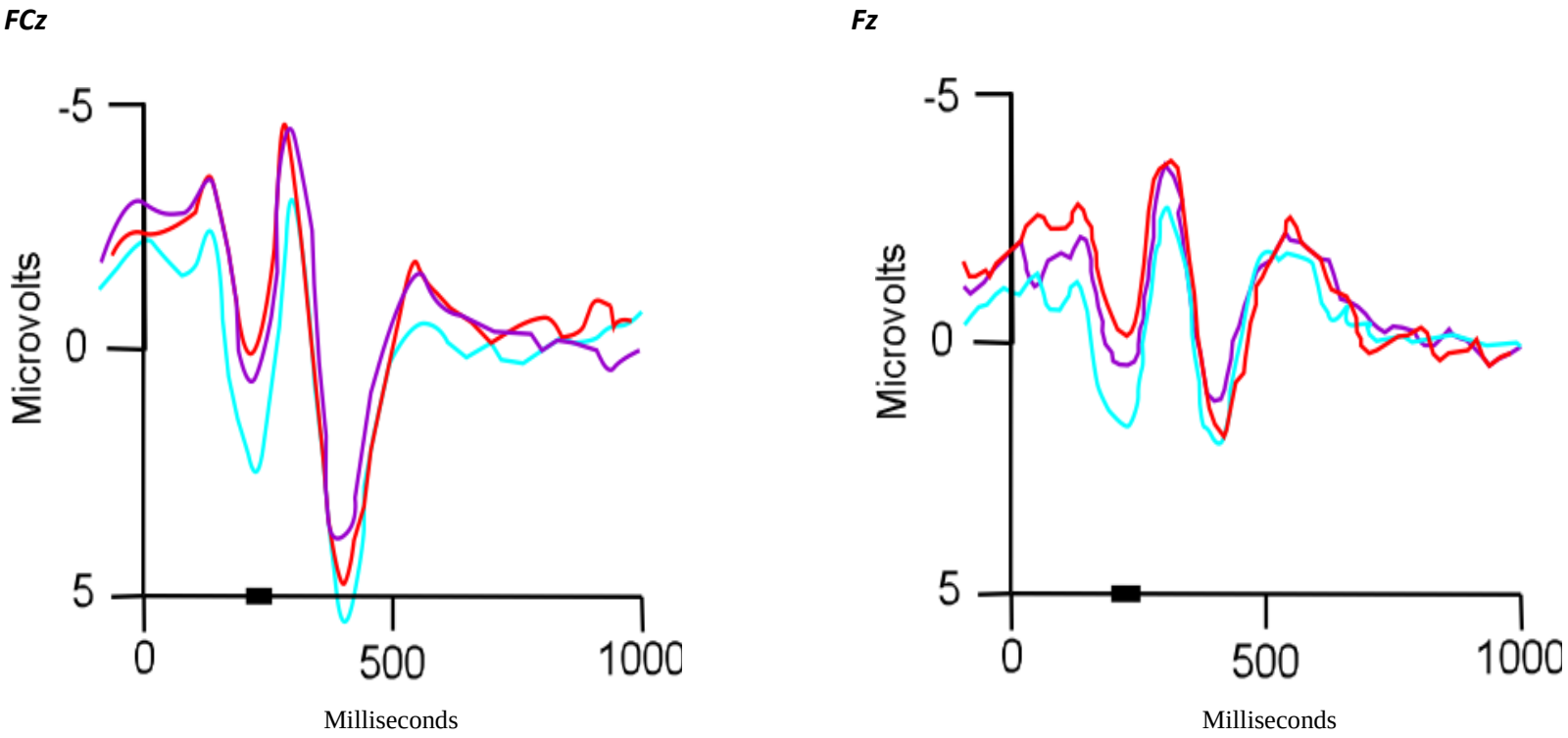

Fig. 6.6: Depicts the waveforms from FCz and Fz (negative plotted up). The time course of the various components can be observed in the grand averaged data from each user group. The significant differences between ecstasy users and drug naïve controls in the P2 component can be observed in Fz from the epoch of 200–250ms (ecstasy users shown in blue, polydrug controls in purple and drug naïve controls in red). Also the magnitude and time course of the significant differences in mean amplitude in the P2 component between ecstasy users and both other control groups can be observed in FCz.

Given the heavy use of cannabis in the ecstasy user group in particular, multiple regression analyses were conducted on the data, to observe whether level use of ecstasy (after controlling for cannabis use) was a predictor of amplitude at the electrodes Fz and FCz. In the first regression, amplitude at Fz was entered as the dependent variable; in the first step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the second step, the same indices of ecstasy use were entered as predictors. The overall regression model accounted for a non-significant 9.5% (R^2 = 0.10, R^2 adjusted = -0.01, $F(6,52)=0.91$, $p>0.05$) of the variance in Fz amplitude. Cannabis use indices (step 1) did not predict a significant amount of variance in Fz amplitude (R^2 = 0.04, R^2 adjusted = -0.01, $F(3,55)=0.83$, $p>0.05$). With none of the three cannabis use variables predicting Fz amplitude; frequency of use (β=-0.70, $p>0.05$), total lifetime dose (β=0.06, $p>0.05$) and amount smoked in the last 30 days (β=-0.72, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in Fz amplitude, after controlling for cannabis use indices (R^2change=0.05, *F-change* (3,52)=1.00, $p>.05$). Frequency of use (β=-0.01, $p>0.05$), last 30 day use (β=0.34, $p>0.05$) and lifetime dose (β=0.01, $p>0.05$) were not significant predictors.

In the second regression, amplitude at FCz was entered as the dependent variable and predictors entered as above. The overall regression model accounted for 14.2% (non-significant) (R^2 = 0.14, R^2 adjusted = 0.04, $F(6,52)=1.43$, $p>0.05$) of the variance in FCz amplitude. Cannabis use indices (step 1) did not predict a significant amount of variance in FCz amplitude (R^2 = 0.06, R^2 adjusted = -0.01, $F(3,55)=1.09$, $p>0.05$). None of the three cannabis use variables predicted FCz amplitude; frequency of use (β=0.60, $p>0.05$), total lifetime dose (β=-0.25, $p>0.05$) and amount smoked in the last 30 days (β=-0.44, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in FCz amplitude, after controlling for cannabis use indices (R^2change=0.09, *F-change* (3,52)=1.72,

p>.05). Frequency of use (β=06, *p*>0.05) and lifetime dose (β=0.04, *p*>0.05) were not significant predictors. However last 30 days use was a significant predictor of amplitude (β=0.42, *p*<0.05), with greater use associated with increased amplitude.

Number-Letter: Mean amplitudes for each condition and electrode are given in Table 6.7. Due to some participants not completing the task and some unusable EEG data 6 participants are excluded from statistical analysis on the EEG data, 4 from the drug naïve group (n=16) and 2 from the ecstasy user group (n=18).

Table 6.7: Mean amplitudes (μvolts) across components, for each electrode measured during the number-letter task.

	P3									
	PO7	*PO3*	*O1*	*Oz*	*POz*	*PO8*	*PO4*	*O2*		
Ecstasy users	1.25 (2.13)	2.59 (1.63)	0.68 (1.84)†	0.40 (1.52)	2.67 (2.37)†	1.63 (2.58)	2.24 (1.70)	0.37 (1.76)		
Polydrug controls	1.94 (2.05)	2.57 (1.54)	0.71 (1.90) †	0.27 (1.72)†	2.27 (0.94)†	0.92 (3.11)	1.94 (1.77)†	0.32 (2.82)		
Drug naïve controls	2.03 (2.20)	3.56 (2.61)	2.37 (2.41)	1.66 (2.33)	4.50 (3.26)	1.56 (3.49)	3.61 (2.75)	1.93 (2.72)		
	N2									
									P7	*P8*
Ecstasy users	-2.56 (0.61)	-0.90 (0.46)	-1.58 (0.53)	-0.15 (0.48)	0.45 (0.60)	-0.66 (0.78)	-0.06 (0.60)	-0.48 (0.60)	-2.81 (0.46)	-1.36 (0.63)
Polydrug controls	-2.08 (0.57)	-0.70 (0.44)	-1.17 (0.50)	0.47 (0.45)	0.81 (0.57)	-1.44 (0.74)	0.12 (0.57)	0.39 (0.57)	-1.88 (0.44)	-0.87 (0.60)
Drug naïve controls	-0.60 (0.64)	0.05 (0.50)	0.20 (0.56)	0.70 (0.51)	1.09 (0.64)	-0.45 (0.82)	0.57 (0.64)	0.56 (0.63)	-0.66 (0.49)	-0.13 (0.67)
	P2									
	FC3	*FC1*	*Fz*	*FC4*	*FC2*	*FCz*	*Cz*			
Ecstasy users	-0.18 (4.22)	1.07 (1.38)	1.54 (1.63)*†	1.66 (1.33)	1.57 (1.24)	2.10 (1.45)*†	1.78 (1.24)*†			
Polydrug controls	0.95 (1.18)	0.65 (1.34)	0.37 (1.84)	1.61 (1.64)	1.10 (1.69)	0.64 (1.43)	0.45 (2.34)			
Drug naïve controls	0.96 (2.30)	0.74 (1.86)	0.28 (1.12)	0.88 (1.53)	0.59 (1.29)	0.59 (1.71)	0.47 (1.63)			

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Mixed ANOVA of mean amplitudes at component P3 (290-400ms) revealed a significant main effect of electrode site $F(4.04, 206.02)=15.78$, $p<.01$, though the electrode by user group interaction was non-significant $F(8.08, 206.02)=0.99$, $p>.05$. There was however a significant main effect of group $F(2,51)=3.35$, $p<.05$. To further explore this difference, a series of one-way ANOVAs were conducted. This yielded significant effect of

group at electrode O1 $F(2,51)=3.80$, $p<.05$, with post-hoc tests indicating that both drug using groups had a significantly diminished mean amplitude compared to drug naïve participants ($p<.05$), the two drug user groups did not differ from each other ($p>.05$). There were also significant differences at electrode POz $F(2,51)=4.56$, $p<.05$, and again, post-hoc analysis showed that both drug groups had significantly lower mean amplitude than drug naïve participants ($p<.05$). The two drug user groups did not differ from each other ($p>.05$). Significant differences were also apparent at PO4 $F(2,51)=3.11$, $p<.05$, with post-hoc tests indicating that polydrug users had significantly lower mean amplitude than drug naive participants ($p<.05$). Differences at electrode Oz were approaching significance $F(2,51)=2.88$, $p=.07$. Post-hoc analysis showed that polydrug users had significantly lower mean amplitude than drug naïve controls ($p<.05$). The grand average waveforms for each group (users, polydrug controls and drug naïve controls), for the electrodes showing significant differences in the P3 component can be observed in Figure 6.7.

Figure 6.7. Grand average waveforms for the 3 groups across electrodes: O1, Oz, POz and PO4 (correct switches)

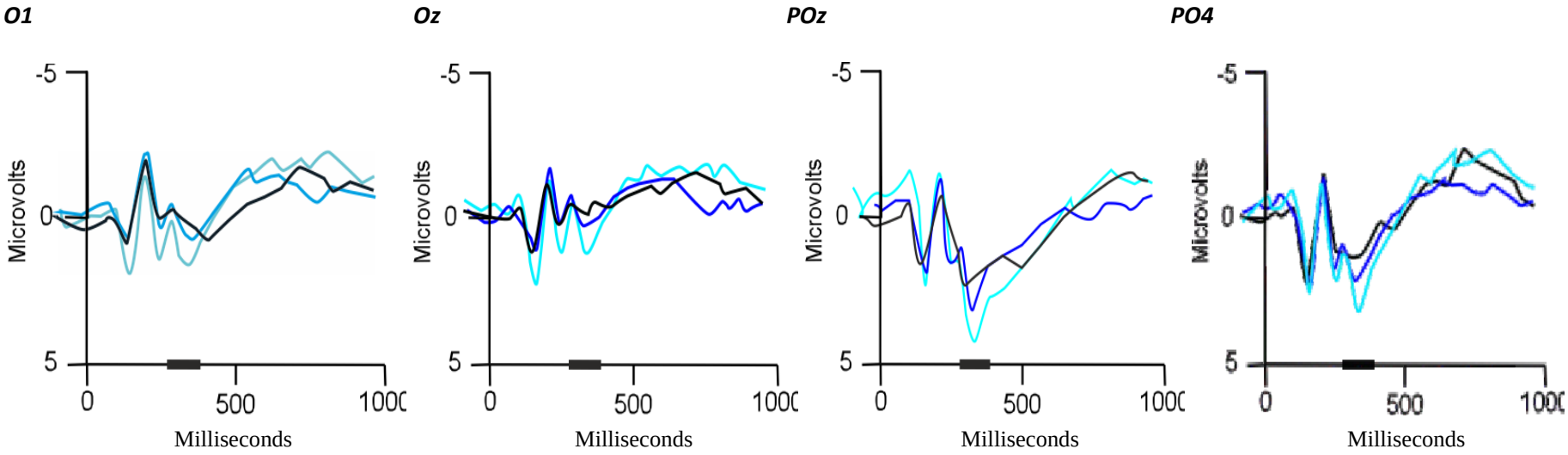

Fig. 6.7: Depicts the waveforms from electrodes that showed significant group differences in the P3 component. Ecstasy users are displayed in blue, polydrug users are displayed in black and drug naïve controls are displayed in lilac. These waveforms are from grand averaged data from each user group. The significant differences between drug naïve controls and both drug user groups can be seen in O1and POz (290-400ms). Differences between polydrug users and drug naïve participants can be seen in Oz and PO4.

Regression analyses were conducted on the four electrodes showing differences, to observe whether level use of ecstasy (after controlling for cannabis use) was a predictor of amplitude at the electrodes O1, POz, PO4 and Oz. In the first regression, amplitude at O1 was entered as the dependent variable; in the first step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the second step, the same indices of ecstasy use were entered as predictors. The overall regression model accounted for a significant 24.4% (R^2 = 0.24, R^2 adjusted = 0.15, $F(6,47)=2.53$, $p<0.05$) of the variance in O1 amplitude. However, cannabis use indices (step 1) did not predict a significant amount of the variance in O1 amplitude (R^2 = 0.10, R^2 adjusted = 0.04, $F(3,50)=1.80$, $p>0.05$); Cannabis use variables; frequency of use (β=-0.72, $p>0.05$) and amount smoked in the last 30 days (β=1.11, $p>0.05$) did not predict O1 amplitude, however total lifetime dose (β=-1.11, $p<0.01$), was a significant predictor (greater use = lower amplitude). The ecstasy use indices (step 2) predicted a significant amount of variance in O1 amplitude, after controlling for cannabis use indices (R^2change=0.147, *F-change* (3,47)=3.05, $p<.05$). Specifically lifetime ecstasy dose was a significant predictor (β=0.74, $p<.01$) with greater ecstasy use being associated with increased amplitude**.** However frequency of use (β=0.02, $p>0.05$) and last 30 day use (β=0.02, $p>0.05$) were not significant predictors.

A second regression was conducted with amplitude at POz entered as the dependent variable. The overall regression model accounted for a non-significant 6% (R^2 = 0.06, R^2 adjusted = -0.06, $F(6,47)=4.96$, $p>0.05$) of the variance in POz amplitude. Cannabis use indices (step 1) did not predict a significant amount of the variance in POz amplitude (R^2 = 0.05, R^2 adjusted = -0.01, $F(3,50)=0.92$, $p>0.05$); none of the three cannabis use variables predicted POz amplitude; frequency of use (β=-0.15, $p>0.05$), total lifetime dose (β=-0.42, $p>0.05$) and amount smoked in the last 30 days (β=0.41, $p>0.05$). The ecstasy use indices

(step 2) did not predict significant amount of variance in POz amplitude, after controlling for cannabis use indices (R^2change=0.01, *F-change* (3,47)=0.12, p>.05). None of the ecstasy use variables predicted POz amplitude; frequency of use (β=0.06, p>0.05), total lifetime dose (β=0.07, p>0.05) and last 30 day use (β=-0.08, p>0.05).

Amplitude at PO4 was entered as the dependent variable in the third regression. The overall regression model accounted for a non-significant 7.7% (R^2 = 0.08, R^2 adjusted = -0.04, F(6,47)=0.65, p>0.05) of the variance in PO4 amplitude. Cannabis use indices (step 1) did not predict a significant amount of the variance in PO4 amplitude (R^2 = 0.06, R^2 adjusted = 0.01, F(3,50)=1.09, p>0.05); none of the three cannabis use variables predicted PO4 amplitude; frequency of use (β=-0.50, p>0.05), total lifetime dose (β=-0.07, p>0.05) and amount smoked in the last 30 days (β=0.53, p>0.05). The ecstasy use indices (step 2) did not predict significant amount of variance in PO4 amplitude, after controlling for cannabis use indices (R^2change=0.02, *F-change* (3,47)=0.26, p>.05). None of the ecstasy use variables predicted PO4 amplitude; frequency of use (β=0.09, p>0.05), total lifetime dose (β=-0.17, p>0.05) and last 30 day use (β=-0.13, p>0.05).

Amplitude at Oz was entered as the dependent variable in the 4th regression. The steps entered were consistent with the previous regressions. The overall regression model accounted for 20.3% (R^2 = 0.20, R^2 adjusted = 0.10, F(6,47)=1.99, p=0.09) of the variance in Oz amplitude (approaching significance). Cannabis use indices (step 1) did not predict a significant amount of the variance in Oz amplitude (R^2 = 0.11, R^2 adjusted = 0.06, F(3,50)=2.11, p>0.05); however total lifetime joints (β=-0.95, p<0.05) significantly predicted Oz amplitude (increased dose associated with decreased amplitude), and amount smoked in the last 30 days approached significance (β=1.12, p=0.06) (increased use associated with increased amplitude). Frequency of use did not predict Oz amplitude (β=-0.54, p>0.05). The

ecstasy use indices (step 2) did not predict significant amount of variance in Oz amplitude, after controlling for cannabis use indices (R^2change=0.09, *F-change* (3,47)=1.77, $p>.05$). The ecstasy use variables frequency of use (β=0.09, $p>0.05$) and last 30 day use (β=-0.17, $p>0.05$) did not predict Oz amplitude; however total lifetime dose (β=0.46, $p=0.08$) was approaching significance (increased use associated with increased amplitude).

Mixed ANOVA of mean amplitudes at component N2 (170-220) revealed a significant main effect of electrode $F(4.27, 217.82)=12.23$, $p<.01$. The electrode by user group interaction $F(8.54, 217.82)=0.76$, $p>.05$, and the main effect of group $F(2,51)=1.83$, $p>.05$, were however non-significant so this component is not discussed further.

At component P2 (200-250ms) Mixed ANOVA revealed a non-significant effect of electrode $F(3.30, 168.44)=1.60$, $p>.05$, though the electrode by user group interaction was significant $F(6.61, 168.44)=2.12$, $p<.05$. The main effect of group was not significant for this component $F(2,51)=2.11$, $p>.05$. To further explore the nature of the significant interaction, a series of one way ANOVAs were used. These yielded significant group differences at electrode Fz $F(2,51)=3.52$, $p<.05$, with post-hoc analysis showing that ecstasy users had significantly greater mean amplitude compared to both other groups ($p<.05$); at electrode FCz $F(2,51)=5.66$, $p<.01$, with ecstasy users having significantly greater mean amplitude than both other groups ($p<.05$); and at electrode Cz $F(2,51)=3.14$, $p<.05$, with ecstasy users showing greater amplitude than both other groups ($p<.05$). Inspection of Table 6.7 suggests that for all the electrodes, ecstasy users have higher mean P2 amplitudes than the other two groups, with the exception of electrode FC3, where the opposite pattern is seen. The grand average waveforms of each group, for the electrodes showing significant differences in the P2 component can be observed in Figure 6.8.

Figure 6.8. Grand average waveforms for the 3 groups across electrodes: Fz, FCz and Cz (P2 correct switches)

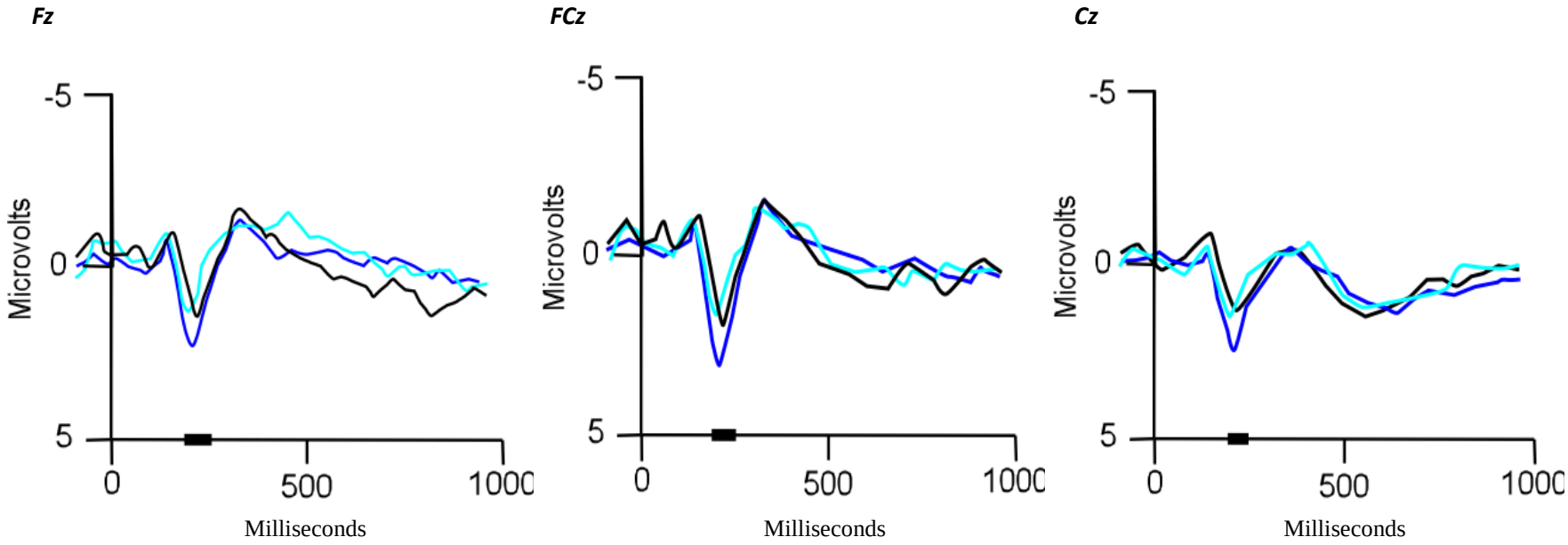

Fig. 6.8: Depicts the waveforms from electrodes that showed significant group differences in the P2 component. Ecstasy users are displayed in blue, polydrug users are displayed in black and drug naïve controls are displayed in lilac. The significant differences between ecstasy users and both control groups can be seen in Fz, FCz and Cz between 200-250ms.

Regression analyses were conducted on the three electrodes showing differences, to observe whether level use of ecstasy (after controlling for cannabis use) was a predictor of amplitude at the electrodes Fz, FCz and Cz. In the first regression, amplitude at Fz was entered as the dependent variable; in the first step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the second step, the same indices of ecstasy use were entered as predictors. The overall regression model accounted for a non-significant 16.5% (R^2 = 0.16, R^2 adjusted = 0.06, $F(6,47)=1.55$, $p>0.05$) of the variance in Fz amplitude. Cannabis use indices (step 1) did not predict a significant amount of the variance in Fz amplitude, although this was approaching significance (R^2 = 0.12, R^2 adjusted = 0.07, $F(3,50)=2.34$, $p=0.09$); none of the three cannabis use variables predicted Fz amplitude; frequency of use (β=0.45, $p>0.05$), total lifetime dose (β=-0.23, $p>0.05$) and amount smoked in the last 30 days (β=-0.19, $p>0.05$). The ecstasy use indices (step 2) did not predict significant amount of variance in Fz amplitude, after controlling for cannabis use indices (R^2change=0.04, *F-change* (3,47)=0.79, $p>.05$). None of the ecstasy use variables predicted Fz amplitude; frequency of use (β=0.00, $p>0.05$), total lifetime dose (β=-0.16, $p>0.05$) and last 30 day use (β=0.32, $p>0.05$).

FCz was entered as the dependent variable in the second regression. This overall regression model accounted for a significant 22.4% (R^2 = 0.22, R^2 adjusted = 12.5, $F(6,47)=2.26$, $p<0.05$) of the variance in FCz amplitude. Cannabis use indices (step 1) predicted a significant amount of the variance in FCz amplitude (R^2 = 0.14, R^2 adjusted = 0.09, $F(3, 50)=2.80$, $p<0.05$); However none of the three individual cannabis use variables predicted FCz amplitude; frequency of use (β=0.43, $p>0.05$), total lifetime dose (β=-0.46, $p>0.05$) and amount smoked in the last 30 days (β=-0.17, $p>0.05$). The ecstasy use indices (step 2) did not predict significant amount of variance in FCz amplitude, after controlling for cannabis use indices (R^2change=0.08, *F-change* (3,47)=1.62, $p>.05$). Individual ecstasy use

variables did not predict FCz amplitude; frequency of use (β=0.03, p>0.05), and last 30 day use (β=0.32, p>0.05), however total lifetime dose was approaching significance (β=0.46, p=0.08) with increased use being associated with increased amplitude.

In the third regression Cz was entered as the dependent variable. This overall regression model accounted for a non-significant 5% (R^2 = 0.05, R^2 adjusted = -0.07, F(6,47)=0.43, p>0.05) of the variance in Cz amplitude. Cannabis use indices (step 1) did not predict a significant amount of the variance in Cz amplitude (R^2 = 0.03, R^2 adjusted = -0.03, F(3,50)=0.47, p>0.05); None of the three individual cannabis use variables predicted Cz amplitude; frequency of use (β=0.18, p>0.05), total lifetime dose (β=-0.39, p>0.05) and amount smoked in the last 30 days (β=-0.04, p>0.05). The ecstasy use indices (step 2) did not predict significant amount of variance in Cz amplitude, after controlling for cannabis use indices (R ²change=0.03, *F-change* (3,47)=0.42, p>.05). None of the ecstasy use variables predicted Cz amplitude; frequency of use (β=0.08, p>0.05), total lifetime dose (β=-0.26, p>.05).and last 30 day use (β=0.49, p>0.05).

Semantic Association: Mean amplitudes for each condition and electrode are given in Table 6.8. Due to some unusable EEG data, 1 participant is excluded from statistical analysis on the EEG data, from the drug naïve group (n=19).

Table 6.8: Mean amplitudes (μvolts) across components, for each electrode measured for the semantic association task.

User group	PO7	PO3	O1	Oz	POz	PO8	PO4	O2
	P3 high association							
Ecstasy users	2.95 (4.02)	3.94 (2.42)	2.76 (3.01)	1.60 (3.24)	4.19 (2.34)	4.40 (3.23)	4.94 (2.99)	3.31 (3.59)
Polydrug controls	4.12 (2.83)	2.97 (2.57)	3.52 (4.60)	3.04 (3.40)	3.28 (2.27)	5.72 (3.78)	4.05 (3.27)	3.32 (3.32)
Drug naïve controls	3.62 (3.19)	3.92 (2.39)	2.84 (3.17)	2.13 (3.14)	3.42 (2.56)	5.35 (3.40)	4.00 (2.21)	3.27 (2.75)
	P3 low association							
Ecstasy users	3.48 (3.74)	4.60 (2.74)	2.32 (3.57)	1.79 (2.90)	4.46 (2.40)	3.78 (2.92)	4.98 (3.33)	2.97 (3.39)
Polydrug controls	4.16 (3.29)	3.44 (3.16)	2.27 (4.40)	2.70 (2.85)	3.89 (2.26)	5.92 (4.61)	4.41 (3.67)	3.14 (3.90)
Drug naïve controls	4.32 (3.42)	4.40 (2.64)	3.08 (3.46)	2.22 (3.21)	3.93 (2.80)	6.07 (3.32)	4.54 (2.69)	3.78 (2.92)
	N2 high association							
Ecstasy users	-1.66 (3.41)	-1.32 (3.55)	-2.19 (3.53)	-2.51 (3.45)	-0.70 (4.01)	-1.41 (3.20)	-1.04 (3.69)	-2.17 (4.12)
Polydrug Controls	-2.06 (4.57)	-2.37 (2.90)††	-2.41 (4.36)	-0.59 (4.19)	-0.58 (2.42)	-0.09 (4.11)	-0.44 (2.57)	-1.15 (3.44)
Drug naïve controls	-0.58 (3.11)	0.67 (2.95)	-0.36 (3.43)	0.09 (3.78)	1.22 (3.03)	1.12 (3.50)	0.82 (3.19)	0.33 (3.51)
	N2 low association							
Ecstasy users	-1.40 (3.55)	-0.95 (3.76)	-2.76 (4.19)	-1.98 (3.57)	-0.17 (3.62)	-1.56 (3.53)†	-1.01 (3.67)	-2.40 (3.75)†
Polydrug controls	-1.69 (4.69)	-1.55 (3.15)	-2.11 (4.47)	-1.09 (3.43)	-0.28 (2.16)	-0.22 (4.02)	-0.29 (1.91)	-0.10 (3.71)
Drug naïve controls	0.01 (3.67)	0.65 (2.97)	-0.33 (4.07)	-0.99 (3.62)	1.55 (2.91)	1.30 (3.58)	0.91 (2.73)	0.67 (3.48)
	P2 high association							
	Fz	**FCz**	**FC1**	**FC2**	**Cz**	**C1**	**C2**	
Ecstasy users	0.55 (2.15)	1.49 (2.04)	1.15 (1.79)	0.86 (2.09)	1.91 (1.48)	0.89 (2.13)	0.77 (1.53)	
Polydrug controls	1.07 (1.98)	1.85 (1.54)	1.28 (1.52)	1.05 (1.87)	1.53 (1.73)	0.87 (1.87)	0.40 (1.64)	
Drug naïve controls	-0.10 (2.43)	0.78 (2.43)	0.61 (1.45)	0.03 (2.75)	0.59 (2.78)	0.22 (2.03)	-0.22 (2.72)	
	P2 low association							
Ecstasy users	0.87 (2.59)	1.64 (2.39)	1.33 (2.54)	0.83 (2.75)	1.55 (1.97)	1.06 (1.45)	0.93 (1.40)	
Polydrug controls	0.51 (1.42)	1.72 (1.62)	1.49 (2.81)	0.98 (1.74)	1.38 (1.77)	0.88 (1.99)	0.62 (1.79)	
Drug naïve controls	0.14 (1.90)	0.72 (2.16)	0.54 (1.98)	0.41 (1.79)	0.81 (2.41)	0.39 (2.22)	0.54 (1.97)	

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

A mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty (high association and low association) and site (PO7, PO3, O1, OZ, POZ, , PO4, PO8 and O2) on the P3 component revealed no main effect of difficulty $F(1,56)$ 0.71, $p>.05$, no difficulty by group interaction $F(2,56)=0.60$, $p>.05$, no main effect of site $F(4.18, 233.99)=13.97$, $p>.05$, no difficulty by site interaction $F(3.42, 191.64)=1.56$, $p>.05$ and no difficulty by site by group interaction $F(6.84, 191.64)=0.61$, $p>.05$. However there was a significant site by user group interaction $F(8.36, 233.99)=1.65$, $p<.05$. There were no significant between group effects $F(2,56)=0.74$, $p>.05$, so these were not investigated further. To further explore the site by user group interaction, a series of univariate ANOVAs were run with group as the between groups variable and amplitude at the various sites as the dependent variable. This yielded no significant differences between the three groups and no significant post-hoc comparisons, $p>.05$ in all cases.

A mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty (high association and low association) and site (PO7, PO3, O1, OZ, POZ, , PO4, PO8 and O2) on the N2 component revealed no main effect of difficulty $F(1,56)=1.05$, $p>.05$, no difficulty by group interaction $F(2,56)=0.04$, $p>.05$, no main effect of site $F(3.82, 213.92)=6.37$, $p>.05$, no site by group interaction $F(7.64, 213.92)=1.10$, $p>.05$, no difficulty by site interaction $F(4.78, 267.40)=0.81$, $p>.05$ and no difficulty by site by group interaction $F(9.55, 267.40)=0.73$, $p>.05$. Between group differences approached significance $F(2,56)=2.78$, $p=.07$. In line with a priori predictions and to further explore this trend on the N2 component a series of univariate ANOVAs were conducted. These revealed significant between group differences at electrode PO3 in the high association condition $F(2,56)=4.68$, $p<.05$, post-hoc analysis revealed that polydrug controls were significantly different (greater negativity) to drug naïve controls at this electrode ($p<.01$). Significant between group differences were also observed at electrode O2 in the low association condition $F(2,56)=3.45$,

$p<.05$), post-hoc analysis revealed that ecstasy users differed significantly (greater negativity) from drug naive controls here ($p<.05$). Between group differences were also approaching significance at PO8 in the low association condition $F(2,56)=2.89$, $p=.06$, again post-hoc analysis showed that ecstasy users were significantly different (greater negativity) from drug naïve controls here ($p<.05$). Ecstasy users and polydrug controls did not differ significantly from one another at these three sites ($p>.05$). In all cases, ecstasy users showed a greater negativity than drug naïve controls (Table 6.8). The grand average waveforms of each group, for the electrodes showing significant differences in the N2 component can be observed in Figures 6.9 and 6.10.

Figure 6.9. Grand average waveforms for the three groups across electrode PO3 on the high association condition of the semantic association task.

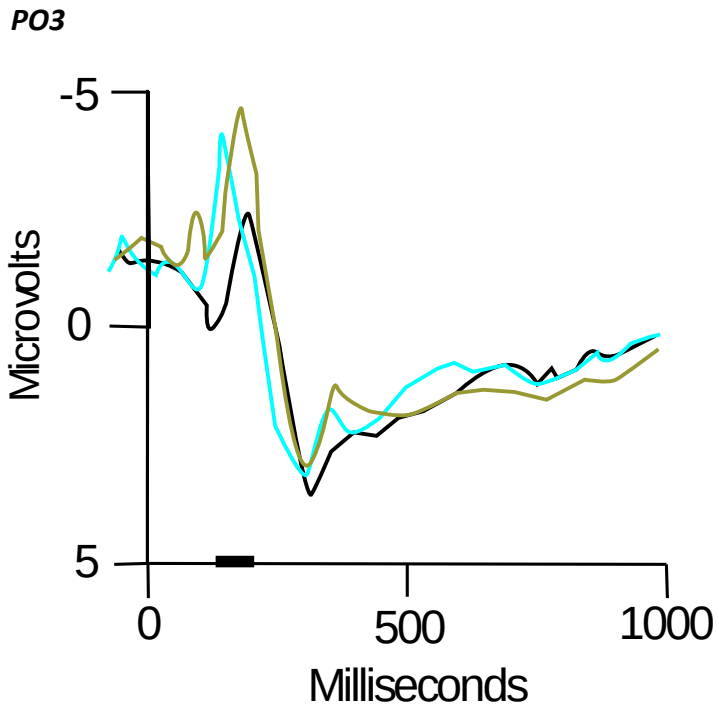

Fig. 6.9: Depicts the grand average waveform for each user group (ecstasy users in blue, polydrug users in green and drug naïve controls in black) at PO3 for the high association condition. Significant differences in mean amplitude in the N2component (120-200ms) between polydrug users and drug naïve controls can be observed.

Figure 6.10. Grand average waveforms for the three groups across electrodes O2 and PO8 on the low association condition of the semantic association task.

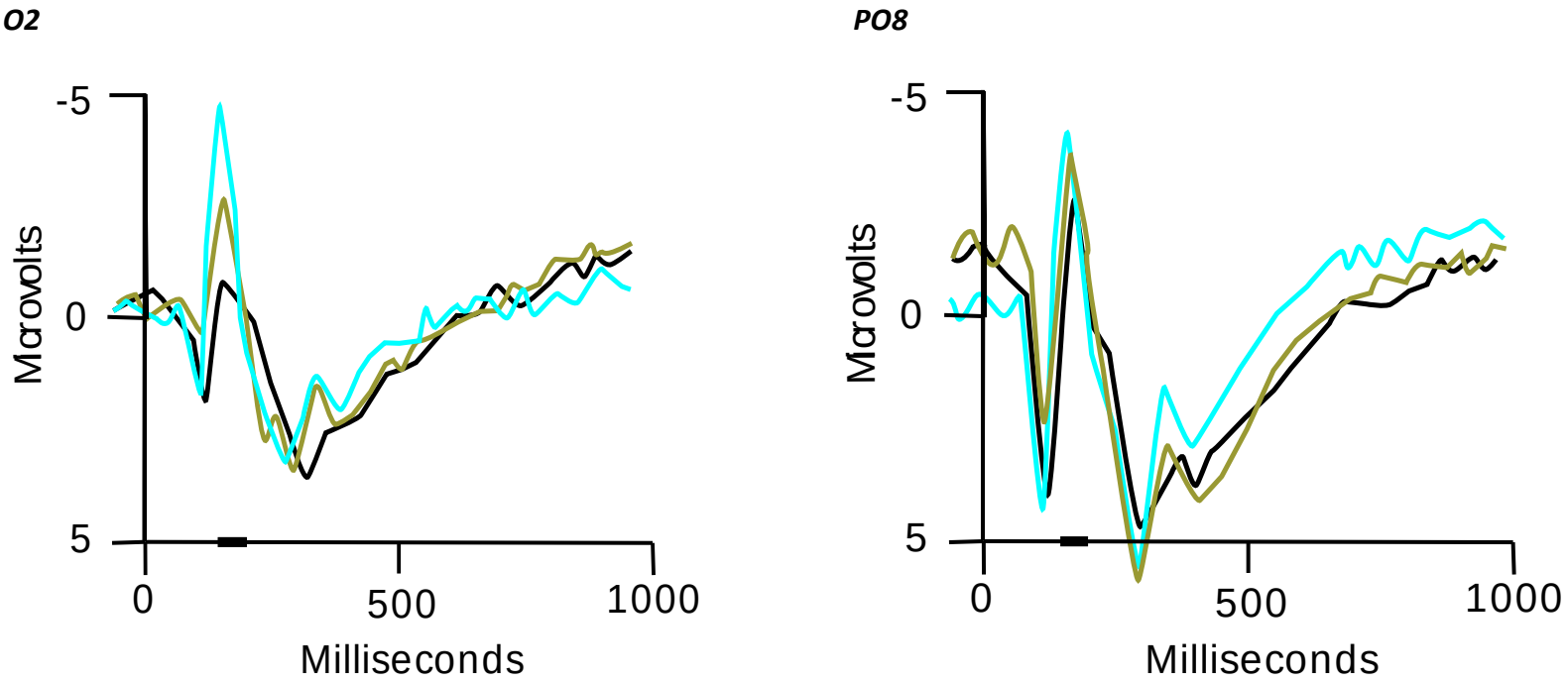

Fig. 6.10: Depicts the grand average waveforms for each user group (ecstasy users in blue, polydrug users in green and drug naïve controls in black) for O2 and PO8 during the low association condition of the task.. Significant differences in mean amplitude, in the N2component (120-190ms), between ecstasy users and drug naïve controls can be observed at both electrodes.

Regression analyses were conducted on the three electrodes showing significant differences, to observe whether level use of ecstasy (after controlling for cannabis use) was a predictor of amplitude at the electrodes PO3 (high association), O2 (low association) and PO8 (low association). In the first regression, amplitude at PO3 (high association) was entered as the dependent variable; in the first step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the second step, the same indices of ecstasy use were entered as predictors. The overall regression model accounted for a significant 24.9% (R^2 = 0.25, R^2 adjusted = 0.16, $F(6,52)=2.88$, $p<0.05$) of the variance in PO3 amplitude. Cannabis use indices (step 1) did not predict a significant amount of the variance in PO3 amplitude, although this was approaching significance (R^2 = 0.12, R^2 adjusted = 0.08, $F(3,55)=2.59$, $p=0.06$); lifetime dose of cannabis significantly predicted PO3 amplitude (β=-1.13, $p<0.01$) (higher dose associated with more negative amplitude), however frequency of use (β=0.24, $p<0.05$) and amount smoked in the last 30 days (β=0.29, $p>0.05$) did not. The ecstasy use indices (step 2) predicted a significant amount of variance in PO3 amplitude, after controlling for cannabis use indices (R^2change=0.13, *F-change* (3,52)=2.90, $p<.05$). Specifically total lifetime dose predicted PO3 amplitude (β=0.56, $p<0.01$) (higher dose associated with higher amplitude). However frequency of use (β=0.11, $p>0.05$) and last 30 day use (β=0.22, $p>0.05$) did not significantly predict PO3 amplitude.

For the second regression amplitude at O2 (low association) was entered as the dependent variable. This overall regression model accounted for a significant 21.9% of the variance in O2 amplitude (R^2 = 0.22, R^2 adjusted = 0.13, $F(6,52)=2.43$, $p<0.05$). Cannabis use indices (step 1) did not predict a significant amount of the variance in O2 amplitude, although this was approaching significance (R^2 = 0.12, R^2 adjusted = 0.07, $F(3,55)=2.42$, $p=0.08$); lifetime dose of cannabis significantly predicted O2 amplitude (β=-0.91, $p<0.01$), with

greater use associated with greater negativity, however frequency of use (β=-0.01, *p*>0.05) and amount smoked in the last 30 days (β=0.08, *p*>0.05) did not. The ecstasy use indices (step 2) predicted variance in O2 amplitude that was approaching significance, after controlling for cannabis use indices (R^2change=0.10, *F-change* (3,52)=2.28, *p*=.09). Total lifetime dose predicted O2 amplitude (β=0.51, *p*<0.05) with greater use associated with higher amplitude. However frequency of use (β=-0.13, *p*>0.05) and last 30 day use (β=0.28, *p*>0.05) did not significantly predict PO3 amplitude.

PO8 was entered as the dependent variable in the third regression. This regression model accounted for a non-significant 6% of the variance in PO8 amplitude (R^2 = 0.06, R^2 adjusted = -0.05, *F*(6,52)=0.58, *p*>0.05). Cannabis use indices (step 1) did not predict a significant amount of the variance in PO8 amplitude (R^2 = 0.05, R^2 adjusted = -0.00, *F*(3,55)=0.98, *p*>.05); none of the individual cannabis use variables significantly predicted PO8 amplitude; frequency of use (β=-0.03, *p*>0.05), lifetime dose of cannabis (β=-0.30, *p*>0.05), and amount smoked in the last 30 days (β=-0.08, *p*>0.05). The ecstasy use indices (step 2) did not predict a significant amount of variance in PO8 amplitude, after controlling for cannabis use indices (R^2change=0.01, *F-change* (3,52)=0.23, *p*>.05). None of the individual ecstasy use variables significantly predicted PO8 amplitude; frequency of use (β=-0.05, *p*>0.05) total lifetime dose (β=-0.19, *p*>0.05) and last 30 day use (β=0.05, *p*>0.05).

A mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty (high association and low association) and site (FZ, FCZ, FC1, FC2, CZ, C1 and C2) was conducted on the mean amplitudes across the epochs measured (170-230ms in both conditions) for the P2 component. This revealed, no main effect of difficulty *F*(1,56)=0.32, *p*>.05, no difficulty by group interaction *F*(2,56)=0.35, *p*>.05, no main effect of site *F*(4.21, 236.03)=5.22, *p*>.05, no site by group interaction *F*(8.43, 236.03)=0.26, *p*>.05, no difficulty

by site interaction $F(4.85, 271.44)=0.51$, $p>.05$ and no difficulty by site by group interaction $F(9.69, 271.44)=0.48$, $p>.05$. Between group differences were also non-significant $F(2,56)=1.68$, $p>.05$.

Updating: Mean amplitudes for each condition, ERP component and electrode are given in Tables 6.9, 6.10, and 6.11. Due to some unusable EEG data, one participant from the drug naïve group (n=19), one participant from the ecstasy users group (n=19) and two participants from the polydrug group (n=18) have their data excluded from statistical analysis.

Table 6.9: Mean amplitudes (μvolts) at each electrode measured for the P3 component (n-back task)

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
N = 0 electrode:			
P5	1.83 (2.23)	1.36 (3.11)	1.54 (1.89)
P7	0.16 (3.17)	-0.00 (4.17)	0.66 (2.60)
PO7	1.88 (3.18)	2.29 (3.23)	2.35 (3.15)
PO3	2.30 (2.50)	2.18 (2.16)	3.69 (3.06)
O1	0.85 (3.04)	0.51 (2.41)	1.30 (4.24)
Oz	-0.00 (2.25)	-0.17 (3.07)	0.71 (4.68)
POz	1.98 (3.14)	2.06 (1.84)	3.04 (3.43)
P6	2.74 (1.84)	3.10 (2.96)	2.60 (2.06)
P8	2.02 (3.25)	2.43 (4.10)	1.71 (1.97)
PO8	3.07 (2.66)	3.83 (4.11)	2.30 (4.08)
PO4	3.10 (2.85)	3.53 (2.51)	3.22 (3.17)
O2	1.23 (3.51)	1.52 (3.15)	1.26 (4.47)
N = 2 electrode:			
P5	1.78 (2.47)	0.62 (3.13)	1.49 (2.69)
P7	0.42 (3.22)	-0.54 (3.26)	0.05 (2.80)
PO7	2.09 (3.96)	0.62 (3.05)	1.71 (3.08)
PO3	2.82 (2.76)	2.23 (2.31)	3.21 (3.30)
O1	1.27 (4.19)	-0.20 (3.10)	1.19 (4.20)
Oz	0.06 (2.22)	0.86 (2.04)	0.81 (4.06)
POz	2.03 (2.31)	2.34 (1.55)	3.82 (3.81)
P6	2.70 (2.24)	2.97 (2.04)	2.53 (1.52)
P8	2.05 (2.76)	2.54 (3.38)	1.98 (1.62)
PO8	3.73 (3.24)	3.91 (3.63)	3.36 (2.94)
PO4	2.93 (2.54)	3.61 (2.00)	3.71 (3.08)
O2	1.33 (3.26)	2.29 (3.06)	1.66 (4.32)
N = 4 electrode:			
P5	2.64 (3.03)	1.28 (2.80)	1.92 (2.68)
P7	1.01 (3.93)	0.05 (2.76)	0.62 (3.06)
PO7	3.17 (4.37)	1.97 (2.82)	2.08 (3.32)
PO3	4.10 (3.11)	2.94 (2.39)	3.54 (2.67)
O1	2.45 (4.03)	1.43 (3.13)	1.69 (5.04)
Oz	1.05 (3.70)	1.18 (3.45)	0.51 (4.44)
POz	3.28 (3.88)	2.20 (2.18)	4.53 (3.87)
P6	2.05 (2.80)	3.43 (2.05)	2.95 (2.29)
P8	2.08 (3.68)	3.05 (3.73)	2.00 (2.72)
PO8	3.83 (3.21)	4.77 (4.09)	3.00 (2.92)
PO4	4.22 (3.52)	3.88 (2.74)	3.95 (2.84)
O2	1.64 (4.00)	2.52 (3.34)	2.25 (4.58)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Table 6.10: Mean amplitudes (μvolts) for each electrode measured for the N2 component.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
N = 0 electrode:			
P6	-0.90 (1.89)	-0.47 (20.6)	0.04 (1.45)
P8	-1.78 (2.68)	-0.98 (2.38)	-0.43 (2.40)
PO8	-2.45 (3.55)	-1.45 (3.23)	-0.95 (3.47)
N=2 electrode:			
P6	-0.48 (1.77)	-0.41 (1.78)	-0.03 (1.45)
P8	-1.17 (3.05)	-1.37 (2.82)	-0.99 (2.40)
PO8	-1.65 (3.41)	-1.87 (3.63)	-0.76 (3.37)
N=4 electrode:			
P6	-1.41 (2.17)	0.07 (2.42)	0.29 (1.57)
P8	-1.67 (3.61)	-0.86 (3.03)	-0.62 (2.51)
PO8	-1.73 (4.40)	-1.15 (4.10)	-0.88 (2.80)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Table 6.11: Mean amplitudes (μvolts) for each electrode measured for the P2 component.

	Ecstasy users	Polydrug controls	Drug naïve controls
N = 0 electrode:			
F1	1.49 (1.76)	0.69 (2.46)	0.60 (1.32)
F3	0.34 (1.68)	0.05 (2.32)	0.38 (1.57)
FC1	2.05 (1.58)	1.19 (2.24)	1.37 (1.87)
Fz	1.51 (1.65)	1.15 (2.51)	0.85 (2.22)
F2	1.61 (2.07)	1.07 (3.01)	0.42 (2.69)
F4	1.54 (1.96)	1.42 (2.47)	1.38 (2.69)
FC2	2.74 (1.87)	1.76 (3.30)	1.22 (2.00)
FCz	3.02 (1.51)	1.84 (2.84)	1.72 (2.48)
C2	2.72 (1.67)	1.58 (2.10)	0.67 (2.11)
N = 2 electrode:			
F1	1.27 (2.37)	1.06 (2.40)	0.94 (1.68)
F3	0.31 (1.81)	0.15 (2.40)	0.12 (1.82)
FC1	1.46 (1.79)	0.99 (2.19)	1.26 (1.65)
Fz	1.59 (3.15)	1.56 (2.29)	0.71 (1.29)
F2	1.15 (3.42)	1.06 (1.24)	-0.09 (1.87)
F4	1.42 (2.38)	1.73 (2.29)	0.22 (1.80)
FC2	2.13 (2.52)	1.52 (2.30)	1.51 (1.96)
FCz	2.03 (3.99)	2.07 (2.24)	1.23 (2.00)
C2	1.62 (2.62)	1.27 (2.65)	0.35 (2.11)
N = 4 electrode:			
F1	1.21 (1.60)	0.58 (1.89)	0.32 (2.03)
F3	0.74 (2.12)	0.12 (2.88)	0.30 (1.73)
FC1	1.95 (1.75)	1.18 (2.21)	1.12 (2.07)
Fz	1.71 (2.66)	0.90 (2.29)	1.30 (2.02)
F2	0.77 (4.64)	0.56 (3.14)	0.16 (2.14)
F4	1.01 (3.01)	0.96 (2.56)	0.50 (2.27)
FC2	1.59 (4.38)	1.19 (2.47)	1.33 (2.43)
FCz	3.57 (2.08)	1.47 (2.62)	1.71 (1.92)
C2	2.12 (2.52)	1.68 (3.30)	0.74 (1.60)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

For the P3 component, a mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty (n=0, n=2 and n=4) and site (P5, P7, PO7, PO3, O1, Oz, POz, P6, P8, PO8, PO4 and O2) revealed mixed ANOVA revealed there was a significant main effect of difficulty indicating that mean amplitudes differed according to condition $F(2,52)=7.79$, $p<.01$. Inspection of Table 6.9 reveals that this was because amplitude increases in line with difficulty. There was also a significant main effect of electrode $F(4.48, 237.40)=16.77$, $p<.01$. The difficulty by user group, electrode by user group, difficulty by

electrode and difficulty by electrode by user group interactions were all non-significant (p>.05 in all cases). There was no significant effect of user group $F(2,53)=0.03$, p>.05. Therefore this component will not be discussed further.

A mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty and site (P6, P9 and PO8) on the N2 component revealed a main effect of electrode $F(2,52)=5.58$, p<.01. The effects of difficulty and the difficulty by user group, electrode by user group, difficulty by electrode and difficulty by electrode by user group interactions were all non-significant p>.05 in all cases. There was no effect of user group $F(2,53)=1.02$, p>.05. Therefore this component will not be discussed further.

For the P2 component, a mixed ANOVA, with between subjects factor of group and within subjects factors of difficulty and site (F1, F3, FC1, Fz, F2, F4, FC2, FCz and C2) showed the effects of difficulty were non-significant $F(2,52)=0.94$, p>.05 as was the difficulty by user group interaction $F(4,106)=0.39$, p>.05. There was however, a main effect of electrode $F(5.32,282.18)=8.92$, p<.01. The electrode by user group, difficulty by electrode and difficulty by electrode by user group interactions were all non-significant, p>.05 in all cases. Finally, there was no significant effect of group $F(2,53)=1.65$, p>.05. As such this component is not discussed further.

Implications of Chapter 6

The ERP results from Chapter 6 support the view that ecstasy/polydrug use does alter cognitive processes involved in the executive functions of inhibitory control, switching and access. Furthermore these findings are independent of gender, age, fluid intelligence, daytime sleepiness, morningness-eveningness types, weekly alcohol intake and state levels of arousal, anxiety and depression. Differences between ecstasy users and both control groups in the P2 component during the Go/NoGo task reflect atypical early processing in ecstasy users (the

implications of which will be discussed in more detail in Chapter 10). Moreover recency of use may play a role in inhibition, given that amount of ecstasy used in the last 30 days was a significant predictor of FCz amplitude after controlling for cannabis use indices. Furthermore a diminished P3 response to switching was observed in ecstasy users and polydrug controls relative to drug naïve controls at several parieto-occipital and occipital electrode sites. The regression analysis on electrode O1 for the P3 component suggested that lifetime cannabis use significantly predicted the diminished P3 amplitude in the first step. Although lifetime ecstasy use was a significant predictor of P3 amplitude after controlling for cannabis use, this appeared to predict amplitude in the opposite direction. This task also yielded ecstasy specific alterations in the P2 component at fronto-central sites, perhaps again reflecting alterations to early processing suggestive of compensatory mechanisms; lifetime dose of ecstasy approached significance for predicting amplitude at FCz in this component. The N2 component during the low association (more difficult) level of the semantic association task showed ecstasy-related differences in comparison to controls at occipital and parieto-occipital sites (O2 and PO8). Ecstasy users were not significantly different from polydrug controls so the results need treating with caution. There were no differences in P3 amplitude between groups in the semantic association task. These results potentially reflect evidence of cognitive reallocation, or compensatory mechanisms in ecstasy/polydrug users to ameliorate behavioural differences, given that there were no between group differences on behavioural performance on these three tasks. Unexpectedly, no between group differences were observed in performance (errors) on the n-back task. However there were reaction time differences indicating that drug naïve controls were significantly slower to respond than polydrug users on the n-back task. Drug naïve controls were also slower than ecstasy users for reaction times on the n-back task (although non-significant). Moreover, inspection of table 6.5 shows that although non-significant, drug naïve participants made fewer errors on

the task than ecstasy users. This data tentatively suggests an accuracy/speed trade off as a function of increased impulsivity in ecstasy users, although these differences are non-significant. Furthermore polydrug controls show the lowest error rates as well as fastest reaction time. There were no between group differences in the ERP components during the updating task, this was contrary to expectations and potential reasons for this will be discussed in Chapter 10. Due to this function showing more consistent deficits in ecstasy users in the literature, it will be explored further in Chapter 7, using alternative tasks and neuroimaging measures.

Chapter 7: fNIRS and Updating

7.1 Chapter Overview

Chapter 6 showed electrophysiological differences in the executive functions of inhibitory control, switching and access. However no such difference was observed with regards to the EEG data from memory updating. This chapter aims to further explore the nature of this executive function in relation to ecstasy use. In this chapter the updating function of the central executive has been assessed behaviourally using letter updating and spatial updating tasks. Furthermore the haemodynamic response to task has been assessed using fNIRS. Twenty ecstasy users, 20 polydrug controls and 20 drug naïve controls were recruited for this study. Behavioural performance on the letter updating task and the spatial updating task was equivalent between the 3 groups. However analysis of fNIRS data showed MDMA related alterations to haemodynamic response that may reflect compensatory functioning.

7.2 Introduction

The Updating component of working memory as previously discussed has been shown to be degraded in ecstasy users. Results from Chapter 6 do not reflect ecstasy-related deficits in this function in the sample studied. However the n-back task has been employed in the ecstasy literature previously and yielded few observable deficits between users and non-users (Gouzoulis-Mayfrank *et al.*, 2003; Daumann, Fimm *et al*., 2003). Nevertheless studies employing this task with haemodynamic neurophysiological measures have observed haemodynamic correlates that reflect subtle cognitive alterations (Daumann, Fimm *et al*., 2003: Daumann, Schnitker *et al*., 2003). Perhaps electrophysiological differences were undetectable in the last chapter due to the variability in response times from the time-locked probe, due to the difficulty of the task. The n-back task that was employed would have required more protracted mental calculations than the tasks assessing the other executive

functions, and as such may have produced a large amount of noise for the time locked ERPs. Haemodynamic response to stimuli is not instantaneous. It is understood that the haemodynamic response occurs over a 10-12 second epoch (Izzetoglu *et al*., 2005; Miezin *et al.,* 2000). As such, activity over a block of trials may be a better way to measure neuronal response. Given that the updating task requires continuous monitoring of information, perhaps measurements of neuronal activity over the entire updating process will provide a greater understanding of how ecstasy affects this process.

fNIRS is an emerging non-invasive neuroimaging tool that measures cerebral blood flow. Due to cerebral blood flow and neuronal activation being closely linked (Villringer & Dirnagl, 1995), fNIRS can be used to assess the haemodynamic response to mental demand. More specifically fNIRS uses wavelengths of light in the near infrared range to assess levels of oxygenated and deoxygenated haemoglobin in the prefrontal cortex (PFC). Areas of the PFC are easily accessed by this type of neuroimaging due to it having a penetration depth of 2-3mm (Firbank *et al*., 1998). As such fNIRS is ideal for observing neurological activation during tasks that load on the (DL)PFC such as executive functioning tasks. Increases in the chromophore oxy-Hb are accepted as reflecting an increase in neuronal activity in certain brain regions (Leff *et al*., 2011). Furthermore it is hypothesised that although blood oxygenation is expected to increase with increased workload, this is only if the participant is engaged in the task, whereas if the task becomes too difficult and attention shifts (as well as performance decline), a decrease in oxygenation will be observed (Izzetoglu *et al.,* 2004). The distribution of the activation response is regionally specific i.e. the cortical regions underlying the voxels at which the activation is observed are responsible for the activation (Leff *et al.,* 2011). Often an increase in oxygenated haemoglobin is coupled with a decrease in deoxygenated haemoglobin (Ehlis *et al.,* 2008; Leff *et al*., 2008; Leff *et al.,* 2011). However, increases in oxy-Hb have also been observed to be coupled with increases in

deoxy-Hb (Hoshi & Tamura, 1993; Sakatani *et al.,* 1999). The relationship between oxygenated and deoxygenated haemoglobin is non-linear and estimates of total haemoglobin are sometimes calculated as a correlate of neuronal activation (Ayaz *et al*., 2012). Total haemoglobin is obtained from summating oxygenated and deoxygenated haemoglobin. As such increases in deoxy-Hb may reflect increased neuronal activity. However oxy-Hb is generally accepted as the best indicator of neuronal activity.

Although there is currently a paucity of empirical research on substance users with this type of technology, there have been studies investigating cognitive impairments associated with neurological disease and psychiatric disorders (Ehlis *et al.,* 2008; Falgatter *et al*., 1997; Hermann *et al.,* 2008). Generally, participants with neurological disease such as Alzheimer's are reported as performing worse on cognitive tasks than healthy controls (Ehlis *et al*., 2008; Herrmann *et al*., 2008), and this is usually coupled with a decrease in oxy-Hb. This technology has also been used as a measure of mental workload in human operators (Ayaz *et al.,* 2012) to improve efficiency of human-machine systems in critical tasks. Although human operators may have similar performance output, fNIRS was incorporated as an additional measure of mental workload, given that increased effort (indexed by increased oxy-Hb) is predictive of future failure. As such operator efficiency could be predicted from their haemodynamic response to demanding tasks.

As well as integrating haemodynamic measures to assess this function in ecstasy using populations. It may be useful to explore this function in greater depth using tasks that are shown in the literature to reliably produce effects in ecstasy users. The letter updating task and the spatial updating task have both yielded behavioural deficits in ecstasy users previously (Montgomery & Fisk, 2008; Montgomery, Fisk, Newcombe & Murphy, 2005). Montgomery, Fisk, Newcombe and Murphy (2005) observed performance deficits in ecstasy

users compared to non-users on the letter updating task. The nature of this deficit was explored further by Montgomery and Fisk (2008), in which, the analyses explored whether the ecstasy/polydrug deficit is more pronounced at specific serial positions, or whether length of sequence was a critical factor in performance. In this study, analyses were also varied for letter span and spatial span. Deficits in ecstasy users were observed for letter updating, in participants with simple spans of 5 and 6 compared to controls, and deficits in spatial updating were observed in ecstasy users with a simple span of 5. This study also suggested that ecstasy users may display deficits in early serial position recall, with those with spatial spans of 4 and 5, and those with letter spans of 6 showing impairment of recall of stimuli at early serial positions. The authors suggest that ecstasy use may lead to greater susceptibility to chunk destruction during the updating process as an explanation of why early serial positions show impairments. Furthermore correlational analysis revealed that indices of ecstasy use such as total lifetime dose and average weekly dose had a significant negative correlation with letter updating and spatial updating performance. The correlations suggest that greater use of ecstasy is associated with poorer updating performance. Moreover indices of cannabis and cocaine use were not significantly correlated with letter or spatial updating performance.

The aim of this study is to fully elucidate the effects of ecstasy on the haemodynamic response to memory updating by using fNIRS in combination with spatial updating and consonant updating tasks. It is predicted that ecstasy users will show performance deficits in the updating tasks as well as alterations to their haemodynamic response relative to polydrug controls and drug naïve controls. However if performance is equivalent, it is predicted that ecstasy users will display evidence of more effortful cognition from their haemodynamic response (increases in oxy-Hb).

7.3 Method

Design:

For analysis of behavioural data and fNIRS data a between groups design was used. The between groups factor was drug user group which consisted of three levels (ecstasy users, polydrug controls and drug naïve controls). Univariate ANOVA was performed on the behavioural data for both letter updating and spatial updating, with composite scores on each task as the dependent variables. The fNIRS data was analysed with univariate ANOVAs using mean oxy and deoxy-Hb changes from baseline as the dependent variables (voxels 1-16). Any significant main effects were further investigated using Tukey's HSD.

Participants:

Twenty ecstasy users (mean age = 21.76, SD = 3.19, 11 = male), 20 polydrug controls (mean age = 19.75, SD = 1.48, 11 = male) and 20 drug naïve controls (mean age = 19.68, SD = 1.89, 9 = male) were recruited via direct approach (e-mail) to Liverpool John Moores University students. Inclusion and exclusion criteria were the same as that in Chapter 6. Indices of ecstasy use were as follows: total lifetime dose 1305.31 tablets ± 4951.61; mean amount used in last 30 days 3.80 tablets ± 4.63, and frequency of use 0.39 times/week ± 0.48.

Materials

Questionnaires

The *Background Drug Use Questionnaire*, sleep quality questionnaire, The ESS, The MEQ, KSS, UMACL, NASA-TLX and Raven's SPM were used, as described in Chapter 6.

Updating Tasks

The letter and spatial updating tasks were carried out as per Montgomery and Fisk (2008), whereby each participant's letter and spatial span were first calculated prior to conducting the updating tasks.

Letter span: Consonants appeared on a computer monitor sequentially and remained on screen for 1.25s each. Following presentation of a sequence of letters, participants were required to recall the order in which the letters appeared. To begin with three sets of two letters were presented, progressing to three sets of three letters, then three sets of four letters and so on up until three sets of seven letters. Participants' span is noted as the largest string of letters they can recall accurately on at least two of the three trials.

Spatial span: Analogous to the letter span task, participants had to recall the positions of highlighted blocks in a Corsi block type arrangement in the order that they were presented. Highlighted blocks appeared on screen for 1.25s each.

Letter updating: Based on running span memory task (Morris & Jones, 1990) consonants appear on the computer screen in random sequences dependent upon the participants calculated letter span. Twenty-four sequences were presented and in each trial participants were unaware of the number of letters that would appear in the sequence (length of list). Participants were required to recall the most recent *n* consonants in the order in which they appeared (where *n* is the participants calculated letter span). There were four sequence lengths; *n*, *n* + 2, *n* + 4 and *n* + 6 and six trials of each length were presented in randomised order.

Spatial updating: This computer based task was again analogous to the letter updating task. Spatial locations were highlighted on a Corsi block type arrangement, in random sequences. Twenty-four trials were presented and again participants were unaware of the length of the sequence being presented each time, with the exception of six trials, in which participants were told how many spatial locations were going to be presented (in each case it was always the participant's span that was the list length for the known length trials). Furthermore participants were required to recall the last *n* (where *n* is the participant's spatial span) positions in the order that they were presented. There were six trials at each list length; known *n*, unknown *n*, *n* + 2, *n* + 4 and *n* + 6 and the order in which these appeared was randomised.

Equipment

Haemodynamic response to task in the PFC was monitored using a continuous wave fNIRS system developed by Drexel University (Philadelphia, PA) and supplied by Biopac systems (Goleta, CA, USA). The fNIR sensor has a temporal resolution of 500ms per scan (2Hz), with a source-detector separation of 2.5cm allowing 1.25cm penetration depth (Ayaz *et al.*, 2012). An fNIR100 control box and data acquisition and visualisation software COBI studio (Drexel university) were used during data collection (as per Ayaz *et al.*, 2011; Ayaz *et al*., 2012) with a serial cable between display and acquisition PCs to identify task markers.

Procedure

Participants were required to attend the lab for a one off session lasting approximately 2.5 hours. Testing sessions commenced at 9am, 11.30am and 2pm, equal numbers of each group were tested at each time. Upon entering the lab participants were given an information sheet explaining what was involved in the study and written consent of their participation was obtained. Following this, participants completed a battery of questionnaires in the following

order; Background drug use questionnaire, sleep quality questionnaire, MEQ, ESS, pre-test KSS, UMACL and Raven's SPM. Participants then completed the letter span task and the spatial span task, the order in which these were given was randomised. The fNIRS headband was then fitted to the participant's forehead. The fNIRS signals were displayed on a desktop computer running COBI studio (Drexel University) in an adjacent room to the testing room. Providing the signals from the fNIRS were stable, a baseline of inactivity was recorded – this involved participants watching a video of planet earth accompanied by soothing music. Following this the letter updating and spatial updating tasks were completed (a baseline was taken prior to each task). After completing the tasks participants completed the post task KSS and post task NASA TLX (one for each task). Finally participants were fully debriefed and were paid £20 in store vouchers. The study was approved by Liverpool John Moores University Research Ethics Committee, and was administered in accordance with the ethical guidelines of the British Psychological Society.

fNIRS Analysis

fNIRS raw data from COBI studio was pre-processed using fnirSoft (Biopac systems; Goleta, CA, USA). All 16 voxels (oxy and deoxy-Hb) were visually inspected for light saturation. Saturated channels were discarded. A high-pass filter (0.1Hz cut off) and a linear phase filter (order of 20) were used to remove high frequency noise and noise due to respiration (Ayaz *et al.*, 2011; Ayaz *et al*., 2012). Using the modified Beer-Lambert law logarithm in fnirSoft (Ayaz *et al*., 2010), total oxy-Hb and deoxy-Hb changes relative to baseline over the entire epoch were calculated for the 16 voxels.

Statistical analysis

Behavioural data were analysed using ANOVA, with drug user group as the between subjects factor and individual task performance score (A composite overall performance

score for each updating task, which was calculated by adding the score on each level of the task e.g. *n*, *n* + 2, *n* + 4 and *n* + 6 in the case of letter updating, and dividing by the number of levels to give a mean score) on each of the updating tasks as the dependent variables**.** For analysis of fNIRS data, mean oxygenated and deoxygenated haemoglobin change from baseline over the entire epoch of each task, at each voxel was calculated. fNIRS data were analysed using ANOVA[2] with drug user group as the between subjects factor and mean oxy and deoxy-Hb change from baseline at each voxel as the dependent variables. Any significant main effects were further explored using post-hoc Tukey's HSD test.

7.4 Results

Socio-demographic information about the participants, sleep measures and scores of anxiety, depression and arousal from the UMACL are shown in Table 7.1. Indices of other drug and alcohol use are displayed in Table 7.2.

[2] Due to small amounts of missing data on different voxels, MANOVA was not appropriate for this analysis.

Table 7.1 – Indices sleep quality, fluid intelligence and socio-demographic variables

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
Males: n, %	11 (55)	11 (55)	9 (45)
Age (SD)	21.76 (3.19)*†	19.75 (1.48)	19.78 (1.90)
University degree: n (%)	3 (15)	2 (10)	1 (5)
Employment status			
Student; n, (%)	19 (95)	20 (100)	20 (100)
Employed; n (%)	1 (5)	0 (0)	0 (0)
Unemployed; n (%)	0 (0)	0 (0)	0 (0)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Ravens Progressive Matrices (maximum 60)	47.88 (5.04)	46.90 (6.61)	49.72 (4.70)
Sleep – Hours/night	7.65 (1.51)	8.18 (1.55)	8.39 (1.23)
ESS Score (maximum 24)	7.18 (3.13)	6.15 (3.63)	6.17 (3.13)
KSS before	5.31 (1.49)†	5.20 (1.47)	4.15 (1.35)
KSS after	6.00 (1.37)	4.79 (1.99)	4.74 (1.88)
MEQ total	40.29 (9.35)	40.70 (9.11)	44.44 (9.67)
UMACL anxiety	7.95 (2.63)	8.10 (1.77)	7.85 (2.39)
UMACL depression	9.35 (2.08)	9.26 (1.85)	8.35 (2.41)
UMACL arousal	16.6 (3.45)†	16.5 (2.96)†	19.35 (3.38)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Table 7.2 – Indices of other drug and alcohol use.

	Ecstasy users		Polydrug controls		Drug naive controls	
	Mean (SD)	***n***	**Mean (SD)**	***n***	**Mean (SD)**	***n***
Cannabis						
Frequency (times/wk)	2.28 (2.86)	16	2.05 (2.43)	19	-	-
Last 30 days (joints)	33.81 (57.61)	16	24.76 (40.27)	17	-	-
Total use (joints)	4183.42 (6353.33)	16	1137.27 (2516.24)	19	2 (0)	1
Cocaine						
Frequency (times/wk)	0.32 (0.47)	12	0.25 (0.21)	5	-	-
Last 30 days (lines)	9.38 (26.30)	13	2.75 (3.20)	4	-	-
Total use (lines)	964.63 (2876.88)	14	42.9 (67.17)	10	-	-
Ketamine						
Frequency (times/wk)	0.39 (0.79)	6	0.08 (0.05)	2	-	-
Last 30 days use (grams)	1.2 (2.68)	5	0.10 (0.14)	2	-	-
Total use (grams)	118.73 (249.71)	9	0.44 (0.47)	5	-	-
Alcohol units p/w	23.05 (28.00)†	20	14.82 (13.15)	20	8.33 (6.15)	20

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

One way ANOVA revealed no significant between group differences on several background variables including; average hours slept per night, total score on the ESS, total score on the MEQ, levels of anxiety, depression and arousal and total score on Raven's SPM. However there were between group differences in age $F(2,53)=4.89$, $p<0.05$, KSS before $F(2,56)=3.94$, $p<0.05$, and arousal $F(2,57)=4.85$, $p<0.05$ and a between group difference approaching significance in post task KSS $F(2,52)=2.84$, $p=0.07$. Post-hoc t-tests revealed that the ecstasy user group was significantly older than both other groups ($p<.05$ in both cases). Ecstasy users were significantly more sleepy prior to testing than drug naïve controls ($p<.05$) but not polydrug users, and drug naïve controls had significantly higher

levels of arousal in comparison to both drug user groups ($p<.05$) in both cases. The post-hoc t-tests on the post task KSS were not significant ($p>.05$).

t-tests on indices of drug use aside from ecstasy between the ecstasy user group and polydrug controls revealed that ecstasy users had smoked a greater mean lifetime amount of joints compared to polydrug controls (4183.42±6353.33 compared to 1137.27±2516.24) and this difference was approaching significance $t(18.95)=1.80$, $p=.09$ (Levene's test was significant so degrees of freedom have been adjusted accordingly). However there were no significant between group differences in frequency of cannabis, cocaine or ketamine use, nor were there significant between group differences in lifetime total doses for cocaine or ketamine, or last 30 day totals for cannabis, cocaine or ketamine. Nevertheless, as can be observed in Table 7.2, the ecstasy user group can be described as polydrug users.

A one way ANOVA on average weekly alcohol consumption revealed a significant between group difference $F(2,57)=3.29$, $p<0.05$. Multiple comparisons revealed that the ecstasy users consumed significantly more alcohol than drug naïve controls on a weekly basis ($p<.05$), there were no significant differences between ecstasy users and polydrug controls, or polydrug controls and drug naïve controls in weekly alcohol consumption.

Behavioural data analysis

Performance on the updating tasks was compared using overall performance scores on each task. For letter updating there were four levels of difficulty depending on the length of the sequence presented; the easiest being *n* (where *n* is the participants letter span and only *n* amount of letters are presented in the sequence) followed by *n* + 2 (list length is two letters greater than participants span), then *n* + 4 (list length is four letters greater than participants span), and finally *n* + 6 (list length is six letters greater than participants span). In each case, participants had to recall the last *n* amount of letters in the sequence, in order. Points were

awarded for a correctly identified letter recalled, in the correct position of the sequence. The spatial updating task follows the same structure. However there is an extra level of difficulty in that it has a known *n* sequence (where participants have to recall their span *(n)* amount of spatial locations, and they are informed that only *n* amount of spatial locations will be presented) and an unknown *n* sequence (whereby participants have to recall *n* amount of spatial locations, however they are not informed of the sequence length prior to the trial).

The overall performance scores were a composite of performance on each level of difficulty of the task, relative to the participant's span, divided by the number of levels of difficulty, to give a mean score. For example, if a participant had a span of five on the letter updating task, this would yield five responses on each trail. Therefore, for each level of difficulty on the task, their total score would be divided by their span (in this case five) to give a mean score of performance on each level of difficulty. To attain an overall performance score, mean totals from each difficulty level are added together and divided by the number of levels of difficulty (4 for letter updating, and 5 for spatial updating).

Task data is displayed in Tables 7.3 and 7.4. ANOVA revealed no between group differences on overall performance of letter updating $F(2,56)=1.21$, $p>.05$, or spatial updating $F(2,56)=1.13$, $p>.05$. Mixed ANOVA with list length as within groups and user group as between groups was conducted on letter and spatial span separately. As there were no significant differences aside from a within groups effect of length, for brevity, a total composite score is reported here.

Table 7.3: Means and SDs of performance on the letter updating task.

	Ecstasy users	Polydrug controls	Drug naïve controls
Sequence length	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
n	4.97 (0.98)	4.85 (0.78)	5.04 (0.75)
n + 2	3.68 (0.96)	3.76 (1.22)	4.34 (1.02)
n + 4	3.50 (1.10)	3.68 (0.94)	3.93 (1.09)
n + 6	3.53 (1.03)	3.75 (1.23)	3.83 (1.10)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Table 7.4: Means and SDs of performance on the spatial updating task.

	Ecstasy users	Polydrug controls	Drug naïve controls
Sequence length	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Kn n	4.85 (1.32)	5.09 (0.73)	5.01 (0.74)
Ukn n	4.70 (1.00)	5.00 (0.91)	4.70 (1.06)
n + 2	3.38 (1.41)	3.44 (0.74)	3.36 (1.32)
n + 4	3.46 (1.44)	4.03 (0.92)	3.07 (1.42)
n + 6	3.47 (1.31)	3.90 (0.98)	3.34 (1.52)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

fNIRS Analysis

Mean averages of oxy and deoxy Hb changes from baseline for the letter updating task are displayed in table 7.5, and oxy and deoxy-Hb changes from baseline for the spatial updating task are displayed table 7.6. Due to inappropriate recording of baselines, nine participant's data was excluded from analysis (3 ecstasy users, 5 polydrug controls and 1 drug naïve control). Furthermore channels 4 and 6 failed to obtain any data throughout this experiment, so these channels are omitted from analysis.

Letter Updating:

Table 7.5: Mean oxy-Hb and deoxy-Hb changes from baseline (μmolar) for letter updating.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
V1 oxy	-0.15 (1.38)	-0.39 (0.65)	-0.09 (1.28)
V2 oxy	0.93 (1.72)**	-0.60 (0.98) †	0.67 (1.53)
V3 oxy	-0.03 (1.38)	-0.08 (0.39)	0.60 (1.14)
V4 oxy			
V5 oxy	0.01 (1.29)	-0.30 (0.62)	0.15 (1.35)
V6 oxy			
V7 oxy	0.30 (1.37)	-0.55 (1.01)	-0.52 (1.44)
V8 oxy	0.42 (1.56)	-0.32 (1.14)	0.15 (1.99)
V9 oxy	0.26 (2.14)	-0.55 (1.10)	-0.40 (1.48)
V10 oxy	0.50 (1.76)	-0.23 (1.12)	-0.30 (1.95)
V11 oxy	-0.05 (1.33)	-0.12 (0.77)	-0.25 (1.32)
V12 oxy	1.13 (2.07)*†	-0.12 (0.93)	-0.40 (1.22)
V13 oxy	0.31 (1.46)	0.05 (0.69)	0.49 (0.96)
V14 oxy	0.94 (1.67)**	-0.50 (1.15)	0.09 (1.48)
V15 oxy	0.31 (0.94)	-0.41 (0.64)†	0.39 (1.32)
V16 oxy	1.49 (3.27)*	-0.19 (0.99)	0.44 (1.60)
V1 deoxy	0.46 (1.79)*	-0.81 (0.81)	-0.60 (1.30
V2 deoxy	0.39 (1.58)	-0.62 (0.91)	-0.11 (1.12)
V3 deoxy	0.04 (1.18)	-0.29 (0.86)	0.10 (1.07)
V4 deoxy			
V5 deoxy	0.12 (0.97)	-0.27 (1.00)	-0.42 (1.24)
V6 deoxy			
V7 deoxy	0.34 (1.26)	-0.60 (1.22)	-0.75 (1.45)
V8 deoxy	0.43 (1.37)	-0.54 (1.02)	-0.61 (1.33)
V9 deoxy	0.33 (1.76)	-0.44 (1.16)	-0.64 (1.21)
V10 deoxy	0.36 (1.27)	-0.47 (1.15)	-0.71 (1.36)
V11 deoxy	0.02 (1.14)	-0.45 (0.91)	-0.48 (1.16)
V12 deoxy	0.79 (1.88)†	-0.27 (0.97)	-0.76 (1.18)
V13 deoxy	0.02 (1.03)	-0.51 (0.86)	-0.18 (0.98)
V14 deoxy	0.26 (1.22)	-0.49 (0.95)	-0.54 (1.04)
V15 deoxy	0.17 (1.24)	-0.82 (0.86)	-0.52 (1.30)
V16 deoxy	1.22 (3.24)	-0.42 (1.12)	-0.28 (1.29)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Univariate ANOVA on the oxy-Hb data during the letter updating task revealed significant between group differences at several voxels; V2 $F(2,44)=4.62$, $p<.05$, V12 $F(2,45)=4.80$, $p<.01$ and V14 $F(2,47)=4.15$, $p<.05$. Group differences also approached significance at V15 $F(2,45)=2.90$, $p=.07$ and V16 $F(2,47)=2.49$, $p=.09$. There were no significant differences at any of the other voxels measured ($p>.05$).

Planned comparisons revealed that ecstasy users had significantly increased oxy-Hb compared to polydrug controls at V2 ($p<.01$), drug naïve controls also had significantly increased oxy-Hb compared to polydrug controls at V2 ($p<.05$). Ecstasy users and drug naïve controls were not significantly different to one another at this voxel ($p>.05$). At V12, ecstasy users displayed significantly increased oxy-Hb compared to both control groups ($p<.05$ in both cases). Polydrug controls and drug naïve controls did not differ significantly at V12. At V14, ecstasy users displayed a significant increase in oxy-Hb relative to polydrug controls ($p<.01$), but the difference between ecstasy users and drug naïve controls was non-significant ($p>.05$), as was the difference between the two control groups ($p>.05$). At V15, ecstasy users displayed increased oxy-Hb compared to polydrug controls that was approaching significance ($p=.07$). However, drug naïve controls showed the greatest increase in oxy-Hb at this voxel, and the difference between drug naïve controls and polydrug controls was significant ($p<.05$). Differences between ecstasy users and drug naïve controls were non-significant ($p>.05$). At V16, ecstasy users had significantly increased oxy-Hb compared to polydrug controls ($p<.05$) Differences between drug naïve controls and both other groups were non-significant ($p>.05$).

Due to the groups showing differences in age and pre-test sleepiness and given the heavy use of cannabis in the ecstasy user group, multiple regression analyses were conducted on Voxel 12, at which ecstasy users showed significant increases in oxy-Hb compared to both other groups. This was conducted to observe whether ecstasy use indices predicted oxy-Hb increase after controlling for age, sleepiness and cannabis use. Oxy-Hb level at V12 was entered as the dependent variable. In the first step, age and pre-test KSS score were entered as predictors. In the second step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the third step the same indices of ecstasy use were entered as predictors.

The overall regression model accounted for a significant 46.9% (R^2 = 0.47, R^2 adjusted = 0.34, $F(8,34)=3.75$, $p<0.01$) of the variance in oxy-Hb. Age and pre-test sleepiness (step 1) did not predict a significant amount of variance in oxy-Hb (R^2 = 0.05, R^2 adjusted = 0.00, $F(2,40)=1.01$, $p>0.05$). Cannabis use indices (step 2) did not predict a significant amount of variance oxy-Hb after controlling for age and pre-test sleepiness (R^2change=0.05, *F-change* (3,37)=0.63, $p>.05$). Individual indices - frequency of use (β=-0.71, $p>0.05$) and total lifetime dose (β=-0.02, $p>0.05$) did not predict oxy-Hb, however amount smoked in the last 30 days was a significant predictor (β=-1.00, $p<0.05$), with increased use being associated with reduced oxy-Hb. The ecstasy use indices (step 2) predicted a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2change=0.37, *F-change* (3,34)=7.99, $p<.05$). Specifically frequency of use (β=0.97, $p<0.05$) and last 30 day use (β=-0.49, $p<0.05$), were significant predictors of variance in oxy-Hb. Lifetime dose (β=0.14, $p>0.05$) was not a significant predictor. It would appear that frequency of use is the most important predictor here as increased frequency is associated with increased level of oxy-Hb.

ANOVA on the deoxy-Hb data revealed significant between group differences at voxels; V1 $F(2,44)=3.79$, $p<.05$, V7 $F(2,46)=3.41$, $p<.05$, V8 $F(2,44)=3.39$, $p<.05$ and V12 $F(2,45)=5.15$, $p<.01$. Group differences were also approaching significance at V10 $F(2,41)=3.04$, $p=.06$, V14 $F(2,47)=2.94$, $p=.06$, V15 $F(2,45)=2.97$, $p=.06$ and V16 $F(2,46)=3.01$, $p=.06$. There were no significant differences at any of the other voxels measured ($p>.05$).

Planned comparisons revealed that ecstasy users displayed a significant increase in deoxy-Hb compared to drug naïve controls at voxel 12 ($p<.01$). At V12 ecstasy users also showed increased deoxy-Hb compared to polydrug controls that was approaching significance ($p=.09$). At V7, ecstasy users showed a strong trend for increased deoxy-Hb compared to drug naïve controls ($p=.05$), the difference between ecstasy users and polydrug controls was non-significant ($p>.05$). At V1 ecstasy users showed significantly greater deoxy-Hb compared to polydrug controls ($p<.05$), and greater deoxy-Hb compared to drug naïve controls that was approaching significance ($p=.08$). At V8 ecstasy users displayed greater deoxy-Hb that was approaching significance to drug naïve controls ($p=.06$) and polydrug controls ($p=.09$). Ecstasy users displayed greater deoxy-Hb than drug naïve controls that was approaching significance at V10 ($p=.06$) and V14 ($p=.09$). Finally ecstasy users also displayed greater deoxy-Hb compared to polydrug controls that was approaching significance at V15 ($p=.06$) and V16 ($p=.08$). These were all that the 2 tailed level.

Regression analyses (using the same steps and predictor variables as earlier) were conducted for voxels showing significant ecstasy-related increases in deoxy-Hb - V1 and V12.

With deoxy-Hb at voxel 1 as the dependent variable the overall regression model accounted for a non-significant 13.8% ($R^2 = 0.14$, R^2 adjusted = 0.06, $F(8,34)=0.68$, $p>0.05$) of the variance in deoxy-Hb. Age and pre-test sleepiness (step 1) did not predict a significant

amount of variance in deoxy-Hb (R^2 = 0.03, R^2 adjusted = -0.02, $F(2,40)=0.51$, $p>0.05$). Cannabis use indices (step 2) did not predict a significant amount of variance in V1 deoxy-Hb after controlling for age and pre-test sleepiness (R^2change=0.02, *F-change* (3,37)=0.27, $p>.05$). None of the individual cannabis indices were significant predictors; frequency of use (β=0.01, $p>0.05$), total lifetime dose (β=-0.12, $p>0.05$) and amount smoked in the last 30 days (β=-0.13, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in V1 deoxygenation, after controlling for cannabis use indices (R^2change=0.09, *F-change* (3,34)=1.22, $p>.05$). Individual indices; frequency of use (β=0.23, $p>0.05$), lifetime dose (β=-0.19, $p>0.05$) and last 30 day use (β=0.20, $p>0.05$) did not significantly predict V1 deoxygenation.

With deoxy-Hb at V12 as the DV the overall regression model accounted for a non-significant 26.8% (R^2 = 0.27, R^2 adjusted = 0.10, $F(8,34)=1.56$, $p>0.05$) of the variance in deoxygenation. Age and pre-test sleepiness (step 1) did not predict a significant amount of variance in deoxygenation (R^2 = 0.02, R^2 adjusted = -0.03, $F(2,40)=0.37$, $p>0.05$). Cannabis use indices (step 2) did not predict a significant amount of variance in V12 deoxygenation after controlling for age and pre-test sleepiness (R^2change=0.03, *F-change* (3,37)=0.33, $p>.05$). None of the individual cannabis indices were significant predictors; frequency of use (β=0.57, $p>0.05$), total lifetime dose (β=-0.18, $p>0.05$) and amount smoked in the last 30 days (β=-0.76, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in V12 deoxy-Hb, after controlling for cannabis use indices (R^2change=0.22, *F-change* (3,34)=3.47, $p>.05$). Individual indices; lifetime dose (β=-0.21, $p>0.05$) and last 30 day use (β=0.12, $p>0.05$) did not significantly predict V12 deoxygenation. However frequency of use (β=0.74, $p<0.05$) was a significant predictor of V12 deoxygenation with increased frequency of use being associated with increased deoxygenation.

Spatial Updating:

Table 7.6: Mean oxy-Hb and deoxy-Hb changes from baseline (μmolar) for spatial updating.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
	Mean (SD)	Mean (SD)	Mean (SD)
V1 oxy	0.91 (1.68)*	-0.81 (1.36)†	0.70 (1.42)
V2 oxy	1.06 (1.79)*	-0.61 (1.18)†	0.76 (1.15)
V3 oxy	0.53 (1.33)	-0.11 (0.70)	0.74 (1.23)
V4 oxy			
V5 oxy	0.57 (1.74)	-0.11 (0.85)	0.71 (1.86)
V6 oxy			
V7 oxy	0.39 (1.72)	-0.14 (0.85)	0.05 (1.54)
V8 oxy	1.05 (1.80)*†	-0.31 (1.04)	-0.06 (1.28)
V9 oxy	0.08 (1.73)	-0.32 (0.75)	0.05 (1.57)
V10 oxy	0.59 (1.80)	-0.42 (0.85)	-0.11 (1.41)
V11 oxy	0.34 (1.65)	-0.30 (0.72)	0.54 (1.34)
V12 oxy	0.64 (1.64)*	-0.44 (0.93)	0.06 (1.35)
V13 oxy	0.26 (1.51)	-0.19 (1.13)	0.26 (1.11)
V14 oxy	0.36 (1.79)	-0.63 (1.30)	0.13 (1.50)
V15 oxy	0.12 (1.30)	-0.34 (1.09)	0.22 (1.07)
V16 oxy	0.47 (1.07)	-0.24 (1.17)†	0.83 (1.31)
V1 deoxy	0.07 (1.78)	-0.41 (0.80)	0.61 (1.15)
V2 deoxy	0.12 (0.68)	-0.26 (0.72)	0.37 (0.97)
V3 deoxy	0.09 (1.29)	-0.18 (0.77)	0.74 (1.15)
V4 deoxy			
V5 deoxy	0.22 (1.39)	-0.05 (0.69)	0.69 (1.19)
V6 deoxy			
V7 deoxy	0.13 (1.27)	-0.14 (0.88)	0.51 (1.29)
V8 deoxy	0.34 (0.99)	-0.06 (0.68)	0.32 (1.23)
V9 deoxy	-0.13 (1.21)	-0.20 (0.75)	0.56 (1.50)
V10 deoxy	0.27 (0.60)	-0.14 (0.69)	0.18 (1.11)
V11 deoxy	-0.03 (1.22)	-0.01 (0.48)	0.61 (1.36)
V12 deoxy	0.16 (1.61)	-0.19 (0.58)	0.36 (1.45)
V13 deoxy	-0.05 (1.43)	-0.40 (0.88)	0.39 (0.92)
V14 deoxy	-0.06 (1.78)	-0.38 (0.85)	0.06 (1.18)
V15 deoxy	-0.45 (1.80)	-0.45 (0.96)	0.24 (0.96)
V16 deoxy	-0.09 (1.18)	-0.40 (1.04)	0.37 (1.00)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Univariate ANOVA on the oxy-Hb data, revealed significant between group differences at voxels; V1 $F(2,42)=5.89$, $p<.01$, V2 $F(2,42)=5.88$, $p<.01$, V8 $F(2,42)=3.94$, $p<.05$ and V16 $F(2,46)=3.47$, $p<.05$. Between group differences were also approaching

significance at V12 $F(2,44)=2.54$, $p=.09$. There were no significant between group differences at any of the other voxels measured ($p>.05$)

Pairwise comparisons revealed that at V1 polydrug users had significantly lower oxy-Hb that both other groups ($p<.05$), however the difference between ecstasy users and drug naïve controls was non-significant ($p>.05$). At V2 polydrug users had significantly lower oxy-Hb than both other groups ($p<.05$), however the difference between ecstasy users and drug naïve controls was non-significant. At V8 ecstasy users had significantly increased oxy-Hb relative to polydrug controls and drug naïve controls ($p<.05$). At V16 polydrug users had significantly reduced oxy-Hb relative to drug naïve controls ($p<.05$). Ecstasy users did not differ from either control group significantly ($p>.05$ in both cases). At V12 ecstasy users had significantly increased oxy-Hb compared to polydrug users ($p<.05$) although they were not significantly different to drug naïve controls ($p>.05$). Polydrug users and drug naïve controls did not differ from one another significantly at this voxel.

A regression analysis with the same steps and indices entered as the letter updating regressions with oxy-Hb at V8 as the dependent variable was conducted. This overall regression model accounted for a non-significant 0.8% ($R^2 = 0.08$, R^2 adjusted = -0.15, $F(8,31)=0.35$, $p>0.05$) of the variance in oxy-Hb. Age and pre-test sleepiness (step 1) did not predict a significant amount of variance in oxy-Hb ($R^2 = 0.00$, R^2 adjusted = -0.05, $F(2,37)=0.00$, $p>0.05$). Cannabis use indices (step 2) did not predict a significant amount of variance in V8 oxygenation after controlling for age and pre-test sleepiness (R^2change=0.01, *F-change* (3,34)=0.09, $p>.05$). None of the individual cannabis indices were significant predictors; frequency of use ($\beta=0.58$, $p>0.05$), total lifetime dose ($\beta=-0.07$, $p>0.05$) and amount smoked in the last 30 days ($\beta=-0.70$, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in V8 oxy-Hb, after controlling for cannabis use

indices (R^2change=0.08, *F-change* (3,31)=.0.85, $p>.05$). Individual indices; frequency of use (β=0.45, $p>0.05$), lifetime dose (β=0.02, $p>0.05$) and last 30 day use (β=-0.29, $p>0.05$) did not significantly predict V8 oxy-Hb level.

ANOVA on the deoxy-Hb data during the spatial updating task revealed no significant between group differences at any voxel ($p>.05$ in each case). However differences were approaching significance at voxels V1 $F(2,44)=2.63$, $p=.08$ and V3 $F(2,41)=2.59$, $p=.09$. Pairwise comparisons revealed that polydrug users showed decreased deoxy-Hb compared to drug naïve controls that was approaching significance at V1 ($p=.07$) and V3 ($p=.09$). Ecstasy users did not differ significantly from either group at either of these voxels.

7.5 Discussion and summary

Analysis of performance data in Chapter 7 suggest that ecstasy users perform equivalently to non-users on letter updating and spatial updating. However the haemodynamic response data suggest that ecstasy users may be engaged in more effortful cognition to attenuate performance differences. During the letter updating task ecstasy users showed significantly increased oxy-Hb compared to both control groups at voxel 12, located over the right medial PFC. Furthermore regression analysis revealed that ecstasy use indices predicted a significant amount of the variance in oxy-Hb at this voxel after controlling for age, sleepiness and cannabis use variables; specifically, frequency of use predicted increased oxy-Hb level. Furthermore ecstasy users showed increases in deoxy-Hb from baseline compared to drug naïve controls at V12, this difference was also approaching significance at V1, V7 and V8. Ecstasy users also showed significantly increased deoxy-Hb from baseline compared to polydrug controls at V1 and this was approaching significance at V12 and V8. This indicates that ecstasy users were engaged in more effortful cognition and were perhaps relying on additional cognitive resources. Regression analyses on deoxy-Hb data, were

generally non-significant, although frequency of MDMA use predicted deoxy-Hb at V12, suggesting that more frequent use lead to greater deoxygenation.

During spatial updating, the oxy-Hb data again revealed that ecstasy users showed significantly increased oxy-Hb compared to both control groups at voxel 8, pertaining to the left medial prefrontal cortex. Ecstasy use indices did not significantly predict oxygenation at this voxel after controlling for age, sleepiness and cannabis use indices in a regression analysis. Nevertheless the between group differences are evidence that increased cognitive effort is displayed in ecstasy users compared to non-users during spatial updating. The implications of which will be discussed in greater detail in Chapter 10.

Chapter 8: fNIRS response to switching, inhibition and access

8.1 Chapter overview

Chapter 7 showed that ecstasy users show greater haemodynamic activation than control groups when performing memory updating tasks. This chapter assessed the haemodynamic response to the other three executive functions – inhibition, switching and access. Twenty ecstasy users and 20 controls (polydrug users and drug naïve participants) completed a random letter generation (RLG) task (inhibition), a number-letter task (switching) and an oral variant of the CWFT (access). No performance differences were observed between groups on any of the tasks. However significant increases in oxy-Hb from baseline were observed in ecstasy users relative to controls at various sites on every level of every task. Significant increases in deoxy-Hb were also observed in ecstasy users relative to controls at various sites during the CWFT and the RLG task.

8.2 Introduction

As previously discussed research in cognitive psychology suggests that the central executive of working memory is not a unified construct and is comprised of separable functions including memory updating, mental set switching, inhibitory control and access to semantic memory (Fisk and Sharp, 2004; Miyake *et al*., 2000). In ecstasy users, tasks that are proposed to tap the executive function of mental set switching show equivocal results. Dafters (2006) observed ecstasy users to be impaired on a switch component of the Stroop task compared to cannabis users and non-users and Dafters *et al.* (1999) found MDMA use to be negatively correlated with performance on the BADS rule shift cards test; however, the majority of studies suggest that MDMA users are unimpaired on this function (Fox *et al.*,, 2001; Back-Madruga *et al*., 2003: Montgomery, Fisk, Newcombe & Murphy, 2005). Chapter 6 in this thesis examined performance on the number-letter task in combination with ERP analysis. Atypicalities in the P2 component in ecstasy users compared to both polydrug and

drug naïve controls despite equivalent behavioural performance, as well as drug related alterations to the P3 response (Roberts *et al.,* 2013c in press). This highlights the importance of using more sensitive measures of cognitive performance, such as EEG or fNIRS, to gain a clearer understanding of the mechanisms underpinning cognitive deficits in substance users, as behavioural measures alone may not be sensitive enough to detect subtle ecstasy-related cognitive deficits in switching.

The effects of ecstasy use on inhibition have been reviewed in Chapters 3 and 6. As with switching, results of behavioural studies are mixed though the general consensus is that this executive function is relatively robust to ecstasy-related decline. Halpern *et al.,* (2004) found ecstasy-related deficits on the Stroop task in a relatively pure ecstasy using sample, however this was not replicated in a follow up study (Halpern *et al*., 2011). Moreover, using this task more studies have observed ecstasy users to be unimpaired at inhibition (Back-Madruga *et al.,* 2003; Gouzoulis-Mayfrank *et al*., 2000). Using the RLG task, Wareing *et al*. (2000) observed performance deficits in ecstasy users compared to non-users, though these findings were not replicated by Fisk *et al*. (2004), using a larger sample and more effective controls for concomitant use of other drugs. Nevertheless it has been suggested that depletion of Serotonin (5-HT) and impairment of other executive functions may lead to poor inhibitory control (Morgan *et al.,* 2006). Moreover, the use of neurophysiological measures may be necessary to better understand the impact of MDMA on these cognitive processes. For example Roberts and Garavan (2010) observed no performance deficits in ecstasy users on a Go/NoGo task, however fMRI data revealed that users showed increased frontal and parietal BOLD activation during successful inhibitions and hyperactivity of temporal, frontal and cingulate regions during commission errors. Furthermore ERP data in this thesis from Chapter 6 support the view that recreational ecstasy use may lead to subtle cognitive alterations during this executive function that are more readily detected in neuroimaging data

than behavioural data. Atypicalities in early processing (P2 component), during a Go/NoGo task suggest that compensatory cognitive mechanisms were being employed to enable equivalent behavioural performance to controls (Roberts *et al*., 2013a).

While results for switching and inhibitory control are mixed, there appears to be more evidence in support of impaired access in ecstasy users. The COWA task has yielded deficits in ecstasy users compared to controls (Bhattachary & Powell, 2001; Fox *et al.,* 2002), though this is not always a consistent finding (Halpern *et al.,* 2004). Ecstasy users do appear to perform at a consistently lower level than controls when the written variant of this task is used (CWFT). The CWFT is understood to be more complex than the COWA task as it requires participants to name words that fit specific criteria (e.g. four-letter words beginning with the letter C) and therefore places more demand on the central executive. Using this task, ecstasy-related impairments have been more consistently reported (Montgomery *et al.*, 2007; Montgomery, Fisk, Newcombe & Murphy, 2005). Raj *et al.* (2010) investigated the BOLD response to a semantic retrieval task of ecstasy users and suggested that MDMA exposure results in reduced BOLD response in neuronal areas relating to verbal memory. Furthermore Chapter 6 of this thesis reported evidence of abnormal executive functioning in ecstasy users (the N2 ERP component) despite the absence of behavioural differences in a semantic association paradigm (Roberts *et al*., 2013b).

Consequently studies into cognitive deficits associated with ecstasy use are increasingly employing neuroimaging techniques to gain insight into processes underlying such deficits. Burgess *et al*., (2011) observed differences in ecstasy users' ERPs in a late positive component over left parietal scalp sites in a recall task that had yielded no significant performance deficits. The amelioration of this late positivity in ecstasy users is accepted as a durable abnormality in processing that would not have been detected by behavioural

measures alone. fMRI studies (e.g. Daumann, Fimm *et al.*, 2003) have reported increased cortical activity in ecstasy users to compensate for behavioural differences. Furthermore Moeller *et al*. (2004) report increases in blood volume in ecstasy users relative to controls in several brain regions including the medial frontal gyrus and the thalamus, during a delayed memory task. These fMRI studies show increases in cortical activity and blood volume that are understood to reflect compensatory mechanisms in ecstasy users. In effect, ecstasy users are working harder to achieve the same result behaviourally. Moreover results from Chapter 7 in this thesis, indicated that differences in haemodynamic response (increases in oxy-Hb and deoxy-Hb) were apparent in ecstasy users compared to controls, despite equivalent behavioural performance. The findings from Chapter 7 suggest that ecstasy users were engaged in more effortful cognition (recruitment of additional resources) to attenuate performance deficits, in letter updating and spatial updating.

The present chapter sought to investigate the cerebral haemodynamic response (using fNIRS) to three executive functioning tasks in ecstasy users and non-user controls. The three executive functions investigated were mental set switching, access and inhibitory control. Based on previous research that has yielded the most interesting results and compatibility with the technology being used, the following tasks have been used to assess each executive function – the number-letter task (switching), the CWFT (access) and RLG (inhibition). Performance and haemodynamic response were measured on each task. It was hypothesised that although performance on each of the executive functions may be equivalent between groups, differences will be observed in oxy-Hb and deoxy-Hb haemoglobin changes from baseline, in line with findings from Chapter 7, that reflect increased cognitive effort (increased oxy-Hb and deoxy-Hb) in ecstasy users.

8.3 Method

Participants:

Twenty ecstasy users (mean age = 21.85±2.76; 13 = male) and 20 non-user controls (mean age = 20.89±2.05; 7 = male) were recruited via email to university students. Inclusion criteria for the experiment was similar to that in Chapters 6 and 7, except due to practical constraints only one control group was recruited that were ecstasy naïve (although the majority of participants in the control group were drug naïve, so there is limited drug use in the non-user group). Indices of ecstasy use were as follows: total lifetime dose 431.75 tablets ± 885.08; mean amount used in last 30 days 2.55 tablets ± 3.23, and frequency of use 0.37 times/week ± 0.51.

Materials

Questionnaires:

The *Background Drug Use Questionnaire*, The ESS, The MEQ, KSS, UMACL, NASA-TLX and Raven's SPM were used, as described in Chapter 6.

Executive function tasks:

Random Letter Generation (inhibition) (Baddeley, 1966)

Participants were presented with a bar on the screen that alternated between two positions at a set pace, cueing participants to generate a letter. Participants had to produce 100 letters in each block of the task. There were three blocks and each block represented a different production rate (one letter every 4, 2, and 1-seconds). Participants were instructed to avoid alphabetical sequences, repetition of sequences of letters and to produce each letter with the same overall frequency. Presentation of blocks was randomised and participants' responses were recorded onto a cassette deck with a built in microphone. Four scores were

generated – the number of alphabetically ordered pairs, the number of repeat sequences, "redundancy" (the extent to which all letters are produced equally with 0% being truly random) and the number of letters produced. A high score on the first three indicates poor performance whereas the opposite is true in the fourth case**.** All scores were standardised and a single score for each random generation measure was obtained by calculating the mean standardised scores for the three production rates (as per Montgomery, Fisk, Newcombe & Murphy, 2005)

The Number Letter Task (switching) (Rogers & Monsell, 1995) – as described in Chapter 6.

The Chicago Word Fluency Task (access) (Thurstone, 1938)

A variation of the original Thurstone word fluency task, this task consisted of three blocks in which participants had to verbally produce as many words as they could in one minute. In the first block (semantic fluency), participants were instructed to name as many animals as they could during the time period. Following this they were instructed to produce as many words possible beginning with the letter "S", and in the third and final block they were required to name as many four letter words beginning with the letter "C" as possible (word fluency). Participants were informed that place names, people's names and plurals were prohibited. Responses were recorded on a cassette deck with a built in microphone. Scores for each of the fluency tasks were counted as the number of appropriate words in each case.

Equipment

A continuous wave fNIRS system (developed by Drexel University, Philadelphia, PA) supplied by Biopac systems (Goleta, CA, USA) was used for collecting haemodynamic response to task data from the PFC as described in Chapter 7.

Procedure

Participants attended the lab for a single session lasting approximately 2 hours. Testing sessions commenced at 9am, 11am and 1pm and 3pm, with equal numbers of each group tested at each session time. Upon arrival participants were given an information sheet explaining what was involved in the study, and written consent was obtained. Questionnaires were administered in the following order: background drug use questionnaire, MEQ, ESS, pre-test KSS, UMACL and Raven's SPM. The fNIRS headband was then fitted to the participant's forehead. fNIRS signals were displayed on a desktop computer running COBI studio (Drexel University) in an adjacent room to the testing room. Once stability of fNIRS signals was obtained, a baseline of inactivity was recorded. Baselines were recorded prior to each task. Participants watched a video of planet earth accompanied by soothing music and the baselines were recorded during this period. Participants then completed one of the three tasks (number-letter task, CWFT or RLG). After each task participants completed the NASA TLX then the process was repeated for the other tasks starting with baseline recording. Task order was randomised. After all three tasks had been completed participants were administered the post task KSS. Participants were fully debriefed after the testing procedure and were paid £20 in store vouchers. The study was approved by Liverpool John Moores University Research Ethics Committee, and was administered in accordance with the ethical guidelines of the British Psychological Society.

fNIRS analysis

Pre-processing and analysis followed the same procedure as that described in Chapter 7. fNIRS data was analysed using ANOVA[3] in blocks of discrete epochs on each task. The number-letter task was analysed over two switching blocks, the CWFT had three blocks (animals, "S" letter words and four-letter long words beginning with "C") and RLG had three blocks where the speed at which letters had to be produced differed (every 4, 2, or 1-seconds). Due to the various blocks in these tasks relating to the level of difficulty of the task, mean oxy-Hb and deoxy-Hb changes from baseline at each channel were calculated for each block.

8.4 Results

Socio-demographic information about the participants, sleep measures and scores of anxiety, depression and arousal are shown in Table 8.1. Indices of other drug and alcohol use are displayed in Table 8.2.

[3] Due to small amounts of missing data on different voxels, MANOVA was not appropriate for this analysis.

Table 8.1- Indices sleep quality, fluid intelligence and socio-demographic variables

	Ecstasy users	**Non-users**
Males: n, (%)	13 (65)	8 (40)
Age (SD)	21.85 (2.76)	20.89 (2.05)
University degree: n (%)	4 (20)	5 (25)
Employment status		
Student; n, (%)	17 (85)	20 (100)
Employed; n (%)	2 (10)	0 (0)
Unemployed; n (%)	1 (5)	0 (0)
	Mean (SD)	**Mean (SD)**
Ravens Progressive Matrices (maximum 60)	47.20 (5.64)	48.00 (6.79)
ESS Score (maximum 24)	5.00 (2.81)	5.25 (2.81)
KSS before	4.30 (1.49)	4.75 (1.74)
KSS after	5.33 (2.15)	4.06 (2.05)
MEQ total	45.33 (9.31)	50.00 (9.95)
UWIST anxiety	8.70 (2.56)	8.75 (2.24)
UWIST depression	9.05 (3.22)	8.70 (2.00)
UWIST arousal	17.35 (5.38)	17.75 (3.29)

*Indicates a significant difference from controls at the .05 level, and ** at the .01 level.

Table 8.2: Indices of Drug use

	Ecstasy users	
	Mean (SD)	***n***
Cannabis		
Frequency (times/wk)	1.42 (1.94)	19
Last 30 days (joints)	23.03 (40.19)	19
Total use (joints)	1607.88 (2212.54)	19
Cocaine		
Frequency (times/wk)	1.15 (2.96)	11
Last 30 days (lines)	6.42 (14.80)	12
Total use (lines)	294.64 (465.18)	14
Ketamine		
Frequency (times/wk)	0.24 (0.32)	10
Last 30 days use (grams)	0.33 (0.71)	9
Total use (grams)	7.16 (9.56)	11

t-tests on background variables revealed there was no significant difference between the two groups in age $t(36)=1.21$, $p>.05$, total scores on the ESS $t(37)=-0.28$, $p>.05$, MEQ $t(30)=-.1.37$, $p>.05$, Raven's SPM $t(38)=-0.41$, $p>.05$, pre-test KSS $t(38)=-0.88$, $p>.05$, post-test KSS $t(26)=1.59$, $p>.05$, or levels of arousal $t(38)=-0.28$, $p>.05$, depression $t(38)=0.41$, $p>.05$ and anxiety $t(38)=-0.07$, $p>.05$. However ecstasy users did drink significantly more units of alcohol per week than non-users (18.6±11.91 units p/w compared to 9.75±8.63 units p/w) $t(38)=2.71$, $p<.01$, and it is clear from Table 8.2 that there is concomitant drug use this cohort. Perhaps it would be more appropriate to call the current sample of ecstasy users, ecstasy/polydrug users.

Behavioural Data Analysis: See Table 8.3 for descriptive statistics.

Random Letter Generation: Standardised scores for alphabetically ordered pairs, repeat sequences and redundancy were added together and the standardised score for the number of letters produced was subtracted from this total, this new total was then divided by four, to give a single standardised performance score for each rate (1s, 2s and 4s) for each

participant. MANOVA was conducted on the performance scores for this task, this revealed no significant main effect of group, $F(3,36)=0.85$, $p>.05$ for Pillai's trace. Univariate ANOVA revealed no significant between group differences on performance at each individual rate; 1s $F(1,38)=0.01$, $p>.05$, 2s $F(1,38)=1.75$, $p>.05$ or 4s $F(1,38)=0.93$, $p>.05$.

Number-Letter Task: Incorrect answers were given a score of 0 and were not investigated any further. Responses before 200ms and after 4000ms were excluded from analysis as these represent pre-emptive responding and loss of concentration respectively and individual reaction times that were 3 standard deviations or more above the individual mean were excluded. The mean percentage of outliers discarded from each group were: ecstasy users 1.51 ±0.73, drug naïve 1.48 ±0.91, there were no between group differences in amount of outliers $F(1,37)=0.02$, $p>.05$. One participant from the ecstasy user group had an incomplete dataset and therefore was excluded from the final analysis. ANOVA revealed no significant between groups difference on switch cost $F(1,37)=0.31$, $p>.05$.

Chicago Word Fluency Task: A mixed ANOVA was conducted on the CWFT data with group as the between subjects variable and level of difficulty as the within subjects variable (the easiest being "animals" followed by words beginning with "S" and the most difficult being 4 letter words beginning with "C"). There was a significant main effect of difficulty on the task $F(1.59, 60.23)=158.33$, $p<.01$ (the sphericity assumption was violated so Greenhouse-Geisser adjusted stats are reported), however there was no group by difficulty interaction $F(1.59, 60.23)=0.75$, $p>.05$. Furthermore there were no significant between group differences in performance on the task $F(1,38)=0.64$, $p>.05$.

Table 8.3: Means and SDs for behavioural measures for ecstasy users and non-users

	Ecstasy users Mean (SD)	*Non-users* Mean (SD)
RLG 4-second rate		
Redundancy	0.087 (0.02)	0.087 (0.02)
Repeat Sequences	11.70 (6.13)	11.10 (3.00)
Alphabetical Sequences	5.10 (3.61)	4.30 (2.36)
Number of Letters Produced	97.15 (11.38)	99.95 (0.22)
RLG 2-second rate		
Redundancy	0.093 (0.03)	0.095 (0.02)
Repeat Sequences	14.90 (7.35)	14.30 (3.98)
Alphabetical Sequences	8.65 (8.22)	6.75 (3.43)
Number of Letters Produced	95.50 (11.94)	99.20 (1.01)
RLG 1-second rate		
Redundancy	0.113 (0.03)	0.11 (0.03)
Repeat Sequences	13.95 (5.99)	15.75 (6.16)
Alphabetical Sequences	9.95 (4.38)	10.50 (4.50)
Number of Letters Produced	82.60 (15.09)	86.95 (12.66)
Number/letter Switch Cost (ms)	358.61 (161.79)	323.40 (227.34)
CWFT		
Animals	42.10 (9.24)	38.55 (7.27)
Words beginning with “S”	37.95 (11.26)	35.75 (11.49)
4 letter words beginning with “C”	15.45 (7.12)	15.55 (8.17)

*Indicates a significant difference from controls at the .05 level, and ** at the .01 level.

fNIRS Analysis

RLG: Changes in oxy-Hb and deoxy-Hb from baseline are displayed in table 8.4.

Table 8.4: Mean changes in oxy-Hb and deoxy-Hb (μmolar) from baseline, for ecstasy users and non-user controls during the random letter generation task.

	Ecstasy users			Non-users		
	RLG4	**RLG2**	**RLG1**	**RLG4**	**RLG2**	**RLG1**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
V1oxy	0.56 (0.84)	0.78 (1.32)	0.85 (1.27)	1.04 (1.13)	0.74 (0.86)	1.16 (1.51)
V2oxy	0.10 (1.82)	0.52 (1.49)	0.57 (1.44)	0.29 (1.36)	0.13 (1.49)	0.58 (1.59)
V3oxy	0.29 (1.07)	0.36 (1.03)	0.55 (1.17)	0.28 (0.94)	0.04 (0.82)	0.53 (1.45)
V4oxy	0.23 (1.42)	0.75 (1.44)*	1.14 (1.83)	-0.19 (0.70)	-0.36 (0.76)	0.27 (1.12)
V5oxy	0.18 (1.63)	0.30 (1.81)	0.50 (1.82)	0.54 (1.08)	0.34 (1.26)	0.55 (1.53)
V6oxy	0.06 (1.74)	0.37 (1.88)	0.33 (2.18)	0.16 (1.23)	0.24 (1.81)	0.40 (1.56)
V7oxy	0.25 (1.61)	0.32 (1.34)	0.35 (1.80)	0.67 (1.22)	0.49 (1.50)	0.71 (1.53)
V8oxy	0.84 (2.12)	1.31 (1.84)	1.22 (2.47)	0.49 (1.37)	0.53 (1.95)	0.64 (1.57)
V9oxy	0.47 (1.62)	0.42 (1.76)	0.62 (1.88)	0.52 (1.32)	0.21 (1.48)	0.56 (1.55)
V10oxy	1.21 (2.17)*	1.37 (2.31)*	1.11 (2.66)	0.14 (1.22)	-0.02 (1.85)	0.23 (1.59)
V11oxy	0.47 (1.17)	0.88 (1.34)	0.96 (1.26)	0.42 (1.08)	-0.02 (1.25)	0.34 (1.19)
V12oxy	0.49 (1.62)	0.84 (1.35)*	0.97 (1.62)*	-0.07 (0.91)	-0.17 (1.40)	0.08 (1.24)
V13oxy	0.41 (1.09)	0.37 (1.77)	1.03 (1.34)	0.35 (1.25)	0.37 (1.20)	0.44 (1.34)
V14oxy	0.51 (1.74)	0.64 (1.65)	0.93 (1.68)*	0.00 (1.08)	-0.27 (1.65)	-0.06 (1.52)
V15oxy	0.45 (1.30)	0.43 (1.82)	0.68 (1.53)	0.56 (1.40)	0.35 (1.17)	0.61 (1.79)
V16oxy	0.51 (1.43)	0.50 (1.71)	0.82 (1.60)	0.07 (0.84)	0.10 (1.14)	0.30 (1.13)
V1deoxy	0.17 (1.27)	0.13 (1.83)	0.04 (1.49)	-0.34 (0.96)	-0.55 (2.00)	-0.38 (1.02)
V2deoxy	-0.14 (1.46)	0.03 (1.55)*	-0.03 (1.29)*	-0.83 (0.98)	-1.12 (1.65)	-0.90 (1.17)
V3deoxy	0.18 (1.19)*	-0.01 (1.39)	-0.01 (1.10)*	-0.79 (1.33)	-0.94 (2.11)	-0.81 (1.66)
V4deoxy	0.10 (1.58)*	0.30 (1.55)*	0.49 (1.59)**	-1.45 (1.71)	-1.91 (2.91)	-1.59 (1.59)
V5deoxy	0.24 (1.37)*	0.14 (1.31)	0.25 (1.35)*	-0.48 (1.22)	-0.66 (1.91)	-0.60 (1.56)
V6deoxy	0.16 (1.32)	0.18 (1.34)	0.16 (1.32)	-0.40 (1.38)	-0.45 (2.11)	-0.33 (1.59)
V7deoxy	0.25 (1.70)	0.10 (1.73)	0.11 (1.54)	0.03 (0.88)	-0.12 (1.45)	-0.06 (1.15)
V8deoxy	0.31 (1.52)	0.55 (2.54)	0.21 (1.53)	-0.25 (1.25)	-0.36 (1.79)	-0.30 (1.35)
V9deoxy	0.25 (1.70)	0.15 (1.76)	0.03 (1.57)	-0.22 (1.35)	-0.49 (2.18)	-0.29 (1.46)
V10deoxy	0.53 (1.92)	0.61 (2.76)	0.17 (1.84)	-0.49 (1.79)	-0.72 (2.43)	-0.60 (1.90)
V11deoxy	0.18 (2.09)	0.28 (1.96)*	0.16 (1.80)*	-1.07 (1.86)	-1.53 (2.97)	-1.34 (2.21)
V12deoxy	0.50 (1.91)	0.45 (1.86)	0.45 (1.88)	-0.29 (0.62)	-0.28 (1.06)	-0.42 (1.08)
V13deoxy	0.34 (1.46)**	0.43 (1.56)*	0.57 (1.57)**	-0.72 (1.18)	-0.85 (1.91)	-0.83 (1.61)
V14deoxy	0.66 (2.09)*	0.61 (1.84)*	0.71 (1.98)**	-0.27 (0.62)	-0.35 (1.00)	-0.51 (0.83)
V15deoxy	0.56 (1.75)*	1.10 (3.99)*	0.53 (1.64)**	-0.62 (1.41)	-0.98 (2.52)	-0.84 (1.67)
V16deoxy	0.20 (1.44)*	-0.09 (1.79)	0.04 (1.82)	-0.89 (1.42)	-0.85 (1.98)	-0.88 (1.65)

*Indicates a significant difference from controls at the .05 level, and ** at the .01 level

ANOVA on oxy-Hb change from baseline on the first level of difficulty of the task (4s rate) revealed that ecstasy users showed increased oxy-Hb compared to controls at V10 $F(1,30)=2.96$, $p<.05$ and this difference was approaching significance at V1 $F(1,33)=2.00$, $p=.08$. There were no significant differences at any of the other voxels measured ($p>.05$).

There were also significant differences in the amount of deoxy-Hb change from baseline at V3 $F(1,35)=5.42$, $p<.05$, V4 $F(1,16)=3.90$, $p<.05$, V5 $F(1,36)=2.92$, $p<.05$, V13 $F(1,36)=6.11$, $p<.01$, V14 $F(1,34)=3.11$, $p<.05$, V15 $F(1,34)=4.93$, $p<.05$ and V16 $F(1,35)=5.37$, $p<.05$, whereby ecstasy users showed greater deoxygenation than controls. This difference was also approaching significance at V2 $F(1,32)=2.64$, $p=.06$, V10 $F(1,30)=2.44$, $p=.06$, V11 $F(1,20)=2.19$, $p=.08$ and V12 $F(1,31)=2.37$, $p=.07$. No other differences were observed for the other voxels measured ($p>.05$ in each case).

At the 2nd level of difficulty in this task (2s rate) ANOVA revealed between group differences in oxy-Hb at V4 $F(1,16)=3.47$, $p<.05$, V10 $F(1,30)=3.52$, $p<.05$, and V12 $F(1,31)=4.45$, $p<.05$ whereby ecstasy users showed greater oxy-Hb increase from baseline than controls. This difference was approaching significance at V11 $F(1,20)=2.65$, $p=.06$ and V14 $F(1,34)=2.75$, $p=.06$. There were no differences at any other voxels ($p>.05$). ANOVA on deoxy-Hb changes at the 2-s rate revealed that ecstasy users showed significantly greater deoxy-Hb increase than controls at V2 $F(1,32)=4.33$, $p<.05$, V4 $F(1,16)=4.47$, $p<.05$, V11 $F(1,20)=2.84$, $p<.05$, V13 $F(1,36)=5.12$, $p<.05$, V14 $F(1,34)=3.67$, $p<.05$ and V15 $F(1,34)=3.48$, $p<.05$. This difference was also approaching significance at V3 $F(1,35)=2.56$, $p=.06$, V5 $F(1,36)=2.27$, $p=.07$, V10 $F(1,30)=2.10$, $p=.08$ and V12 $F(1,31)=1.83$, $p<.09$. No other significant differences were observed ($p>.05$ in each case).

For the 3rd and most difficult level of the task (1s rate) ANOVA revealed that ecstasy users displayed significantly increased oxy-Hb from baseline relative to controls at V12 $F(1,31)=3.08$, $p<.05$ and V14 $F(1,34)=3.42$, $p<.05$. This difference was also approaching significance at V13 $F(1,36)=1.83$, $p=.09$. There were no other significant differences at any of the voxels measured ($p>.05$ in each case). ANOVA on the deoxy-Hb data in this block revealed that ecstasy users displayed significantly greater deoxy-Hb than controls at V2

$F(1,32)=4.24$, $p<.05$, V3 $F(1,35)=3.07$, $p<.05$, V4 $F(1,16)=7.20$, $p<.01$, V5 $F(1,36)=3.18$, $p<.05$, V11 $F(1,20)=3.05$, $p<.05$, V13 $F(1,36)=7.28$, $p<.01$, V14 $F(1,34)=5.55$, $p<.01$ and V15 $F(1,34)=6.14$, $p<.01$. This difference was also approaching significance at V12 $F(1,31)=2.50$, $p=.06$ and V16 $F(1,35)=2.59$, $p=.06$. There were no significant differences at the other voxels measured ($p>.05$ in each case).

Overall these results show a general increase in oxy-Hb and deoxy-Hb from baseline for ecstasy users compared to controls that is significant at several voxels in each level of the task.

Multiple regression analyses were conducted on all voxels showing significant between group differences in oxy-Hb and deoxy-Hb. This was conducted to observe whether ecstasy use indices predicted oxy-Hb and deoxy-Hb increase after controlling for cannabis use. Values of oxy-Hb or deoxy-Hb (μmolar) were entered as dependent variables. In step one indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days), in step two the same indices of ecstasy use were entered as predictors. The results from these regression analyses can be seen in Appendix 1, for brevity, only regressions yielding notable results are reported here.

Using deoxy-Hb at V14 during the 4s rate as the dependent variable, this overall regression model accounted for a significant 43.8% ($R^2 = 0.44$, R^2 adjusted = 0.32, $F(6,29)=3.76$, $p<0.01$) of the variance in deoxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in V14 deoxygenation ($R^2 = 0.05$, R^2 adjusted = -0.04, $F(3,32)=0.56$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use ($\beta=-0.10$, $p>0.05$), total lifetime dose ($\beta=0.24$, $p>0.05$) and amount smoked in the last 30 days ($\beta=-0.39$, $p>0.05$). The ecstasy use indices (step 2) did, however, predict a significant amount of variance in V14 deoxy-Hb, after controlling for

cannabis use indices (R^2 change=0.39, *F-change* (3,29)=6.66, $p<.01$). Individual indices; frequency of use (β=-0.42, $p<0.05$) and last 30 day use (β=0.82, $p<0.05$) predicted V14 deoxy-Hb level at the 4s rate, with increased frequency being associated with decreased deoxy-Hb and increased last 30 day use being associated with increased deoxy-Hb. Lifetime dose (β=0.02, $p>0.05$) was not a significant predictor.

Using deoxy-Hb at V14 during the 2s rate as the dependent variable this overall regression model accounted for a non-significant 25.1% (R^2 = 0.25, R^2 adjusted = 0.10, $F(6,29)=1.62$, $p>0.05$) of the variance in deoxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in V14 deoxygenation (R^2 = 0.03, R^2 adjusted = -0.07, $F(3,32)=0.28$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=-0.01, $p>0.05$), total lifetime dose (β=0.24, $p>0.05$) and amount smoked in the last 30 days (β=-0.31, $p>0.05$). There was a strong trend for ecstasy use indices (step 2) to predict variance in V14 deoxy-Hb, after controlling for cannabis use indices (R^2 change=0.23, *F-change* (3,29)=2.92, $p=.05$). Individual indices; frequency of use (β=-0.35, $p>0.05$) and lifetime dose (β=0.04, $p>0.05$) did not predict V14 deoxy-Hb level at the 2s rate, whereas last 30 day use (β=0.62, $p<0.01$) was a significant predictor, with increased use being associated with increased deoxy-Hb.

Using oxy-Hb at V12 during the 1s rate as the dependent variable this overall regression model accounted for a significant 38.9% (R^2 = 0.39, R^2 adjusted = 0.25, $F(6,26)=2.76$, $p<0.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in V12 oxy-Hb (R^2 = 0.23, R^2 adjusted = 0.15, $F(3,29)=2.90$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=0.73, $p>0.05$), total lifetime dose (β=0.39, $p>0.05$) and amount smoked in the last 30 days (β=-0.31, $p>0.05$). Ecstasy use indices (step 2) did not predict a significant amount of

variance in V12 oxy-Hb, after controlling for cannabis use indices (R^2 change=0.16, *F-change* (3,26)=2.25, p>.05). Individual indices; frequency of use (β=-0.27, p>0.05) and lifetime dose (β=0.03, p>0.05) did not predict V12 oxy-Hb increase at the 1s rate. However last 30 day use (β=0.52, p<0.05) was a significant predictor with increased use being associated with increased oxy-Hb.

Using oxy-Hb level at V14 during the 1s rate as the dependent variable this overall regression model accounted for a significant 33.8% (R^2 = 0.34, R^2 adjusted = 0.20, F(6, 29)=2.47, p<0.05) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in V14 oxy-Hb (R^2 = 0.18, R^2 adjusted = 0.11, F(3,32)=2.38, p>0.05). None of the individual cannabis use indices were significant predictors; frequency of use (β=0.11, p>0.05), total lifetime dose (β=0.16, p>0.05) and amount smoked in the last 30 days (β=0.21, p>0.05). Ecstasy use indices (step 2) did not predict a significant amount of variance in V14 oxy-Hb, after controlling for cannabis use indices (R^2 change=0.16, *F-change* (3,29)=2.28, p>.05). Individual indices; frequency of use (β=-0.27, p>0.05) and lifetime dose (β=-0.19, p>0.05) did not predict V14 oxy-Hb level at the 1s rate. However, last 30 day use (β=0.49, p<0.05) was a significant predictor, with increased use being associated with increased oxy-Hb change.

Using deoxy-Hb at V14 during the 1st rate as the dependent variable this overall regression model accounted for a non-significant 30.7% (R^2 = 0.31, R^2 adjusted = 0.16, F(6, 29)=2.14, p>0.05) of the variance in deoxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in V14 deoxy-Hb (R^2 = 0.03, R^2 adjusted = -0.06, F(3,32)=0.34, p>0.05). None of the individual cannabis use indices were significant predictors; frequency of use (β=-0.09, p>0.05), total lifetime dose (β=0.38, p>0.05) and amount smoked in the last 30 days (β=-0.25, p>0.05). However, ecstasy use indices (step 2)

did predict a significant amount of variance in V14 deoxy-Hb, after controlling for cannabis use indices (R^2 change=0.28, *F-change* (3,29)=3.85, $p<.05$). Individual indices; frequency of use (β=-0.37, $p>0.05$) and lifetime dose (β=-0.86, $p>0.05$) did not predict V14 deoxy-Hb level at the 1s rate. However, last 30 day use (β=0.69, $p<0.01$) was a significant predictor, with increased use being associated with increased deoxy-Hb level.

Number-Letter Task: The fNIRS data from the two switching blocks of this task is displayed in table 8.5.

Table 8.5: Mean changes in oxy-Hb and deoxy-Hb (μmolar) from baseline, for ecstasy users and non-user controls on the switching blocks of the number/letter task.

	Ecstasy users		**Non-users**	
	NL1	**NL2**	**NL1**	**NL2**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
V1oxy	0.71 (1.78)	0.57 (1.74)	1.07 (2.37)	1.22 (2.53)
V2oxy	1.59 (2.57)	1.52 (2.50)	1.10 (1.45)	1.45 (1.51)
V3oxy	0.89 (1.70)	0.78 (1.73)	0.22 (1.37)	0.24 (1.38)
V4oxy	1.26 (2.17)	1.17 (2.23)	0.57 (1.47)	0.76 (1.40)
V5oxy	1.36 (2.57)*	1.29 (2.49)	0.10 (1.43)	0.26 (1.47)
V6oxy	1.70 (3.38)	1.68 (3.44)	0.57 (1.10)	0.57 (1.14)
V7oxy	0.95 (3.15)	0.89 (3.03)	0.69 (2.03)	0.96 (2.27)
V8oxy	3.33 (7.04)	3.51 (7.09)	1.11 (1.72)	1.52 (1.80)
V9oxy	1.05 (2.12)	0.91 (2.08)	0.27 (1.81)	0.60 (1.97)
V10oxy	1.26 (2.76)	1.60 (3.07)	0.88 (3.33)	1.36 (3.30)
V11oxy	0.64 (2.75)	0.52 (2.75)	0.40 (1.70)	0.78 (1.61)
V12oxy	0.59 (2.61)	0.48 (2.66)	0.50 (1.58)	0.80 (1.42)
V13oxy	1.34 (2.49)	1.29 (2.42)	0.39 (1.06)	0.49 (1.22)
V14oxy	0.90 (1.42)	0.88 (1.44)	0.66 (1.32)	0.81 (1.29)
V15oxy	0.94 (1.91)	0.83 (2.23)	1.11 (1.69)	1.21 (1.83)
V16oxy	1.22 (2.02)	1.20 (2.02)	1.05 (1.25)	1.11 (1.33)
V1deoxy	-0.21 (2.23)	-0.20 (2.29)	1.21 (3.57)	0.75 (2.78)
V2deoxy	0.17 (1.97)	0.28 (2.05)	0.35 (1.71)	0.11 (1.27)
V3deoxy	-0.17 (1.54)	-0.13 (1.52)	0.60 (1.96)	0.21 (0.91)
V4deoxy	0.43 (1.65)	0.45 (1.68)	-0.01 (0.96)	0.05 (1.06)
V5deoxy	0.39 (1.81)	0.45 (1.86)	0.45 (2.03)	0.09 (1.04)
V6deoxy	0.54 (1.98)	0.60 (2.03)	0.33 (2.03)	-0.06 (1.04)
V7deoxy	0.69 (3.35)	0.74 (3.41)	0.73 (2.87)	0.30 (1.79)
V8deoxy	2.25 (5.52)	2.63 (5.56)	1.18 (2.48)	1.02 (2.23)
V9deoxy	0.10 (1.81)	0.11 (1.75)	0.64 (2.85)	0.17 (1.68)
V10deoxy	0.63 (2.25)	1.14 (2.82)	0.95 (2.87)	0.70 (2.59)
V11deoxy	-0.32 (1.55)	-0.27 (1.60)	0.88 (2.83)	0.18 (1.23)
V12deoxy	-0.46 (1.09)	-0.44 (1.05)	0.28 (2.09)	-0.06 (1.05)
V13deoxy	-0.16 (1.27)	-0.12 (1.28)	0.36 (2.34)	-0.10 (1.03)
V14deoxy	-0.40 (1.49)	-0.31 (1.50)	0.08 (1.73)	-0.22 (1.00)
V15deoxy	-0.33 (1.53)	-0.34 (1.54)	0.91 (3.19)	0.43 (2.22)
V16deoxy	-0.32 (1.17)	-0.20 (1.27)	0.07 (1.78)	-0.28 (1.12)

*Indicates a significant difference from controls at the .05 level, and ** at the .01 level

Using the oxy-Hb data, ANOVA revealed that in the first switching block ecstasy users showed a significant increase in oxy-Hb compared to non-user controls at voxel 5 $F(1,36)=3.57$, $p<.05$, and this difference was approaching significance at voxels 6 $F(1,36)=1.99$, $p=.08$ and 13 $F(1,35)=2.41$, $p=.06$. There were no significant differences at any of the other voxels measured ($p>.05$ in all cases).

There were no significant differences in the deoxy-Hb data at any of the voxels measured. However, there were differences that approached significance at V1 $F(1,35)=2.02$, $p=.08$, V11 $F(1,26)=2.03$, $p=.08$ and V15 $F(1,35)=2.15$, $p=.08$, whereby ecstasy users had decreased deoxy-Hb compared to controls.

ANOVA on the oxy-Hb data during the second switching block revealed that ecstasy users displayed greater oxy-Hb at V5 compared to controls and this difference was approaching significance $F(1,35)=2.37$, $p=.07$. There were no significant differences at any voxels ($p>.05$). Analysis of the deoxy-Hb change during the second switching block also revealed no significant between group differences on any of the voxels measured ($p>.05$ in each case).

Multiple regression analyses were conducted on the voxel (V5) showing significant between group differences in oxy-Hb. Oxy-Hb (μmolar) level at V5 during block 1 of the number-letter task was entered as the dependent variable. In step one indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days), in step two the same indices of ecstasy use were entered as predictors. The overall regression model accounted for a non-significant 18.8% ($R^2 = 0.19$, R^2 adjusted = 0.03, $F(6,31)=1.20$, $p>0.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb ($R^2 = 0.15$, R^2 adjusted = 0.08, $F(3,34)=2.07$, $p>0.05$). Individual cannabis use indices; frequency of use ($\beta=-0.07$, $p>0.05$)

and amount smoked in the last 30 days (β=-0.12, p>0.05), did not significantly predict oxy-Hb. However total lifetime dose (β=0.56, p<0.05) was a significant predictor, with increased dose being associated with increased oxy-Hb. Ecstasy use indices (step 2) did not predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.03, *F-change* (3,31)=0.43, p>.05). None of the individual ecstasy use indices were significant predictors of oxy-Hb at V5 during the first switching block; frequency of use (β=0.07, p>0.05) and lifetime dose (β=-0.16, p>0.05) and last 30 day use (β=-0.17, p>0.05).

CWFT: Mean oxy-Hb and deoxy-Hb changes from baseline are displayed in table 8.6.

Table 8.6. Mean changes in oxy-Hb and deoxy-Hb (μmolar) from baseline, for ecstasy users and non-users for the CWFT.

	Ecstasy users			Non-users		
	CWFT1	**CWFT2**	**CWFT3**	**CWFT1**	**CWFT2**	**CWFT3**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
V1oxy	0.80 (1.42)	0.71 (1.24)	0.78 (1.33)	1.23 (1.31)	1.12 (1.46)	1.05 (2.48)
V2oxy	2.36 (2.12)*	1.84 (2.24)*	1.59 (2.02)*	1.04 (1.35)	0.82 (1.30)	0.50 (1.49)
V3oxy	1.45 (1.65)*	1.08 (1.46)	0.94 (1.51)*	0.68 (1.00)	0.45 (0.90)	0.14 (1.35)
V4oxy	2.03 (1.99)*	1.99 (1.75)*	2.07 (1.83)*	0.67 (1.54)	0.31 (1.58)	0.36 (1.70)
V5oxy	1.27 (1.61)	0.83 (1.62)	0.63 (2.13)	0.67 (1.37)	0.47 (1.10)	0.12 (1.33)
V6oxy	1.67 (2.62)	1.24 (2.36)	1.19 (2.21)	1.07 (2.11)	0.59 (1.62)	0.30 (1.80)
V7oxy	0.93 (1.65)	0.90 (1.44)	0.68 (1.71)	0.79 (1.53)	0.95 (1.55)	0.66 (1.62)
V8oxy	1.71 (2.61)	1.72 (2.56)	1.70 (2.34)	1.18 (2.42)	0.93 (1.97)	0.60 (2.41)
V9oxy	1.05 (1.70)	0.87 (1.63)	0.47 (2.08)	0.79 (1.53)	0.74 (1.59)	0.30 (1.65)
V10oxy	2.54 (2.38)*	2.59 (2.30)**	2.17 (2.29)**	1.04 (2.35)	0.60 (1.69)	-0.06 (1.99)
V11oxy	1.35 (1.94)	1.00 (1.56)	0.76 (1.66)	0.56 (1.23)	0.60 (1.09)	0.17 (1.31)
V12oxy	1.48 (2.33)	1.38 (2.35)*	1.49 (1.79)**	0.37 (1.78)	0.13 (1.75)	-0.32 (1.82)
V13oxy	1.26 (1.48)	0.78 (1.51)	0.65 (1.55)	0.81 (1.09)	0.57 (1.15)	0.21 (1.25)
V14oxy	1.08 (1.80)	0.76 (1.66)	0.74 (1.41)	0.60 (1.62)	0.42 (1.64)	0.08 (1.87)
V15oxy	0.83 (1.56)	0.61 (1.36)	0.58 (1.40)	1.27 (2.19)	1.36 (2.41)	1.14 (2.25)
V16oxy	1.83 (1.90)	1.38 (2.41)	1.24 (1.29)	1.16 (1.60)	1.21 (1.61)	0.86 (1.78)
V1deoxy	0.06 (2.79)	0.60 (3.42)	0.94 (3.60)	-0.19 (1.40)	-0.07 (1.50)	0.52 (2.39)
V2deoxy	0.70 (2.01)*	0.77 (2.74)*	0.88 (2.83)	-0.32 (1.19)	-0.42 (1.21)	-0.17 (1.17)
V3deoxy	0.47 (2.39)	0.61 (2.78)	0.82 (2.99)	-0.14 (1.06)	-0.18 (1.00)	0.14 (1.10)
V4deoxy	0.89 (2.83)	1.34 (3.37)	1.42 (3.71)	-0.38 (1.14)	-0.26 (1.16)	0.07 (1.07)
V5deoxy	0.45 (2.39)	0.54 (2.76)	0.64 (2.98)	-0.10 (0.92)	-0.19 (1.01)	0.03 (1.05)
V6deoxy	0.22 (2.36)	0.43 (2.67)	0.45 (2.77)	-0.01 (1.27)	-0.14 (1.20)	0.13 (1.29)
V7deoxy	0.27 (2.53)	0.82 (2.94)	0.93 (3.16)	-0.11 (1.25)	0.26 (1.49)	0.51 (1.42)
V8deoxy	-0.01 (2.59)	0.60 (3.03)	0.84 (3.14)	0.18 (1.59)	0.14 (1.57)	0.32 (1.78)
V9deoxy	0.66 (2.90)	1.06 (3.37)	1.03 (3.56)	0.16 (1.42)	0.29 (1.66)	0.49 (1.54)
V10deoxy	0.49 (2.74)	1.21 (3.54)	1.25 (3.73)	0.19 (1.33)	0.04 (1.48)	0.07 (1.42)
V11deoxy	0.57 (3.04)	0.71 (3.48)	0.65 (3.80)	-0.01 (1.49)	-0.01 (1.47)	0.21 (1.40)
V12deoxy	0.25 (2.51)	0.44 (2.89)	0.56 (3.10)	-0.48 (2.10)	-0.51 (1.98)	-0.33 (1.95)
V13deoxy	0.33 (2.34)	0.38 (2.66)	0.53 (2.89)	-0.12 (1.11)	-0.16 (1.03)	0.11 (0.98)
V14deoxy	-0.12 (2.09)	0.10 (2.36)	0.29 (2.50)	-0.84 (1.56)	-0.78 (1.50)	-0.51 (1.48)
V15deoxy	0.35 (2.73)	0.43 (3.06)	0.57 (3.37)	0.13 (1.81)	0.31 (1.79)	0.58 (1.71)
V16deoxy	0.42 (2.30)	0.24 (2.46)	0.47 (2.56)	-0.22 (0.99)	-0.19 (1.04)	-0.07 (0.96)

*Indicates a significant difference from controls at the .05 level, and ** at the .01 level.

Analysis of oxy-Hb change in block one of the CWFT ("animals") revealed that ecstasy users displayed a significant increase in oxy-Hb compared to controls at V2 $F(1,37)=5.27$, $p<.05$, V3 $F(1,36)=2.94$, $p<.05$, V4 $F(1,18)=2.91$, $p<.05$ and V10 $F(1,31)=3.28$, $p<.05$. All other differences were non-significant ($p>.05$). ANOVA on the deoxy-Hb data revealed that ecstasy users showed greater deoxygenation compared to controls at V2 $F(1,37)=3.63$, $p<.05$. No other differences were observed here for any other voxel measured ($p>.05$ in each case).

The more difficult criteria of naming words beginning with the letter "S" yielded significant increased oxy-Hb change in ecstasy users relative to controls at V2 $F(1,37)=2.98$, $p<.05$, V4 $F(1,18)=5.09$, $p<.05$, V10 $F(1,31)=8.06$, $p<.01$ and V12 $F(1,33)=3.14$, $p>.05$. This difference was also approaching significance at V3 $F(1,36)=2.49$, $p=.06$. There were no significant between group differences at any of the other voxels measured ($p>.05$ in each case). ANOVA on deoxy-Hb change during this block of the task revealed ecstasy users had significantly greater deoxy-Hb at V2 $F(1,37)=3.05$, $p<.05$, with trends at V4 $F(1,18)=2.01$, $p=.09$ and V14 $F(1,37)=1.94$, $p=.09$. There were no significant differences at any of the other voxels measured ($p>.05$ in each case).

In the 3rd and most difficult block of this task ANOVA on oxy-Hb change from baseline revealed that ecstasy users displayed a significant increase in oxy-Hb compared to controls at V2 $F(1,37)=3.65$, $p<.05$, V3 $F(1,36)=2.96$, $p<.05$, V4 $F(1,18)=4.69$, $p<.05$, V10 $F(1,31)=9.01$, $p<.01$ and V12 $F(1,33)=8.68$, $p<.01$. There were no significant differences at any of the other voxels measured ($p>.05$ in each case). There were no significant between group differences in deoxy-Hb change during this part of the task ($p>.05$ in all cases). However ecstasy users displayed a greater deoxygenation compared to controls that was

approaching significance at V2 $F(1,37)=2.26$, $p=.07$. There were no significant differences at any of the other voxels measured ($p>.05$ in each case).

As with the data from the other two tasks, multiple regression analyses were conducted on the fNIRS data, at voxels showing between group differences, to observe whether ecstasy use predicted oxy/deoxy-Hb level after controlling for cannabis use. Oxy-Hb/deoxy-Hb (µmolar) change level was entered as the dependent variable in each case. In step one indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days), in step two the same indices of ecstasy use were entered as predictors. The results from these regression analyses can be seen in Appendix 2, for brevity, only regressions yielding notable results are reported here.

Using oxy-Hb at V3 during the first block of the task (animals) as the dependent variable, this overall regression model accounted for 30.8% of the variance in oxy-Hb and this was approaching significance ($R^2 = 0.31$, R^2 adjusted = 0.17, $F(6,31)=2.30$, $p=0.06$). Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb ($R^2 = 0.10$, R^2 adjusted = 0.02, $F(3,34)=1.28$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use ($\beta=0.40$, $p>0.05$), total lifetime dose ($\beta=-0.29$, $p>0.05$) and amount smoked in the last 30 days ($\beta=-0.19$, $p>0.05$). The ecstasy use indices (step 2) did, however, predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.21, *F-change* (3,31)=3.07, $p<.05$). Individual indices; last 30 day use ($\beta=-0.28$, $p>0.05$) and lifetime dose ($\beta=0.37$, $p>0.05$) did not predict oxy-Hb. However frequency of use ($\beta=0.44$, $p<0.05$) was a significant predictor with increased frequency being associated with increased oxy-Hb.

Using oxy-Hb at V4 during the first block of the task (animals) as the dependent variable, this overall regression model accounted for a non-significant 52.4% ($R^2 = 0.52$, R^2

adjusted = 0.30, $F(6,13)=2.38$, $p>.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb (R^2 = 0.13, R^2 adjusted = -0.03, $F(3,16)=0.80$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=-0.04, $p>0.05$), total lifetime dose (β=0.54, $p>0.05$) and amount smoked in the last 30 days (β=-0.38, $p>0.05$). The ecstasy use indices (step 2) did, however, predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.39, *F-change* (3,13)=3.58, $p<.05$). Individual indices; frequency of use (β=0.62, $p<0.05$) and lifetime dose (β=0.46, $p<0.05$) did significantly predict oxy-Hb level, with increased frequency and increased dose being associated with increased oxy-Hb. However last 30 day use (β=-0.35, $p>0.05$) was not a significant predictor.

Using deoxy-Hb at V2 during the first block of the task (animals) as the dependent variable, this overall regression model accounted for a non-significant 14.8% (R^2 = 0.15, R^2 adjusted = -0.01, $F(6, 32)=0.92$, $p>.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in deoxy-Hb (R^2 = 0.03, R^2 adjusted = -0.01, $F(3,35)=0.35$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=0.05, $p>0.05$), total lifetime dose (β=-0.14, $p>0.05$) and amount smoked in the last 30 days (β=-0.01, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in deoxy-Hb, after controlling for cannabis use indices (R^2 change=0.12, *F-change* (3,32)=1.48, $p>.05$). Individual indices; frequency of use (β=0.44, $p>0.05$) and last 30 day use (β=-0.28, $p>0.05$) did not predict deoxy-Hb level. However lifetime dose (β=0.34, $p=0.06$) approached significance as a predictor, with increased use being associated with increased deoxy-Hb.

Using oxy-Hb at V4 during the second block of the task (S letter words) as the dependent variable, this overall regression model accounted for a non-significant 52.1% (R^2 =

0.52, R^2 adjusted = 0.30, $F(6, 13)=2.35$, $p>.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb (R^2 = 0.31, R^2 adjusted = 0.18, $F(3,16)=2.35$, $p>0.05$). Individual cannabis use indices; frequency of use (β=-.029, $p>0.05$), and amount smoked in the last 30 days (β=-0.47, $p>0.05$) did not predict oxy-Hb, however total lifetime dose (β=0.91, $p<0.05$) was a significant predictor, with increased use being associated with increased oxy-Hb. The ecstasy use indices (step 2) did not predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.22, *F-change* (3,13)=1.94, $p>.05$). Individual indices; frequency of use (β=-0.03, $p>0.05$) and last 30 day use (β=0.29, $p>0.05$) did not predict oxy-Hb. However lifetime dose (β=0.42, $p=0.06$) approached significance as a predictor, with increased use being associated with increased oxy-Hb change.

Using deoxy-Hb at V2 during the second block of the task (S letter words) as the dependent variable, this overall regression model accounted for a non-significant 14.1% (R^2 = 0.14, R^2 adjusted = -0.02, $F(6, 32)=2.35$, $p>.05$) of the variance in deoxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in deoxy-Hb (R^2 = 0.03, R^2 adjusted = -0.05, $F(3,35)=0.35$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=-0.08, $p>0.05$), total lifetime dose (β=-0.15, $p>0.05$) and amount smoked in the last 30 days (β=0.04, $p>0.05$). The ecstasy use indices (step 2) did not predict a significant amount of variance in deoxy-Hb, after controlling for cannabis use indices (R^2 change=0.11, *F-change* (3,32)=1.38, $p>.05$). Individual indices; frequency of use (β=-0.00, $p>0.05$) and last 30 day use (β=0.06, $p>0.05$) did not predict deoxy-Hb. However lifetime dose (β=0.44, $p=0.06$) approached significance as a predictor, with increased use being associated with increased deoxy-Hb.

Using oxy-Hb at V3 during the third block of the task (4 letter C words) as the dependent variable, this overall regression model accounted for a non-significant 26.7% (R^2 = 0.27, R^2 adjusted = 0.13, $F(6, 31)=1.88$, $p>.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb (R^2 = 0.03, R^2 adjusted = -0.06, $F(3,34)=0.33$, $p>0.05$). None of the individual cannabis use indices were significant predictors; frequency of use (β=0.01, $p>0.05$), total lifetime dose (β=-0.02, $p>0.05$) and amount smoked in the last 30 days (β=-0.05, $p>0.05$). The ecstasy use indices (step 2) did predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.24, *F-change* (3,31)=3.36, $p<.05$). Individual indices; lifetime dose (β=0.29, $p>0.05$) and last 30 day use (β=-0.09, $p>0.05$) did not predict oxy-Hb level. However frequency of use (β=0.48, $p<0.05$) was a significant predictor, with increased use being associated with increased oxy-Hb.

Using oxy-Hb at V4 during the third block of the task (4 letter C words) as the dependent variable, this overall regression model accounted for a non-significant 46.3% (R^2 = 0.46, R^2 adjusted = 0.22, $F(6, 13)=1.87$, $p>.05$) of the variance in oxy-Hb. Cannabis use indices (step 1) did not predict a significant amount of variance in oxy-Hb (R^2 = 0.27, R^2 adjusted = 0.14, $F(3,16)=2.01$, $p>0.05$). However individual cannabis use indices: total lifetime dose (β=0.94, $p<0.05$) and amount smoked in the last 30 days (β=-1.17, $p<0.05$) were significant predictors of oxy-Hb, with increased lifetime dose being associated with increased oxy-Hb and increased amount smoked in last 30 days being associated with decreased oxy-Hb. Frequency of use (β=0.31, $p>0.05$), was not a significant predictor. The ecstasy use indices (step 2) did not predict a significant amount of variance in oxy-Hb, after controlling for cannabis use indices (R^2 change=0.19, *F-change* (3,13)=1.53, $p>.05$). None of the individual ecstasy use indices predicted oxygenation at V4; frequency of use (β=-0.07, $p>0.05$) lifetime dose (β=0.33, $p>0.05$) and last 30 day use (β=0.39, $p>0.05$).

Implications of Chapter 8

The results from this chapter indicate that ecstasy users show an increase in effortful cognition during all three executive functioning tasks, despite having similar behavioural output to controls. Ecstasy users displayed significantly increased oxy-Hb changes from baseline relative to controls over several voxels during the inhibition (RLG) task. At the slowest rate (4second rate), that is understood to be the easiest level of the task, increases were observed in V10 relating to the right medial PFC in ecstasy users. As difficulty increased, a more pronounced difference between ecstasy users and controls was observed. During the second block of the task (2 second rate) ecstasy users displayed significant increases in oxy-Hb relative to controls at voxels 4, 10 and 12. This indicates a bilateral induction of oxy-Hb increase. At the most difficult level of the task (1 second rate), ecstasy users displayed significant increases in oxy-Hb in voxels relating to inferior parts of the right medial PFC and right DLPFC (V12 and V14). Although, this is a less pronounced difference than in block two, there were complimentary increases in deoxy-Hb that suggest more pronounced differences between users and non-users as a function of difficulty. A total of eight voxels, showed significant between group differences in deoxy-Hb at the one second rate, compared to six voxels at the two second rate and seven voxels at the four second rate. In each case, increases in deoxy-Hb, were observed over the breadth of the prefrontal cortex, suggesting that induction of haemoglobin in ecstasy users during inhibition is bilateral. The majority of the regression analyses, on voxels showing significant between group differences, to observe whether ecstasy use predicted oxy and deoxy-Hb increases after controlling for cannabis use indices, were non-significant. However ecstasy use indices predicted a significant amount of the variance in deoxy-Hb at voxel 14, in the four second rate of the task. Specifically frequency of use and last 30 days use were significant predictors, with increased frequency being associated with reduced deoxy-Hb, and increased last 30 day use being

associated with increased deoxy-Hb. Last 30 days use was also a significant predictor of oxy-Hb increase at V12 and V14 in the 1 second rate block, with increased use being associated with increased oxygenation. Last 30 days use also predicted deoxy-Hb increase at V14 at the two and one second rates. The results from regression analyses suggest that recency of ecstasy use may play an important role in the observed cognitive function alterations during inhibition.

Ecstasy users displayed increases in oxy-Hb relative to controls in voxels pertaining to the left medial PFC during switching. Indeed voxel 5 in the first switching block saw significant increases in oxygenated haemoglobin for ecstasy users relative to controls. Moreover V5 also displayed differences in block two that were approaching significance. Regression analyses did not show ecstasy use indices as significant predictors of oxy-Hb change at V5 (block 1) after controlling for cannabis use. However lifetime dose of cannabis was a significant predictor of oxygenation at V5, in block one, with increased use being associated with increased oxy-Hb. Increases in oxy-Hb relative to controls were observed consistently in several sites over the left DLPFC and right PFC during tasks that tap the executive function of "access". Furthermore the number of voxels showing differences here increased as a function of difficulty. Ecstasy users also displayed significant increases in deoxy-Hb compared to controls at V2 relating to the left DLPFC in blocks one (semantic fluency – "animals") and two (first level of word fluency – "s" letter words). Frequency of ecstasy use was a significant predictor of oxy-Hb after controlling for cannabis use indices at V3 and V4 of block one and V3 in block three. Increased frequency is associated with increased oxy-Hb at these sites. Lifetime dose of ecstasy was also a significant predictor of oxy-Hb at V4 in block one. Lifetime dose was also approaching significance (p=.06 in each case) as a predictor of oxy-Hb at V4 in block two and of deoxy-Hb in V2 in blocks one and two. Again increased lifetime dose was associated with increases in oxy and deoxy-Hb.

Lifetime dose of cannabis was also a significant predictor of oxy-Hb at V4 in blocks two and three. These results suggest that frequency of use and lifetime dose of ecstasy may play a role in neurocognitive alterations associated with access.

Thus far it appears that ecstasy users are engaged in more effortful cognition in terms of haemodynamic response than non-users, during executive function tasks. This is consistent across Chapters 7 and 8. Furthermore the results from this chapter corroborate the results from Chapter 6 that suggest atypical processing of inhibition, switching and access in ecstasy users. All three chapters thus far have a dissociation between behavioural output and cognitive effort reflecting neuroimaging measures' greater sensitivity to cognitive impairment, and the results are consistent with ecstasy users relying on recruitment of additional resources to attenuate performance deficits. The next chapter will assess the haemodynamic response to a multitasking paradigm alongside cortisol sampling data as a measure of the integrity of the HPA axis in ecstasy users.

Chapter 9: fNIRS multitasking and neuroendocrine response

9.1 Chapter overview

Thus far it appears that fNIRS has provided clear observable differences in relation to effortful cognition exhibited between ecstasy users and controls. This chapter further explores haemodynamic response in ecstasy users relative to controls. However this time a multi-tasking paradigm has been employed. Furthermore a diurnal cortisol profile has been completed by all participants to assess the integrity of the HPA axis. Twenty ecstasy users, 17 polydrug controls and 19 drug naïve controls were recruited for this study. Again performance on the task was equivalent between groups. However, fNIRS data show differences in haemodynamic response to task between groups. The cortisol profiling data show generally increased levels of cortisol in ecstasy users compared to controls, which was significant compared to both control groups at time 3 on day 1 of the study protocol and also significantly increased compared to polydrug controls at time 1 of day 1 of the protocol.

9.2 Introduction

Recreational drug use is argued to be detrimental to normal physiological and psychological functioning. As documented in this thesis, working memory deficits – particularly those associated with higher level executive functioning tasks appear to be most prominent in the literature (Fisk et al., 2004; Montgomery, Fisk, Newcombe & Murphy, 2005). However ecstasy produces its acute psychological and physiological effects by being a powerful indirect serotonin agonist, whilst also having stimulatory effects on dopamine amongst other neurotransmitters (McDowell & Kleber, 1994). After exposure, rebound neurotransmitter depletion is common, leading to anhedonia (Curran & Travill, 1997), amongst other psychobiological alterations to cognition sleep and mood (Parrott & Lasky,

1998; Parrott, 2006). Repeated exposure of MDMA may lead to long lasting effects on monoamine mediated psychobiological functions.

MDMA's agonist action on serotonin also leads to stimulation of the hypothalamic-adrenal-axis (HPA) axis, resulting in altered neuroendocrine function (Parrott *et al.*, 2008). The neurohormone cortisol is understood to be produced in response to stress, and has been used as an indicator of neuroendocrine function. In ecstasy users, acute effects of MDMA combined with dancing in hot environments, have been reported to increase salivary cortisol levels by up to 800% compared to clubbing without taking the drug (Parrott *et al.*, 2008). This combination of drug use and prolonged dancing in hot environments is proposed to have an interactive effect on psychobiological functions, which has been termed the Bioenergetic Stress Model of recreational MDMA use (Parrott, 2006; Parrott *et al*., 2008). Moreover MDMA has been described as an acute metabolic stressor, due to its actions on cortisol (Parrott, 2006; Parrott *et al.*, 2008). Further evidence for acute increases of cortisol after MDMA use comes from de la Torre *et al*. (2000) who observed marked elevation of plasma cortisol and prolactin after doses of MDMA that are equivalent to recreational doses (50-150mg). Peak cortisol concentration was observed 2 hours post ingestion. Harris *et al*. (2002) report similar significant increases in plasma cortisol after administration (1.5mg/kg) of MDMA in humans.

The most marked increases in cortisol have been observed in the field environment where recreational MDMA users are predominantly using these drugs, such as night clubs (Parrott *et al.*, 2008). It is in these 'real world' situations where ecstasy users are exposed to multiple stimulatory factors (heat, crowding, loud music, intense light), which can cause high levels of bioenergetic stress (Parrott, 2009). Moreover, it has been suggested that repeated

exposure to such hyperstimulation will have cumulative effects and result in chronic bioenergetic distress (Parrott, 2006; Parrott, 2009).

Gerra *et al.,* (2000) investigated long lasting effects of MDMA use on cortisol and prolactin. Ecstasy users' basal cortisol levels appeared equivalent to controls, three weeks post MDMA exposure. However a significantly reduced cortisol response to D-fenfluramine challenge was observed in ecstasy users, three weeks post administration, though cortisol responses appeared to normalise after 12 months abstinence. The same research group (Gerra *et al.,* 2001) observed significantly elevated baseline cortisol levels in MDMA users, who had been free from MDMA for at least three weeks, compared to controls. A possibility for this increase was suggested to be MDMA related alterations to basal HPA-axis function, due to serotonergic changes produced by repeated MDMA exposure. Basal cortisol plasma levels were again observed to be elevated in drug free ecstasy users compared to controls in a study by Gerra *et al*. (2003); ecstasy users and controls were exposed to psychosocial stressors (Stroop interference task, mental arithmetic and public speaking) and MDMA users showed a blunted cortisol response to psychological stress compared to controls. It was suggested that increased basal levels of cortisol may reflect increased worry about the tasks and perception of them being more stressful. Alternatively, perhaps elevated basal cortisol due to MDMA exposure exhausts HPA axis leading to blunted responses to stress (Gerra *et al*., 2003). Nevertheless there is evidently a complex relationship between drug use and HPA-axis function.

Cortisol release follows an established diurnal pattern, increasing rapidly within the first 30 minutes of awakening (cortisol awakening response) (Pruessner *et al.,* 1997), and remaining elevated for up to 60 minutes. Following this a general decline in cortisol levels throughout the day is normal. Hyperactivity of the HPA-axis has real health implications,

given that it is associated with susceptibility to infectious disease (Sapolsky, 1996) and depression (Wong *et al*., 2000). Cortisol also increases as a response to stress and allostatic load in normal individuals (McEwen, 1998). Thus investigation of diurnal cortisol profiles in ecstasy users in the days leading up to a psychological stressor may yield more information about basal cortisol levels and stress reactivity.

Wetherell *et al.*, (2012) recently investigated psychological stress reactivity in ecstasy users and controls using a multi-tasking stressor framework (including tasks that require executive function resources). Self-reported feelings of calmness were significantly reduced in ecstasy users compared to drug naïve controls in response to the stressor task. This is suggestive that ecstasy use can have long lasting ill effects on the psychological response to stress. This in turn has real life implications for recreational drug users and also warrants further investigation.

The aims of this study were to investigate changes in prefrontal blood oxygenation in response to a demanding task in ecstasy users, polydrug controls and drug naïve controls. The acute stressor is provided in the form of a multitasking stressor task (Purple Research Solutions, UK), with four higher-level processing tasks (Stroop task, two visual monitoring tasks and mental arithmetic). The cerebral hemodynamic response to conducting several tasks at once was measured as well as performance on the task behaviourally. Moreover a diurnal cortisol profile was obtained from all participants in the day preceding the multitasking stressor and the test day. Pre and post test samples were also collected (saliva samples) to assess cortisol levels in response to a psychological stressor. It was hypothesised that performance on the multitasking stressor task may be equivalent, as with other measures of performance in this thesis, but MDMA users will again show increased haemodynamic response to the task, reflective of increased cognitive effort. Furthermore in line with

previous research on cortisol, it was predicted that ecstasy users will show increased basal cortisol levels from their diurnal cortisol profiles and as such will not display marked cortisol increase in response to the multitasking stressor.

9.3 Method

Participants:

Twenty ecstasy users (mean age = 21.61, SD = 0.52, 12 = male), 17 non-ecstasy, polydrug controls (mean age = 21.23, SD = 0.79, 12 = male) and 19 drug naïve controls (mean age = 21.60, SD = 0.84, 6 = male) were recruited via direct approach (e-mail) to Liverpool John Moores University students. Inclusion criteria were the same as that in Chapter 6. Indices of ecstasy use were as follows: total lifetime dose 253.86 tablets ± 376.20; mean amount used in last 30 days 2 tablets ± 3.46, and frequency of use 0.22 times/week ± 0.21.

Materials

Questionnaires:

The Background Drug Use Questionnaire, NASA-TLX and Raven's SPM were used, as described in Chapter 6.

The *SAI VAS (State Anxiety Inventory – Visual Analogue Scale)* was completed pre and post testing period, this comprises 6 statements (I feel calm, I feel tense, I feel upset, I feel relaxed, I feel content, I feel worried) and participants have to indicate on a 100mm line how much they agree with the statement, ranging from 0 – not at all, to 100 – very much. To attain a measure of perceived stress in the lab, the *Perceived Stress Scale (PSS4)* was used. This is a four item scale that asks participants about their perceived stress, for example "How

often have you felt difficulties were piling up so high that you could not overcome them?" Participants are required to respond from 0 = Never, to 4 = very often.

The *HADS (Hospital Anxiety and Depression Scale)* (Zigmond & Snaith, 1983), is a 14-item scale with seven items relating to anxiety and seven items relating to depression. This scale includes items such as "I still enjoy things that I used to enjoy" and participants are required to state how much they agree with this from for example 1 = definitely as much, to 4 = hardly at all. A high score for the anxiety related items reflects high levels of anxiety and a high level on depression related items reflect high levels of depression.

Multitasking stress test

The multi-tasking framework (Purple Research Solutions, UK) is a PC run platform used to elicit acute psychological stress (Wetherell & Sidgreaves, 2005). The same combination of four stressor modules (Stroop, mental arithmetic, tracking/target area – visual monitoring and warning/rising bars – visual monitoring) was used for all participants, at a medium intensity workload. The task requires participants to attend to the four different components/modules of the task simultaneously. The instructions on screen inform participants of how points are scored and the participants were instructed to achieve the highest score possible. The set of tasks included a mental arithmetic task whereby participants were required to calculate a series of 2 x 3 digit addition sums; visual monitoring (target area) whereby participants monitor the position of a moving cursor and reset this cursor when it entered a points zone; a second visual monitoring module (rising bars) comprises of a set of six bars that rise towards a target line at varying speeds. Once the bars reach the target, participants select the order in which the bars reached the target, fastest first. Finally a Stroop task module involved colour names appearing onscreen in various colours,

participants had to select the colour the word appeared in, rather than read the word. For more information on the different modules of the framework, see Wetherell and Sidgreaves (2005)

Equipment

fNIRS: Haemodynamic response to task in the PFC was monitored as in Chapter 7.

Cortisol: Saliva samples were obtained by instructing participants to chew on a salivette (Sarstedt Ltd, Germany) for one minute. Participants collected labelled salivettes from the laboratory prior to commencing the study. Sample 1 was taken on day 1 on awakening, sample 2 was taken 30 minutes after waking, sample 3 was taken in the afternoon between 1-3 pm and a fourth sample was taken in the evening (between 9-11pm). The following day (test day) a sample was again taken upon waking, a second sample was taken 30 minutes later, a third sample was taken upon entering the lab for testing, a fourth sample was taken post-test and a final sample was collected in the evening (between 9-11pm). Saliva samples were frozen until they were assayed for salivary cortisol using Neogen cortisol ELISA kits (Neogen Corporation, USA). Assays were conducted by Northumbria University**.**

Procedure

Participants were required to attend the lab on two occasions. Upon entering the lab for the first day participants were informed of what the study would entail and written consent was obtained. Participants were given questionnaires in the following order: background drug use questionnaire, PSS4, HADS and Raven's SPM and informed of the saliva sampling protocol (outlined above). The following day, participants did not attend the lab, but did collect cortisol samples (day 1 of cortisol profile protocol). One day later (test day, day 2 of sampling protocol), a pre-task SAI-VAS and HADS were given upon entering the lab, and a pre–test cortisol sample was taken. After this the fNIRS sensor pad was

attached to the participants' forehead whilst they read instructions on how to complete the task. Participants were then instructed to complete a two minute practise trial of the task after which any questions that the participant had about the task could be answered. The fNIRS signals were displayed on a desktop computer running COBI studio (Drexel University) in an adjacent room to the testing room. Providing the signals from the fNIRS were stable, a baseline of inactivity was recorded before the participants were instructed to complete a 20 minute session of the multi-tasking stressor task on a desktop computer running the purple framework (Purple Solutions, UK). After the 20 minutes had elapsed, participants completed a post task SAI-VAS and gave post-test cortisol sample. The NASA TLX was also completed post task. Finally participants were fully debriefed and were paid £20 in store vouchers. The study was approved by Liverpool John Moores University Research Ethics Committee, and was administered in accordance with the ethical guidelines of the British Psychological Society.

fNIRS analysis

Pre-processing and analysis followed the same procedure as that described in Chapter 7.

Statistical analysis

Behavioural data was analysed using ANOVA with group as the between subjects factor and total scores on each component of the task (Stroop, mental arithmetic, tracking/target area – visual monitoring and warning/rising bars – visual monitoring) as well as overall score on the task as the dependent variables. ANOVA[4] was conducted on oxy-Hb

[4] Due to small amounts of missing data on different voxels, MANOVA was not appropriate for this analysis.

and deoxy-Hb changes from baseline (µmolar) at each voxel, for the whole epoch of the multitasking test (20 minutes). ANOVA was also conducted on the cortisol data[5].

Any significant main effects were further explored using post-hoc Tukey's HSD test.

9.4 Results

Perceived stress scores, HADS scores and pre and post task SAI-VAS scores are displayed in Table 9.1. Indices of other drug and alcohol use are displayed in Table 9.2**.**

Table 9.1: Indices sleep quality, fluid intelligence and socio-demographic variables

	Ecstasy Users	**Polydrug Controls**	**Drug Naïve Controls**
Males: n, (%)	13 (65)	13 (76)	6 (32)
Age (SD)	21.61 (2.20)	21.23 (2.83)	21.60(3.27)
University degree: n (%)	5 (25)	5 (30)	3 (15)
Employment status			
Student; n, (%)	18 (90)	11 (65)	18 (95)
Employed; n (%)	1 (5)	6 (35)	3 (15)
Unemployed; n (%)	1 (5)	0 (0)	1 (5)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Ravens Progressive Matrices (maximum 60)	49.70 (5.12)	51.82 (5.42)	49.58 (6.94)
PSS4 (1)	1.55 (0.83)	1.41 (0.94)	1.32 (0.95)
PSS4 (2)	1.90 (0.55)	1.82 (0.64)	2.00 (0.67)
PSS4 (3)	2.45 (0.60)	2.35 (0.49)	2.37 (0.50)
PSS4 (4)	1.45 (0.89)	1.41 (0.80)	1.21 (0.79)
HADS anxiety Day 1	17.92 (1.64)	18.13 (1.46)	18.50 (1.62)
HADS depression Day 1	10.80 (1.97)	10.57 (1.70)	10.06 (2.88)
HADS anxiety Day 2	10.14 (3.53)	9.93 (2.55)	9.39 (2.97)
HADS depression Day 2	10.33 (2.13)	10.79 (2.83)	9.17 (2.62)
SAIVAS pre calm	63.80 (24.25)	84.06 (10.29)	79.00 (19.44)
SAIVAS post calm	70.00 (17.27)	74.24 (30.68)	78.37 (20.28)
SAIVAS pre tense	20.30 (15.89)	15.71 (19.09)	16.14 (16.84)
SAIVAS post tense	25.10 (15.97)	22.35 (24.89)	14.32 (16.24)
SAIVAS pre upset	11.70 (9.59)	14.65 (23.17)	11.00 (11.69)
SAIVAS post upset	12.50 (9.55)	8.00 (9.97)	10.37 (10.65)
SAIVAS pre relaxed	66.05 (20.35)	68.29 (28.76)	79.47 (16.52)
SAIVAS post relaxed	64.30 (17.93)	69.00 (29.54)	78.89 (16.70)
SAIVAS pre content	71.60 (16.54)	76.76 (21.33)	74.21 (24.67)
SAIVAS post content	71.25 (11.84)	82.00 (14.90)	73.89 (21.21)
SAIVAS pre worried	22.40 (17.27)	19.12 (24.76)	14.79 (17.69)
SAIVAS post worried	19.70 (12.68)	13.71 (17.75)	12.37 (13.80)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

[5] Due to missing data at each time point it was not appropriate to perform mixed ANOVA on the cortisol data.

Table 9.2: Indices of drug use.

	Ecstasy Users		Polydrug Controls		Drug Naïve Controls	
	Mean (SD)	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**n**
Cannabis						
Frequency (times/wk)	2.74 (2.81)*	20	1.11 (1.56)	16	-	-
Last 30 days (joints)	46.56 (59.89)	17	19.34 (46.36)	16	-	-
Total use (joints)	3613.80 (4469.70)	20	1562.96 (3021.05)	17	-	-
Cocaine						
Frequency (times/wk)	0.06 (0.08)	2	0.05 (0.06)	2	-	-
Last 30 days (lines)	0.00 (0.00)	2	0.00 (0.00)	2	-	-
Total use (lines)	415.00 (43.84)	2	7.50 (0.71)	2	-	-
Ketamine						
Frequency (times/wk)	0.19 (0.19)	5	-	-	-	-
Last 30 days use (grams)	0.00 (0.00)	5	-	-	-	-
Total use (grams)	21.72 (16.90)	5	-	-	-	-
Alcohol units p/w	13.20 (6.68)	20	12.44 (9.70)	16	6.99 (9.14)	19

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

One way ANOVA revealed that there were no significant between group differences on measures such as age and fluid intelligence or on measures of perceived stress (PSS4 pretesting), (p>.05 in all cases). Pre and post task SAI-VAS scores for each of the six subscales (calm, tense, relaxed, content, upset and worried) were analysed using a mixed ANOVA, with user group as the between subject factor and time point (pre/post-test) as the within subjects factor. Using the score on the visual analogue scale as the DV, for *calm* there was no significant main effect of time point $F(1,53)=0.19$, $p>.05$, no time point by group interaction $F(2,53)=1.97$, $p>.05$, but there was a strong trend for main effect of group $F(2,53)=3.08$, $p=05$. Pairwise comparisons showed that ecstasy users reported feeling less calm than both other groups overall ($p<.05$) in both cases. For *tense* there was a strong trend for main effect of time point $F(1,53)=3.95$, $p=.05$, with all three groups showing increases in tenseness post-task. There was no time point by group interaction $F(2,53)=0.32$, $p>.05$. There was no main effect of group $F(2,53)=1.75$, $p>.05$.

The subscale *upset* showed no main effect of time point $F(1,53)=1.69$, $p>.05$, no time point by group interaction $F(2,53)=1.82$, $p>.05$ and no main effect of group $F(2,53)=0.07$, $p>.05$. The subscale of *relaxed* also showed no main effect of time point $F(1,53)=0.03$, $p>.05$ and no time point by group interaction $F(2,53)=0.05$, $p>.05$, but does show a significant main effect of group $F(2,53)=3.04$, $p<.05$, pairwise comparisons revealed that drug naïve controls were significantly more relaxed than ecstasy users ($p<.05$). The *content* subscale revealed no significant main effect of time point $F(1,53)=0.25$, $p>.05$, no time point by group interaction $F(2,53)=0.33$, $p>.05$ and no main effect of group $F(2,53)=1.39$, $p>.05$. Finally, *worried* revealed a main effect of time point, that was approaching significance $F(2,53)=3.04$, $p=06$, with worry being greatest pre task, but no time point by group interaction $F(2,53)=0.27$, $p>.05$ and there was no main effect of group $F(2,53)=1.06$, $p>.05$.

Mixed ANOVA was also performed on the HADS with user group as the between subject factor and time point (day1/test day awakening) as within subjects. For anxiety there was a significant main effect of time point $F(1,44)=232.12$, $p<.05$ with reduced anxiety on the day of testing. There was however no group by time point interaction $F(2,44)=0.53$, $p>.05$, or main effect of group $F(2,44)=0.02$, $p>.05$. For depression there was no main effect of time point $F(1,44)=1.98$, $p<.05$, no time point by group interaction $F(2,44)=1.41$, $p>.05$ and no main effect of group $F(2,44)=1.14$, $p>.05$.

ANOVA revealed a significant between group difference in the amount of alcohol consumed (weekly) $F(2,52)=3.28$, $p<.05$. Pairwise comparisons revealed a strong trend for ecstasy users to drink more than drug naïve controls $p=.05$. t-tests between ecstasy users and polydrug controls on drug use other than ecstasy revealed that ecstasy users reported smoking cannabis more frequently than polydrug controls (2.74 ± 2.81 compared to 1.11 ± 1.56) $t(30.74)=2.20$, $p<.05$ (Levene's test was significant so degrees of freedom have been adjusted accordingly). However there were no differences in total lifetime joints smoked or total joints smoked in the last 30 days. The ecstasy user group showed greater total cocaine use (415 ± 43.84 compared to 7.5 ± 0.71) though only 2 participants in each group reported taking cocaine. Ketamine was used by 5 participants in the ecstasy user group, though there were no polydrug users who reported using ketamine, so a statistical comparison cannot be made. However as seen in Table 9.2, the ecstasy user group can be considered a polydrug user group. No differences were observed on other drug intake variables.

Behavioural data analysis

Due to eight participants (4 ecstasy users, 3 polydrug users and 1 drug naïve control) not following instructions correctly on the Stroop task and consistently answering incorrectly, their data on this component of the task was not analysed any further. These participants are also excluded from fNIRS analysis. Performance data can be observed in table 9.3.

Table 9.3: Performance data (means and SDs of total scores) multitasking components.

	Ecstasy users	**Polydrug controls**	**Drug naïve controls**
	Mean	**Mean**	**Mean**
Stroop	4443.75 (1653.38)	4222.14 (1683.38)	4500.28 (2545.14)
Warning	550.50 (43.71)	566.47 (28.93)	533.16 (141.07)
Tracking	392.80 (112.39)	437.29 (58.23)	386.11 (203.88)
Maths	414.35 (235.65)	463.65 (230.06)	371.05 (293.16)
Total	5847.75 (1721.07)	5691.29 (1727.09)	6382.22 (2357.42)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

Univariate ANOVA with a between subjects factor of group and score on task component as the dependent variables revealed that there were no significant differences between groups on any of the components of the task; Stroop $F(2,45)=0.08$, $p>.05$; Maths $F(2,53)=0.56$, $p>.05$; Tracking/target are visual monitoring $F(2,53)=0.50$, $p>.05$. Levene's statistic was violated on the warning/rising bars scores, therefore an independent samples

Kruskall-Wallace test was conducted. This revealed that there were no significant differences between ecstasy users (rank = 560), polydrug controls (rank = 570) and drug naïve controls (rank = 580) on this component of the task; (H(2) =1.43, p>.05). On the composite total score, ANOVA revealed no significant between group differences F(2,45)=0.55, p>.05.

Post task NASA TLX scores were analysed using MANOVA. This revealed no overall between group differences in task load F(12,96)=1.25, p>.05 for Pillai's trace, nor any between group differences on the individual sub-scales (Mental demand; F(2,52)=1.32, p>.05, Physical demand; F(2,52)=0.11, p>.05, Temporal demand; F(2,52)=0.10, p>.05, Effort; F(2,52)=1.97, p>.05, Performance; F(2,52)=2.39, p>.05, Frustration; F(2,52)=2.65, p>.05).

fNIRS Analysis

Averaged oxy-Hb and deoxy-Hb changes (μmolar) from baseline are displayed in table 9.4. A series of ANOVAs were used to assess group differences in changes from baseline.

Table 9.4: Oxy-Hb and deoxy-Hb changes from baseline (μmolar) for each group during the multitasking test.

	Ecstasy users Mean (SD)	Polydrug controls Mean (SD)	Drug naïve controls Mean (SD)
V1oxy	1.03 (2.27)	0.85 (1.17)	1.06 (0.91)
V2oxy	0.34 (0.88) †	1.22 (1.16)	1.51 (1.18)
V3oxy	0.68 (1.85)	0.69 (1.02)	0.79 (0.82)
V4oxy	0.26 (2.25)	1.40 (1.22)	0.92 (1.01)
V5oxy	0.22 (2.12)	0.02 (1.75)	1.12 (0.91)
V6oxy	0.23 (2.57)	1.36 (1.35)	1.06 (1.09)
V7oxy	0.22 (1.56)	0.20 (1.44)	0.78 (1.14)
V8oxy	0.37 (2.56)	0.91 (1.38)	1.14 (0.86)
V9oxy	-0.02 (1.65)	0.13 (1.11)	0.65 (1.53)
V10oxy	0.20 (2.06)	0.99 (1.36)	1.11 (1.52)
V11oxy	0.75 (1.84)	0.32 (0.97)	0.38 (1.31)
V12oxy	-0.06 (1.90)	0.58 (1.29)	1.05 (0.98)
V13oxy	0.51 (1.77)	0.94 (1.66)	1.10 (1.06)
V14oxy	-0.27 (1.61)** †	1.74 (2.06)	1.37 (1.13)
V15oxy	0.58 (1.82)	1.01 (1.33)	0.55 (1.01)
V16oxy	0.17 (1.33) †	1.20 (1.55)	1.35 (1.19)
V1deoxy	-0.74 (1.00)	0.41 (2.02) †	-0.81 (0.67)
V2deoxy	-1.11 (0.77)*	0.15 (1.65)	-0.75 (0.80)
V3deoxy	-0.12 (1.28)	-0.08 (1.03)	-0.59 (0.57)
V4deoxy	-0.67 (1.58)	0.42 (1.63) †	-1.24 (1.12)
V5deoxy	-0.23 (1.44)	-0.54 (1.34)	-0.31 (0.68)
V6deoxy	-0.64 (1.71)	0.31 (1.50)	-0.48 (1.44)
V7deoxy	-0.26 (0.69)	-0.10 (1.68)	-0.46 (0.52)
V8deoxy	-0.33 (1.67)	0.13 (1.78)	-0.88 (1.29)
V9deoxy	-0.66 (1.04)	-0.02 (1.26)	-0.48 (0.84)
V10deoxy	-0.83 (1.41)	0.15 (1.93)	-1.08 (1.42)
V11deoxy	-0.51 (1.04)	0.44 (2.45)	-0.55 (1.03)
V12deoxy	-1.07 (1.22)*	0.71 (2.03) †	-1.09 (1.03)
V13deoxy	-0.49 (1.01)	0.08 (1.02)	-0.20 (0.61)
V14deoxy	-1.28 (1.21)**	0.28 (1.65)	-0.80 (1.14)
V15deoxy	-0.62 (1.50)	-0.00 (1.10)	-0.74 (0.54)
V16deoxy	-0.88 (1.09)	0.08 (1.81)	-0.76 (0.80)

*Indicates a significant difference from polydrug controls at the .05 level, and ** at the .01 level;
† indicates a significant difference from drug naïve controls at the .05 level and †† at the .01 level.

ANOVA revealed significant between group differences in average oxy-Hb changes at voxel 2 $F(2,43)=4.78$, $p<.05$; V14 $F(2,43)=6.37$, $p<.01$ and V16 $F(2,42)=3.32$, $p<.05$. There were no significant between group differences at any of the other voxels measures ($p>.05$).

Pairwise comparisons revealed that at V2 ecstasy users showed a significantly reduced oxy-Hb change compared to drug naïve controls ($p<.05$). At V14 ecstasy users show significantly lower oxy-Hb than both polydrug controls ($p<.01$) and drug naïve controls ($p<.05$). At V16 ecstasy users again show significantly lower oxy-Hb than drug naïve controls ($p<.05$).

ANOVA on deoxy-Hb changes from baseline revealed significant between group differences at V1 $F(2,42)=3.96$, $p<.05$, V2 $F(2,43)=4.71$, $p<.05$, V4 $F(2,30)=3.66$, $p<.05$, V12 $F(2,30)=5.04$, $p<.05$ and V14, $F(2,43)=5.09$, $p<.01$. There were no significant between group differences at any of the other voxels measured ($p>.05$).

Pairwise comparisons revealed that at V1, polydrug controls showed significantly greater deoxy-Hb than drug naïve controls ($p<.05$), and this difference approached significance compared to ecstasy users ($p=.07$). At V2, polydrug controls showed significantly greater deoxy-Hb increase than ecstasy users ($p<.05$) and this difference approached significance compared to drug naïve controls ($p=.08$). At V4 polydrug controls showed significantly increased deoxy-Hb compared to drug naïve controls ($p<.05$). At V12 polydrug controls showed significantly increased deoxy-Hb compared to both ecstasy users and drug naïve controls ($p<.05$ in both cases) and at V14 polydrug controls showed significantly greater deoxy-Hb compared to ecstasy users ($p<.01$). Ecstasy users and drug naïve controls did not differ significantly from each other at any of these voxels.

Cortisol Analysis

Mean salivary cortisol levels for each group over the time course of the sampling protocol can be observed in figure 9.1.

Figure 9.1. Diurnal cortisol profile for each group over the two-day protocol.

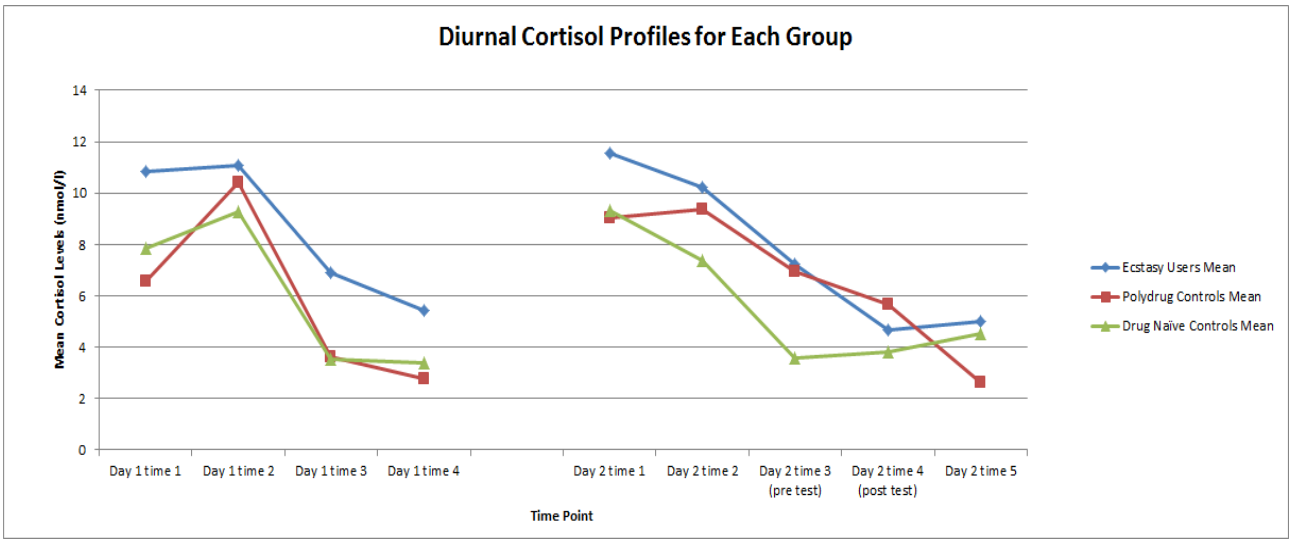

Fig. 9.1: Depicts mean salivary cortisol levels (nmol/l) for each group over the time course for the two day protocol. Note the steep increase in cortisol levels 30 minutes after waking on day 1, then gradual decline throughout the day. This increase was not as pronounced in day 2, perhaps reflecting elevated cortisol levels at waking itself, due to anxiety about attending the lab to undertake a stressor task. On day1 ecstasy users' cortisol levels remain elevated throughout the day compared to control groups. This is significant compared to both groups at time 3 on day 1. Ecstasy users had significantly increased cortisol levels at time 1 of day 1 compared to polydrug controls.

ANOVA was conducted for cortisol levels at each time point. Significant between group differences were observed at day 1 time 3 $F(2,45) = 3.60$, $p<.05$, and between group differences were approaching significance at day 1 time 1 $F(2,44) = 2.92$, p=.06. There were no significant between group differences in salivary cortisol at any of the other time points measured ($p>.05$ in each case). Planned comparisons revealed that at day 1 time 3 ecstasy users had significantly increased cortisol levels compared to both other groups ($p<.05$ in each case). At day 1 time 1, ecstasy users had significantly increased cortisol levels compared to polydrug controls ($p<.05$), there was no significant difference between ecstasy users and drug

controls at this time (p>.05). Furthermore, as can be observed in figure 9.1, ecstasy users and polydrug users display decreased cortisol level post task compared to pre task.

9.5 Implications of Chapter 9

The results from this chapter show that there were no performance differences between groups on any of the subscales of multitasking, or on total score for the task. In addition, there were no significant differences on self-report measures of perceived workload (indexed by the NASA – TLX). There were differences between groups in their haemodynamic response to the task. However these were contrary to expectations. Analysis of oxy-Hb change from baseline revealed that ecstasy users showed a blunted increase compared to controls in response to the task. Indeed, at voxel 14 (pertaining to the right DLPFC) ecstasy users showed significantly less oxy-Hb compared to both control groups. Moreover drug naïve controls displayed a significant increase in oxy-Hb from baseline compared to ecstasy users at V2 (left DLPFC), and V16 (right DLPFC). Polydrug controls showed the greatest increase in deoxy-Hb. This was significant compared to ecstasy users at V2, 12 and 14, and both ecstasy users and drug naïve controls at V12. Polydrug controls also showed increases compared to drug naïve controls at V1 and V4. There were no differences between ecstasy users and drug naïve controls in deoxy-Hb at any voxel. These results provide difficulty for interpretation given what has preceded them in this thesis. Perhaps the individual tasks that comprise the multitasking framework were not executive function specific, rather they require other neuronal areas for performance. If this is the case, perhaps ecstasy users showed a decrease in oxy-Hb compared to controls in the PFC due to reallocating resources to other brain regions. This will be discussed in greater depth in Chapter 10. However, the act of multitasking itself, should load on the central executive.

The results from cortisol sampling were more in line with predictions and current knowledge of MDMA's effects on the HPA axis. Observation of figure 9.1 shows a general elevation in salivary cortisol levels in ecstasy users. However it was only at time 3 on day one that ecstasy users displayed significantly increased cortisol levels compared to both other groups. Ecstasy users did however show significant increase in salivary cortisol levels compared to polydrug controls at time 1 day 1. These results reflect increased basal cortisol levels in MDMA users relative to controls. Ecstasy users report significantly reduced feelings of calmness, on the day of testing compared to both control groups on the SAI-VAS, and significantly reduced feelings of being relaxed than drug naïve controls. Waking cortisol levels are increased in control groups on the day of the test, by a greater amount than the ecstasy user group. However the ecstasy users still show the greatest levels of cortisol at this point. Perhaps such a high level of cortisol upon waking, reflects elevated anxiety about undertaking the stressor task, and this increased level of cortisol is unsustainable, hence a continuing drop in cortisol levels (as in day 1) rather than an increase post task. The following chapter will discuss these results, along with the other results from the empirical chapters in this thesis in greater depth.

10. General Discussion

The aim of the current thesis was to examine the neurophysiological response to executive functioning in MDMA users. This was investigated using EEG and fNIRS, to aid the current understanding of MDMA related cognitive deficits, due to potential serotonergic neurotoxicity. Degradation to the serotonin system via repeated use of MDMA may also have profound implications for other psychobiological functions. To this end a secondary aim of this thesis was to examine neuroendocrine function in ecstasy users, and their neurohormonal response to stressful events.

Chapter 6 of this thesis investigated the executive functions of inhibitory control, switching, updating and, access to semantic memory and their behavioural and electrophysiological correlates. Background variables such as fluid intelligence, age, measures of sleep (apart from pre-test differences between polydrug controls and ecstasy users on KSS), level of arousal, depression and anxiety showed no significant differences between ecstasy users, polydrug controls and drug naïve controls. There were no behavioural differences between groups in terms of number of errors for the Go/NoGo, N-back task and semantic association tasks and there were no switch cost differences on the number-letter task. No between group differences were observed in terms of reaction time on the semantic association task. Furthermore ecstasy users did not differ significantly to the control groups with respect to subjective mental workload on any of the tasks. However there were differences in reaction time data on the n-back task, whereby drug naïve controls were significantly slower to respond than polydrug controls. Although ecstasy users were not significantly different in terms of reaction times compared to either control group, they did show generally increased reaction times compared to drug naïve controls. Furthermore the error count was generally higher for ecstasy users compared to controls (although not

significant) perhaps reflecting an accuracy versus speed trade off, that reflects increased impulsivity in ecstasy users. The results here are non-significant however, and the polydrug users show less errors than drug naïve controls as well as faster reaction times, so this interpretation needs treating with caution. The lack of performance differences between groups in response inhibition and switching was to be expected. Several studies have shown that ecstasy users appear unimpaired at inhibitory control (Gouzoulis-Mayfrank *et al*., 2003; Hanson & Luciana, 2010; Roberts & Garavan, 2010) and mental set switching (Back-Madruga *et al*., 2003; Fox *et al*., 2001; Montgomery, Fisk, Newcombe & Murphy, 2005). It was predicted that ecstasy users may show performance deficits in the semantic association task. However, ecstasy users have been reported to display unimpaired performance in tasks that assess access previously (Bedi & Redman, 2008; Halpern *et al.*, 2004). Furthermore, it was expected that ecstasy users may show performance deficits on the n-back task. Nevertheless, the lack of performance impairment in updating on this task is in line with previous research (Daumann, Fimm *et al.*, 2003). Moreover as has been stipulated previously, it was a central aim of this thesis to examine the neurophysiological response to executive functioning tasks, as this may be more sensitive in exposing cognitive deficits.

Despite the lack of between-group differences on behavioural measures, there were differences in EEG measures in line with our predictions that reflect atypical processing in ecstasy users in response to executive function tasks involving inhibitory control, switching and access. The following parts of this discussion will summarise the findings from EEG and their relationship to the existing literature and implications for each executive function separately. First to be discussed are the findings from the inhibitory control (Go/NoGo) task.

The ERP results from performing the Go/NoGo task are suggestive of changes in attentional processes between the components involved in early inhibition processing (P2).

Ecstasy users exhibited significantly higher mean amplitudes than both control groups at anterior midline site FCz and significantly higher amplitudes than drug-naïve controls at another anterior midline site Fz. Furthermore regression analyses revealed that the amount of ecstasy consumed in the last 30 days was a significant predictor of FCz amplitude after controlling for cannabis use, suggesting that recent use of ecstasy may play a role in response inhibition. It is interesting to observe such differences in the P2 component, given that it has been suggested that problems with early orienting or preparation may have consequences for later processing stages (Pliszka *et al.,* 2000). Differences in this component have been observed previously in attention deficit hyperactivity disorder (ADHD) subjects (Johnstone *et al.,* 2001; Lazzaro *et al*., 2001) who display greater amplitude in this component relative to controls. This has been interpreted as atypical inhibition of sensory input in ADHD subjects (Johnstone *et al*., 2001). In addition, research has shown that the P2 component is elevated in unexpected versus expected inhibition trials (Gajewski *et al*., 2008). Research has also investigated the P2 component in inhibitory control in high and low functional impulsives (i.e. individuals whose impulsivity may facilitate performance). High functional impulsives show an increase in P2 amplitude as a function of task demand (higher demand=increased amplitude) whereas low functional impulsives do not (Fritzsche *et al.,* 2011). Taken together, this suggests a number of explanations for the elevation of P2 during performance of this function. Firstly, ecstasy users have elevated impulsivity compared to nonusers and this impulsivity may be masking performance deficits. Fritzsche *et al*. (2011) suggest that this steeper P2 slope, as seen in the ecstasy-polydrug users, reflects earlier and more efficient evaluation of stimuli as a result of impulsivity. This seems a reasonable assumption given that elevated impulsivity has been noted in ecstasy users in previous research (e.g**.** Butler and Montgomery, 2004**).** The heightened P2 has been shown to be associated with stimulus evaluation and response (Gajewski *et al.,* 2008). It is notable that Gajewski *et al.,* (2008) only

reported elevated P2 when they increased the demands of their task, which tentatively suggests that, in the present study, the task was more demanding for ecstasy users. Secondly in line with the ADHD research cited above, the atypical early inhibitory processing displayed in the P2 ERP component in ecstasy users could be due to recruitment of additional compensatory resources, similar to the increased activity in prefrontal areas associated with executive functioning deficits in Alzheimer's disease patients (Grady, *et al.,* 2003; Saykin *et al*., 1998; Woodard *et al*., 1998). This proposal could also help explain the lack of observed behavioural differences on the task. Moreover the recruitment of additional resources at this early stage in processing could offset any further waveform modulation at later processing stages.

Although some previous studies report differences between ecstasy users and controls in the P3 component on a Go/NoGo task (Gamma *et al.,* 2005), these have conceded that between-group differences were lower after age, education level and cannabis use were controlled for. Debates have arisen about the contribution of the P3 and N2 components in response inhibition. For example, although often cited as being reflections of inhibitory control (Kok, 1986; Kopp *et al.,* 1996), the N2 has also been argued to have a role in conflict monitoring, rather than response inhibition (Donkers and Boxtel, 2004; Nieuwenhuis *et al.,* 2003). Furthermore, the P3 has been suggested to be insensitive to performance differences in inhibitory control and not necessarily involved in response inhibition (Falkenstein *et al.,* 1999; Kopp *et al.,* 1996). If this is the case then perhaps the task used in the current study, which was employed due to it tapping the executive function of inhibitory control only, would not highlight any differences in these components.

Moving on to mental set switching in Chapter 6, ERP data during the number-letter task also provide support for MDMA related disturbances to cognitive processing. The P3

component, thought to play an important role in the allocation of attentional resources and as such an important role in the ability to switch between mental sets, showed significant between group differences at several parieto-occipital and occipital electrode sites. Drug naïve controls displayed significantly higher mean amplitude in this component compared to ecstasy users as well as polydrug controls at O1 and POz. A diminished P3 component is thought to reflect cognitive impairment, and as such these findings are consistent with those of Casco *et al*. (2005) and Mejias *et al*. (2005) who observed reduced P3 in ecstasy users compared to controls in other cognitive tasks. Interestingly, the polydrug control group appear to have a reduced P3 in a further two sites (PO4 and Oz) compared to drug naïve controls, suggesting some evidence of atypical processing that is related to the use of drugs in general and not just ecstasy i.e. a polydrug effect. Furthermore, it has been suggested previously that concomitant cannabis use may account in part or fully for cognitive deficits observed in ecstasy users (Dafters *et al.,* 2004; Gamma *et al.,* 2005). Further to this point the regression analyses suggested that lifetime dose of cannabis significantly predicted lower mean amplitude at O1 and Oz. Although polydrug users did not differ from ecstasy users in amplitude at PO4 and Oz, these results provide evidence for cannabis contributing to processing atypicalities in mental set switching.

Ecstasy specific differences were also apparent in the P2 component, involved in early processing of stimuli. It was observed that ecstasy users displayed a significantly higher mean amplitude than both control groups at frontal, central and fronto-central sites; Fz, Cz and FCz. Atypicalities at this early stage of processing in ecstasy users provide evidence that suggest additional resources are being recruited as a compensatory mechanism as described above. Perhaps additional recruitment of resources at this stage allowed for similar results behaviourally, despite diminished P3 amplitude at a later stage of processing. These ERP

results are in line with our predictions and suggest evidence for an ecstasy/polydrug effect on the degradation of the executive function of mental set switching.

In the semantic association task, there were no main effects of difficulty or site, or any interactions with these and group, or difficulty by site by group for the N2 component. There were however between group trends that warranted further exploration. In the low association condition of the task ecstasy users displayed a significantly larger negativity in the N2 component compared to drug naïve controls in occipital electrode site O2 and parieto-occipital electrode PO8 although non-ecstasy polydrug users did not differ from either group.

The supposedly easier high association condition showed significant differences in negativity at the N2 component in polydrug controls compared to drug naïve controls at parieto-occipital site PO3. Components that reflect positivity's (P2 and P3) showed no main effects of difficulty or site, or any interactions with these and group, or difficulty by site by group (except in P3 where there was a site by group interaction) and there were no between group differences. Thus these components are less informative about access to semantic memory in ecstasy users. However, the group difference in the N2 component does provide some interesting points to consider. The N2 component has been reported as having a source in the anterior cingulate cortex (Bekker *et al.*, 2005; Nieuwenhuis *et al.*, 2003) and to reflect neural processes engaged during conflict monitoring, thus being increased in high conflict trials (Yeung & Cohen, 2006), for example when incongruence between targets and cues/distracters elicits a conflict of response in a Stroop task (Kopp *et al.*, 1996). Firstly, considering why the N2 was more pronounced in ecstasy users compared to drug naïve controls in those trials where there was a lower semantic association between target and cue words, it is possible that at this level of processing, the ecstasy users required the recruitment of additional resources in order to access the semantic network of long term memory

compared to drug naïve controls. Previous research has provided evidence that ecstasy users' performance can be more greatly impaired under higher task difficulty. For example Montgomery, Fisk, Newcombe and Murphy (2005) observed a decline in performance in a word fluency task when more rules were imposed, suggesting that deficits are more prominent in tasks that place more demand on the central executive. Given that participants reported no perceived differences in cognitive effort on the NASA-TLX it is possible that compensatory cognitive processing at neurological sites is correcting for deficits in executive function to eradicate behavioural differences and other research reporting null results, with respect to performance may reflect similar reallocation of cognitive resources. This aspect of the results was in line with our predictions.

Ecstasy users did not show significant differences to controls on the high association condition of the task. It is generally accepted (Jefferies *et al*., 2004; Rossell *et al.*, 2001; Shiffrin & Schneider, 1977) that information processing involves two modes of processing: automatic and controlled. Controlled processing, unlike automatic processing, involves selectively and consciously attending to a stimulus, suggesting that controlled processing involves higher level mental processes. As such automatic processing is proposed to rely on long-term memory, whilst controlled processing loads more on working memory (Jeffries *et al.* 2004), suggesting separable neural substrates. Indeed Rossell *et al.* (2001) used fMRI to investigate differences in effortful and automatic processing in a similar lexical decision priming experiment, and found that distinct sub regions of the anterior cingulate cortex showed activation dependent on the processing type involved. The N2 component in a semantic classification task was argued to reflect controlled processing by Ritter *et al.* (1982). This could help explain why the magnitude of effects was larger under the more difficult low association condition, as this was more effortful and as such required recruitment of additional resources. Furthermore it has been observed that patients with mild head injuries

will display greater N2 amplitudes whilst performing at a similar level of performance on cognitive tasks (Rugg *et al.,* 1993). This is proposed as evidence for reallocation of cognitive resources to cope with task demands and to achieve similar performance.

While the above discusses possible N2-related differences in access, it is possible that the N2 here reflects changes in other cognitive processes additional to semantic access (See Folstein & van Petten, 2008, for a review). The N2 in the current study was prominent in more posterior electrodes which Suwazono *et al*. (2000) suggest is reflective of increased attention demands in the visual cortex required for stimulus processing. In the study by Suwazono *et al.* (2000) posterior N2 was eliminated by eliminating target novelty (i.e. making targets completely predictable). Luck and Hillyard (1994) investigated subcomponents of the N2 component using visual search tasks. It was found that the bilateral posterior N2 as seen in the present study was related to visual search and target probability, with an increased posterior N2 when participants could not predict a target before presentation. Taken together this provides evidence that in the present study the posterior N2 may reflect increased demands on visual search and maintenance of visual representations, with greater negativity in ecstasy polydrug users showing that they require increased attentional resources for this. Durable abnormalities of the N2 component observed over occipital and parieto-occipital sites of drug users compared to drug naïve controls are indicative of compensatory mechanisms, or reallocation of cognitive resources to attenuate any observable behavioural differences caused by ecstasy-related disturbances to traditional processing of semantic information and allocation of attention during visual search.

Contrary to expectations, the n-back task yielded no observable ERP differences between groups. It was predicted that ecstasy users would show alterations to ERP components that reflect cognitive impairment/compensatory mechanisms, given that

performance was not significantly reduced. Furthermore, previous research combining this task with neuroimaging methods has suggested neuronal alterations despite equivalent performance (Daumann, Fimm *et al.,* 2003). One possible explanation for the current lack of differences may be the design of the n-back task employed. In several ERP studies using the n-back task, a paradigm is employed whereby participants are presented with a series of stimuli (letters or numbers) and are required to respond when a stimulus in the series matches a stimulus presented *n* stimuli back in the series (Chen *et al.,* 2008; Watter *et al.,* 2001). This would therefore elicit an ERP on each response, whereas the task used in the current thesis required participants to select (from a display of numbers) which number was presented *n* digits back. In this case the task is more difficult and requires more protracted mental processing to calculate which digit was presented. As such this task may lend itself better to imaging methods that are not time-locked, but rather evaluate the induction of neuronal activity over time, for example ERD/ERS or measures or haemodynamic response such as fMRI/fNIRS. Indeed the ecstasy-related activation differences observed by Daumann, Fimm *et al*. (2003), were observed in an fMRI study.

To summarise the results from chapter 6, the ERP evidence suggests that ecstasy/polydrug users are showing evidence of atypical cognitive processing during tasks that require response inhibition, switching of the mental set and access of semantic/long term memory stores. The durable abnormalities observed in these tasks in P2 and N2 components, in line with predictions, reflect potential recruitment of additional resources to attenuate behavioural differences. Whereas the diminished P3 response associated with mental set switching may reflect general cognitive deficiencies that have been observed in ecstasy using populations in the past. The lack of MDMA related differences in the ERPs elicited from the n-back task are contrary to expectations. However the next part of this discussion focuses on

the results from haemodynamic response to memory updating tasks, which provide more evidence consistent with expectations.

Chapter 7 investigated the haemodynamic response to memory updating using letter updating and spatial updating tasks. Functional near-infrared spectroscopy was employed to assess the haemodynamic response to task in ecstasy users, polydrug controls and drug naïve controls. To summarise the results, performance was equivalent between groups on both updating measures. Whilst this was again contrary to expectations due to previous research suggesting that ecstasy users were consistently impaired on this function, it was in line with results from updating performance in Chapter 6. Furthermore it was predicted that in the absence of behavioural differences, haemodynamic measures would provide evidence of ecstasy users being engaged in more effortful cognition as an index of cognitive reallocation/compensatory mechanisms. The results from fNIRS showed that during the letter-updating task ecstasy users showed significant increases in oxy-Hb from baseline compared to both control groups at voxel 12, situated over the right medial PFC. Furthermore significant increases in deoxy-Hb were observed in ecstasy users relative to drug naïve controls at V12. At V12 the difference was approaching significance compared to polydrug users. At V7 ecstasy users had greater deoxy-Hb compared to drug naïve controls that was approaching significance. At V1 ecstasy users displayed significant increases in deoxy-Hb compared to polydrug users, and differences compared to drug naïve controls that were approaching significance and at V8 ecstasy users showed greater deoxy-Hb compared to both control groups that was approaching significance. These voxels are located across the breadth of the PFC showing a bilateral haemodynamic response to letter updating. This is consistent with previous neuroimaging studies that suggest memory updating requires bilateral neuronal response (e.g. Collette *et al*., 2007). During the spatial updating task ecstasy users showed significant increases in oxy-Hb compared to both groups at voxel 8, situated over the left

medial prefrontal cortex. No significant between group differences were observed for deoxy-Hb during the spatial updating task. Regression analyses were generally non-significant. However frequency of MDMA use predicted oxy-Hb and deoxy-Hb at V12 during letter updating, suggesting that frequency of use may affect haemodynamic response to memory updating.

Increases in oxy-Hb compared to both control groups, are indicative of increased cognitive effort displayed by ecstasy users to attenuate behavioural differences and are in line with expectations. These results suggest that although performing at a similar level, the tasks were more demanding for ecstasy users. Increases in oxygenated haemoglobin are understood to reflect increases in neuronal activity (Leff *et al.,* 2011), and levels of oxy-Hb increase with increased demand (Izzetoglu *et al.,* 2004). The importance of measuring haemodynamic response to tasks where subjects perform at a similar level behaviourally has been explored previously in human operators (for example, air traffic control operators – Ayaz *et al*., 2012). Such studies highlight the dissociation between cognitive effort and performance output, arguing that performance can be maintained at necessary levels via increased mental effort or perhaps strategic alterations. However increased mental workload is also suggested to be predictive of future performance failure (with increased demand or task changes). Increases in oxy-Hb are accepted as increases in cognitive effort despite behaviourally similar performance, and can be used as an assessment of operators' ability (Ayaz *et al.,* 2012). This is an interesting distinction to make, as in previous studies neurological disorders are coupled with task performance deficits and reductions in oxy-Hb (Ehlis *et al*., 2008). However given that the current sample does not suffer from neurological impairment it is not appropriate to compare results from this sample with those in studies such as that conducted by Ehlis *et al.* (2008). The explanation of increasing cognitive effort to maintain similar behavioural

performance is more applicable and may reflect recruitment of additional cognitive resources compared to controls and predict future cognitive decline.

The post-hoc statistical comparisons of means for the deoxy-Hb data in this chapter were assessed at the 2 tailed level. This is because it is suggested that during neurovascular coupling, increases in cerebral blood flow and cerebral blood volume are caused by reductions in local glucose and oxygen due to increased consumption of these from the local capillary bed (Irani *et al.,* 2007). Thus increases in oxygenated haemoglobin delivery will outweigh consumption leading to an excess of oxygenated haemoglobin in the activated area (Fox *et al.,* 1988). The excess of oxy-Hb is argued to cause decreases in deoxy-Hb, however this has been the subject of much debate, as equally, increases in deoxy-Hb may be observed in the capillary bed due to increased oxygen consumption (Irani *et al.,* 2007). Previous research has suggested that increases in oxy-Hb are often complimented by a decrease in deoxy-Hb in the same area (Ehlis *et al.,* 2008; Leff *et al*., 2008). However oxygenated and deoxygenated haemoglobin do not necessarily have a linear relationship, rather they are separate sources of haemodynamic response. Furthermore several studies have shown increases in deoxy-Hb alongside increases in oxy-Hb (Hoshi & Tamura, 1993; Sakatani *et al*., 1999). As such deoxy-Hb appears to be a less reliable measure of neuronal activation than oxy-Hb in fNIRS. Nevertheless these results are better understood as an increase in total haemoglobin to the areas of the prefrontal cortex that are involved in this executive function, given that total-Hb is understood to be the sum of oxy-Hb and deoxy-Hb (Steinbrink *et al*., 2006).

To summarise results from Chapter 7, there is evidence from fNIRS that ecstasy users are engaged in more effortful cognition indexed by increases in oxy-Hb to areas of the prefrontal cortex in letter-updating (V12 in the right medial PFC) and spatial updating (V8

left medial PFC). Increases in neuronal activation, reflect a compensatory mechanism due to degradation of 5HT neurons in the PFC via MDMA use. Furthermore this may predict future cognitive failure with increasing task demands. This is in line with our predictions and supports previous research suggesting that ecstasy users show performance deficits in updating (Montgomery & Fisk, 2008; Montgomery, Fisk, Newcombe & Murphy, 2005). Moreover, these results are consistent with neural activation changes that have been observed in ecstasy users during memory updating tasks in fMRI studies that are suggested to reflect MDMA-induced neurotoxicity (Daumann, Fimm *et al.*, 2003; Daumann, Schnitker *et al.*, 2003; Daumann, Fischermann, Heekeren *et al*., 2004).

Chapter 8 investigated the effects of ecstasy/MDMA on the haemodynamic response to the remaining executive functions that were not covered in Chapter 7; inhibition, switching and access. Ecstasy users were compared to a non-ecstasy control group (largely of drug naïve participants) on performance of RLG, number-letter task and CWFT and their haemodynamic response was assessed using fNIRS. The ecstasy users in this sample did not differ significantly from controls in fluid intelligence, sleep measures or levels of arousal, depression or anxiety. However, they did report drinking significantly more alcohol per week than controls and due to their concomitant use of other drugs, it may be more adequate to refer to them as polydrug users.

As in previous chapters behavioural data did not yield any significant differences at any level of any of the tasks used. However, as predicted ecstasy users did display alterations to neuronal activation on all three tasks used, which is consistent with findings from Chapter 6. Typically ecstasy users displayed increases in oxy-Hb compared to controls that reflect increases in effortful cognition, which is in line with findings from Chapter 7. Furthermore

increases in deoxy-Hb were again observed in ecstasy users relative to controls during the RLG task and the CWFT.

Inhibitory control was measured using RLG and analysis of fNIRS data during this task revealed that on the easier level of the task (generation rate of 4s), ecstasy users showed significant increases in oxy-Hb at one voxel (V10) in the right medial PFC and one voxel that was approaching significance in the left DLPFC (V1). However deoxy-Hb was also increased in ecstasy users relative to controls at several voxels relating the left DLPFC and the right DLPFC. When difficulty was increased (2 second rate) a stronger haemodynamic response was observed in the ecstasy user group. Increased levels of oxy-Hb were observed in V4 relating to the left DLPFC, and V10 and V12 relating to the (inferior) right medial PFC compared to controls. A further two voxels (V1 and V14) also approached significance. Significantly more deoxy-Hb was observed in six voxels in ecstasy users compared to controls, covering the spectrum of the PFC. This marked increase in significantly different voxels, suggest that neuronal activation is increasing as a function of difficulty. Again in the most difficult block of the task (generation rate of 1s) ecstasy users display significant increases in oxy-Hb at two voxels V12 (located on the inferior part on the right medial PFC) and V14 (relating to the right DLPFC), with a third (V13) approaching significance. This was a less pronounced difference than at the two second rate, however there was an increase in the number of voxels showing increased deoxy-Hb (a total of 8 voxels, primarily relating to the left DLPFC and right DLPFC, with a further two approaching significance).This shows a general increase in neuronal activity at this rate, if we consider that the total amount of haemoglobin to the prefrontal cortex appears increased. This supports the existing evidence that suggests ecstasy users are more greatly affected when greater cognitive load is placed upon them (Wareing *et al*., 2000; Montgomery, Fisk, Newcombe & Murphy, 2005).

The increase in neuronal activation observed in this inhibitory control task is bilateral and suggests that ecstasy polydrug users find this task more difficult than non-users. Meta-analysis of neuroimaging data during cognitive functions suggest a network of PFC regions are regularly active, including bilateral activation of the DLPFC, inferior frontal cortex and anterior cingulate cortex (Duncan & Owen, 2000). Interestingly, a review of lesion studies (Aron *et al.,* 2004), suggested that although the network of PFC areas described above is necessary for inhibitory control, the right inferior frontal cortex is of particular importance in this function. This is consistent with the current results that observe consistently increased oxy-Hb in inferior voxels relating to the right of the PFC (V10 and V12), for ecstasy users. If MDMA damages 5-HT neurons, which are abundant in the PFC, it is logical that these areas that are necessary for performing executive tasks would require additional resources, or would show increased activation as a function of increased demand. This is further support for the argument that ecstasy users are recruiting additional resources to perform at a similar level as controls on the task. This supports results from the ERP data on inhibition from Chapter 6 (Roberts *et al*., 2013a), which suggests atypical processing, despite equivalent behavioural performance. These results are also in agreement with those of Roberts and Garavan (2010), who observed that ecstasy users displayed increased frontal and temporal BOLD activation compared to controls during a Go/NoGo task, in an fMRI study. Morgan *et al*. (2006) suggested that depletion of serotonin and impairment of other executive functions may lead to poor inhibitory control. Taken together these results potentially reflect evidence of MDMA related serotonergic neurotoxicity. The regression analyses on the present dataset showed that last 30 day use significantly predicted oxy-Hb increase in voxels 12 and 14 during the one second rate of the task, after controlling for cannabis use indices. This is indicative of recency of MDMA use having implications for inhibitory control. Indeed Hoshi *et al.* (2007) observed impaired inhibitory control in ecstasy users which they suggest is

related to recency of use, given that current users were impaired, but former users were not. It is suggested that abstention may lead to recovery of this function. Furthermore, last 30 day use also predicted P2 amplitude during the Go/NoGo task in Chapter 6, this provides further evidence that recency of use may be an important factor for inhibitory control.

In the first switching block of the number-letter task ecstasy users displayed significantly more oxy-Hb than drug naïve controls in voxel 5 relating to the left medial PFC. Increases in oxy-Hb were also approaching significance at V6 and V13. In the second block ecstasy users showed increased oxy-Hb compared to controls that was approaching significance at V5 again. There were no significant differences in the deoxy-Hb data. Nevertheless, significant increases in oxy-Hb again suggest that ecstasy users are engaging in more effortful cognition during mental set switching than non-users. The increase in oxy-Hb, typically in the left medial PFC reflects recruitment of additional resources to attenuate performance deficits. This is consistent with our predictions, and findings of atypical processing during this task in Chapter 6 (Roberts *et al.,* 2013c in press). This reflects an increase in cognitive effort at the same level of performance that may predict future cognitive failures (Ayaz *et al*., 2012). Interestingly, the regression analysis conducted on oxy-Hb increase at V5, showed that lifetime dose of cannabis significantly predicted oxy-Hb increase. Furthermore none of the ecstasy use indices predicted level of oxy-Hb after controlling for cannabis use indices. This is particularly salient, given the observed contribution of cannabis to the diminished P3 response during this executive function in Chapter 6. Dafters and Hoshi (2004) have previously highlighted the contribution of cannabis to memory performance in MDMA users. However work from the same lab (Dafters 2006), suggested that MDMA users (who also used cannabis) were impaired in switching performance compared to cannabis only users and drug naïve controls. The contribution of cannabis to the results on switching in this thesis highlight the importance of understanding potential drug interactions that may affect

cognition. It is advised that the results here are treated with caution and are described as polydrug effects due to the concomitant use of other drugs. However the interaction between MDMA use and cannabis use appears to be particularly salient in relation to task switching.

fNIRS analysis during the CWFT (access) yielded some interesting results; as predicted, ecstasy users displayed increases in oxy-Hb compared to controls in three voxels relating to the left DLPFC as well as one voxel relating to the right medial PFC on what is considered to be the easiest level of difficulty on the task (naming animals). As difficulty increased, ecstasy users displayed a significantly greater increase in oxy-Hb relative to controls at two voxels, and a third approaching significance relating to the left DLPFC and a further two voxels relating to the right medial PFC. This increase in oxygenation is complimented by an increase in deoxy-Hb compared to controls at V2 (and V4 that was approaching significance) in the left DLPFC and V14 in the right DLPFC that was approaching significance. In the final and most difficult phase of the task (4 letter words beginning with C) ecstasy users displayed significant increases in oxy-Hb compared to controls at three voxels (and a further 1 voxel approaching significance) that pertain to the left DLPFC and two voxels relating to the right medial PFC.

Thus ecstasy users show consistently increased levels of oxy-Hb in the LDLPFC and RPFC regions during the access executive function. Moreover the haemodynamic response to the task increases with task difficulty, with ecstasy users displaying more significant differences in oxy-Hb in more voxels as the task progresses. This supports previous arguments that ecstasy users perform worse as cognitive demand increases (Wareing *et al*., 2000). Further to this point Montgomery, Fisk, Newcombe and Murphy (2005) observed that ecstasy users perform worse in word fluency tasks as more rules are imposed, as a function of difficulty. Although the current task elicited no behavioural differences, oxy-Hb differences

were more pronounced as a function of difficulty, suggesting that, in agreement with Montgomery, Fisk, Newcombe and Murphy (2005), ecstasy users are showing a greater departure from normal cognitive functioning as difficulty increases. This highlights the greater sensitivity of neurophysiological measures to detect cognitive impairment. Compensatory mechanisms may explain the lack of behavioural differences observed using this task in the literature (Bedi & Redman, 2008; Halpern *et al.,* 2004; Morgan *et al*., 2002). Especially if we consider that these studies, employed simpler word fluency measures, than those yielding performance differences (Montgomery, Fisk, Newcombe & Murphy, 2005; Montgomery *et al*., 2007). Moreover Montgomery, Fisk, Newcombe and Murphy (2005) used a much longer time frame than the task employed in this thesis (and those in the studies mentioned above), suggesting that longer periods of sustained load on the central executive produce more pronounced effects. This is consistent with the current findings, as has been previously stated, the increased neuronal activity that reflects increased cognitive effort to attain equivalent performance, potentially predicts future failure with increasing task demand (Ayaz *et al.,* 2012).

Increases in oxy-Hb to both the left and right hemispheres reflect the need for more cognitive resources to attenuate behavioural performance decline and that this effect is bilateral. It is interesting to note this consistent increase in oxy-Hb in the left DLPFC and right medial PFC over all three levels of CWFT, as these areas have been implicated in semantic and word fluency previously. Stuss *et al.* (1998) observed that in patients with brain lesions, those to the left DLPFC caused the most severe impairments on letter based word fluency measures. The same lesion sites produced impairments in category based fluency, but so did lesions to right medial and DLPFC regions. Indeed the left inferior frontal gyrus, has been consistently associated with semantic and phonologic processing in functional neuroimaging studies (Costafreda *et al.,* 2006), so it is interesting that these areas should

show the greatest differences in the word fluency task in this thesis. These areas appear to be working much harder in ecstasy users compared to controls to achieve similar performance. Likewise, Raj *et al*. (2010) observed that ecstasy users displayed cognitive processing aberrations that relate to areas of the DLPFC during semantic recognition, despite equivalent task performance, in an fMRI study, that is broadly consistent with the present findings. This potentially reflects MDMA induced neurotoxicity. The results from this study were in line with our predictions as well as our interpretation of the results from Chapter 6 with ERP correlates of semantic retrieval (Roberts *et al.*, 2013b). Furthermore regression analyses suggested that frequency of use and lifetime dose of ecstasy predicted oxy-Hb levels after controlling for cannabis use indices at voxels (V3 and V4) relating to the left DLPFC.

Due to ecstasy using populations invariably using other recreational drugs, as well as drinking significantly more alcohol than controls on weekly estimates, it cannot be ruled out that other drugs, or alcohol or concomitant use of substances with ecstasy are not responsible for the effects observed in this study. Nevertheless the ecstasy using sample did show a differential pattern of PFC activation on the CWFT and RLG compared to non-users, which is indicative of cognitive impairment in ecstasy using populations. This study provides evidence of atypical executive functioning in ecstasy users compared to controls on tasks relating to the executive functions of mental set switching, access and inhibitory control. All three tasks invoke an increased haemodynamic response in ecstasy users that is bilateral in the prefrontal cortex. These results show that ecstasy users are engaged in more effortful cognition than non-users to achieve equivalent performance. This is indicative of recruitment of additional cognitive resources in the prefrontal cortex, and perhaps predictive of future cognitive decline.

Chapters 6, 7 and 8 provide evidence of altered cognitive processing in ecstasy users relative to controls during executive functioning tasks that reflect compensatory mechanisms. As previously noted in this thesis, differences in activation observed from neuroimaging measures, despite equivalent performance reflect the increased sensitivity of neuroimaging techniques to detect cognitive deficits than behavioural measures alone. Indeed, information provided in this thesis may help explain the inconsistency in results from previous behavioural studies in this area. This point is interesting to consider as the executive functioning tasks in this thesis yielded no behavioural differences between ecstasy users and controls throughout. This is not surprising given that the tasks used in this thesis are relatively simple tasks of executive function and have been inconsistent in producing behavioural effects in the literature.

There is however, a wealth of literature in other areas of memory and cognition that show ecstasy related performance differences, particularly with tasks that involve higher cognitive processing and mental reasoning. For example McCann *et al.* (1999) observed ecstasy users to perform worse than controls in the Logical Reasoning task (correctly identifying statements that accurately describe transformational grammar, from active/passive, positive/negative statements), yet performance on several simpler cognitive tasks was equivalent. These results lead the authors to suggest that ecstasy related alterations to cognitive function are quite subtle and are only detected on sensitive tasks that place high demand on the central executive. Montgomery, Fisk, Newcombe, Wareing and Murphy (2005) observed MDMA users to be significantly impaired in syllogistic reasoning relative to controls. Syllogistic reasoning involves participants drawing inferences from a set of premises. Reasoning is suggested to be the most cognitively demanding of all intellectual abilities and requires operation of working memory processes (Montgomery, Fisk, Newcombe, Wareing & Murphy, 2005). Therefore consistency in these measures, that are

more demanding than simpler executive measures, yielding ecstasy related performance differences may be expected. Indeed, Fisk *et al*. (2005) observed reasoning deficits in ecstasy users relative to controls, particularly when problems are more difficult. These studies offer consistent MDMA related difficulties in thinking and reasoning, that Fisk *et al.* (2005) conclude is a result of working memory limitations.

Immediate and delayed recall has been investigated extensively in ecstasy users. Early reports showed differences between ecstasy users (novice and regular users) and controls, in both immediate and delayed memory that suggest performance deficits in ecstasy users (Parrott *et al.*, 1998). However, this study was criticised for not reporting use of other illicit drugs (Parrott, 2000). The Rivermead Behavioural Memory Task (RBMT) was used to investigate immediate and delayed memory in ecstasy users, polydrug controls and drug naïve controls by Morgan (1999). This task requires a passage of prose to be remembered and to be recalled as accurately as possible, immediately and then again 40-50 minutes later. This study observed that ecstasy users recalled significantly less prose than both control groups in both immediate and delayed recall conditions. Morgan (1999) suggests that impaired recall performance may be an early sign of global, age related cognitive impairment, due to 5-HT function declining with age. The author argues that ecstasy use may lead to exacerbation of cognitive ageing. Similarly, Morgan (2002) observed ecstasy users (current and former) to be impaired in recall on the RBMT, particularly in the delayed recall condition compared to non-user controls. The results from this study suggest that ecstasy has protracted neuropsychological impairment, due to abstinence not reversing recall deficits. A longitudinal study by Zakzanis *et al*. (2003) suggested that continued ecstasy use was associated with progressive decline in immediate and delayed recall. Furthermore, after observing ecstasy users performing poorly in immediate and delayed recall, McCardle *et al.* (2004) posit that

ecstasy users have problems coding information into long term memory and are less able to focus attention on complex tasks.

Studies into declarative memory have also frequently observed ecstasy related deficits. Measures of 'everyday' memory, in particular prospective memory (remembering to do something in the future) appear to have consistent impairment following regular ecstasy use (Heffernan *et al.*, 2001; Zakzanis *et al*., 2003). Prospective memory is understood to be underpinned by central executive resources. Measures of prospective memory produce more consistent evidence of behavioural deficits than simple executive tasks. Perhaps, such robust impairment behaviourally in declarative memory, higher cognitive processing and mental reasoning reflect how ecstasy related deficits are more apparent when greater demand is placed on the central executive. This is consistent with findings from fNIRS data in Chapter 8, which suggests haemodynamic response is greater when task difficulty increases, as well as other studies that have observed greater ecstasy related performance deficits as difficulty increases (Montgomery, Fisk, Newcombe & Murphy, 2005; Wareing *et al.*, 2000). Furthermore, such behavioural tests as those mentioned above are markedly more complex than the relatively simple executive tasks employed throughout this thesis, which may explain the lack of performance deficits in the current sample. With this in mind, it may be interesting to observe neurophysiological correlates from fNIRS and EEG in future research of these more complex measures of cognitive processing.

McCann *et al.* (1999) observed that cognitive function differences between ecstasy users and non-users are quite subtle, and performance differences are only observed in tasks that are more sensitive than those used in this thesis. However, they argue that this may lead users to unwittingly continue MDMA use, oblivious to potential cumulative damage. It is also suggested that difficulties may become manifest with aging and diminished neuronal

reserve. Indeed studies from the clinical literature into ageing and dementia may aid the current understanding of the implications of the effects of increased cognitive load. For example, it has been reported that early prefrontal connectivity abnormalities are modulated by cognitive demand in pre Huntingdon's disease subjects (Wolf *et al.,* 2008). Furthermore impairment in verbal memory and executive function are associated with the development of dementia in those with Parkinson's disease (Levy *et al*., 2002). Moreover studies into cognitive ageing and prospective memory suggest that event-based prospective memory tasks that require greater cognitive demand produce significantly larger age effects than event-based prospective memory tasks that rely on more automatic processes (Henry *et al.,* 2004). Taken together the results from the current thesis that suggest that ecstasy users are engaged in more effortful cognition to perform at similar levels to controls, and that this can be modulated as a function of cognitive load, suggest that ecstasy use may exacerbate cognitive ageing.

Chapter 9 investigated the effects of ecstasy use on neuroendocrine function and neurohormonal response to stress, using a multitasking stressor task. Performance on the task and the haemodynamic response was assessed, between ecstasy users, polydrug controls and drug naïve controls. A diurnal cortisol profile was obtained from salivary cortisol samples over a two day period, as well as pre and post stressor. The ecstasy users in this study did not differ significantly from controls on background variables such as perceived stress, fluid intelligence or age. Nor did they differ significantly on any of the individual components that made up the multi-tasking stressor task. There were also no significant between group differences on perceived workload as measured by the NASA TLX. There were however differences on subscales of the SAI VAS, indicating that ecstasy users felt less calm than both other groups overall and less relaxed than drug naïve controls. Furthermore as to be expected,

all groups showed a decrease in worry post task. Analysis of the HADS showed no significant differences between groups on states of anxiety or depression.

Despite an absence of between group differences on behavioural measures the fNIRS data revealed several significant differences that are worthy of discussion. Ecstasy users displayed a significant reduction in oxy-Hb compared to both polydrug users and drug naïve controls at voxel 14. At voxels 2 and 16, ecstasy users had significantly less oxy-Hb relative to drug naïve controls. As such the results infer reduced activation of the right dorsolateral prefrontal cortex in ecstasy users compared to both control groups. Polydrug users displayed significant increases in deoxy-Hb compared to ecstasy users at voxels 2 and 14 and to both ecstasy users and drug naïve controls at V12. The results from the fNIRS data are contrary to expectation and are also in opposition to those observed from fNIRS data in Chapters 7 and 8. Although reductions in oxy-Hb have been associated with cognitive impairment in fNIRS studies previously (e.g. Ehlis *et al*., 2008; Herrmann *et al*., 2008), these are usually coupled with task performance deficits. As the ecstasy user group in this experiment performed to similar levels to controls, and given the interpretation of fNIRS results from Chapter 7 and 8, this does not seem an adequate explanation of the fNIRS results during multi-tasking.

One possible explanation for these findings could be that the individual tasks that comprise the multi-tasking paradigm are not executive tasks and perhaps do not load on the PFC. Indeed two of the component tasks are visual monitoring tasks, which may induce more activation of the visual cortex in the occipital lobe. Neuroimaging studies that have attempted to define the neural substrates of mental arithmetic, suggest a widespread network of activation that includes activation of the lateral prefrontal cortex, cingulate cortex, occipital cortex and of particular salience the parietal cortex (Kong *et al*., 2005). Indeed Grabner *et al.* (2007) observed that activation in the parietal cortex (particularly the left angular gyrus)

predicts mathematical competence using fMRI. Although the prefrontal cortex does appear to play a role in mathematical calculation (Burbaud *et al*., 1995), it is reported to lie in a circuit involving bilateral intraparietal, prefrontal and anterior cingulate components (Chochon *et al.*, 1999). The final component of the multi-tasking framework is a Stroop task which measures inhibition (see Chapter 3.2.2). Potentially the diversity of the tasks used would require widespread neuronal network activation; this may lead ecstasy users to reallocate resources to other brain regions that this task requires that are not measured by prefrontal fNIRS. If this is the case it may be possible that a reduction in oxy-Hb to the PFC reflects cognitive decline. For example, when resources are reallocated to other regions, the PFC shows a deficit in activation due to exhausted resources. However if this were the case it may be expected that performance on the subcomponent of the Stroop task would decline, which is not the case. So the results are difficult to interpret in terms of our hypothesis. However, it is noteworthy that 7 of the 8 participants' data that were excluded from analysis of the Stroop task module, due to incorrect interpretation of instructions were drug users (4 ecstasy users). It has been observed previously that ecstasy users make more errors when completing a web based questionnaire compared to other drug users and drug naïve controls (Rodgers *et al*., 2003). Therefore it is possible that there are deficits in the processing of instructional information associated with ecstasy use.

The results from analysis of salivary cortisol were more in line with our hypotheses. It was observed that ecstasy users displayed significant increases in cortisol levels compared to both other groups at time three of day one of the cortisol profiling protocol. Furthermore they exhibited greater cortisol upon waking (time 1) on day one of the cortisol profile compared to polydrug controls. These results reflect that ecstasy users show elevated basal cortisol levels compared to healthy controls, suggesting that MDMA use has adverse effects on the HPA-axis. This is in agreement with previous reports of acute, on drug elevation of cortisol level in

ecstasy users (Parrott *et al*., 2008), as well as longer lasting basal increases in plasma cortisol secretion observed in ecstasy users (Gerra *et al.,* 2003). Gerra *et al*., (2003) report a blunted cortisol response to stress in ecstasy users comparative to controls from plasma cortisol levels, the results from post task salivary cortisol analysis in our sample suggest that the current sample had steadily declining cortisol levels throughout the day. Perhaps the multi-tasking framework used in this thesis was not sufficiently stressful to observe elevated cortisol levels post task. Alternatively, perhaps the HPA-axis becomes exhausted, due to high basal levels of cortisol, and as such appears unresponsive to experimental stressors. This is a potential explanation of the current findings that is in concordance with suggestions by Gerra *et al*. (2003). In summary it appears that ecstasy users display increases in basal salivary cortisol levels, which reflects damage to the integrity of the HPA-axis. Repeated doses of the acute metabolic stressor MDMA (Parrott, 2006; Parrott *et al*., 2008), hyperstimulation (from crowding, intense lighting, heat) and prolonged physical activity (dancing), may have cumulative effects, resulting in chronic bioenergetic distress (Parrott, 2006; Parrott, 2009). Such effects may lead to long lasting alterations to neuroendocrine function, which is potentially what is being observed in the current ecstasy using sample.

Limitations:

Unlike the relatively "pure" MDMA user groups in the two studies by Halpern *et al.* (2004 & 2011)**,** the ecstasy user groups in this thesis tended to take several other drugs – in particular cannabis. Although attempts to control for this have been made with the addition of a polydrug control group in three out of the four experimental chapters, it was apparent that the ecstasy user group generally smoked more cannabis and consumed more cocaine than polydrug users in each case. This is problematic for our results as cocaine has been shown to have strong associations with deficits in inhibitory control (Fillmore & Rush, 2002). As such

any observed differences could still be attributed to the use of these other drugs, or indeed a synergistic effect of concomitant use of other drugs. Furthermore, in Chapter 6 there were 9 participants in the ecstasy user group who reported using ketamine in the last 30 days. This is potentially problematic for the interpretation of the results given the association between ketamine use and executive function deficits in humans (for a review see Morgan & Curran, 2006). Specifically, switching has been shown to be impaired in animals with ketamine exposure (Stoet & Snyder, 2006). The polydrug control group showed a diminished P3 response compared to drug naïve controls in several sites in the switching task, also in the semantic association task ecstasy users, although showing greater negativities in the N2 in the low association condition of the task, compared to drug naïve controls, were not significantly different to polydrug controls. Regression analyses were conducted to try and control for cannabis use (as this was the primary co-used substance in the ecstasy using samples). However statistical analysis and polydrug control groups cannot account for potentially additive effects that concurrent drug use may have. Perhaps it would be more accurate to call the observed effects "polydrug effects". With this in mind, it can also not be ruled out that premorbid factors do not predict drug use, and that such factors (for example differences in sensation seeking) contribute to the observed differences in the present thesis. In addition, the self-reporting of psychological state is potentially problematic and in future research, a structured psychiatric assessment may be more appropriate. Tobacco use was also not controlled for in this thesis, there has been previous research to suggest that tobacco smoking has an effect on EEG measures (Gilbert *et al.,* 2004; Illan & Polich, 2001). In particular abstinence from tobacco is associated with performance and activity decline that can last for up to 31 days (Gilbert *et al*., 2004). However, smokers were permitted to smoke tobacco on the day of testing so this is unlikely to have affected the results reported here. The quasi-experimental design employed in each study also means it cannot be ruled out that the

differences observed are not the result of factors other than drug use. Attempts have been made to control for many of these such as sleep patterns, sleepiness, fluid intelligence, age, state mood and residual intoxication of drugs. In most cases there were no between group differences on these measures. However in Chapter 7, ecstasy users were significantly older than both control groups and also reported being sleepier than drug naïve controls prior to testing. These factors were entered into the regression analyses for this chapter and did not predict significant amounts of variance in haemodynamic response in any case. Residual intoxication of alcohol was self-report, but in future studies it would be advantageous to verify this with a breathalyser to ensure no residual alcohol intoxication. Self-report measures for background drug use are also problematic, however because of the legal status of the drugs consumed, this remains the most appropriate measure of background drug use, and is also the most commonly used in this area of research (Fox *et al*., 2001; Montgomery *et al.*,2010). The purity of the tablets consumed by the current set of participants as well as the strength of the cannabis being consumed is questionable. However, Parrott (2004) reported that the purity of ecstasy tablets collected from amnesty bins in nightclubs in the UK is approaching 100%. However if this is not the case then this raises additional concerns over the magnitude of cognitive deficits incurred (Montgomery *et al.,* 2010). Furthermore although confirmation of drug abstinence (apart from cannabis) was sought through collecting urine samples from participants in every experiment, these have only been analysed from the participants in Chapter 6. This is due to the analysis being performed at NHS hospitals, and relying on the time of our collaborator. Thus confirmation of abstinence from illicit drug use (apart from cannabis) for 7 days prior to testing, again relies upon self-report for Chapters 7, 8 and 9. However many of the published research articles in this area do not report objective measures of drug use (Montgomery, Fisk, Newcombe & Murphy, 2005; Fisk & Montgomery 2009; Burgess *et al.,* 2011).

Implications:

There are several implications from the research conducted in this thesis, firstly, the neuroimaging results suggest that ecstasy users are employing compensatory mechanisms to make up for shortcomings in executive functioning. Neuronal activity aberrations are suggestive of potential serotonergic neurotoxicity due to repeated pharmacological interference to the serotonin system by administration of MDMA. It has been suggested that the evidence of compensatory mechanisms and increased effortful cognition predict future failures. This is in line with the suggestion that MDMA use potentially exacerbates cognitive ageing. Furthermore the atypicalities in processing observed in this thesis also reflect cognitive inflexibility. These have real world implications as cognitive flexibility is a desirable trait in every-day life. Serotonin is involved in the regulation of many psychobiological functions; as such degradation to the serotonin system from MDMA use has other psychobiological implications that stretch further than cognitive deficits. As already shown in this thesis, MDMA use may have adverse effects on neuroendocrine function. Neurohormonal activation has a role in moderating immune responses. Indeed increased basal cortisol levels reflect elevated levels of physiological stress. Such physiological stress can lead to exacerbation of infectious disease (Sapolsky, 1996). Furthermore pronounced and sustained load on the HPA-axis can increase incidence of depression (Wong *et al*., 2000). Work related stress, along with anxiety and depression accounted for the majority of sick days from work taken in 2011/2012 (Labour Force Survey for 2011/2012, HSE)**.** Indeed Connor (2004) has already reported MDMA's effects as a stressor on the immune system. Therefore findings of this thesis could be used for educational purposes and could inform prospective users or individuals who have used ecstasy in the past of the potential harms in terms of neurohormonal changes and cognitive function alterations before consideration of use.

The research from this thesis has implications for cognitive and psychobiological health and well-being. It contributes to the existing knowledge about MDMA's potential as a selective serotonergic neurotoxin; as such it can aid the current understanding and treatment of drug related disorders involving HPA axis dysfunction, and cognitive decline. The results are useful for health education and have potential use in harm reduction strategies and interventions for drug use disorders. This is of particular salience given the government's recent discussions about drug reclassification, information provided in this thesis could prove beneficial when assessing the relative harms of ecstasy for reclassification under the Misuse of Drugs Act (MDA).

Future Research:

There are a number of suggestions for future research that arise from the current thesis. Firstly, in Chapters 6 and 8 it was observed that ecstasy users show differences to controls in their neurophysiological response to inhibition. In both cases there were significant results from regression analyses to suggest that recency of use may play a role in this executive function. It would be interesting to conduct research into this function with ecstasy users that have been abstinent from drug use for a reasonably long period of time, for example 12 months. Hoshi *et al.,* (2007) conducted behavioural research into this area and observed former users to be unimpaired compared to current users. Morgan *et al*. (2002) also compared former users to current users and controls, observing little performance differences. However as has been shown in this thesis, neuroimaging techniques provide more sensitive measures of impairment and greater information about the nature of such impairments. Thus it would be beneficial to observe former users performance on inhibitory control relative to current users and controls in combination with fNIRS, EEG and fMRI. Indeed Reneman, Lavalaye *et al*. (2001) have reported that MDMA's neurotoxic effects on SERT may be reversible after

long periods of abstinence. A multi-faceted neuroimaging approach combining several methods with strict inclusion criteria for group assignment might help address the contribution to prolonged abstinence to this function.

In the switching tasks in chapters 6 and 8, there is evidence to suggest that cannabis may be an important factor in ecstasy-related neuronal activation differences for this function. Indeed the contribution on drug use besides ecstasy is always problematic for research in this area. Few studies have the purity of the ecstasy user group observed in Halpern *et al.*'s 2004 and 2011 studies. Concomitant drug use is at least partially controlled for in the literature with the inclusion of non-ecstasy polydrug users and various statistical controls. However, ideally future research should concentrate on sampling techniques that recruit participants that are ecstasy only users, cannabis only users etc. This may help aid the understanding of the relative contributions of each drug to potential cognitive deficits. That said, in the real-world environment of recreational drug use, where individuals are using ecstasy, it appears that use is frequently coupled with co-use of other drugs. As such the results from studies such as those in this thesis do offer validity in terms of understanding the nature of drug interactions and the effects arising from drug use that is commonplace in recreational environments.

The use of fNIRS in this thesis has been beneficial in aiding our understanding of PFC activation in executive functioning tasks, and MDMA related changes to haemodynamic responses in these areas. However this technology in the current thesis has been limited to the PFC only. The whole head fNIRS systems would provide more information about haemodynamic response across the cortex. This may be advantageous for gaining information about connections with other neural areas that may be involved in some working memory tasks. Indeed this may provide more information that is necessary to understand the results

from the multi-tasking neuronal response. To this end, it may also be beneficial to incorporate fMRI into some of the protocols in the current thesis. fNIRS is a valuable and upcoming portable technology that is easy to set up and is more robust to noise and movement artefacts than fMRI. However it does not have the spatial resolution to provide the rich information about specific neuronal areas showing activation during tasks across the whole brain that fMRI affords. fMRI has been employed in the past to observe ecstasy-related changes to neuronal activation during updating (Daumann, Fischermann, Heekeren *et al*., 2004), inhibitory control (Roberts & Garavan, 2010) and semantic recognition (Raj *et al*., 2010). However, future research could use this technology with greater task specificity such as that employed by Raj *et al.* (2010) and with control groups (including drug naïve, cannabis only, former drug users) that may provide more complete assessment of the executive functions used in all three of the aforementioned studies.

The interesting results that have been observed in this thesis from salivary cortisol levels in ecstasy users also have scope for future research. The long lasting effects of bioenergetic stress on the HPA axis could be explored by conducting diurnal cortisol profiles on participants who report taking ecstasy in environments with high levels of sensory stimulation (hot environments, crowding, and intense lighting) and prolonged dancing, and those who report taking the drug in more ambient environments with less physiological stress.

Although this thesis did attempt to control for a number of background factors that may influence results (intelligence, sleep, age etc.), it may be that there are other individual differences and lifestyle variables that have not been controlled for that may contribute to the effects observed. Future research should aim to focus on protective factors (such as that mentioned in the previous paragraph), to observe whether these may be useful in reducing harm when on drug. Regular breaks from dancing, adequate nutrition and more research into

the effects of bingeing and higher nightly doses may lead future research to produce neuroprotective strategies for drug users.

Thesis Summary:

This thesis sought to evaluate the neurophysiological response to executive functions in a sample of recreational ecstasy users. This was investigated using Miyake *et al.*'s (2000) conceptual framework of executive functioning with the additions made by Fisk and Sharp (2004) with EEG and fNIRS. It was found that ecstasy users show atypical processing in ERP components during inhibition, switching and access tasks that reflect cognitive impairment/compensatory mechanisms. Furthermore the haemodynamic response to each of the four proposed executive functions was altered in ecstasy users reflecting increased cognitive effort and recruitment of additional resources as a result of potential degradation to the serotonin system via MDMA related neurotoxicity. In most cases neuronal activation changes appear to be due to ecstasy. However cannabis use emerged as an important predictor of ERP amplitude and oxygenated haemoglobin increase during switching. The changes to neuronal activation reflect compensatory mechanisms/reallocation of cognitive resources to enable equivalent performance to controls in executive functioning as a whole.

There is also evidence of MDMA related alterations to HPA-axis function, whereby increased salivary cortisol levels in ecstasy users from diurnal cortisol profiles reflect elevated basal stress levels. The neurophysiological changes observed in this thesis suggest that ecstasy is damaging to the human brain. This is likely to be a result of damage to the serotonergic system. As such results from this thesis should be used to educate individuals considering using ecstasy.

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Appendices

Appendix 1

The table below shows results from regression analyses on voxels showing significant between group differences during the RLG task in Chapter 8. Cannabis use indices and ecstasy use indices are entered as predictors of oxy-Hb and deoxy-Hb increases from baseline (μmolar).

DV	IV	R^2	ΔR^2	F-change	B	SE	β
	Step 1						
4s V10 oxy	Cannabis frequency of use	0.12	0.03	1.29	0.22	0.26	0.20
	Lifetime dose (cannabis)				0.00	0.00	-0.18
	Last 30 days dose (cannabis)				0.01	0.02	0.09
4s V3 deoxy	Cannabis frequency of use	0.06	-0.03	0.65	0.08	0.19	0.09
	Lifetime dose (cannabis)				1.51	0.00	0.02
	Last 30 days dose (cannabis)				-0.01	0.01	-0.30
4s V4 deoxy	Cannabis frequency of use	0.07	-0.13	0.34	0.07	0.56	0.06
	Lifetime dose (cannabis)				0.00	0.00	0.46
	Last 30 days dose (cannabis)				-0.03	0.03	-0.79
4s V5 deoxy	Cannabis frequency of use	0.10	0.03	1.31	0.19	0.18	0.22
	Lifetime dose (cannabis)				1.94	0.00	0.03
	Last 30 days dose (cannabis)				-0.02	0.01	-0.47
4s V13 deoxy	Cannabis frequency of use	0.09	0.01	1.16	0.15	0.20	0.16
	Lifetime dose (cannabis)				0.00	0.00	0.19
	Last 30 days dose (cannabis)				-0.02	0.01	-0.48
4s V14 deoxy	Cannabis frequency of use	0.50	-0.04	0.56	-0.10	0.19	-0.10
	Lifetime dose (cannabis)				0.00	0.00	0.24
	Last 30 days dose (cannabis)				-0.02	0.01	-0.39

4s V15 deoxy	Cannabis frequency of use	0.50	-0.04	0.56	-0.27	0.43	-0.21
	Lifetime dose (cannabis)				0.00	0.00	0.19
	Last 30 days dose (cannabis)				-0.00	0.02	-0.03
4s V16 deoxy	Cannabis frequency of use	0.09	0.00	1.05	-0.08	0.22	-0.08
	Lifetime dose (cannabis)				0.00	0.00	0.36
	Last 30 days dose (cannabis)				-0.01	0.01	-0.25
2s V4 oxy	Cannabis frequency of use	0.43	0.30	3.47*	-0.40	0.35	-0.50
	Lifetime dose (cannabis)				0.00	0.00	0.59
	Last 30 days dose (cannabis)				0.01	0.02	0.39
2s V10 oxy	Cannabis frequency of use	0.09	-0.01	0.88	0.24	0.32	0.18
	Lifetime dose (cannabis)				-7.73	0.00	-0.06
	Last 30 days dose (cannabis)				0.01	0.02	0.08
2s V12 oxy	Cannabis frequency of use	0.09	-0.01	0.91	-0.09	0.20	-0.10
	Lifetime dose (cannabis)				6.04	0.00	0.08
	Last 30 days dose (cannabis)				0.01	0.01	0.23
2s V14 oxy	Cannabis frequency of use	0.07	-0.02	0.75	0.04	0.24	0.04
	Lifetime dose (cannabis)				0.00	0.00	0.15
	Last 30 days dose (cannabis)				0.01	0.02	0.10
2s V2 deoxy	Cannabis frequency of use	0.12	0.03	1.34	0.04	0.25	0.04
	Lifetime dose (cannabis)				8.30	0.00	0.09
	Last 30 days dose (cannabis)				-0.02	0.02	-0.41
2s V4 deoxy	Cannabis frequency of use	0.05	-0.15	0.26	-0.02	0.81	-0.01
	Lifetime dose (cannabis)				0.00	0.00	0.50
	Last 30 days dose (cannabis)				-0.03	0.04	-0.50

2s V11 deoxy	Cannabis frequency of use	0.04	-0.12	0.24	0.00	0.41	0.00
	Lifetime dose (cannabis)				0.00	0.00	0.32
	Last 30 days dose (cannabis)				-0.04	0.03	-0.57
2s V13 deoxy	Cannabis frequency of use	0.07	-0.02	0.82	0.23	0.27	0.19
	Lifetime dose (cannabis)				0.00	0.00	0.15
	Last 30 days dose (cannabis)				-0.02	0.02	-0.37
2s V14 deoxy	Cannabis frequency of use	0.03	-0.07	0.28	-0.01	0.21	-0.01
	Lifetime dose (cannabis)				0.00	0.00	0.24
	Last 30 days dose (cannabis)				-0.02	0.01	-0.31
2s V15 deoxy	Cannabis frequency of use	0.01	-0.08	0.11	0.16	0.93	0.06
	Lifetime dose (cannabis)				0.00	0.00	0.06
	Last 30 days dose (cannabis)				-0.02	0.04	-0.15
1s V12 oxy	Cannabis frequency of use	0.23	0.15	2.90*	0.07	0.19	0.07
	Lifetime dose (cannabis)				0.00	0.00	-0.17
	Last 30 days dose (cannabis)				0.02	0.01	0.39
1s V14 oxy	Cannabis frequency of use	0.18	0.11	2.38	0.11	0.21	0.11
	Lifetime dose (cannabis)				0.00	0.00	0.16
	Last 30 days dose (cannabis)				0.01	0.01	0.21
1s V2 deoxy	Cannabis frequency of use	0.10	0.01	1.12	-0.01	0.20	-0.01
	Lifetime dose (cannabis)				8.12	0.00	0.12
	Last 30 days dose (cannabis)				-0.01	0.01	-0.34
1s V3 deoxy	Cannabis frequency of use	0.03	-0.05	0.38	0.11	0.21	0.12
	Lifetime dose (cannabis)				-9.90	0.00	-0.01
	Last 30 days dose (cannabis)				-0.01	0.01	-0.16

1s V4 deoxy	Cannabis frequency of use	0.18	0.00	1.02	-0.52	0.57	-0.46
	Lifetime dose (cannabis)				0.00	0.00	0.65
	Last 30 days dose (cannabis)				-0.00	0.03	-0.12
1s V5 deoxy	Cannabis frequency of use	0.11	0.03	1.36	0.34	0.21	0.35
	Lifetime dose (cannabis)				4.46	0.00	0.05
	Last 30 days dose (cannabis)				-0.02	0.01	-0.45
1s V11 deoxy	Cannabis frequency of use	0.03	-0.13	0.21	0.01	0.33	0.01
	Lifetime dose (cannabis)				0.00	0.00	0.29
	Last 30 days dose (cannabis)				-0.03	0.02	-0.56
1s V13 deoxy	Cannabis frequency of use	0.07	-0.02	0.81	0.24	0.25	0.22
	Lifetime dose (cannabis)				0.00	0.00	0.20
	Last 30 days dose (cannabis)				-0.02	0.02	-0.37
1s V14 deoxy	Cannabis frequency of use	0.03	-0.06	0.34	-0.09	0.21	-0.09
	Lifetime dose (cannabis)				0.00	0.00	0.38
	Last 30 days dose (cannabis)				-0.01	0.01	0.25
1s V15 deoxy	Cannabis frequency of use	0.08	-0.01	0.87	-0.51	0.45	-0.37
	Lifetime dose (cannabis)				0.00	0.00	0.20
	Last 30 days dose (cannabis)				0.01	0.02	0.21
	Step 2						
4s V10 oxy	Ecstasy frequency of use	0.21	0.02	0.91	-0.01	0.92	-0.00
	Lifetime dose (tablets)				0.00	0.00	-0.07
	Last 30 days dose (tablets)				0.20	0.15	0.31
4s V3 deoxy	Ecstasy frequency of use	0.14	-0.03	0.99	0.16	0.69	0.05
	Lifetime dose (tablets)				0.00	0.00	0.28

	Last 30 days dose (tablets)				0.08	0.11	0.16
4s V4 deoxy	Ecstasy frequency of use	0.34	-0.03	1.47	-0.34	1.15	-0.10
	Lifetime dose (tablets)				0.00	0.00	0.38
	Last 30 days dose (tablets)				0.31	0.23	0.50
4s V5 deoxy	Ecstasy frequency of use	0.19	0.04	1.13	0.12	0.67	0.04
	Lifetime dose (tablets)				0.00	0.00	0.25
	Last 30 days dose (tablets)				0.10	0.12	0.20
4s V13 deoxy	Ecstasy frequency of use	0.16	-0.00	0.82	-0.03	0.73	-0.01
	Lifetime dose (tablets)				0.00	0.00	0.13
	Last 30 days dose (tablets)				0.13	0.12	0.25
4s V14 deoxy	Ecstasy frequency of use	0.44	0.32	6.66**	-1.63	0.69	-0.42*
	Lifetime dose (tablets)				9.37	0.00	0.04
	Last 30 days dose (tablets)				0.49	0.11	0.82**
4s V15 deoxy	Ecstasy frequency of use	0.09	-0.10	0.38	0.02	0.91	0.00
	Lifetime dose (tablets)				0.00	0.00	0.10
	Last 30 days dose (tablets)				0.11	0.15	0.18
4s V16 deoxy	Ecstasy frequency of use	0.16	-0.01	0.07	-0.45	0.78	-0.12
	Lifetime dose (tablets)				0.00	0.00	0.06
	Last 30 days dose (tablets)				0.19	0.13	0.34
2s V4 oxy	Ecstasy frequency of use	0.53	0.28	0.84	-0.87	0.72	-0.36
	Lifetime dose (tablets)				0.00	0.00	0.12
	Last 30 days dose (tablets)				0.22	0.14	0.47
2s V10 oxy	Ecstasy frequency of use	0.12	-0.09	0.36	0.64	1.17	0.13
	Lifetime dose (tablets)				0.00	0.00	-0.13

	Last 30 days dose (tablets)				0.06	0.19	0.08
2s V12 oxy	Ecstasy frequency of use	0.19	0.00	1.12	-0.39	0.74	-0.12
	Lifetime dose (tablets)				1.47	0.00	-0.01
	Last 30 days dose (tablets)				0.21	0.12	0.40
2s V14 oxy	Ecstasy frequency of use	0.14	-0.04	0.84	-0.41	0.88	-0.10
	Lifetime dose (tablets)				0.00	0.00	-0.16
	Last 30 days dose (tablets)				0.20	0.14	0.32
2s V2 deoxy	Ecstasy frequency of use	0.18	-0.00	0.70	-0.06	0.91	-0.01
	Lifetime dose (tablets)				0.00	0.00	0.24
	Last 30 days dose (tablets)				0.12	0.15	0.19
2s V4 deoxy	Ecstasy frequency of use	0.24	-0.18	0.87	0.06	1.66	0.01
	Lifetime dose (tablets)				0.00	0.00	0.30
	Last 30 days dose (tablets)				0.29	0.33	0.36
2s V11 deoxy	Ecstasy frequency of use	0.27	-0.03	1.55	3.12	2.63	0.36
	Lifetime dose (tablets)				0.00	0.00	0.31
	Last 30 days dose (tablets)				0.05	0.25	0.06
2s V13 deoxy	Ecstasy frequency of use	0.10	-0.07	0.37	-0.04	0.98	-0.01
	Lifetime dose (tablets)				0.00	0.00	0.13
	Last 30 days dose (tablets)				0.11	0.16	0.16
2s V14 deoxy	Ecstasy frequency of use	0.25	0.10	2.92	-1.31	0.76	-0.35
	Lifetime dose (tablets)				9.77	0.00	0.04
	Last 30 days dose (tablets)				0.36	0.12	0.62**
2s V15 deoxy	Ecstasy frequency of use	0.02	-0.18	0.12	0.11	1.95	0.01
	Lifetime dose (tablets)				0.00	0.00	0.08

	Last 30 days dose (tablets)				0.10	0.31	0.08
1s V12 oxy	Ecstasy frequency of use	0.39	0.25	2.25	-0.93	0.67	-0.27
	Lifetime dose (tablets)				6.03	0.00	0.03
	Last 30 days dose (tablets)				0.28	0.11	0.52*
1s V14 oxy	Ecstasy frequency of use	0.34	0.20	2.28*	-1.06	0.76	-0.27
	Lifetime dose (tablets)				0.00	0.00	-0.19
	Last 30 days dose (tablets)				0.30	0.12	0.49*
1s V2 deoxy	Ecstasy frequency of use	0.12	-0.07	0.22	0.07	0.73	0.02
	Lifetime dose (tablets)				0.00	0.00	0.18
	Last 30 days dose (tablets)				0.02	0.12	0.04
1s V3 deoxy	Ecstasy frequency of use	0.09	-0.09	0.66	-0.02	0.77	-0.01
	Lifetime dose (tablets)				0.00	0.00	0.29
	Last 30 days dose (tablets)				0.06	0.12	0.11
1s V4 deoxy	Ecstasy frequency of use	0.39	0.05	1.24	0.00	1.16	0.00
	Lifetime dose (tablets)				0.00	0.00	0.34
	Last 30 days dose (tablets)				0.24	0.23	0.37
1s V5 deoxy	Ecstasy frequency of use	0.15	-0.02	0.50	-0.14	0.78	-0.04
	Lifetime dose (tablets)				0.00	0.00	0.17
	Last 30 days dose (tablets)				0.10	0.12	0.18
1s V11 deoxy	Ecstasy frequency of use	0.29	0.01	1.80	1.94	2.08	0.28
	Lifetime dose (tablets)				0.00	0.00	0.40
	Last 30 days dose (tablets)				0.07	0.20	0.11
1s V13 deoxy	Ecstasy frequency of use	0.11	-0.07	0.46	-0.23	0.91	-0.06
	Lifetime dose (tablets)				0.00	0.00	0.09
	Last 30 days dose (tablets)				0.15	0.15	0.23

1s V14 deoxy	Ecstasy frequency of use	0.31	0.16	3.85	-1.44	0.77	-0.37
	Lifetime dose (tablets)				0.00	0.00	-0.09
	Last 30 days dose (tablets)				0.42	0.12	0.69**
1s V15 deoxy	Ecstasy frequency of use	0.11	-0.07	0.40	0.11	0.95	0.03
	Lifetime dose (tablets)				0.00	0.00	0.07
	Last 30 days dose (tablets)				0.11	0.15	0.17

*Indicates significance at the .05 level, and ** at the .01 level.

Appendix 2

The table below shows results from regression analyses on voxels showing significant between group differences during the CWFT in Chapter 8. Cannabis use indices and ecstasy use indices are entered as predictors of oxy-Hb and deoxy-Hb increases from baseline (μmolar).

DV	IV	R^2	ΔR^2	F-change	B	SE	β
	Step 1						
V2 animals oxy	Cannabis frequency of use	0.02	-0.07	0.22	0.17	0.27	0.14
	Lifetime dose (cannabis)				0.00	0.00	-0.11
	Last 30 days dose (cannabis)				-0.01	0.02	-0.08
V3 animals oxy	Cannabis frequency of use	0.10	0.02	1.28	0.37	0.18	0.40
	Lifetime dose (cannabis)				0.00	0.00	-0.29
	Last 30 days dose (cannabis)				-0.01	0.01	-0.19
V4 animals oxy	Cannabis frequency of use	0.13	-0.03	0.80	-0.05	0.49	-0.04
	Lifetime dose (cannabis)				0.00	0.00	0.54
	Last 30 days dose (cannabis)				-0.02	0.02	-0.38
V10 animals oxy	Cannabis frequency of use	0.15	0.06	1.70	0.47	0.34	0.32
	Lifetime dose (cannabis)				0.00	0.00	0.36
	Last 30 days dose (cannabis)				-0.04	0.03	-0.55
V2 animals deoxy	Cannabis frequency of use	0.03	-0.05	0.35	0.06	0.25	0.05
	Lifetime dose (cannabis)				0.00	0.00	-0.14
	Last 30 days dose (cannabis)				-0.00	0.02	-0.01
V2 “S” words oxy	Cannabis frequency of use	0.01	-0.07	0.16	0.00	0.28	0.00
	Lifetime dose (cannabis)				-4.06	0.00	-0.04
	Last 30 days dose (cannabis)				-0.00	0.02	-0.02

V4 “S” words oxy	Cannabis frequency of use	0.31	0.18	2.35	-0.34	0.48	-0.29
	Lifetime dose (cannabis)				0.00	0.00	0.91**
	Last 30 days dose (cannabis)				-0.02	0.02	-0.47
V10 “S” words oxy	Cannabis frequency of use	0.13	0.04	1.48	0.45	0.31	0.33
	Lifetime dose (cannabis)				0.00	0.00	0.30
	Last 30 days dose (cannabis)				-0.03	0.03	-0.46
V12 “S” words oxy	Cannabis frequency of use	0.30	0.23	4.31**	0.42	0.28	0.32
	Lifetime dose (cannabis)				0.00	0.00	-0.42
	Last 30 days dose (cannabis)				0.03	0.02	0.44
V2 “S” words deoxy	Cannabis frequency of use	0.03	-0.05	0.35	-0.11	0.31	-0.08
	Lifetime dose (cannabis)				0.00	0.00	-0.15
	Last 30 days dose (cannabis)				0.00	0.02	0.04
V2 “C” words oxy	Cannabis frequency of use	0.03	-0.06	0.32	-0.08	0.28	-0.07
	Lifetime dose (cannabis)				8.30	0.00	0.08
	Last 30 days dose (cannabis)				-0.01	0.02	-0.09
V3 “C” words oxy	Cannabis frequency of use	0.03	-0.06	0.33	0.01	0.20	0.01
	Lifetime dose (cannabis)				-1.41	0.00	-0.02
	Last 30 days dose (cannabis)				-0.00	0.01	-0.05
V4 “C” words oxy	Cannabis frequency of use	0.27	0.14	2.01	0.38	0.54	0.31
	Lifetime dose (cannabis)				0.00	0.00	0.94*
	Last 30 days dose (cannabis)				-0.06	0.03	-1.17*
V10 “C” words oxy	Cannabis frequency of use	0.08	-0.02	0.84	0.32	0.35	0.22
	Lifetime dose (cannabis)				0.00	0.00	0.26
	Last 30 days dose (cannabis)				-0.03	0.03	-0.36

V12 “C” words oxy	Cannabis frequency of use	0.21	0.13	2.71	0.26	0.27	0.21
	Lifetime dose (cannabis)				0.00	0.00	-0.18
	Last 30 days dose (cannabis)				0.02	0.02	0.34
	Step 2						
V2 animals oxy	Ecstasy frequency of use	0.12	-0.04	1.29	1.64	0.98	0.36
	Lifetime dose (ta-0.blets)				0.00	0.00	0.21
	Last 30 days dose (tablets)				-0.16	0.16	-0.23
V3 animals oxy	Ecstasy frequency of use	0.31	0.17	3.07	1.52	0.66	0.44*
	Lifetime dose (tablets)				0.00	0.00	0.37
	Last 30 days dose (tablets)				-0.15	0.11	-0.28
V4 animals oxy	Ecstasy frequency of use	0.52	0.30	3.58*	2.22	1.00	0.62*
	Lifetime dose (tablets)				0.00	0.00	0.46*
	Last 30 days dose (tablets)				-0.23	0.20	-0.35
V10 animals oxy	Ecstasy frequency of use	0.27	0.10	1.36	0.97	1.48	0.17
	Lifetime dose (tablets)				0.00	0.00	0.34
	Last 30 days dose (tablets)				-0.19	0.30	-0.18
V2 animals deoxy	Ecstasy frequency of use	0.15	-0.01	1.48	0.25	0.89	0.06
	Lifetime dose (tablets)				0.00	0.00	0.44
	Last 30 days dose (tablets)				0.03	0.14	0.04
V2 “S” words oxy	Ecstasy frequency of use	0.04	-0.14	0.34	0.30	1.03	0.07
	Lifetime dose (tablets)				0.00	0.00	0.22
	Last 30 days dose (tablets)				-0.02	0.16	-0.03
V4 “S” words oxy	Ecstasy frequency of use	0.52	0.30	1.94	-0.09	0.99	-0.03

	Lifetime dose (tablets)				0.00	0.00	0.42
	Last 30 days dose (tablets)				0.20	0.20	0.29
V10 “S” words oxy	Ecstasy frequency of use	0.23	0.05	1.11	-0.42	1.37	-0.08
	Lifetime dose (tablets)				0.00	0.00	0.33
	Last 30 days dose (tablets)				0.10	0.28	0.10
V12 “S” words oxy	Ecstasy frequency of use	0.31	0.16	0.22	-0.16	1.23	-0.03
	Lifetime dose (tablets)				0.00	0.00	0.17
	Last 30 days dose (tablets)				0.00	0.24	0.00
V2 “S” words deoxy	Ecstasy frequency of use	0.14	-0.02	1.38	-0.02	1.13	-0.00
	Lifetime dose (tablets)				0.00	0.00	0.44
	Last 30 days dose (tablets)				0.05	0.18	0.06
V2 “C” words oxy	Ecstasy frequency of use	0.04	-0.14	0.19	0.03	1.00	0.01
	Lifetime dose (tablets)				0.00	0.00	0.14
	Last 30 days dose (tablets)				0.05	0.16	0.07
V3 “C” words oxy	Ecstasy frequency of use	0.27	0.13	3.36	1.72	0.71	0.48*
	Lifetime dose (tablets)				0.00	0.00	0.29
	Last 30 days dose (tablets)				-0.05	0.11	-0.09
V4 “C” words oxy	Ecstasy frequency of use	0.46	0.22	1.53	-0.25	1.10	-0.07
	Lifetime dose (tablets)				0.00	0.00	0.33
	Last 30 days dose (tablets)				0.27	0.22	0.39
V10 “C” words oxy	Ecstasy frequency of use	0.17	-0.02	0.95	-0.45	1.54	-0.08
	Lifetime dose (tablets)				0.00	0.00	0.30
	Last 30 days dose (tablets)				0.16	0.31	0.16
V12 “C” words oxy	Ecstasy frequency of use	0.25	0.09	0.48	0.21	1.20	0.04

Lifetime dose (tablets)	0.00	0.00	0.21
Last 30 days dose (tablets)	0.06	0.23	0.07

*Indicates significance at the .05 level, and ** at the .01 level.

Original Paper

Electrophysiological indices of response inhibition in human polydrug users

Psychopharm

Journal of Psychopharmacology
27(9) 779–789

DOI: 10.1177/0269881113492899
jop.sagepub.com
SAGE

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Abstract

Previous research in ecstasy users suggests impairment of various executive functions. In general, the executive function of response inhibition appears unaffected by ecstasy use. Nonetheless, it remains a possibility that cognitive tasks alone are not sensitive enough to pick up subtle changes in function. The current study sought to investigate behavioural measures of response inhibition and their electrophysiological correlates in drug users. Twenty ecstasy polydrug users, 20 non-ecstasy polydrug users and 20 drug naïve controls were recruited. Participants completed questionnaires about their background drug use, sleep quality, fluid intelligence and mood state. Each individual also completed a Go/NoGo response inhibition task whilst electroencephalography (EEG) measures were recorded. Analysis of variance (ANOVA) revealed that there were no between-group differences on the behavioural measure of response inhibition. Multivariate analysis of variance (MANOVA) revealed no main effect of group across midline electrodes for the P3, N2 and P2 components. Univariate ANOVA revealed significant between-group differences in the P2 component with the ecstasy user group having a significantly higher mean amplitude than drug naïve controls at two midline frontal electrodes: at Fz and significantly higher mean amplitude than both control groups at FCz. The present study provides evidence of atypical early processing in ecstasy users that is suggestive of compensatory mechanisms ameliorating any behavioural differences.

Keywords

Ecstasy, memory, executive function

Introduction

Use of the recreational drug 3, 4- methylendioxymethamphetamine (MDMA; 'ecstasy') has remained stable over recent years despite growing concern about long-term effects of the drug. The stability in use may reflect a decrease in purity and a rise in the use of comparable legal highs (non-illicit amphetamine analogues and psychedelics). However, recent media reports suggest that the purity of the drug is increasing and with the change in legal status of legal highs (according to UK law most ecstasy substitutes have been reclassified as class B illicit drugs), ecstasy remains a public health concern.

The acute psychological and physiological effects of ecstasy are primarily caused by serotonin (5HT) and dopamine agonism amongst other neurotransmitters (McDowell and Kleber, 1994). During acute regular use it may be expected that ecstasy causes downregulation of serotonin receptors as seen in animal models (e.g. Reneman et al., 2002). However, following periods of chronic use compensatory upregulation of 5-HT_{2A} receptors is seen in the human brain suggesting an attempt to maintain homeostasis after neurotoxicity (Di Iorio et al., 2012; Reneman et al., 2002; Urban et al., 2012). Such neurotoxicity has been observed in several animal studies (Molliver et al., 1990; Ricaurte et al., 1988), and in humans reductions in 5-HT, 5-hydroxyindoleacetic acid (5HIAA), tryptophan hydroxylase and loss of 5-HT reuptake sites and neuronal transporters are documented (Parrott, 2002). Research has observed 5-HT system impairments in currently abstinent ecstasy users (Gerra et al., 2000), with neuroendocrine alterations (responses to cortisol and prolactin) being attributed to use of MDMA. In addition, several studies have reported degradation of the serotonin system in abstinent users with decreased cortical serotonin binding compared to nonusers (Erritzoe et al., 2011; Kish et al., 2010; McCann et al., 2008). Given the involvement of serotonin in the regulation of several physiological functions including sleep, mood and cognition, it is reasonable to postulate that depletion of serotonin in certain brain regions may account for ecstasy-related disturbances in mood and cognition (Montgomery et al., 2005a).

Areas that are involved in working memory such as the dorsolateral prefrontal cortex are richly innervated with 5-HT receptors: therefore degradation to the serotonergic system via ecstasy use could lead to deficits in cognitive processes maintained by these forebrain structures. Significant deficits have been observed in ecstasy users compared to nonusers in components of working memory such as visuospatial working memory span (Wareing et al., 2004), access to semantic memory and memory updating (Fisk et al., 2004; Montgomery et al., 2005b). Furthermore, ecstasy users perform poorly in information processing tasks when cognitive demand is high (Wareing et al., 2000). It has been suggested (Cole et al., 2002) that sleep (among other possible lifestyle variables), or lack of it, may exacerbate or indeed be causal of cognitive deficits observed in ecstasy-using

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populations. Furthermore, several characteristics of sleep, such as sleep quality, length of sleep (hours) and related changes in alertness have been reported to be altered in ecstasy users relative to controls (Allen et al., 1993). However such deficits appear to have little mediating effect on ecstasy-related cognitive deficits (Montgomery et al., 2010) and more recently Blagrove et al. (2011) have found no evidence for ecstasy impairing the memory consolidation phase of sleep.

When looking at executive functioning in ecstasy users, some functions appear to be more affected than others, with the updating function of the executive being particularly susceptible to ecstasy use (Montgomery et al., 2005a; Montgomery and Fisk, 2008) along with access to long term memory (Montgomery et al., 2005a). Inhibitory control and set switching appear to be more robust to ecstasy-related deficits: however recent research in ecstasy users suggests that even in the absence of behavioural differences, ecstasy users may show electrophysiological differences related to task demands (Burgess et al., 2011). Such paradoxical effects can be seen in the implicit cognition literature where heavy drug users can show altered electrophysiological responses to drug stimuli in the absence of behavioural differences (Petit et al., 2012). Consequently, participants in previous studies reporting null results on behavioural measurements may not necessarily be exhibiting 'normal' functioning. The present study therefore sought to assess response inhibition in ecstasy users through behavioural and electrophysiological assessments of performance.

Inhibitory control, or the inhibition of prepotent or dominant responses, has been assessed in ecstasy users previously. The Stroop task has been used in several studies to observe whether ecstasy use affects inhibition performance (Back-Madruga et al., 2004; Gouzoulis-Mayfrank et al., 2000; Morgan et al., 2002), with all studies reporting no ecstasy-related impairment. Wareing et al. (2000) employed random letter generation to assess inhibition in ecstasy users and did observe performance deficits in ecstasy users compared to nonusers. However, further studies from the same laboratory (Fisk et al., 2004) did not replicate this. A review by Murphy et al. (2009) stipulated that the literature on inhibition in ecstasy users was unclear, although there is little evidence to suggest ecstasy-related impairments here. Furthermore, any perceived impairment is often obscured by confounding variables such as polydrug use and although the use of analysis of covariance (ANCOVA) and regression are usually employed to statistically control for this, the majority of findings in the literature need to be interpreted with some degree of caution. Previous studies using a cued Go/NoGo task with ecstasy users (e.g. Gouzoulis-Mayfrank et al., 2003), have observed little difference in performance on the task between nonusers, moderate users and heavy users. However, it has been suggested that 5-HT depletion, as well as impaired executive functions may play a role in inhibitory control (Morgan, 2006). One study that has been conducted on ecstasy users with minimal exposure to other drugs (Halpern et al., 2004) reported that heavy use of MDMA led to notable impairments in inhibition and impulsivity.

Although much of the research on behavioural tasks assessing inhibitory control in ecstasy users has provided inconclusive evidence, perhaps such cases where no differences have been observed can be attributed to compensatory mechanisms. Various mental strategies could be compensating for the more commonly-used areas for inhibitory control that result in undetectable differences behaviourally. This has been observed in cannabis users previously, whereby in a task assessing spatial working memory, behavioural measures indicate that the processes involved are intact. However, analysis of regional brain activity using functional magnetic resonance imaging (fMRI) suggests that there is an increase in activation in regions usually involved in spatial working memory tasks as well as additional activation in regions not normally associated with this type of task (Kanayama et al., 2004). Similarly, Jager et al. (2006) observed that cannabis users showed alterations to left superior parietal cortex activity, from analysing fMRI data, despite equivalent performance to controls on a working memory task. Both of the preceding studies suggest that drug-related deficits in cognition could be compensated by differences in brain activity during performance.

Neuroimaging techniques such as electroencephalography (EEG) may be useful in providing a clearer indication of possible alterations of normal cognitive functioning. Indeed, such techniques are used in other clinical samples. For example, in patients with Alzheimer's disease, neuroimaging shows that patients exhibit increases in prefrontal activity in comparison to controls during executive function tasks. Saykin et al. (1998) observed that Alzheimer's patients displayed additional activation in frontal regions which they postulated reflects recruitment of additional resources from local and remote regions when conducting a semantic memory task. Moreover, Woodard et al. (1998) observed that on tasks that require rehearsing list information, Alzheimer's patients would display a shift in processing resources recruited from more anterior regions as cognitive load increases. This was interpreted as recruitment of additional resources due to increased demand on the frontal cortex. Compensatory reallocation in Alzheimer's disease patients was investigated further by Grady et al. (2003). Using positron emission tomography (PET) they observed that patients employed a unique network of resources in the DLPFC compared to controls, which they infer as being evidence for additional/compensatory neural mechanisms being recruited. Such resources facilitate performance by supplementing the degraded primary neuronal pathways involved in executive functioning.

In event related potential (ERP) research, cognitive impairment is associated with alterations to the P3 amplitude or latency, due to the P3 being involved in processing of stimuli. Due to the Go/NoGo task requiring continuous attention to the stimuli in order to respond to a stimulus (Go) or to withhold/inhibit a response (NoGo), it is useful for measuring processing and attentional capacity in ERPs (Smith et al., 2004). The P3 component, although a significant component in many cognitive tasks due to its involvement in attentional processing, does not appear to have a consistent role in response inhibition. This is possibly due to this component occurring relatively late in terms of the stages of processing and therefore perhaps not in the initial early inhibition processes. The N2 component is observed to be involved in inhibition as this component has been suggested to reflect stimulus discrimination (Ritter et al., 1982) and is therefore an important measure of response inhibition. Kok and colleagues (2004) suggest the N2 component shows greater amplitude in trials where inhibition of response is required (no go) than no inhibition (go) trials. Moreover amplitude of N2 is more prominent in unsuccessful inhibition trials. The N2 component is associated with errors (i.e. 'error negativity' or Ne), and is sensitive to monitoring errors. This has been suggested to be a product of activity in medial frontal regions such as the anterior cingulate (Bekker et al., 2005). The P2 wave can be observed at anterior and central sites, and elicits a larger response to simple target features that are relatively infrequent (Luck and

Hillyard, 1994). This component precedes the N2 and is suggested to be involved in the initial inhibition from further processing in target stimuli (Hansen and Hillyard, 1998).

EEG studies in ecstasy users

Differences between ecstasy users and controls have been observed in P3 components. Casco et al. (2005) observed a reduction in P3 amplitude in both heavy and moderate ecstasy user groups compared to controls in visual evoked potentials (VEPs) pertaining to a simple discrimination task, though no differences in latency were observed. However Mejias et al. (2005) report longer P3 latencies for detection of target stimuli in a visual oddball task, suggesting reduced cognitive processing. De Sola et al. (2008) assessed the relationship between cognitive function in ecstasy users and P3 ERPs. Here a more predictable difference between ecstasy users and healthy controls was observed with ecstasy users showing a negatively correlated latency in P3s and semantic word fluency and verbal memory. Furthermore, a reduced P3 amplitude was also observed in ecstasy polydrug users compared to non-drug controls and cannabis users, although this was non-significant. Although, the delayed latency in cognitive processing was consistent with other behavioural studies of ecstasy users, the P3 ERPs still fell within the normal range and thus failed to reflect electrophysiological differences in cognitive processing. Despite this, sub-clinical deficits are often observed so further investigation is warranted.

More recently, Burgess et al. (2011) looked at ERPs as evidence for selective impairment of verbal recollection in currently abstinent recreational MDMA/polydrug users. Interestingly, there appeared to be no significant differences between ecstasy users, polydrug controls and drug naïve controls on the behavioural tasks (memory tasks which involved recognition of words and faces). However, the ecstasy user group showed attenuation of late positivity over left parietal scalp sites, which is a component associated with the memory process of recollection. The finding of ecstasy users showing a durable abnormality in this ERP component exemplifies how EEG is a much more sensitive measure of cognitive impairment than behavioural measures alone. This point is further elucidated by Nulsen et al. (2011) wherein ecstasy users displayed alternative patterns of activity in ERPs compared to drug naïve and polydrug controls in short term and working memory tasks, despite no significant behavioural differences.

The aim of the current study was to observe whether there are any behavioural or electrophysiological differences between ecstasy users and controls in a task measuring inhibitory control (Go/NoGo). In view of the previous literature it is predicted that any behavioural differences will be negligible, however observable differences in components of the elicited ERPs are predicted in line with compensatory mechanisms. More specifically, it is envisaged that ecstasy polydrug users in particular will differ from both controls and non-ecstasy polydrug users. As such, this study aims to characterise the nature of ecstasy's effects on cognitive processes involved in inhibition of a response.

Method

Design

In all analyses, the between-groups factor was drug user group with three levels (ecstasy user, non-ecstasy polydrug user and drug naïve controls). Univariate ANOVA was conducted on the behavioural data with the composite scores on the Go/NoGo (NoGo errors) as the dependent variable. ERP data was analysed using multivariate analysis of variance (MANOVA) with drug user group as the between-subjects factor and mean amplitude of the three ERP components at electrode sites Fz (frontal midline), FCz (frontal central midline), Cz (central midline), CPz (central posterior midline) and Pz (posterior midline) as the dependent variables.

Participants

Twenty ecstasy users (mean age=23.95 years, standard deviation (SD)=2.50, 10=male), 20 non-drug user controls (mean age=23.10 years, SD=2.94, 7=male) and 20 non-ecstasy drug user controls (mean age=22.58 years, SD=3.45, 9=male) were recruited via direct approach to university students, and the snowball technique (Solowij et al., 1992).

For inclusion in the study, participants had to be aged between 18–29 years and not have any neurological impairment. For inclusion in the ecstasy user group, participants had to have taken ecstasy/MDMA on five or more occasions. Indices of ecstasy use were as follows: total lifetime dose 177.65 tablets±301.73; mean amount used in the last 30 days 0.6 tablets±2.26, and frequency of use 0.24 times/week±0.42. Furthermore, for inclusion in both control groups participants must have never used ecstasy/MDMA, however all other illicit substances were permitted for the non-ecstasy poly drug user control group.

All participants were asked to abstain from consuming ecstasy for a minimum of seven days prior to testing and urine samples were collected upon arrival to the lab to be sent away for urinary analysis of metabolites, to ensure abstinence had occurred. Participants were also requested to abstain from use of other illicit drugs for a minimum of 24 h prior to participating and ideally seven days.

Materials

Several questionnaires were issued to participants upon entering the lab. These included a background drug use questionnaire, which provides the researcher with indices of drug use patterns and other lifestyle variables. In this questionnaire comprehensive details of ecstasy use as well as other illicit drug use are requested, such as first and last drug use, patterns of drug use, frequencies and doses over time. Using a method employed by Montgomery et al. (2005b), estimates of total lifetime drug use of each drug were calculated. Totals for last 30 days drug use as well as weekly drug use estimates were also calculated. This questionnaire also sought information about health, age, years of education and changes to mood and cognition amongst other lifestyle variables.

Measures of sleep quality

Several questionnaires investigating sleep quality and alertness were employed to investigate any possible relationship between sleep quality and cognition. These include a sleep quality questionnaire, exploring typical quantities of sleep (how many hours slept typically, how many hours over the last three nights) and level of quality of sleep. The Epworth Sleepiness Scale (ESS; Johns, 1991), explores the chances of dozing or falling asleep in

various situations. A high total score here is indicative of increased subjective daytime sleepiness. The Morningness-Eveningness Questionnaire (MEQ; Terman et al., 2001) is a self assessment of morningness-eveningness in human circadian rhythms (originally developed by Horne and Östberg, 1976). A high score on this questionnaire is indicative of a morning type person and a low score is indicative of an evening type person. Finally the Karolinska Sleepiness Scale (Akerstedt and Gillberg, 1990), is a self assessment of sleepiness at the current moment in time, therefore this can be administered at different time points of the experiment to assess sleepiness,

State mood

State anxiety, arousal and depression were measured using scales devised by Fisk and Warr (1996). Participants were required to rate on a five-point Likert scale from 1=not at all, to 5=extremely, how they were feeling at the time of testing. A high score on each subscale indicates increased hedonic tone/anxiety/arousal.

North American Space Agency-Task Load Index (NASA-TLX, Hart and Staveland, 1988)

This is a multi-dimensional scale, consisting of six sub-scales (mental demand, physical demand, temporal demand, personal performance rating, effort and frustration). Participants are required to place a mark on a 100 mm line, indicating where they perceive their demand to be on the scale. These are administered to observe whether there are any differences between ecstasy users and non users in demand perceived by the participant as it has been suggested that ecstasy users may be more susceptible to stress than nonusers (Wetherell et al., 2012).

Inhibitory control

The Go/NoGo task is frequently used in combination with EEG to assess inhibitory control (Gamma et al., 2005; Kok, 1986; Oddy and Barry, 2009). Here, in a simplified version of the task participants are required to 'Go' (press the space bar) when an X appears on the screen: however, they are to inhibit their response 'NoGo', when any other letter appears (W, Y or Z). The task is designed such that 'X' appears 75% of the time and the 'NoGo' letters appear only 25% of the time. This is so that the task builds up a pre-potent response to 'Go'. Furthermore, the first block of the task has 'X' appearing 100% of the time, again to build up a pre-potent/dominant response which participants are required to inhibit. The task therefore comprises of two blocks; a practise block with 60 'Go' trails, followed by an interval and then a larger main block whereby participants are required to attend to 240 trials (180 Go/60 NoGo) lasting a total of approximately 15 min. The task has an inter-trial interval of 1.5 sec and participants had an epoch of 2.5 sec from stimulus onset to respond. Participants were instructed to respond as quickly and as accurately as possible.

Equipment

EEG was recorded using a 64 channel Biosemi Ag-AgCl active-two electrode system (Biosemi B.V., Amsterdam, Netherlands) with pin type electrodes mounted in a stretch-lycra headcap (Biosemi). Electrodes were positioned according to the international 10–20 system. Electrical activity was recorded from the following sites: frontal (FPz, FP1, FP2), anterior-frontal (AFz, AF3, AF4, AF7, AF8), frontal (Fz, F1, F2, F3, F4, F5, F6, F7, F8), frontocentral (FCz, FC1, FC2, FC3, FC4, FC5, FC6), central (Cz, C1, C2, C3, C4, C5, C6), temporal (FT7, FT8, T7, T8, TP7, TP8), parietocentral (CPz, CP1, CP2, CP3, CP4, CP5, CP6), parietal (Pz, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10), occipitoparietal (POz, PO3, PO4, PO7, PO8) and occipital (Oz, O1, O2, Iz). Sigma electrolyte gel was used to ensure contact between scalp and electrodes. Vertical and horizontal electro-occulograms (EOGs) were recorded using bipolar, flat Ag-ACl electrodes positioned above and below the left eye as well as to the outer side of each eye. Data was digitised at a sampling rate of 512 Hz and no filters were applied online so that the data could be visually inspected for noise and offline filtering could be performed.

Procedure

Testing sessions commenced at 0930 or 1330, and equal numbers of participants from each condition were tested in the morning as were in the afternoon. Upon entering the laboratory, participants were given a brief description of the experiment and written consent was obtained. Following this, participants were required to give a urine sample. The urine sample was frozen at –25°C and later transported to the clinical laboratories for analysis. First, participants were required to fill out the battery of questionnaires whilst their head circumference and other details were measured, and an electrode cap and electrodes were fitted. The questionnaires were administered in the following order: Background drug use questionnaire, MEQ, sleep quality questionnaire, mood scale, ESS, Karolinska Sleepiness Scale (pre-test) and fluid intelligence was assessed using Raven's Progressive Matrices (Raven et al., 1998). Following completion of these questionnaires, providing the EEG setup was correct and actiview running, the computerised task was completed on a desktop computer running Inquisit version 3.0.6.0 (Millisecond software, 2011). The NASA-TLX questionnaire was completed after the Go/NoGo task. Upon completion of the tasks a final KSS (after) was administered. Finally participants were fully debriefed and paid £20 in store vouchers. The study was approved by the Ethics Committee of Liverpool John Moores University, and was administered in accordance with the ethical guidelines of the British Psychological Society.

EEG analysis

The EEG data was analysed using brain electrical source analysis (BESA) 5.3 (MEGIS software GmbH, Gräfelfing, Germany). All recordings were visually analysed offline, using high and low pass filters of 0.1 Hz and 40 Hz respectively. Any channels judged to be bad (for example noisy data or many motion artefacts) were replaced by interpolation and all data were EOG-corrected using BESAs primary components analysis (PCA)-based algorithm. All trials judged to be bad after this point were discarded. EEG was segmented into epochs from –500 to 1000 ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (e.g. correct 'Go' responses, correct 'NoGo' responses and incorrect 'NoGo'

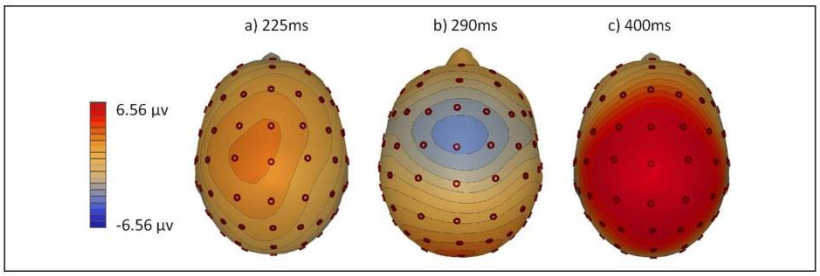

Figure 1. Topographies at midpoints for each component (P2, N2 and P3).
Shows the topographies for the central point of each component, note that this is from grand averages of each group combined. (a) shows a positivity in the P2 component that is clustered around the midline electrodes, (b) shows a negativity in N2 similarly in anterior midline electrodes and (c) shows a strong positivity in the P3 component that has a wide spread of activity that appears to peak at central electrodes.

responses in the Go/NoGo task) could be generated for each individual. Only ERPs for correct responses on the 'NoGo' condition were included in the subsequent analysis. There were 240 trials in the main block of the task, 60 of which were 'NoGo' trials. The mean number of good 'NoGo' trials retained for grand averaging per subject was 51.92 (average of 13.5% rejected trials), after rejecting incorrect trials (5%) and those containing artefacts (8.5%). Grand averages were made for each group (ecstasy user, polydrug user and drug naïve) on each task condition (correct 'Go' responses, correct 'NoGo' responses). The overall P3 response was defined as the mean amplitude between 352 and 452 ms. This window was centred on the positive peak latency and the duration was chosen due to this epoch containing the majority of positive activity for all conditions by observing topographic maps (see Figure 1). Midline electrode activity was obtained in this epoch from electrodes Fz, FCz, Cz, CPz and Pz, as much of the activity could be observed in these sites as well as these midline electrodes being commonly used for this task in the literature (Jonkman 2006; Kato et al., 2009). In addition further components were analysed for between-group differences, including the N2 and P2 components. The N2 (previously observed to be important in inhibitory control tasks of this type) of subjects in response to the inhibitory condition, was defined as the mean amplitude 260 and 330 ms, this epoch was based around the mean local negative peak at midline sites and encompassed the majority of negative activity over all three conditions. The P2 epoch was obtained from using a small, 50 ms epoch (200–250 ms) based around the positive peak from the grand averages of all conditions, directly preceding the N2.

Urinary analysis

Frozen urine samples were delivered to University Hospital Aintree (NHS) and were analysed using solid phase extraction (mixed mode phase) followed by reverse phase high performance liquid chromatography tandem mass spectrometry (HPLC MS/MS) detection using both positive and negative ion multiple reaction monitoring (MRM). Urine specimens were tested for the synthetic cannabinoids (JWH-018, JWH-073, JWH-250, JWH-398, JWH-122, JWH-019, AM-694, WIN 48098 and WIN-55212-2), as well as the 'designer' drugs 'mephedrone', 'methylone' (bk-MDMA), or 'butylone' (bk-MBDB), or 'methedrone' (bk-PMMA), 1-benzylpiperazine, trifluoromethylphenylpiperazine (TFMPP), meta-Chlorophenylpiperazine (mCPP), and methylenedioxypyrovalerone (MDPV). In addition they were tested for a series of 12 piperazine compounds, 4 β-keto amphetamines, a series of 11 methcathinone compounds, 4-fluoroamphetamine, bupropion and the hallucinogenic amphetamines: DOB ('bromo-STP' or 'brolamphetamine'), DOC and DOI and 'traditional' drugs of abuse: amphetamine(s) including MDMA, MDA. and MDEA, barbiturates, benzodiazepines, tetrahydrocannabinol (THC), and cannabinoids, buprenorphine, cocaine and metabolites, methadone and metabolites, opiates and opioids (morphine, codeine, dihydrocodeine, tramadol, d-propoxyphene, oxymorphone and oxycodone), lysergic acid diethylamide (LSD), gammahydroxybutyrate (GHB), (and the lactone precursor), psilocybin, ketamine and methaqualone.

Statistical analysis

Data were analysed using MANOVA with drug user group as the between-subjects factor and the mean amplitudes at the five midline electrodes observed (Fz, FCz, Cz, CPz and Pz) as the dependent variables. Any significant effects between groups or electrodes were further analysed with a Tukey HSD test, to observe pairwise differences.

Results

Socio-demographic information about the participants, anxiety, depression and arousal scores from the mood scale and sleep measures are shown in Table 1. Indices of other drug and alcohol use are displayed in Table 2.

One way analysis of variance (ANOVA) revealed that there were no significant between-group differences on measures such as age, average hours sleep per night, total score on the ESS, MEQ total score, post test Karolinska Sleepiness Scale, levels of arousal, depression and anxiety or total score on Ravens Progressive Matrices. However there were between-group differences in the pre-testing Karolinska Sleepiness Scale (i.e. how sleepy the participants felt before the test battery) $F(2, 56)=3.78$, $p=0.03$, post-hoc Tukey's test revealed that the ecstasy polydrug users felt significantly more sleepy prior to testing than the polydrug control group ($p=0.03$) but not the drug naïve control group.

Table 1. Indices of sleep quality, fluid intelligence and socio-demographic variables.

Males: *n*, %	Ecstasy users		Non-ecstasy drug users		Drug naïve controls	
	10 (50)		9 (45)		7 (35)	
	Mean	SD	Mean	SD	Mean	SD
Age	23.94	2.50	22.58	3.45	23.10	2.94
University degree; *n* (%)	14 (70)		12 (60)		11 (55)	
Employment status						
Student; *n* (%)	12 (60)		14 (70)		17 (85)	
Employed; *n* (%)	4 (20)		4 (20)		3 (15)	
Unemployed; *n* (%)	4 (20)		2 (10)		0 (0)	
Ravens Progressive Matrices (maximum 60)	48.68	5.96	48.35	5.83	51.35	5.01
Sleep; hours/night	7.13	1.91	7.8	1.39	7.05	1.16
ESS; score (maximum 24)	6.5	3.3	6.7	3.15	6.5	3.32
KSS before	5.05	1.93	3.75	1.48	4.79	1.23
KSS after	6.53	2.03	5.85	1.53	6.56	1.46
MEQ total	42.10	10.15	45.70	9.40	47.90	8.30
Mood anxiety	11.4	4.08	12.44	2.18	11.75	2.12
Mood depression	13.1	3.91	12.61	2.4	12.1	3.14
Mood arousal	19.7	4.54	20.5	3.68 18	20.1	3.02

ESS: Epworth Sleepiness Scale; KSS: Karolinska Sleepiness Scale; MEQ: Morningness-Eveningness Questionnaire; SD: standard deviation.

Table 2. Indices of other drug use.

	Ecstasy users			Non-ecstasy drug users			Drug naïve controls		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Cannabis									
Frequency (times/wk)	2.67	3.24	12	0.95	1.9	13	–	–	–
Last 30 days (joints)	32.77	53.75	15	6.09	15.34	17	–	–	–
Total use (joints)	5057.88	7504.30	16	1091.71	2531.65	19	–	–	–
Cocaine									
Frequency (times/wk)	0.15	0.14	11	0.27	0.34	2	–	–	–
Last 30 days (lines)	0.4	1.12	15	1.60	3.58	5	–	–	–
Total use (lines)	813.97	1940.19	16	107.30	208.43	5	–	–	–
Ketamine									
Frequency (times/wk)	0.26	0.42	5	0.02	–	1	–	–	–
Last 30 days use (grams)	1	2.65	9	–	–	–	–	–	–
Total use (grams)	31.26	70.61	11	1.13	1.62	3	–	–	–
Alcohol units per wk	15.33	15.29	20	10.53	8.37	20	9.93	11.58	20

SD: standard deviation.

Use of *t*-tests between the ecstasy user group and the polydrug non-ecstasy group revealed that the ecstasy user group had a significantly larger lifetime total of cannabis joints smoked (5057.88 ±7504.30) than the non ecstasy drug users (1091.71± 531.65) $t(17.88)=2.02$, $p=0.03$ (Levene's test was significant so degrees of freedom have been adjusted accordingly). The ecstasy users had also smoked significantly more joints within the last 30 days (32.77±53.75 compared to 6.09 ±15.34) $t(16.01)=1.86$, $p=0.05$. There were however no differences between these two groups on other drug intake variables. However, as can be seen from the table, the ecstasy user group can be described as polydrug users.

Urinary analysis

As participants were asked to remain abstinent before attending the lab, relatively low levels of drug metabolites were found. Three ecstasy users' urine contained THC (mean 0.0083 mg/L±0.01185), Δ-9-THC (0.16 mg/L±0.18 mg/L), 11-hydroxy-Δ-9THC (0.003 mg/L±0.003). One ecstasy user's urine also contained 1-benzopiperazine (0.84 mg/L) and TFMPP (0.18 mg/L). One participant in the polydrug group had cannabis metabolites in their urine, specifically THC (0.001 mg/L), Δ-9-THC (0.41 mg/L) and 11-hydroxy-Δ-9THC (0.002 mg/L). As such, we re-ran all main analyses excluding the participants who had metabolites in their urine. This

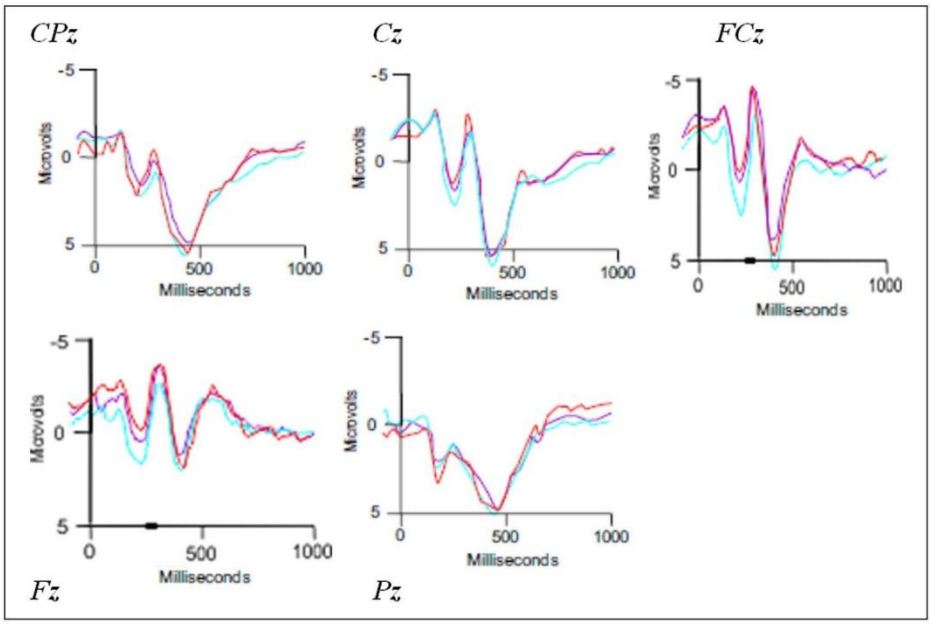

Figure 2. Grand average waveforms for the three groups across electrodes: CPz, Cz, FCz and Fz.(correct trials on Go/NoGo task).
Depicts the waveforms from each electrode measured (negative plotted up). As such the time course of the various components can be observed. These waveforms are from grand averaged data from each user group. The significant differences of ecstasy users compared to drug naïve controls in the P2 component can be observed in Fz from the epoch of 200–250 ms (ecstasy users shown in blue, polydrug users in purple and drug naïve controls in red). Also the magnitude and time course of the significant differences in mean amp in the P2 component between ecstasy users and both other control groups can be observed in FCz. (ERP waveforms created using CorelDrawX5).

did not affect the significant and non-significant results and so the analyses reported below contain all participants.

Behavioural data analysis

The Go/NoGo task was programmed in Inquisit version 3.0.6.0 (Millisecond software, 2011) and was analysed using SPSS 17. Incorrect answers in each case were given a score of 0. Therefore an error count could be performed on each of the datasets. Further to this, mean reaction times were calculated for correct 'Go' responses. Reaction time was not an applicable measure for correct 'NoGo' responses. Univariate ANOVA revealed that there was no significant difference between groups in performance on this task $F(2,57)=1.15$, $p=0.33$. The mean 'NoGo' errors (i.e. responding to a letter other than an X that required no response/an inhibition of response) were used as the measure of performance in this case (Ecstasy users: 2.7±1.95, polydrug users: 3.4±2.80, drug naïve: 4.35±4.92). However the mean 'Go' reaction time (ms) between groups was also non-significant $F(2,57)=0.35$, $p=0.71$ (Ecstasy users: 362.47±42.60, polydrug users; 372.60±62.92, drug naïve; 356.59±74.08)

Post-task NASA TLX scores were analysed using a MANOVA. This revealed no overall between-group differences in task load $F(12,102)=0.52$, $p=0.90$, nor any between-group differences on the individual sub-scales(Mental demand; $F(2,55)=0.15$, $p=0.86$, Physical demand; $F(2,55)=0.71$, $p=0.50$, Temporal demand; $F(2,55)=1.11$, $p=0.34$, Effort; $F(2,55)=0.09$, $p=0.92$, Performance; $F(2,55)=0.45$, $p=0.64$, Frustration; $F(2,55)=0.01$, $p=0.99$)

ERP analysis

The grand averages for each group (users, polydrug nonusers and drug naïve controls) can be observed at each electrode measured in Figure 2. Mean amplitudes for each condition and electrode are given in Table 3. Due to some unusable EEG data, one participant is excluded from statistical analysis on the EEG data, from the drug naïve group ($n=19$).

MANOVA of mean amplitudes at component P3 (352–452 ms) revealed no significant main effect of group on activity across the five electrodes measured $F(10,106)=0.35$, $p=0.96$. Moreover univariate tests yielded no significant differences at any of the individual electrode sites $p>0.05$ in all cases. Similarly multivariate analysis of variance of mean amplitudes at component N2 (260–330 ms) revealed no significant main effect of group on activity across the five electrodes measured $F(8,108)=0.78$, $p=0.62$, as well as the univariate tests yielding no significant between group differences at individual electrode sites $p>0.05$ in all cases.

Table 3. Mean amplitudes across components, for each electrode measured.

User group	CPz	Cz	FCz	Fz	Pz`
	P2				
Ecstasy user	2.17 (1.82)	1.94(2.69)	2.08 (2.15)[a]	1.43 (2.13)	1.45 (1.84)
Polydrug (non user)	1.3 (1.28)	1.16 (1.9)	0.29 (2.22)	0.40 (1.94)	1.43 (1.92
Drug naïve	1.49 (3.24)	0.84 (2.1)	−0.14 (2.12)	−0.30 (1.79)[b]	1.64 (2.51)
	N2				
Ecstasy user	1.38 (2.43)	−0.58 (3.60)	−1.92 (3.27)	−2.00 (2.14)	2.66 (1.72)
Polydrug (non user)	0.78 (2.67)	−0.82 (2.95)	−3.21 (3.33)	−2.87 (2.96)	2.16 (2.61)
Drug naïve	0.41 (3.50)	−1.42 (4.37)	−3.44 (4.33)	−3.12 (3.20)	2.16 (2.43)
	P3				
Ecstasy user	4.93 (2.15)	5.04 (2.82)	4.06 (2.22)	1.05 (1.74)	4.29 (1.95
Polydrug (non user)	4.07 (2.83)	4.56 (4.20)	2.91 (3.93)	0.49 (3.06)	3.76 (2.50)
Drug naïve	4.76 (2.65)	5.12 (2.77)	3.59 (3.23)	0.93 (3.12)	4.35 (2.10)

Table 3 shows the mean amplitude for each electrode on the three different ERP components for all three groups. Ecstasy users differed significantly from drug naïve controls at electrode site Fz. Furthermore the ecstasy users differed from polydrug users and drug naïve controls at electrode FCz ([a]p=0.03 and p=0.007 respectively, [b]p=0.02).

MANOVA (with a full Bonferroni correction) for the P2 component (200–250ms) revealed the main effect on mean amplitudes was non-significant, $F(8,108)=1.62$, $p=0.13$, though there were a number of significant univariate effects at individual electrodes in line with our predictions. At electrode FCz $F(2,56)=5.81$, $p=0.005$ and also electrode Fz $F(2,56)=3.84$, $p=0.02$. Post-hoc Tukey's test revealed that the ecstasy users differed significantly from drug naïve controls at electrode site Fz ($p=0.02$). Furthermore the ecstasy users differed from polydrug users and drug naïve controls at electrode FCz ($p=0.03$ and $p=0.007$ respectively). Given the heavy use of cannabis in the ecstasy user group in particular, multiple regression analyses were conducted on the behavioural data, to observe whether level use of ecstasy (after controlling for cannabis use) was a predictor of amplitude at the electrodes Fz and FCz. In the first regression, amplitude at Fz was entered as the dependent variable; in the first step indices of cannabis use were entered as predictors (frequency of use, total lifetime dose, amount smoked in the last 30 days) and in the second step, the same indices of ecstasy use were entered as predictors. The overall regression model accounted for 13.8% of the variance in Fz amplitude. In the first step, cannabis use indices were not significant predictors (R^2 change=0.04, $F(3,55)=0.82$, $p=0.48$) (β=−0.19; 0.09; 0.06 for cannabis use indices respectively, $p>0.05$ in all cases). After controlling for cannabis use, total ecstasy use predicted an additional 10% of variance in Fz amplitude (R^2change=0.10, $F(6,52)=0.91$, $p=0.49$) (β=−0.01; 0.01; 0.34 for ecstasy use indices respectively). While no individual indices of drug use emerged as significant predictors, amount of ecstasy used in the last 30 days approached significance $t(52)=1.68$, $p=0.09$. In the second regression, amplitude at FCz was entered as the dependent variable and predictors entered as above. The overall regression model accounted for 19.8% of the variance in FCz amplitude. In the first step, cannabis use indices were not significant predictors (R^2 change=0.06, $F(3,55)=1.09$, $p=0.36$) (β=−0.05; 0.19; 0.41 for cannabis use indices respectively as above, $p>0.05$ in all cases). After controlling for cannabis use, total ecstasy use predicted an additional 14.2% of variance in FCz amplitude (R^2change=0.14, $F(6,52)=1.42$, $p=0.22$) (β=−0.06; 0.03; 0.42 for ecstasy use indices respectively). Amount of ecstasy used in the last 30 days emerged as the only significant predictor of amplitude at FCz $t(52)=2.11$, $p=0.04$.

Discussion

The current study aimed to examine inhibitory processing in ecstasy polydrug users, with a task that focuses on the inhibition of a pre-potent response. The control groups did not differ from the ecstasy users on many of the background variables such as fluid intelligence, age, measures of sleep, levels of arousal, depression and anxiety. Nor did they differ on behavioural measures of performance on the inhibition task, such as number of failures to inhibit their response or reaction time in responding to targets that elicit a response ('Go'). Furthermore, the ecstasy users showed no differences in comparison to controls on perceived workload as measured by NASA-TLX.

Despite the lack of between-group differences on behavioural measures, there were differences in EEG measures suggestive of changes in attentional processes between the components involved in early inhibition processing (P2). Ecstasy users exhibited significantly higher mean amplitudes than both control groups at anterior midline site FCz and significantly higher amplitudes than drug-naïve controls at another anterior midline site Fz. It is interesting to observe such differences in the P2 component, given that it has been suggested that problems with early orienting or preparation may have consequences for later processing stages (Pliszka et al., 2000). Differences in this component have been observed previously in attention deficit hyperactivity disorder (ADHD) subjects (Jonhstone et al., 2001; Lazzaro et al., 2001), with the ADHD subjects displaying greater amplitude in this component relative to controls. This has been interpreted as atypical inhibition of sensory input in ADHD subjects (Johnstone et al., 2001). In addition, research has shown that the P2 component is elevated in unexpected versus expected inhibition trials (Gajewski et al., 2008). Research has also investigated the P2 component in inhibitory control in high and low functional impulsives (i.e. individuals whose impulsivity may facilitate performance). High functional impulsives show an increase in P2 amplitude as a function of task demand (higher demand=increased amplitude)

whereas low functional impulsives do not (Fritzsche et al., 2011). Taken together, this suggests a number of explanations for the elevation of P2 in the present study. Firstly, ecstasy users have elevated impulsivity compared to nonusers and this impulsivity may be masking performance deficits. Fritzsche et al. (2011) suggest that this steeper P2 slope, as seen in the ecstasy-polydrug users, reflects earlier and more efficient evaluation of stimuli as a result of impulsivity. This seems a reasonable assumption given that elevated impulsivity has been noted in ecstasy users in previous research (e.g. Butler and Montgomery, 2004). The heightened P2 has been shown to be associated with stimulus evaluation and response (Gajewski et al., 2008). It is also worthy of note that Gajewski et al. (2008) only noticed the elevated P2 when they increased the demands of their task, which tentatively suggests that, in the present study, the task was more demanding for ecstasy users. Secondly, in line with the ADHD research cited above, the atypical early inhibitory processing displayed in the P2 ERP component in ecstasy users, could be due to recruitment of additional compensatory resources, similar to the increased activity in prefrontal areas associated with executive functioning deficits in Alzheimer's disease patients (Grady, et al., 2003; Saykin et al., 1998; Woodard et al., 1998). This proposal could also help explain the lack of observed behavioural differences on the task. Perhaps the recruitment of additional resources at this early stage in processing could offset any further waveform modulation at later processing stages. Particularly as this was a simplified Go/NoGo task, a temporal shift to the left in attention may not be surprising.

Although some previous studies report differences between ecstasy users and controls in the P3 component on a Go/NoGo task (Gamma et al., 2005), these have conceded that between-group differences were lower after age, education level and cannabis use were controlled for. Moreover, in Gamma et al.'s study they suggest that ecstasy users have lower P3 amplitudes in comparison to controls as a result of disinhibition. Conversely the present study observed that ecstasy users had a higher (although non-significant) P3 mean amplitude at the majority of midline electrodes compared to controls. Again, this suggests that the P2 related compensatory mechanisms might be obscuring any behavioural differences in a Go/NoGo where participants are instructed to answer as quickly and as accurately as possible (such as in the present study). Gamma et al. (2005) instructed participants to take their time and answer as accurately as possible. Perhaps this would lead to negligible behavioural differences, but could perhaps also contribute to differences in the ERP. For example, if a speeded response is not required, the lowered P3 amplitude reflects a generic cognitive deficiency in users, whereas if the task is speeded it requires instant recruitment of resources and also an increase in early processing. This may explain why the P2 was so prominent in the current ecstasy user sample.

The absence of between-group differences observed in the P3 component as well as the N2 component may be explained by the differences mentioned above. However both of these components were still clearly observed in all conditions of this task. Debates have arisen about the contribution of these two components in response inhibition. For example, although often cited as being reflections of inhibitory control (Kok, 1986; Kopp et al., 1996), the N2 has also been argued to have a role in conflict monitoring, rather than response inhibition (Donkers and Boxtel, 2004; Nieuwenhuis et al., 2003). Furthermore, the P3 has been suggested to be insensitive to performance differences in inhibitory control and not necessarily involved in response inhibition (Falkenstein et al., 1999; Kopp et al., 1996). If this is the case then perhaps the task used in the current study, which was employed due to it tapping the executive function of inhibitory control only, would not highlight any differences in these components.

As with many other studies in this area, there are several limitations. Although the use of other drugs was controlled for, the ecstasy user group did smoke significantly more cannabis than the polydrug control group. Furthermore the ecstasy user group also reported consuming more cocaine than the polydrug control group. This is problematic for our results as cocaine has been shown to have strong associations with deficits in inhibitory control (Fillmore and Rush, 2002). In summary, perhaps it would be better to conclude that any effects were as a result of polydrug use. Indeed, aside from use in the last 30 days, ecstasy and cannabis use indices were equally poor predictors of mean amplitudes at the midline electrodes. In addition, a quasi-experimental design was employed and as such there may be some individual differences that belie the effects other than drug use. Many of these have been attempted to be controlled for, such as sleep quality, fluid intelligence and levels of arousal, depression and anxiety. Further to this, self-reported background drug use has been used to attain a description of quantity of drug use. However, this is problematic and recall here may not be completely accurate. Especially given the memory implications of drug use, however due to the legal status of the drug, this is the most appropriate method of investigating drug use and executive function, this method is also commonly used in the literature (Fox et al., 2001; Montgomery et al., 2005b, 2010). Purity and content of drugs consumed is also potentially problematic, though Parrott (2004) reported that ecstasy tablets collected from amnesty bins in nightclubs in the UK are approaching 100% purity. Additionally, biological analysis supports the presence of MDMA in both saliva samples of users (Parrott et al., 2008) and hair samples (Scholey et al., 2011), with the latter showing a very high correlation between self reports of usage and presence of MDMA metabolites in hair. Nevertheless, if this is incorrect and the purity is much lower, perhaps this raises additional concerns over the magnitude of cognitive effects observed (Montgomery et al., 2010).

The present study provides evidence for differences in electrophysiology as a result of ecstasy/polydrug use. Electrophysiological differences in early processing of response inhibition are suggestive of compensatory mechanisms employed to attenuate behavioural differences due to ecstasy related disturbances in normal processing of information.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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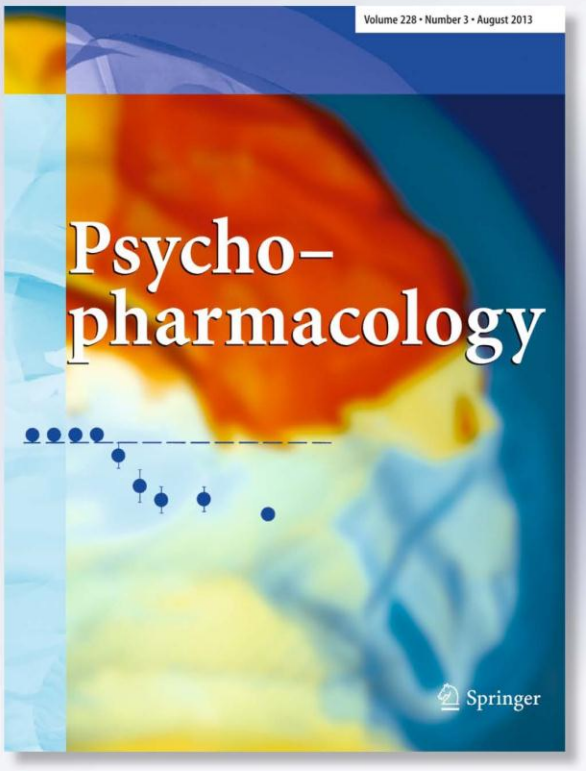
Psychopharmacology

ISSN 0033-3158
Volume 228
Number 3

Psychopharmacology (2013)
228:375-388
DOI 10.1007/s00213-013-3044-6



Springer

Psychopharmacology (2013) 228:375–388
DOI 10.1007/s00213-013-3044-6

ORIGINAL INVESTIGATION

ERP evidence suggests executive dysfunction in ecstasy polydrug users

C. A. Roberts · S. H. Fairclough · J. E. Fisk · F. Tames · C. Montgomery

Received: 11 October 2012 / Accepted: 18 February 2013 / Published online: 27 March 2013

Abstract
Background Deficits in executive functions such as access to semantic/long-term memory have been shown in ecstasy users in previous research. Equally, there have been many reports of equivocal findings in this area. The current study sought to further investigate behavioural and electro-physiological measures of this executive function in ecstasy users.
Method Twenty ecstasy–polydrug users, 20 non-ecstasy–polydrug users and 20 drug-naïve controls were recruited. Participants completed background questionnaires about their drug use, sleep quality, fluid intelligence and mood state. Each individual also completed a semantic retrieval task whilst 64 channel Electroencephalography (EEG) measures were recorded.
Results Analysis of Variance (ANOVA) revealed no between-group differences in behavioural performance on the task. Mixed ANOVA on event-related potential (ERP) components P2, N2 and P3 revealed significant between-group differences in the N2 component. Subsequent exploratory univariate ANOVAs on the N2 component revealed marginally significant between-group differences, generally showing greater negativity at occipito-parietal electrodes in ecstasy users compared to drug-naïve controls. Despite absence of behavioural differences, differences in N2 magnitude are evidence of abnormal executive functioning in ecstasy–polydrug users.

Keywords Ecstasy · *Cannabis* · Executive function

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Introduction

Recreational drug ecstasy/3,4-methylenedioxymethamphetamine (MDMA) is a potent indirect monoaminergic agonist, which is structurally similar to amphetamine and mescaline (Morgan 2000). Acute psychological and physiological effects include feelings of euphoria and empathy, increased energy, dilated pupils and tight jaw (trismus) (Davison and Parrott 1997) and are thought to result primarily from serotonin and dopamine agonism (McDowell and Kleber 1994). However "ecstasy" has been classed under the novel pharmacological category of entactogens owing to its unique psychoactive profile that can be differentiated from classic hallucinogens and stimulants (Morgan 2000).

Cognitive deficits have been reported amongst ecstasy users, and use is associated with working memory and executive functioning impairments (Fisk et al. 2004; Montgomery and Fisk 2008). Areas involved in working memory and executive function include the dorsolateral prefrontal cortex (DLPFC) and, dependent on nature of the task, hippocampus. These structures are densely innervated with 5HT neurons (Pazos et al. 1987). As such, degradation to integrity of this system via neurotoxicity or alternatively down-regulation from prolonged use of the drug may result in deficits in cognitive functions that these areas maintain. In addition to this, lack of sleep (amongst other possible lifestyle variables, such as heat and bioenergetic stress) has been suggested to exacerbate or even cause cognitive deficits observed in ecstasy-using populations (Cole et al. 2002). Furthermore, Allen et al. (1993) report that characteristics of sleep, such as sleep quality, quantity of hours and related changes in alertness are altered in ecstasy users relative to controls. More recently, Carhart-Harris et al. (2009) found that current and abstinent ecstasy users who reported little use of "other" drugs reported persistent abnormalities in

sleep patterns and quality. Nonetheless, deficits in memory have been observed to persist after controlling for sleep and other lifestyle variables (Montgomery et al. 2010).

The central executive of working memory has been suggested to comprise four discrete functions rather than being a unified construct (Miyake, et al. 2000). The initial three functions, monitoring and updating of working memory, inhibitory control and mental set shifting proposed by Miyake et al. (2000), were supplemented by a fourth component of "access" to semantic/long-term memory added by Fisk and Sharp (2004). This "access" component involves word fluency and efficiency of lexical access. Retrieval of words and semantics involves ability to access long-term memory store, and efficiency with which this can occur seems to be dependent on areas of the DLPFC (Stuss et al. 1998) as well as other subcortical networks (Jokeit et al. 1998). It may not be entirely surprising that word fluency requires long-term memory activation, and it has been observed that long-term recall is correlated with performance on word fluency tasks (Ruff et al. 1997).

Some studies have been conducted that assess access to semantic memory in ecstasy users using measures such as the Controlled Oral Word Association (COWA) task in which participants have 1 min to vocalise as many words beginning with F, A and S as possible (see Bhattachary and Powell 2001). Some studies using this measure have yielded deficits in ecstasy users compared to controls (Bhattachary and Powell 2001; Fox et al. 2001), whereas others report no such deficits (e.g. Halpern et al. 2004). However, as a written variant of the COWA, the Chicago Word Fluency Test appears to yield more consistent observable deficits in ecstasy users (Montgomery et al. 2005a, b; Montgomery et al. 2007). It remains a possibility that a verbal 1-min retrieval task, with no restrictions upon word type or length, is too simple to require involvement of the central executive and as such ecstasy users may not show any impairment on the COWA. It has been noted that ecstasy users have shown impairments on difficult aspects of tasks, yet appear unaffected on simple tasks that require relatively automatic processing (Fox et al. 2001). Consequently, further investigation of ecstasy-related deficits in access to semantic memory is required.

Neuroimaging techniques such as electroencephalography, positron electron tomography (PET) and functional magnetic resonance imaging (fMRI) are useful tools for this type of investigation. Firstly, it would be beneficial to observe neurophysiological correlates of behavioural deficits and the underlying mechanisms by which this executive function operates. In addition, due to necessity for neurophysiological techniques to reduce body movements and vocalisations so that data is not contaminated with noise, it would be advantageous to develop novel tasks to tap this function as suggested by Murphy et al. (2009). Badre et al. (2005) used fMRI to observe the mechanisms by which semantic retrieval occurs in healthy controls. Participants were presented with a cue word and three target words on a screen for 3 s. Participants were required to either select a target (by using a keyboard) based on a global relation to the cue (i.e., no judgement made about specific features or dimensions of words such as shape, colour and semantics) or alternatively based on a specific semantic feature. Stimuli were matched for word length and frequency of presentation; furthermore, cue–target associative strength was varied. For example, a low semantic associative strength would have a cue word such as "detective" with targets "search, offer and pond" with "search" being the closest to "detective" semantically. An example of high associative strength would be "candle" with targets "flame, arena and goat" and here, it is clear that "candle" and "flame" are strongly associated in meaning and as such represent a high associative strength. Badre et al. (2005) posit that prefrontal cortex mechanisms mediate semantic retrieval and that the ventrolateral prefrontal cortex (VLPFC) and middle temporal cortex are sensitive to association strength.

Whitney et al. (2010) investigated the neuronal network involved in semantic retrieval and processing. Strength of semantic association with the cue word low vs. high was used as the manipulation. Transcranial magnetic stimulation (TMS) in healthy subjects was employed to disrupt processing in the inferior frontal gyrus (IFG) and the posterior middle temporal cortex. Disruption to both of these sites produced attenuation of effective processing of executively demanding processes; however, processing of cue–target stimuli with strong semantic association that are relatively automatic were unaffected by the disruption. It was concluded that there is a network of prefrontal and posterior temporal regions that underlie semantic control and may provide an explanation why ecstasy users may be unaffected in relatively simple semantic retrieval tasks, such as the COWA.

Indeed, electrophysiological measures such as EEG are more sensitive to detecting impairment than behavioural measures alone. For example, certain behavioural differences may be undetectable due to compensatory mechanisms that are employed to provide support for degraded primary mechanisms. This has been observed in patients with Alzheimer's disease (Rossi et al. 2004). Additionally, Saykin et al. (1998) investigated semantic retrieval in Alzheimer's disease patients with use of neuro-imaging and observed that they displayed additional activation in frontal regions compared to controls. This is suggestive of compensatory recruitment of additional resources in order to complete the tasks to a similar standard of performance to controls. Similarly, using fMRI, Kanayama et al. (2004) reported that *Cannabis* users displayed activation of

additional brain regions to those usually observed in a spatial working memory task using fMRI, despite behavioural measures providing no observable differences between them and controls. Here, results from behavioural measures would indicate that processes involved in spatial working memory were intact in *Cannabis* users, yet neuro-imaging data suggest recruitment of additional resources. Furthermore, Jager et al. (2006) have also reported alterations to left superior parietal cortex activity on working memory tasks in *Cannabis* users despite equivalent behavioural performance to controls on the task. Both studies highlight possibility of compensatory brain mechanisms underlying undetectable behavioural differences in working memory tasks in *Cannabis* users. We propose that similar mechanisms could operate in ecstasy users.

EEG is an electro-physiological technique with high temporal resolution that may be beneficial to use in combination with behavioural measures to assess any changes in brain function as a result of ecstasy use in semantic retrieval. Event-related potentials (ERPs) are ideal for investigating tasks that require executive control, as these tasks require rapid executive decisions that are less detectable with other neuro-imaging techniques. Burgess et al. (2011) observed differences between ecstasy users and controls in ERPs in a word recognition task. Ecstasy users showed an attenuation of a late positivity over left parietal scalp sites despite equivalent performance on the task. As such, this is suggested to be a durable abnormality in a specific ERP component associated with recollection in ecstasy users that may not have been detected with behavioural measures alone.

The P3 ERP is a positive spike in neuro-electric activity that occurs around 300 ms after stimulus onset. This component is thought to be involved in higher level cognitive processing and executive functioning. A diminished P3 potential is understood to reflect cognitive dysfunction. Casco et al. (2005) observed a diminished P3 response in a simple discrimination task in moderate and heavy ecstasy users compared to controls in a visually evoked potential study. Furthermore, Gamma et al. (2005) report reductions in the P3 amplitudes of ecstasy users, compared to controls in an inhibitory control (Go–NoGo) task. However, it is reported that this effect is diminished after controlling for age, educational level and *Cannabis* use. Indeed, the authors suggested that the diminished P3 response could be a polydrug effect given the amount of *Cannabis* use reported by ecstasy users in their sample. de Sola et al. (2008) conducted a battery of cognitive tests to investigate P3 differences between ecstasy users, *Cannabis* users and controls, and they found no significant between-group differences on these tasks in P3 latency or amplitude. However, there were significant correlations between P3 latency and lifetime *Cannabis* use, whereas Mejias et al. (2005) report aberrations in the N2 component in ecstasy users compared to controls in a visual oddball task.

The current study aims to characterise the nature of MDMA's effects on cognitive processes involved in accessing semantic memory by using EEG measures during a semantic retrieval task similar to that used by Badre et al. (2005) and Whitney et al. (2010). It is hypothesised that behavioural differences will not be present in trials where there is high association between the cue and target words, but that differences will be observed with weaker semantic associations, as the weaker association condition will be more difficult, requiring induction of the central executive. Similarly, it is hypothesised that there will be differences in electro-physiological measures that reflect higher level processing (e.g., P3 and N2) whereby ecstasy users display aberrant electro-physiological behaviour compared to non-users.

Method

Design

In all analyses, a mixed design was used with a between-groups factor of drug user group with three levels (ecstasy user, non-ecstasy–polydrug user and drug-naïve controls) and associative strength (high vs. low) as a within-subjects factor. Mixed ANOVA was conducted on behavioural data with scores on semantic association tasks as dependent variable. ERP data was analysed using mixed ANOVA with drug user group as between-subjects factor and site of electrode (PO7, PO3, O1, OZ, POZ, PO8, PO4 and O2 for the N2 and P3 components and sites FZ, FCZ, FC1, FC2, CZ, C1 and C2 for the P2 component) as well as associative strength (high vs. low) as within-groups factors. Mean amplitudes at the three ERP components were the dependent variables. Where appropriate, significant main effects were further investigated using univariate ANOVA.

Participants

Twenty ecstasy users (mean age=23.95, SD=0.57, 10 male), 20 non-drug user controls (mean age=23.1, SD=0.66, seven male) and 20 non-ecstasy drug user controls (mean age=22.58, SD=0.79, nine male) were recruited via direct approach to university students and club goers. In terms of statistical power, with 20 participants in each of the three groups, the sample is sufficient to detect a difference between pairs of means of at least 1 standard deviation at alpha=0.05 and beta=0.20 (Hinkle et al. 1994). The additional control group of participants that have not used ecstasy previously, yet have used other illicit substances,

was recruited to address the issue of polydrug use as a potential cause for the cognitive deficits observed.

To avoid age-related deficits in working memory obscuring pharmacologically derived deficits, age range for inclusion was 18–29 years. Inclusion in the ecstasy user group required participants to have taken ecstasy/MDMA on five or more occasions over their lifetime (actual minimum=five ecstasy tablets). Furthermore, for inclusion in both control groups, participants must have never used ecstasy/MDMA; however, all other illicit substances were permitted for the non-ecstasy–polydrug user control group.

All participants were asked to abstain from consuming ecstasy for a minimum of 7 days prior to testing and urine samples were collected upon arrival to the lab for urinary analysis of all drug metabolites to confirm abstinence (after ingestion, MDMA is generally accepted to be detectable in urine for 1–3 days; this is the same for cocaine and amphetamines, with *Cannabis* being detectable for anything up to 95 days; Verstraete 2004). Participants were also requested to abstain from use of other illicit drugs for a minimum of 24 h prior to participating and ideally for 7 days.

Materials

Upon entering the lab, participants completed a background drug use questionnaire, which provides the researcher with indices of drug use patterns and other lifestyle variables. Comprehensive details of ecstasy use as well as other illicit drug use were requested, such as first and last drug use, patterns of drug use, frequencies and doses over time. Using a method employed by Montgomery et al. (2005a, b), estimates of total lifetime drug use of each drug were calculated. Totals for the last 30 days of drug use as well as weekly drug use estimates were also calculated. This questionnaire also sought information about health, age, years of education and perceived changes to mood and cognition, amongst other lifestyle variables.

Measures of sleep quality

Sleep quality and alertness were measured to investigate any possible relationship between sleep quality and cognition using the following questionnaires: a sleep quality questionnaire, exploring typical quantities of sleep (how many hours slept typically, how many hours over the last three nights) and level of quality of sleep. The Epworth Sleepiness Scale (ESS, Johns 1991) explores the chances of dozing or falling asleep in various situations such as "sitting and reading" and "sitting quietly after lunch without alcohol", and is scored from 0=would *never* doze off to 3=*high* chance of dozing. A high total score here is indicative of increased subjective daytime sleepiness. The Morningness–Eveningness Questionnaire (MEQ)—self-assessment version (Terman et al. 2001) is a self-assessment of morningness–eveningness in human circadian rhythms (originally developed by Horne and Östberg (1976)), and a high score on this questionnaire is indicative of a morning type person and a low score is indicative of an evening type person. Finally, the Karolinska sleepiness scale (Akerstedt and Gillberg 1990) is a self-assessment of sleepiness at the current moment in time (ranging from 1=extremely alert to 9=extremely sleepy, fighting sleep, effort to stay awake); therefore, this can be administered at different time points of the experiment to assess sleepiness. As such, this was administered to participants pre- and post-task.

State mood

State anxiety, arousal and depression were measured using scales devised by Fisk and Warr (1996). Participants are required to rate on a five-point Likert scale from 1=not at all to 5=extremely how they are feeling at the time of testing. A high score on each sub-scale indicates increased hedonic tone/anxiety/arousal.

NASA-Task Load Index (Hart & Staveland 1998)

Finally, a questionnaire measuring subjective workload was given post-task. This is a multi-dimensional scale, consisting of six sub-scales (mental demand, physical demand, temporal demand, personal performance rating, effort and frustration). Participants were required to place a mark on a 100-mm line, indicating where they perceive their demand to be on the scale. These are taken to observe whether there are any differences between ecstasy users and non-users in perceived cognitive demand on the task. It has been reported that ecstasy users may be more susceptible to stress than non-users and may thus report increased cognitive effort (Wetherell et al. 2012).

Access to semantic memory

The executive function "access" was assessed using a semantic association task that is based around the tasks used by Whitney et al. (2010) and Badre et al. (2005), whereby two types of semantic judgement that differed in their level of difficulty (high association/low association) were used. In both difficulty levels, participants were presented with a cue word in the centre of a computer monitor followed by three target words, one which had a semantic association with the cue and two distracters. Participants had to decide which of the three target words had the strongest semantic association with the cue word. They selected their answer by pressing

one of three buttons on a response box that corresponded to their position on screen. They were either high association between cue and target words (e.g., candle–flame) or low association (e.g., detective–search). The low association judgement is deemed to be more difficult and require more processing than the relatively automatic high association semantic judgements. As such, the low association between cue and targets leads to a less obvious dissociation from distracters requiring recruitment of additional executive resources in the semantic network (Whitney et al. 2010). The stimuli used were matched for word length, frequency and cue–target association strength (Badre et al. 2005; Whitney et al. 2010) and were kindly provided by Whitney et al. The task consisted of a practise round followed by four blocks of 30 trials, with both high and low association trial types appearing in each block pseudo-randomly (15 of each in each block). The cue word was presented for 1 s in the centre of a computer screen. After this, the three target words appeared below aligned to the left, centre and right of the monitor. Participants were instructed to respond via pressing a button on the response box corresponding to the position of the target on the screen (left, centre and right). The targets remained on screen until a response was made or until the trial timed out (time out set to 8.5 s). An inter-trial interval of 2 s was employed. The task took around 20 min to complete. Participants were instructed to respond as quickly and as accurately as possible.

Equipment

EEG was recorded using a 64-channel BioSemi Ag–AgCl active two-electrode system (Biosemi B.V., Amsterdam, the Netherlands) with pin type electrodes mounted in a stretch lycra headcap (Biosemi) and positioned according to the international 10–20 system. Neuro-electric activity was recorded from the following sites: frontal (FPz, FP1 and FP2), anterior–frontal (AFz, AF3, AF4, AF7 and AF8), frontal (Fz, F1, F2, F3, F4, F5, F6, F7 and F8), fronto-central (FCz, FC1, FC2, FC3, FC4, FC5 and FC6), central (Cz, C1, C2, C3, C4, C5 and C6), temporal (FT7, FT8, T7, T8, TP7 and TP8), parieto-central (CPz, CP1, CP2, CP3, CP4, CP5 and CP6), parietal (Pz, P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10), occipito-parietal (POz, PO3, PO4, PO7 and PO8) and occipital (Oz, O1, O2 and Iz). Sigma electrolyte gel was used to ensure contact between scalp and electrodes. Vertical and horizontal electro-occulograms were recorded using bipolar, flat Ag–ACl electrodes positioned above and below the left eye as well as to the outer side of each eye. Data was digitised at a sampling rate of 512 Hz and no filters were applied online so that the data could be visually inspected for noise and offline filtering could be performed.

Procedure

Testing sessions commenced at 9:30 a.m or 1:30 p.m., and equal numbers of participants from each condition were tested in the morning and the afternoon. Upon entering the lab, participants were given a brief description of the experiment and written informed consent was obtained. Following this, participants gave a urine sample that was immediately frozen at −25 °C. Participants then completed the battery of questionnaires, whilst their head circumference and other details were measured, and an electrode cap and electrodes were fitted. The questionnaires were administered in the following order: background drug use questionnaire, MEQ, sleep quality questionnaire, state mood, ESS, Karolinska sleepiness scale (before) and fluid intelligence was assessed using Raven's progressive matrices (Raven et al. 1998). Following completion of these questionnaires, the EEG setup was checked and, if necessary, modified. Participants then completed the computerised task on a desktop computer running Inquisit version 3.0.6.0 (Millisecond Software 2011). The NASA-TLX questionnaire was completed after the task as was the post-task Karolinska sleepiness scale. Finally, participants were fully debriefed and paid £20 in store vouchers. The study was approved by Liverpool John Moores University Research Ethics Committee and was administered in accordance with the ethical guidelines of the British Psychological Society.

EEG analysis

The EEG data was analysed using BESA 5.3 (MEGIS software GmbH, Gräfelfing, Germany). All recordings were visually analysed offline using high- and low-pass filters of 0.1 and 40 Hz, respectively. Any channels judged to be bad were replaced by interpolation and all data were Electrooculography (EOG) corrected using BESA's Principal Component Analysis (PCA) based algorithm. All trials judged to be bad after this point were discarded. EEG was segmented into epochs from −500 to 1,000 ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (e.g., correct "high associations" and incorrect "high associations" and correct "low associations" and incorrect "low associations") could be generated for each individual. Only ERPs for correct responses were included in the subsequent analysis. There were 120 trials in total, and the mean number of good trials retained for grand averaging per subject was 109.66 (average of 8.6 % rejected trials) after rejecting incorrect trials (6.1 %) and those containing artefacts (2.5 %). Grand averages were made for each group (ecstasy user, polydrug user and drug naïve) on each condition (correct "high associations", correct "low associations"). The overall P3 response was defined as the mean amplitude between 280 and 350 ms for the low association

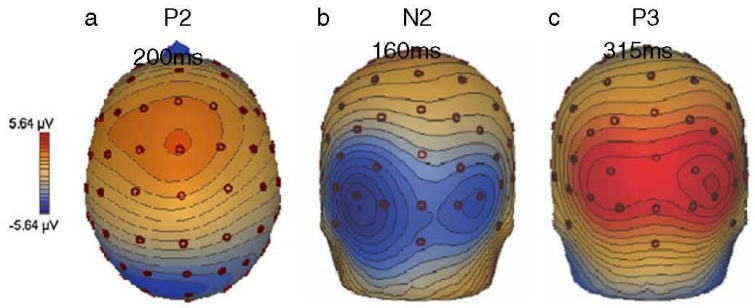

Fig. 1 Topographies for the central point of each component. Note that this is from grand averages of each group combined. **a** Topography of activity in the epoch specified for the P2 component. Note that this component has been isolated at an epoch where there is the most positivity displayed at midline/anterior electrodes. **b** A negativity in N2 notably around posterior and occipital electrodes. **c** Positivity in the P3 component similarly around posterior and occipital electrodes

condition and 250–350 ms for the high association condition. These time windows were centred on positive peak latency and duration was chosen due to this epoch containing majority of positive activity for all conditions by observing topographic maps (see Figs. 1 and 2). Electrode activity was analysed in this epoch from occipito-parietal and occipital electrodes PO7, PO3, O1, OZ, POZ, PO4, PO8 and O2, as the greatest amount of activity in the P3 component could be observed at these sites. Further, components were also analysed for between-group differences, including the N2 and P2 components. The N2 component was also the largest over occipito-parietal and occipital electrodes (PO7, PO3, O1, OZ, POZ, PO4, PO8 and O2) between 120–190 ms in the low association condition and 120–200 ms in the high association condition, and again, epochs were based around the mean local negative peak at these sites and encompassed the majority of negative activity over all three groups. The P2 component was most visible as a positive peak between 170 and 230 ms (for both low and high associations) at anterior and mid-line sites (FZ, FCZ, FC1, FC2, CZ, C1 and C2) and the mean amplitudes at these sites from the epochs based around the peaks from the grand averages of all conditions were analysed.

Urinary analysis

Frozen urine samples were delivered to University Hospitals Aintree and were analysed using solid-phase extraction (mixed-mode phase) followed by reverse-phase HPLC MS/MS detection using both positive and negative ion multiple reaction monitoring (MRM). Urine specimens have been tested for: synthetic cannabinoids (JWH-018, JWH-073, JWH-250, JWH-398, JWH-122, JWH-019, AM-694, WIN 48098 and WIN-55212-2) as well as "designer" drugs "Mephedrone", bk-MDMA or "Methylone", bk-N-methyl-1,3-benzodioxolylbutanamine (MBDB) or "Butylone", bk-Para-Methoxymethamphetamine (PMMA) or "Methedrone", 1-benzylpiperazine, 3-Trifluoromethylphenylpiperazine (TFMPP), mCPP and Methylenedioxypyrovalerone (MDPV). Also tested for were a series of 12 piperazine compounds, four β-keto amphetamines, a series of 11 methcathinone compounds, 4-fluoroamphetamine, bupropion and the hallucinogenic amphetamines: DOB ("bromo-STP" or "Brolamphetamine"), DOC and DOI. Finally, "traditional" drugs of abuse: amphetamine(s) including MDMA, Methylenedioxyamphetamine (MDA)

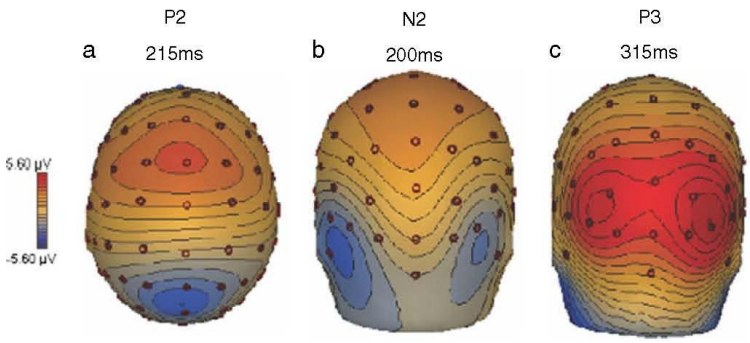

Fig. 2 Topographies for central points of each component. Note that this is from grand averages of each group combined. **a** Topography of activity in the epoch specified for the P2 component. **b** A negativity in N2 notably around posterior and occipital electrodes. **c** Positivity in the P3 component similarly around posterior and occipital electrodes

Table 1 Indices of ecstasy use

Variable	Mean	SD
Total tablets	177.65	301.73
Last 30 days total	0.6	2.26
Frequency of use (times per week) (n=12)	0.24	0.42

and Methylenedioxyethylamphetamine (MDEA), barbiturates, benzodiazepines, THC and cannabinoids, buprenorphine, cocaine and metabolites, methadone and metabolites, opiates and opioids (morphine, codeine, dihydrocodeine, tramadol, d-propoxyphene, oxymorphone and oxycodone), LSD, GHB (and the lactone precursor), psilocybin, ketamine and methaqualone were tested for as well.

Results

Table 1 shows indices of ecstasy use from the ecstasy user group, including total lifetime dose (tablets), total number of tablets taken in the last 30 days and frequency of use (times per week). Participants' socio-demographic information, state mood scores from the mood adjective checklist and sleep measures are shown in Table 2, and indices of other drug and alcohol use are displayed in Table 3.

One-way ANOVA revealed that there were no significant between-group differences on measures such as age, average hours sleep per night, total score on the ESS, MEQ total score, post-test Karolinska sleepiness scale, levels of arousal, depression and anxiety or total score on Raven's progressive matrices. However, there were between-group differences in the pre-testing Karolinska sleepiness scale (i.e., how sleepy the participants felt before the test battery) ($F(2, 58)=3.78$, $p<0.05$), and planned comparison t tests revealed that the ecstasy user group felt significantly more sleepy prior to testing than the polydrug control group ($t(38)=2.39$, $p<0.05$), but not the drug-naïve control group $t(37)=0.50$, $p>0.05$.

t tests between the ecstasy user group and the polydrug–non-ecstasy group revealed that the ecstasy user group had a significantly larger lifetime total of joints smoked than the non-ecstasy drug users (5,057.88 compared to 1,091.71) ($t(17.88)=2.02$, $p<0.05$) (Levene's test was significant so degrees of freedom have been adjusted accordingly). The ecstasy users had also smoked significantly more joints within the last 30 days (32.77 compared to 6.09) ($t(16.01)=1.86$, $p<0.05$). There were, however, no differences between these two groups on other drug intake variables. However, as can be seen from Table 3, the ecstasy user group can be described as polydrug users.

Urinary analysis

Metabolites detected in the urinary analysis are displayed in Table 4. Drug metabolites detected were restricted to cannabinoids, TFMPP and Benzylpiperazine (BZP) and were relatively low level. Exclusion of participants with cannabinoid metabolites in their urine did not change behavioural or

Table 2 Indices of sleep quality, fluid intelligence and socio-demographic variables

	Ecstasy users		Polydrug (non-ecstasy)		Drug-naïve controls	
Males: n, %	10 (50)		9 (45)		7 (35)	
	Mean	SD	Mean	SD	Mean	SD
Age	23.94	2.50	22.58	3.45	23.10	2.94
University degree: n (%)	14 (70)		12 (60)		11 (55)	
Employment status						
Student: n, (%)	12 (60)		14 (70)		17 (85)	
Employed: n (%)	4 (20)		4 (20)		3 (15)	
Unemployed: n (%)	4 (20)		2 (10)		0 (0)	
Raven's progressive matrices (maximum 60)	48.68	5.96	48.35	5.83	51.35	5.01
Sleep (h/night)	7.13	1.91	7.8	1.39	7.05	1.16
ESS score (maximum 24)	6.5	3.3	6.7	3.15	6.5	3.32
KSS before	5.05	1.93	3.75	1.48	4.79	1.23
KSS after	6.53	2.03	5.85	1.53	6.56	1.46
MEQ total	42.10	10.15	45.70	9.40	47.90	8.30
State anxiety	11.4	4.08	12.44	2.18	11.75	2.12
State arousal	19.7	4.54	20.5	3.68	20.1	3.02
State depression	13.1	3.91	12.61	2.4	12.1	3.14

Table 3 Indices of drug use other than ecstasy

	Mean	SD	Number	Mean	SD	Number	Mean	SD	Number
Cannabis									
Frequency (times/week)	2.67	3.24	12	0.95	1.9	13	–	–	–
Last 30 days (joints)	32.77	53.75	15	6.09	15.34	17	–	–	–
Total use (joints)	5,057.88	7,504.30	16	1,091.71	2,531.65	19	–	–	–
Age at 1st use									
Cocaine									
Frequency (times/week)	0.15	0.14	11	0.27	0.34	2	–	–	–
Last 30 days (lines)	0.4	1.12	15	1.60	3.58	5	–	–	–
Total use (lines)	813.97	1,940.19	16	107.30	208.43	5	–	–	–
Age at 1st use									
Ketamine									
Frequency (times/week)	0.26	0.42	5	0.02	–	1	–	–	–
Last 30 days use (g)	1	2.65	9	–	–	–	–	–	–
Total use (g)	31.26	70.61	11	1.13	1.62	3	–	–	–
Age at 1st use									
Alcohol units p/w	15.33	15.29	20	10.53	8.37	20	9.93	11.58	20

electro-physiological analyses so analyses reported include these participants.

Behavioural data analysis

The semantic association was programmed in Inquisit version 3.0.6.0 (Millisecond Software 2011) and was analysed using SPSS (17). Incorrect answers in each case were given a score of 0 and were not investigated any further for reaction time analysis. Mean reaction times were calculated for correct high association trials as well as correct low association trials. Reaction time data reduction involved excluding reaction times less than 200 ms and greater than 5,000 ms as these reaction times represent pre-emptive responding and a loss of concentration, respectively. Furthermore, individual trial reaction times that were more than 3 standard deviations above the individual mean were discarded. The mean percentage of outliers that were discarded from each group were ecstasy users 1.46 (±0.66), polydrug users 1.42 (±1.05) and drug-naïve 1.71 (±0.92), and there were no between-group differences in amount of outliers ($F(2, 57)=0.63$, $p>0.05$).

Performance on the semantic retrieval task was measured both in terms of number of errors made (incorrect responses) and reaction time. A mixed ANOVA was conducted with between-subjects factor of group and within-subjects factor of difficulty (high association and low association). Using error count as the dependent variable, there was no significant effect of difficulty ($F(1, 57)=0.04$, $p>0.05$) (sphericity assumed), no main effect of group ($F(2, 57)=1.56$, $p>0.05$) and no group× difficulty interaction ($F(2, 57)=0.01$, $p>0.05$) (Table 5). Similarly, using reaction time as the dependent variable, no significant between-group differences were observed ($F(2, 57)=0.07$ $p>0.05$). Difficulty and group×difficulty interactions were non-significant ($p>0.05$) in both cases (Table 5).

Post-task NASA-TLX scores were analysed using a multivariate analysis of variance (MANOVA). This revealed no overall between-group differences in perceived demand

Table 4 Amounts of various drug metabolites found in urine samples (mg/L)

		THC	Δ^9 THC	11-Hydroxy-Δ^9-THC	1-Benzylpiperazine	TFMPP
E user	*N*	3	3	3	1	1
	Mean	0.0083	0.16	0.003	0.84	0.18
	SD	0.01185	0.18286	0.00346	–	–
Polydrug	*N*	1	1	1	–	–
	Mean	0.001	0.41	0.0020	–	–
	SD	–	–	–	–	–
Drug naïve	*N*	–	–	–	–	–
	Mean	–	–	–	–	–
	SD	–	–	–	–	–

Table 5 Performance data (means and SDs of error count and reaction times) for all participants in both conditions

	Ecstasy users		Polydrug (non-ecstasy)		Drug naïve	
	Mean	SD	Mean	SD	Mean	SD
High association errors	4.00	2.34	4.60	2.78	5.25	2.77
Low association errors	4.10	2.57	4.60	2.09	5.35	2.92
High association RT (ms)	1,282.26	255.91	1,294.43	354.77	1,209.39	230.89
Low association RT (ms)	1,265.14	250.85	1,294.21	308.44	1,180.39	198.60

($F(12, 104)=0.94$, $p>0.05$) for Pillai's trace or any between-group differences on the individual sub-scales of their subjective perception of subjective workload (mental demand: $F(2, 56)=1.06$, $p>0.05$, physical demand: $F(2, 56)=0.10$, $p>0.05$, temporal demand: $F(2, 56)=1.56$, $p>0.05$, effort; $F(2, 56)=0.48$, $p>.05$, performance: $F(2, 56)=2.62$, $p>0.05$, frustration: $F(2, 56)=0.77$, $p>0.05$).

ERP analysis

The grand average waveforms for each group (users, polydrug non-users and drug-naïve controls) can be observed for electrodes PO7, PO3, O1, OZ, POZ, PO8, PO4 and O2 in Fig. 3 (high association) and Fig. 4 (low association). Mean amplitudes for each condition and electrode are given in Table 6. Due to some unusable EEG data, one participant from the drug-naïve group ($n=19$) is excluded from statistical analysis on the EEG data.

A mixed ANOVA, with between-subjects factor of group and within-subjects factors of difficulty (high association and low association) and site (FZ, FCZ, FC1, FC2, CZ, C1 and C2), was conducted on the mean amplitudes across the epochs measured (170–230 ms in both conditions) for the P2 component. This revealed no main effect of difficulty ($F(1, 56)=0.32$, $p>0.05$), no difficulty × group

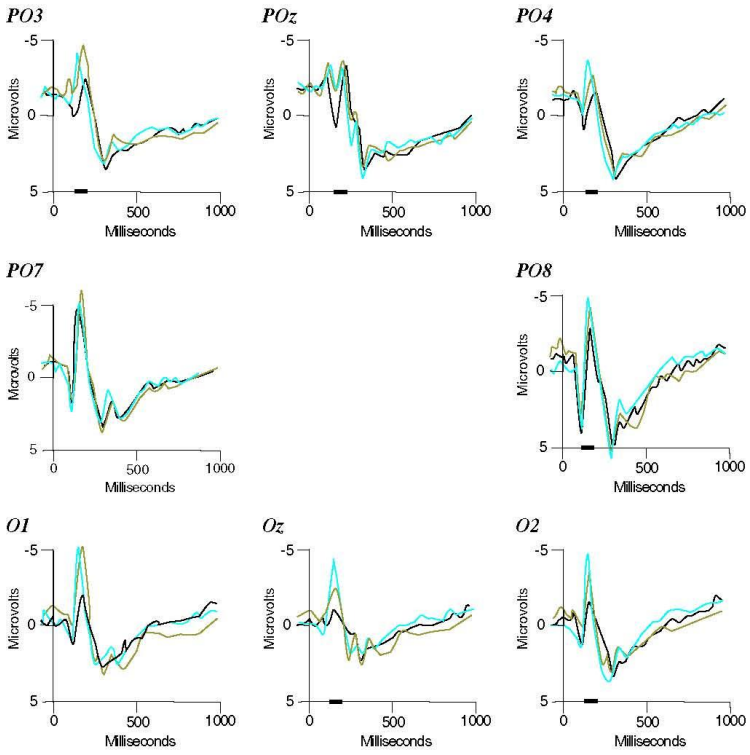

Fig. 3 Depicts the grand average waveforms for each user group from each electrode measured (for the N2 and P3 components) for the high association condition of the task. As such, the time course of the various components can be observed. (Ecstasy users are shown in *blue*, polydrug users in *green* and drug-naïve controls in *black*). Also, the magnitude and time course of the significant differences in mean amp in the N2 component (120–200 ms) between ecstasy users and drug-naïve controls can be observed in PO3, POz, PO4, PO8, Oz and O2. Epochs showing significant differences in the N2 component are emboldened in *black* on the *x* axis

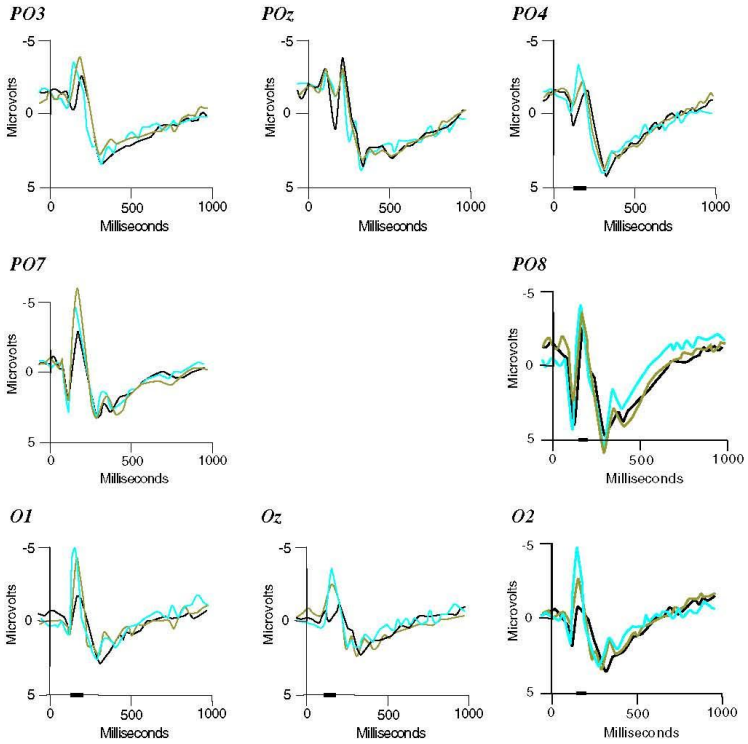

Fig. 4 The grand average waveforms for each user group (ecstasy user in *blue*, polydrug users in *green* and drug-naïve controls in *black*) from each electrode measured (for N2 and P3 components) for the low association condition of the task. The magnitude and time course of the significant differences in mean amp in the N2 component (120–190 ms) between ecstasy users and drug-naïve controls can be observed in PO4, PO8, O1, Oz and O2. Epochs showing significant differences are emboldened in *black* on the *x* axis

interaction ($F(2, 56)=0.35$, $p>0.05$), no main effect of site ($F(4.21, 236.03)=5.22$, $p>0.05$), no site×group interaction ($F(8.43, 236.03)=0.26$, $p>0.05$), no difficulty×site interaction ($F(4.85, 271.44)=0.51$, $p>0.05$) and no difficulty×site×group interaction ($F(9.69, 271.44)=0.48$, $p>0.05$ (degrees of freedom adjusted in line with Greenhouse–Geisser statistic in all cases). Between-group differences were also non-significant ($F(2, 56)=1.68$, $p>0.05$).

A mixed ANOVA, with between-subjects factor of group and within-subjects factors of difficulty (high association and low association) and site (PO7, PO3, O1, OZ, POZ, PO4, PO8 and O2) for the mean amplitudes across the epochs measured (120–190 ms in the low association condition, 120–200 ms in the high association condition) on the N2 component revealed no main effect of difficulty ($F(1, 56)=1.05$, $p>0.05$), no difficulty×group interaction ($F(2, 56)=0.04$, $p>0.05$), no main effect of site ($F(3.82, 213.92)=6.37$, $p>0.05$), no site×group interaction ($F(7.64, 213.92)=1.10$, $p>0.05$), no difficulty×site interaction ($F(4.78, 267.40)=0.81$, $p>0.05$) and no difficulty by site×group interaction ($F(9.55, 267.40)=0.73$, $p>0.05$) (degrees of freedom adjusted in line with Greenhouse–Geisser statistic in all cases). Between-group differences approached significance ($F(2, 56)=2.78$, $p=0.07$). In line with a priori predictions and to further explore this trend on the N2 component, Helmert contrasts were performed. The Helmert contrasts revealed a significant difference between the ecstasy–polydrug user group and the drug-naïve participants (contrast estimate=−2.10, $p<0.05$). Consequently, univariate ANOVA was conducted between drug-naïve participants and ecstasy–polydrug users alone, with amplitude at each site as the dependent variable to explore the significant contrast. In the low association condition, significant differences were observed at electrode site O1 ($F(1,37)=3.37$, $p<0.05$), site OZ ($F(1,37)=3.41$, $p<0.05$), PO8 ($F(1,37)=6.32$, $p<0.01$), PO4 ($F(1,37)=3.42$, $p<0.05$) and O2 ($F(1,37)=7.02$, $p<0.01$). In the high association condition, significant differences were observed at site PO3 ($F(1,37)=3.62$, $p<0.05$), OZ ($F(1,37)=5.05$, $p<0.01$), POZ ($F(1,37)=2.83$, $p<0.05$), PO8 ($F(1,37)=5.56$, $p<0.01$), PO4 ($F(1,37)=2.84$, $p<0.05$) and O2 ($F(1,37)=4.17$, $p<0.05$). In all cases, ecstasy users showed greater negativity than drug-naïve controls (Table 6).

A mixed ANOVA, with between-subjects factor of group and within-subjects factors of difficulty (high association and low association) and site (PO7, PO3, O1, OZ, POZ,

Table 6 Mean amplitudes across components for each electrode measured

User group	P3 high association							
	PO7	PO3	O1	Oz	POz	PO8	PO4	O2
Ecstasy user (20)	2.95 (4.02)	3.94 (2.42)	2.76 (3.01)	1.60 (3.24)	4.19 (2.34)	4.40 (3.23)	4.94 (2.99)	3.31 (3.59)
Polydrug (20)	4.12 (2.83)	2.97 (2.57)	3.52 (4.60)	3.04 (3.40)	3.28 (2.27)	5.72 (3.78)	4.05 (3.27)	3.32 (3.32)
Drug naïve (19)	3.62 (3.19)	3.92 (2.39)	2.84 (3.17)	2.13 (3.14)	3.42 (2.56)	5.35 (3.40)	4.00 (2.21)	3.27 (2.75)
	P3 low association							
Ecstasy user (20)	3.48 (3.74)	4.60 (2.74)	2.32 (3.57)	1.79 (2.90)	4.46 (2.40)	3.78 (2.92)	4.98 (3.33)	2.97 (3.39)
Polydrug (20)	4.16 (3.29)	3.44 (3.16)	2.27 (4.40)	2.70 (2.85)	3.89 (2.26)	5.92 (4.61)	4.41 (3.67)	3.14 (3.90)
Drug naïve (19)	4.32 (3.42)	4.40 (2.64)*	3.08 (3.46)	2.22 (3.21)	3.93 (2.80)	6.07 (3.32)	4.54 (2.69)	3.78 (2.92)
	N2 high association							
Ecstasy user (20)	−1.66 (3.41)	−1.32 (3.55)	−2.19 (3.53)	−2.51 (3.45)	−0.70 (4.01)	−1.41 (3.20)	−1.04 (3.69)	−2.17 (4.12)
Polydrug (20)	−2.06 (4.57)	−2.37 (2.90)*	−2.41 (4.36)	−0.59 (4.19)	−0.58 (2.42)	−0.09 (4.11)	−0.44 (2.57)	−1.15 (3.44)
Drug naïve (19)	−0.58 (3.11)	0.67 (2.95) *	−0.36 (3.43)	0.09 (3.78)	1.22 (3.03)	1.12 (3.50)	0.82 (3.19)	0.33 (3.51)
	N2 low association							
Ecstasy user (20)	−1.40 (3.55)	−0.95 (3.76)	−2.76 (4.19)	−1.98 (3.57)	−0.17 (3.62)	−1.56 (3.53)*	−1.01 (3.67)	−2.40 (3.75)*
Polydrug (20)	−1.69 (4.69)	−1.55 (3.15)	−2.11 (4.47)	−1.09 (3.43)	−0.28 (2.16)	−0.22 (4.02)	−0.29 (1.91)	−0.10 (3.71)
Drug naïve (19)	0.01 (3.67)	0.65 (2.97)	−0.33 (4.07)	−0.99 (3.62)	1.55 (2.91)	1.30 (3.58)*	0.91 (2.73)	0.67 (3.48)*
	P2 high association							
	Fz	FCz	FC1	FC2	Cz	C1	C2	
Ecstasy user (20)	0.55 (2.15)	1.49 (2.04)	1.15 (1.79)	0.86 (2.09)	1.91 (1.48)	0.89 (2.13)	0.77 (1.53)	
Polydrug (20)	1.07 (1.98)	1.85 (1.54)	1.28 (1.52)	1.05 (1.87)	1.53 (1.73)	0.87 (1.87)	0.40 (1.64)	
Drug naïve (19)	−0.10 (2.43)	0.78 (2.43)	0.61 (1.45)	0.03 (2.75)	0.59 (2.78)	0.22 (2.03)	−0.22 (2.72)	
	P2 low association							
Ecstasy user (20)	0.87 (2.59)	1.64 (2.39)	1.33 (2.54)	0.83 (2.75)	1.55 (1.97)	1.06 (1.45)	0.93 (1.40)	
Polydrug (20)	0.51 (1.42)	1.72 (1.62)	1.49 (2.81)	0.98 (1.74)	1.38 (1.77)	0.88 (1.99)	0.62 (1.79)	
Drug naïve (19)	0.14 (1.90)	0.72 (2.16)	0.54 (1.98)	0.41 (1.79)	0.81 (2.41)	0.39 (2.22)	0.54 (1.97)	

*Denote significant main effects at $p<.05$

PO4, PO8 and O2) on the P3 component for the mean amplitudes across the epochs measured (280–350 ms in the low association condition, 250–350 ms in the high association condition) revealed no main effect of difficulty ($F(1, 56)=0.71$, $p>0.05$), no difficulty×group interaction ($F(2, 56)=0.60$, $p>0.05$), no main effect of site ($F(4.18, 233.99)=13.97$, $p>0.05$), no difficulty×site interaction ($F(3.42, 191.64)=1.56$, $p>0.05$) and no difficulty by site×group interaction ($F(6.84, 191.64)=0.61$, $p>0.05$). However, there was a significant site×user group interaction ($F(8.36, 233.99)=1.65$, $p\leq0.05$) (degrees of freedom adjusted with Greenhouse–Geisser statistic in all cases). There were no significant between-group effects ($F(2, 56)=0.74$, $p>0.05$), so these were not investigated further. To further explore the site×user group interaction, a series of univariate ANOVAs were run with group as the between-groups variable and amplitude at the various sites as the dependent variable. This yielded no significant differences between the three groups and no significant post hoc comparisons ($p>0.05$ in all cases).

Discussion

The current study investigated the executive function of access to semantic memory and its behavioural and electro-physiological correlates. Background variables such as fluid intelligence, age, measures of sleep, level of arousal, depression and anxiety showed no significant differences between the three groups. There were no behavioural differences between groups in terms of number of errors on task and reaction time to correct responses. Furthermore, ecstasy users did not differ significantly to the control groups with respect to subjective mental workload.

The electro-physiological data provide support for abnormal executive functioning in ecstasy–polydrug users. In the N2 component, although there were no main effects of difficulty or site, or any interactions with these and group, or difficulty by site by group, there were between-group trends that warranted further exploration. In the low association condition of the task, ecstasy users displayed a significantly larger negativity in the N2 (120–200 ms) component

compared to drug-naïve controls in occipital electrode sites O1, O2 and OZ and occipito-parietal electrode sites PO8 and PO4 compared to drug-naïve controls (Fig. 4), although non-ecstasy–polydrug users did not differ from either group.

The supposedly easier "high association" condition showed significant differences in negativity at the N2 component in ecstasy users compared to drug-naïve controls at occipital electrode sites OZ and O2 as well as occipito-parietal sites PO2, PO3, PO4 and PO8 (Fig. 3). Components that reflect positivities (P2 and P3) showed no main effects of difficulty or site, or any interactions with these and group, or difficulty by site by group (except in P3 where there was a site×group interaction) and there were no between-group differences. Thus, these components are less informative about access to semantic memory in ecstasy users. However, the group difference in the N2 component does provide some interesting points to consider.

The N2 component has been reported as having a source in the anterior cingulate cortex (Bekker et al. 2005; Nieuwenhuis et al. 2003; Van Veen and Carter 2002) and to reflect neural processes engaged during conflict monitoring, thus being increased in high conflict trials (Yeung and Cohne 2006), for example, when incongruence between targets and cues/distracters elicits a conflict of response in a Stroop task (Kopp et al. 1996). Firstly, considering why the N2 was more pronounced in ecstasy users compared to drug-naïve controls in those trials where there was a lower semantic association between target and cue words, it is possible that at this level of processing, the ecstasy users required the recruitment of additional resources in order to access the semantic network of long-term memory compared to drug-naïve controls. Previous research has provided evidence that ecstasy users' performance can be more greatly impaired under higher task difficulty. For example, Montgomery et al. (2005a, b) observed a decline in performance in a word fluency task when more rules were imposed, suggesting that deficits are more prominent in tasks that place more demand on the central executive. Moreover, given that participants reported no perceived differences in cognitive effort on the NASA-TLX, it is possible that compensatory cognitive processing at neurological sites is correcting for deficits in executive function to eradicate behavioural differences and other research reporting null results, with respect to performance, may reflect similar reallocation of cognitive resources. This aspect of the results was in line with our predictions.

However, ecstasy users also displayed greater negativity compared to drug-naïve controls in the high association condition of the task, suggesting perhaps that controlled processing, in general, is impaired regardless of task difficulty. It is generally accepted (Jefferies et al. 2004; Rosell et al. 2001; Shiffrin and Schneider 1977) that information processing involves two modes of processing: automatic and controlled. Controlled processing, unlike automatic processing, involves selectively and consciously attending to a stimulus, suggesting that controlled processing involves higher level mental processes. As such, automatic processing is proposed to rely on long-term memory, whilst controlled processing loads more on working memory (Jefferies et al. 2004), suggesting separable neural substrates. Indeed, Rosell et al. (2001) used fMRI to investigate differences in effortful and automatic processing in a similar lexical decision priming experiment and found that distinct sub-regions of the anterior cingulate cortex showed activation dependent on the processing type involved. The N2 component in a semantic classification task was argued to reflect controlled processing by Ritter et al. (1982). Perhaps any early automatic processing (such as that observed in the P2 component) is unaffected by drug use, whereas such higher level controlled processes are more affected. Indeed, perhaps the N2 is the earliest stage at which controlled processing is detectable, and recruitment of additional resources at this stage could offset any further modulation of the waveform in later components such as the P3. Consequently, whilst we hypothesised that controlled processing and recruitment of the central executive would be elicited only in the low association condition, based on the results, it is likely that the task was demanding enough to recruit executive resources in both conditions.

Whilst the above discusses possible N2-related differences in access to semantic memory, it is indeed possible that the N2 in the present study reflects changes in other cognitive processes additional to semantic access (See Folstein and van Petten 2008, for review). The N2 in the present study was prominent in more posterior electrodes that Suwazono et al. (2000) suggest is reflective of increased attention demands in the visual cortex required for stimulus processing. In this study, posterior N2 was eliminated by eliminating target novelty (i.e., making targets completely predictable). Moreover, Luck and Hillyard (1994) investigated sub-components of the N2 component using visual search tasks. It was found that the bilateral posterior N2 as seen in the present study was related to visual search and target probability, with an increased posterior N2 when participants could not predict a target before presentation. Taken together, this provides tentative evidence that in the present study, the posterior N2 may reflect increased demands on visual search and maintenance of visual representations, with greater negativity in ecstasy–polydrug users, showing that they require increased attentional resources for this.

As with most studies on cognitive deficits relating to ecstasy use, there are some limitations that necessitate a degree of caution when interpreting the data. For example,

despite controlling for the use of other drugs, by introducing a polydrug control group (namely, *Cannabis* users) that had never taken ecstasy, the ecstasy users in this study smoked significantly more *Cannabis* that the polydrug group. The ecstasy user group also reported consuming more cocaine than the polydrug group. As such, any observed differences could still be attributed to the use of these other drugs or, indeed, a synergistic effect of concomitant use of other drugs. Perhaps any effects here could be better described as a result of polydrug use, especially given that ecstasy users, although showing greater negativities in the N2 in both conditions of the task compared to drug-naïve controls were not significantly different to polydrug controls.

Studies that employ quasi-experimental designs cannot exclude the chance that individual differences may belie any observable effect other than drug use. We attempted to control for many of these individual differences, such as sleep quality, fluid intelligence and levels of arousal, depression and anxiety. Another limitation lies in self-reports of background drug use, which may not be complete and accurate due to problematic recall of drug users. However, this is the most appropriate method of investigating drug use and executive function, given the legal status of the drug. Moreover, this method is commonly used in the literature (Fox et al. 2001; Montgomery et al. 2005a, b; Montgomery et al. 2010). Purity of the ecstasy tablets consumed as well as cocaine purity and *Cannabis* strength are all questionable and cannot be guaranteed. However, Parrott (2004) reported that the ecstasy tablets collected from amnesty bins in nightclubs in the UK was approaching 100 % purity. If this is incorrect, however, and purity is, in fact, much lower, perhaps this raises additional concerns about the cognitive effects observed (Montgomery et al. 2010).

The present study provides evidence for changes in electro-physiology in ecstasy/polydrug users. Durable abnormalities of the N2 component observed over occipital and occipito-parietal sites of drug users compared to drug-naïve controls is suggestive of compensatory mechanisms or re-allocation of cognitive resources that are deployed to attenuate any observable behavioural differences caused by ecstasy-related disturbances to traditional processing of semantic information and allocation of attention during visual search.

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Experimental and Clinical Psychopharmacology

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Online First Publication, September 30, 2013. doi: 10.1037/a0034002

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1064-1297/13/$12.00 DOI: 10.1037/a0034002

Electrophysiological Evidence of Atypical Processing Underlying Mental Set Shifting in Ecstasy Polydrug and Polydrug Users

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Executive functioning deficits are reported in ecstasy users. However research into mental set switching has been equivocal, with behavioral studies suggesting the function is preserved. The current study sought to address the issue of switching deficits in ecstasy users by combining behavioral performance with electrophysiological correlates (electroencephalography; EEG). Twenty ecstasy polydrug users, 20 nonecstasy polydrug users, and 20 drug naive controls were recruited. Participants completed questionnaires about their drug use, sleep quality, fluid intelligence, and current mood state. Each participant completed a mental set switching task (the number-letter task) while EEG measures were recorded. Analysis of variance (ANOVA) revealed no between-group differences on performance of the task; however a regression suggested that ecstasy use was a significant predictor for performance, after controlling for cannabis use. Mixed ANOVA revealed a significant effect of group on the P3, with significant differences between both drug groups and naives. There was also an interaction between electrode and group on the P2 component, with ecstasy users differing from both other groups. On the P3 component the results suggest a reduction in positivity at parieto-occipital electrodes for drug users compared to controls. Furthermore a significant increase in negativity in ecstasy users compared to control groups could be observed in several occipito-parietal electrodes at an N2 component as well as observable atypicalities in early processing (P2) displayed by ecstasy users and polydrug controls. The present study provides evidence of atypical processing of attentional shifting in ecstasy and polydrug users. Deficits in this executive function could reflect cognitive inflexibility and paucity of rapid behavioral adjustment, which may be problematic in real world situations.

Keywords: ecstasy, cannabis, executive function, stimulants, cannabis

Mental set switching is the ability to switch attention between task types, whereby a switch between tasks is associated with a performance cost, either in accuracy or time, compared to completing two tasks in succession (Jersild, 1927). Switching reflects cognitive flexibility and is one of the core executive functions outlined in Miyake et al.'s (2000) framework. Executive functions are the mechanisms that underpin the dynamics of human cognition (Miyake et al., 2000) and as such alterations to switching ability may have implications for tasks undertaken in daily life. In ecstasy ("MDMA") users, research in switching is equivocal (Fox, Parrott, & Tuner, 2001; Fox et al., 2002). However tasks used do not always solely assess switching (Fisk & Sharp, 2004). The Wisconsin Card Sort Task (WCST) has been used frequently in ecstasy users to assess switching (Reneman et al., 2006; Thomasius et al., 2003) yielding no ecstasy related deficits. The number-letter task (Rogers & Monsell, 1995) has also been used to assess switching (Montgomery, Fisk, Newcombe, & Murphy, 2005), with no clear ecstasy-related deficit reported. Conversely Halpern et al. (2004) observed deficits in switching using the WCST. Interestingly, the cohort in this sample showed minimal exposure to any other drugs and as such potential confounds from polydrug use were reduced. However in a follow-up study (Halpern et al., 2011) with a larger sample and similar controls for concomitant drug use and other lifestyle variables, no such behavioral deficits in relation to switching were observed. However, Dafters (2006) did observe deficits in ecstasy users over cannabis users and controls, in a task switching version of the Stroop task. As such the impact of MDMA exposure on this executive function remains unclear.

Despite equivocal behavioral results observed in previous research it would not be unreasonable to predict that ecstasy users may show reduced or altered set switching, as executive function is understood to rely heavily on areas of the prefrontal cortex. These frontal structures of the brain are rich in 5-hydroxytryptamine receptor neurons (Pazos, Probst, & Palacios, 1987), therefore potential serotonergic

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neurotoxicity or downregulation from regular use of MDMA may cause disruption to the cognitive processes that these areas maintain. Serotonergic neurotoxicity has been observed in various animal studies, however projecting these findings to humans is problematic (for a review, see Easton & Marsden, 2006). Furthermore any executive function deficits observed with behavioral measures have been criticized because of potential confounds such as lack of sleep (Cole, Sumnall, & Grob, 2002) and concomitant use of other drugs in ecstasy users. Many studies in this area attempt to control for this with the addition of a control group of drug users that have never taken ecstasy (M. J. Morgan, 1998; Reay, Hamilton, Kennedy, & Scholey, 2006).

Progress has been made using electroencephalography (EEG) to observe central serotonin dysfunction in users. Burgess, Venables, Jones, Edwards and Parrott (2011) observed event-related potential (ERP) differences in late positivity over left parietal scalp sites associated with recollection between ecstasy users and controls, despite equivalent behavioral performance. The attenuation of this positivity in ecstasy users is evidence of a durable abnormality that would perhaps not have been identified by behavioral measures alone. There is evidence to suggest that ecstasy users may compensate behavioral differences with increased cortical activity compared to controls in fMRI studies (e.g., Daumann, Fimm, Willmes, Thron, & Gouzoulis-Mayfrank, 2003). Typically cognitive impairment is associated with alterations to the P3 amplitude or latency due to the P3 being involved in higher level processing of stimuli. This component encompasses frontal-parieto network activation (Gaspar et al., 2011), and in normal populations decreases in the amplitude potential reflect increased cognitive load and diminished P3 reflects cognitive dysfunction. Longer latencies and smaller amplitude of the P3 response are indicative of cognitive impairment. Diminished P3 potentials have been observed in heavy and moderate ecstasy user groups in simple discrimination tasks (Casco, Forcella, Beretta, Griego, & Campana, 2005). Gamma, Brandeis, Brandeis, and Vollenweider (2005) reported observable reductions in the P3 amplitudes of ecstasy users in a go/no-go task compared to controls, though this could be a polydrug effect. Conversely de Sola et al. (2008) reported no significant differences between ecstasy users, cannabis users, and controls in P3 latency or amplitude in cognitive tests, though P3 latency was correlated with lifetime cannabis use. Ecstasy users also exhibit longer P3 latencies in detecting targets (Mejias et al., 2005). The P3 component is understood to be associated with the allocation of attentional resources necessary for information processing and also memory function (de Sola et al., 2008) and will consequently be implicated in switching. In normal populations larger P3 is observed in repeat trials compared to switch trials (Karayanidis, Coltheart, Michie, & Murphy, 2003), indicative of a greater amount of available processing resources for nonswitch trials as opposed to switch trials (Goffaux, Phillips, Sinai, & Pushkar, 2006).

The aim of this investigation is to assess the cognitive processes supporting switching in ecstasy polydrug users compared to polydrug users who have not used ecstasy, and nondrug users. It is predicted that although behavioral deficits may not be apparent, differences in ERP components, particularly in the P3 component will emerge, especially as this component is thought to play a role in higher level cognitive processing and is susceptible to degradation with cognitive decline. Specifically, it is predicted that ecstasy-polydrug users will have different electrophysiological responses during the task compared to polydrug users and nonusers consistent with cognitive impairment or reallocation.

Experimental Procedures

Design

In all analyses, the between-groups factor was drug user group with three levels (ecstasy user, nonecstasy polydrug user, and drug naive controls). Univariate analysis of variance (ANOVA) was conducted on the behavioral data with the composite scores on the number-letter task (switch cost) as the dependent variable. ERP data was analyzed using mixed ANOVA with electrode site as within participants, group between participants and amplitude as the DV for each ERP component.

Participants

Twenty ecstasy users (mean age = 23.95, *SD* = 0.57, 10 men), 20 nondrug user controls (mean age = 23.1, *SD* = 0.66, 7 men), and 20 nonecstasy drug user controls (mean age = 22.58, *SD* = 0.79, 9 men) were recruited[1] through direct approach to university students, whereby students were contacted via e-mail or given information about the study during lectures and via the online research participation scheme (SONA systems). Participants were aged between 18 and 29 years and reported no neurological impairments. The ecstasy group must have taken ecstasy/MDMA on five or more occasions. The control group must have never used ecstasy/MDMA; however all other illicit substances were permitted for the nonecstasy polydrug user group. All participants were asked to abstain from consuming ecstasy for a minimum of 7 days prior to testing, and urine samples were collected upon arrival at the lab to confirm abstinence. Participants were also requested to abstain from use of other illicit drugs and alcohol for a minimum of 24 hr prior to participating and ideally 7 days. Tobacco smoking was permitted on the day of testing. All participants reported no current or last year diagnosis of psychological disorders.

Materials

Several questionnaires were issued to participants upon entering the lab. Participants completed a drug use questionnaire in which details of ecstasy use as well as other illicit drug use are requested. Using a method used by Montgomery, Fisk, Wareing, Murphy, and Newcombe (2005) estimates of total lifetime drug use of each drug were calculated. Totals for last 30 days drug use as well as weekly drug use estimates were also calculated.

State Mood

State anxiety, arousal, and hedonic tone (depression) were measured using scales devised by Fisk and Warr (1996). Participants are required to rate on a 5-point Likert scale from 1 (*not at all*) to 5 (*extremely*) how they are feeling at the time of testing. A high score on each subscale indicates increased hedonic tone/anxiety/arousal.

[1] Due to unusable data (from noise and artefacts), 18 ecstasy users, 20 polydrug users, and 16 drug naive controls' EEG data was analyzed.

Ravens Progressive Matrices

Ravens Standard Progressive Matrices (SPM; Raven, Raven, & Court, 1998) was used an indicator of fluid intelligence. This involves a series of problems (five sets of 12, 60 in total), presented as a symbolic sequence. Participants are required to select an appropriate response to complete the sequence from a choice of six options. Successful completion of the task requires an understanding of the parts of the sequence and their interaction with one another. Each block of 12 problems begins with an intuitively simple problem and the problems become progressively more difficult as the task continues.

Mental Set Switching

This executive function was investigated using the number-letter task as per Rogers and Monsell (1995). During this task, number-letter pairs (e.g., B6) are displayed in one of four quadrants on a screen. If the number-letter pair appears in one of the top two quadrants, participants are to attend to the letter and respond to whether it is a vowel or a consonant. If in the bottom two quadrants participants are required to attend to the number and respond to whether it is odd or even. In the first block of trials the number-letter pairs alternate between the top two quadrants; in the second block the pairs alternate between the bottom two quadrants. In the final block, the pairs are presented in anticlockwise rotation; therefore every two responses require a shift in the mental set between letters and numbers. The latency between the trials with the switch and those not requiring a switch is the "switch cost." The task is comprised of six blocks, the first two of which are practice blocks consisting to 62 trials in each. This is followed by four main blocks, each consisting of 64 trials (31 "switch" trials). There were 124 "switch" trials in total. There was an intertrial interval of 1.5 s and participants were allocated an epoch of 5 s to respond. Participants were instructed to respond as quickly and as accurately as possible, and overall the task took around 20 minutes to complete.

Equipment

EEG was recorded using a 64-channel Biosemi Ag-AgCl active-two electrode system (Biosemi B.V, Amsterdam, Netherlands) with pin type electrodes mounted in a stretch-lycra headcap (Biosemi), with electrodes positioned according to the international 10–20 system. Electrical activity was recorded from the following sites: frontal (FPz, FP1, FP2), anterior-frontal (AFz, AF3, AF4, AF7, AF8), frontal (Fz, F1, F2, F3, F4, F5, F6, F7, F8), frontocentral (FCz, FC1, FC2, FC3, FC4, FC5, FC6), central (Cz, C1, C2, C3, C4, C5, C6), temporal (FT7, FT8, T7, T8, TP7, TP8), parietocentral (CPz, CP1, CP2, CP3, CP4, CP5, CP6), parietal (Pz, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10), occipitoparietal (POz, PO3, PO4, PO7, PO8), and occipital (Oz, O1, O2, Iz). Vertical and horizontal electro-occulograms were recorded using bipolar flat Ag-ACl electrodes, positioned above and below the left eye as well as to the outer side of each eye. Sigma electrolyte gel was used to ensure contact between scalp and electrodes. Data was digitized at a sampling rate of 512 Hz and no filters were applied online so that the data could be visually inspected for noise and offline filtering could be performed.

Procedure

Testing sessions commenced at 9.30 a.m. or 1.30 p.m. and equal amounts of participants from each condition were tested in the morning as were in the afternoon. Upon entering the lab participants were given a brief description of the experiment and written consent was obtained. Following this, participants were asked to give a urine sample, which was frozen at −25 Celsius until completion of data collection, when all samples were transported to University Hospitals Aintree for analysis. Participants were then asked to fill out the battery of questionnaires while their head circumference and other details were measured, and an electrode cap and electrodes were fitted. The questionnaires were administered in the following order: Background drug use questionnaire, state mood scale and Raven's SPM (Raven et al., 1998). Following completion of these questionnaires, the EEG and actiview set up was tested and if necessary modified. The computerized task was then completed on a desktop computer running Inquisit version 3.0.6.0 (Millisecond Software, 2011). Finally participants were fully debriefed and paid £20 in store vouchers. The study was approved by Liverpool John Moores University Research Ethics Committee, and administered in accordance with the ethical guidelines of the British Psychological Society.

EEG Analysis: Number-Letter

The EEG data was analyzed using BESA 5.3 (MEGIS Software GmbH, Gräfelfing, Germany). All recordings were visually analyzed offline, using high- and low-pass filters of 0.1 Hz and 40 Hz, respectively. Any channels judged to be bad were replaced by interpolation and all data were electro-oculogram-corrected using BESAs PCA-based algorithm. All trials judged to be bad after this point were discarded. EEG was segmented into epochs from −500 to 1000 ms from time of stimulus onset. Epochs were time-averaged by stimulus type so that ERPs for correctly and incorrectly identified stimuli in each condition of each task (e.g., correct "switches," correct "nonswitches," and incorrect "switches" and "nonswitches") could be generated for each individual. Only ERPs for correct responses on the "switch" condition were included in the subsequent analysis. There were 124 "switch" trials in the entirety of the task. The mean number of good "switch" trials retained for grand averaging per subject was 96.37 (average 22.28% rejected trials), after rejecting incorrect trials (4.48%) and those containing artifacts (17.8%). Grand averages were made for each grouping condition (ecstasy user, polydrug user, and drug naive) on each task condition (correct "switches," correct "nonswitches"). The overall P3 response was defined as the mean amplitude between 290 and 400 ms (this time window was centered on the positive peak latency and the duration was chosen as this epoch contained the majority of positive activity for all conditions). Electrode activity was analyzed in this epoch from occipitoparietal and occipital electrodes POz, PO3, PO4, PO7, PO8, Oz, O1, and O2, as the greatest amount of activity in the P3 component could be observed at these sites. Further components were also analyzed for between group differences, including the N2 and P2 components. The N2 component appeared to be largest over occipital and occipitoparietal sites P7, P8, POz, PO3, PO4, PO7, PO8, Oz, O1, and O2, between 170 and 220 ms, this epoch was based around the mean local negative peak at these sites and encompassed the majority of

negative activity over all 3 conditions. The P2 epoch was most visible as a positive peak between 200 and 250 ms at frontal, frontocentral, and central sites Fz, FCZ, FC1, FC2, FC3, FC4, and Cz. The mean amplitudes at these sites from the epoch based around the positive peak from the grand averages of all conditions were analyzed.

Urinary Analysis

Frozen urine samples were delivered to University Hospitals Aintree and were analyzed using solid phase extraction (mixed mode phase) followed by reverse phase HPLC MS/MS detection using both positive and negative ion multiple reaction monitoring (MRM). urine specimens have been tested for the synthetic cannabinoids (JWH-018, JWH-073, JWH-250, JWH-398, JWH-122, JWH-019, a.m.-694, WIN 48098, and WIN-55212–2), as well as the following "designer" drugs: mephedrone, bk-MDMA or methylone, bk-MBDB or butylone, bk-PMMA or methedrone, 1-benzylpiperazine, TFMPP, mCPP, and MDPV. In addition they were tested for were a series of 12 piperazine compounds, 4 β-keto amphetamines, a series of 11 methcathinone compounds, 4-fluoroamphetamine, bupropion, and the hallucinogenic amphetamines: D.O.B. (bromo-STP or Brolamphetamine), D.O.C. and D.O.I., and "traditional" drugs of abuse: amphetamine(s) including M.D.M.A., M.D.A., and M.D.E.A., barbiturates, benzodiazepines, THC and cannabinoids, buprenorphine, cocaine and metabolites, methadone and metabolites, opiates, and opioids (morphine, codeine, dihydrocodeine, tramadol, d-propoxyphene, oxymorphone, and oxycodone), LSD, G.H.B. (and the Lactone Precursor), psilocybin, ketamine, and methaqualone.

Statistical Analysis

EEG data was analyzed using a mixed ANOVA for each component (P2, N2, P3) with drug user group as the between-subjects factor, electrode sites for the particular component as the within-subject factor, and mean amplitudes at the various components at selected sites as the dependent variable. Any significant interactions or main effects between groups were further analyzed using univariate ANOVA and Tukey's HSD test.

Results

State mood scores, fluid intelligence scores, and drug use variables are displayed in Table 1. One-way ANOVA revealed that there were no significant between-group differences on age, levels of arousal, depression, and anxiety or total score on Ravens SPM. *t* Tests between the ecstasy user group and the polydrug-nonecstasy group revealed that the ecstasy user group had a significantly larger lifetime total of joints smoked than the nonecstasy drug users, $t(17.88) = 2.02$, $p < .05$ (Levene's test was significant so degrees of freedom have been adjusted accordingly). Ecstasy users had also smoked significantly more joints within the last 30 days, $t(16.01) = 1.86$, $p < .05$.

Urinary Analysis

Some drug metabolites were found in participants' urine. Specifically three ecstasy users' urine contained THC (mean 0.0083 mg/L ± 0.01185), Δ-9-THC (0.16 mg/L ± 0.18 mg/L), and 11-hydroxy-Δ-9THC (0.003 mg/L ± 0.003). One ecstasy user's urine also contained 1-benzopiperazine (0.84 mg/L) and TFMPP

Table 1
Background Variables

Variable	Ecstasy users			Nonecstasy drug users			Drug naïve controls		
	M	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*	*n*
RPM (maximum = 60)	48.68	5.96		48.35	5.83		51.35	5.01	
State anxiety	11.4	4.08		12.44	2.18		11.75	2.12	
State depression	13.1	3.91		12.61	2.4		12.1	3.14	
State arousal	19.7	4.54		20.5	3.68		20.1	3.02	
Ecstasy									
Frequency (times/week)	0.24	0.42	12						
Last 30 days (tablets)	0.60	2.26	20						
Total use (tablets)	177.65	301.73	20						
Cannabis									
Frequency (times/wk)	2.67	3.24	12	0.95	1.9	13			
Last 30 days (joints)	32.77*	53.75	15	6.09*	15.34	17			
Total use (joints)	5057.88*	7504.30	16	1091.71*	2531.65	19			
Cocaine									
Frequency (times/week)	0.15	0.14	11	0.27	0.34	2			
Last 30 days (lines)	0.4	1.12	15	1.60	3.58	5			
Total use (lines)	813.97	1940.19	16	107.30	208.43	5			
Ketamine									
Frequency (times/week)	0.26	0.42	5	0.02	—	1			
Last 30 days use (grams)	1	2.65	9						
Total use (grams)	31.26	70.61	11	1.13	1.62	3			
Alcohol units per week	15.33	15.29	20	10.53	8.37	20	9.93	11.58	20
Tobacco cigarettes per day	6.98	6.32	13	7.1	5.48	5	1		1

Note. RPM = Raven's Progressive Matrices.
* Significant difference at $p < .05$.

(0.18 mg/L). One participant in the polydrug group had cannabis metabolites in their urine, specifically THC (0.001 mg/L), Δ-9-THC (0.41 mg/L), and 11-hydroxy-Δ-9THC (0.002 mg/L).[2]

Behavioral Data Analysis

Incorrect answers were given a score of 0 and were not investigated any further. Mean reaction times (RTs) were calculated for correct switch trials as well as correct nonswitch trials so that a switch cost could be calculated. RT data reduction involved excluding RTs less than 200 ms and greater than 4000 ms. Individual trial RTs that were more than 3 standard deviations above the individual mean were discarded. The mean percentage of outliers that were discarded from each group were ecstasy users 1.27 (± 0.73) (rank = 24.58), polydrug users 1.64 (± 0.77) (rank = 33.75), drug naive 6.56 (± 22.0) (rank = 33.18), there were no between-group differences in amount of outliers, $H(2) = 3.53$, $p > .05$.

Switch cost was calculated by subtracting the mean RT from two preliminary blocks with no switching (all letters, followed by all numbers) from the mean RT from the switch trials (from letters to numbers) in the main blocks of the task. One participant in the drug naive group had an incomplete dataset for this task and as such was excluded from analysis. ANOVA revealed that there was no significant difference between groups on switch cost, $F(2, 56) = 0.41$, $p > .05$. Given the heavy use of cannabis in the ecstasy use group in particular, and the possible confounding effects of gender, IQ, and age on performance, a stepwise regression analyses were conducted on the behavioral data, to observe whether level use of ecstasy (after controlling for cannabis use, age, gender, and IQ) was a predictor for performance on the task. In Step 1, age, gender, and IQ (Ravens SPM total score) were entered as predictors. This model accounted for a small and nonsignificant 3.6% of the variance in switch cost; R^2 change = 0.036, $F(3, 52) = 0.65$, $p > .05$ ($\beta = 0.01$; 0.05; 0.18 for gender, age, and IQ, respectively, $p > .05$ in all cases). In Step 2, total lifetime dose of cannabis was entered as a predictor, this model accounted for an additional nonsignificant 0.4% of the variance in switch cost; R^2 change = 0.004, $F(4, 51) = 0.53$, $p > .05$ ($\beta = 0.07$, $p > .05$). In the third step, total lifetime dose of ecstasy was entered as a predictor. This regression model accounted for 15.2% of the variance in switch cost. After controlling for age, gender, IQ, and cannabis use, total ecstasy use predicted an additional 11.2% of variance in switching deficits; R^2 change = 0.11, $F(5, 50) = 1.79$, $p > .05$. Although the overall regression model was nonsignificant, total lifetime dose of ecstasy emerged as the only significant predictor of switch cost after controlling for all of the other variables, meaning participants who had consumed a greater amount of ecstasy showed significantly poorer performance in this task, $\beta = 0.59$; $t(50) = 2.57$, $p < .01$. Furthermore, to investigate the effects of recent cannabis use, we performed the same regression analyses with amount smoked in the last 30 days replacing total lifetime dose as a predictor. In Step 1, age, gender, and IQ (Ravens SPM total score) were entered as predictors. This model accounted for a small and nonsignificant 3.6% of the variance in switch cost; R^2 change = 0.036, $F(3, 52) = 0.65$, $p > .05$ ($\beta = 0.01$, 0.05, and 0.18 for gender, age, and IQ, respectively, $p > .05$ in all cases. In Step 2, number of joints smoked in the last 30 days was entered as a predictor, this model accounted for an additional nonsignificant 0.5% of the variance in switch cost; R^2 change = 0.005, $F(4, 51) = 0.54$, $p > .05$ ($\beta = 0.07$, $p > .05$). In the third step, number of tablets used in the last 30 days was entered as a predictor. This regression model accounted for 8.9% of the variance in switch cost. After controlling for age, gender, IQ, and cannabis use, total ecstasy use predicted an additional 4.8% of variance in switching deficits; R^2 change = 0.048, $F(5, 50) = 0.98$, $p > .05$). There were no significant individual predictors.

ERP Analysis

The grand averages for each group (users, polydrug nonusers, and drug naive controls) can be observed at various electrodes measured for the separate components in Figures 2 and 3. Mean amplitudes for each condition and electrode are given in Table 2. Because of some participants not completing the task and some unusable EEG data, six participants are excluded from statistical analysis on the EEG data, four from the drug naive group ($n = 16$), and two from the ecstasy user group ($n = 18$).

Mixed ANOVA[3] of mean amplitudes at component P3 (290–400 ms) revealed a significant main effect of electrode site, $F(4.04, 206.02) = 15.78$, $p < .001$, though the Electrode × User Group interaction was nonsignificant, $F(5.05, 206.02) = 0.99$, $p > .05$. There was however a significant main effect of group, $F(2, 51) = 3.35$, $p < .05$. To further explore this difference, a series of one-way ANOVAs with group as between participants were conducted. This yielded significant effect of group at electrode O1, $F(2, 51) = 3.80$, $p < .05$, with post hoc tests indicating that both drug using groups differed significantly from drug naive participants ($p < .05$) but not from each other ($p > .05$). There were also significant differences at electrode POz, $F(2, 51) = 4.56$, $p < .05$, and again, post hoc analysis showed that both drug groups differed significantly from drug naive participants ($p < .05$, one-tailed) but not from each other ($p > .05$); There were also significant differences at PO4, $F(2, 51) = 3.11$, $p < .05$, with post hoc tests indicating significant differences between polydrug and naive participants ($p < .05$).

Mixed ANOVA of mean amplitudes at component N2 (170–220) revealed a significant main effect of electrode, $F(4.27, 217.82) = 12.23$, $p < .001$. The Electrode × User Group interaction, $F(8.54) = 217.81) = 0.76$, $p > .05$, and the main effect of group, $F(2, 51) = 1.83$, $p > .05$, were however nonsignificant so this component is not discussed further.

At component P2 (200–250 ms) Mixed ANOVA revealed a nonsignificant effect of electrode, $F(3.30, 168.44) = 1.60$, $p > .05$, though the Electrode × User Group interaction was significant, $F(6.61, 168.44) = 2.12$, $p < .05$. The main effect of group was not significant for this component, $F(2, 51) = 2.11$, $p > .05$. To further explore the nature of the significant interaction, a series of one-way ANOVAs were used. These yielded significant group differences at electrode Fz, $F(2, 51) = 3.52$, $p < .05$, with post hoc analysis showing that ecstasy polydrug users differed from both other groups ($p < .05$); at electrode FCz, $F(2, 51) = 5.66$, $p < .01$, with ecstasy polydrug users differing from both other groups ($p <$

[2] Main analyses were rerun excluding these participants. This did not change the direction or significance of results so analyses in the article pertain to all participants.

[3] In all mixed ANOVAs, Mauchley's test was significant so adjusted degrees of freedom are reported in line with the Greenhouse Geisser statistic.

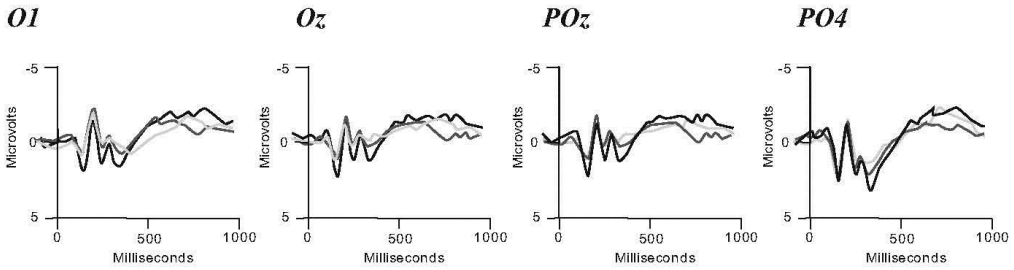

Figure 1. Grand average waveforms for the three groups across electrodes: O1, Oz, POz, and PO4 (correct switches). The figure depicts the waveforms from electrodes that showed significant group differences in the P3 component. Ecstasy users are displayed in blue, polydrug users are displayed in black, and drug naive controls are displayed in lilac. These waveforms are from grand averaged data from each user group. The significant differences between ecstasy users and drug naive controls as well as polydrug users and drug naive controls can be seen in O1, the difference (approaching sig) between polydrug users and naives can be seen in Oz (between 290 and 400ms). The significant differences between these groups in this component can be viewed again in POz, this time the difference between ecstasy users and drug naive controls is approaching significance and finally PO4 depicts the difference between polydrug users and drug naive controls.

.05); and at electrode Cz, $F(2, 51) = 3.14$, $p < .05$, with ecstasy polydrug users differing from both other groups ($p < .05$, one-tailed). Inspection of Table 2 suggests that for all the electrodes, ecstasy users have higher mean P2 amplitudes than the other two groups, with the exception of electrode FC3, where the opposite pattern is seen.

Discussion

The aim of this study was to examine the processes involved in mental set switching in ecstasy users, using a number-letter task. The control groups did not differ from the ecstasy users on the background variables such as fluid intelligence, age, and state mood. Nor did they differ on the number-letter task in terms of number of switch cost or errors. A regression analysis revealed that after controlling for cannabis use, level use of ecstasy predicted performance deficits behaviorally. This is in line with previous research on amphetamine users (Ornstein et al., 2000) that suggests that more chronic use leads to a greater deficit in mental set switching, as well as Halpern et al. (2004)'s original study who observed greater performance deficits on this task in heavy ecstasy users than light users.

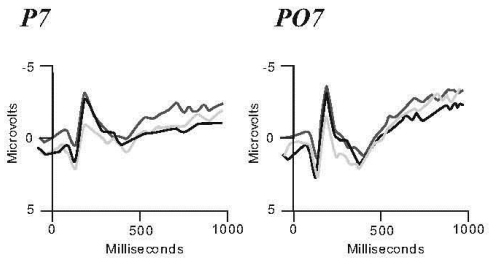

Figure 2. Grand average waveforms for the three groups across electrodes: P7 and PO7 (correct switches). The figure depicts the waveforms from electrodes that showed significant group differences in the N2 component. Ecstasy users are displayed in blue, polydrug users are displayed in black, and drug naive controls are displayed in lilac. P7 shows the significant difference between ecstasy users and drug naive controls, this difference is approaching significance and can be observed in PO7; to observe the difference that is approaching significance in O1, see Figure 1.

The electrophysiological data also provide support for deficits in this executive function with drug use. The P3 component, thought to play an important role in the allocation of attentional resources and as such an important role in the ability to switch between mental sets, showed significant between group differences at several occipito-parietal and occipital electrode sites. Nonusers displayed a significantly higher mean amplitude in this component (290–400 ms) compared to ecstasy users as well as polydrug controls. A diminished P3 component is thought to reflect cognitive impairment, and as such these findings are in line with those of Casco et al. (2005) and Mejias et al. (2005) who have observed reduced P3 in ecstasy users compared to controls in other cognitive tasks. Interestingly, the polydrug control group appears to have a reduced P3 in several areas compared to drug naive controls, suggesting some evidence of atypical processing that is related to the use of drugs in general and not just ecstasy (i.e., a polydrug effect). Furthermore, it has been suggested previously that concomitant cannabis use may account in part or fully for cognitive deficits observed in ecstasy users (Dafters, Hoshi, & Talbot, 2004; Gamma et al., 2005), though in the present study, level of ecstasy use appears to be a more important predictor.

Analysis of the N2 component provided some interesting findings, with ecstasy users and polydrug users generally showing greater negativity compared to drug naive controls at the electrodes measured (occipito-parietal and occipital sites). These differences were only significant between ecstasy users and drug naive controls at site P7, and differences between these groups were approaching significance at two more sites (PO7 and O1). There were, however, no observable differences between ecstasy

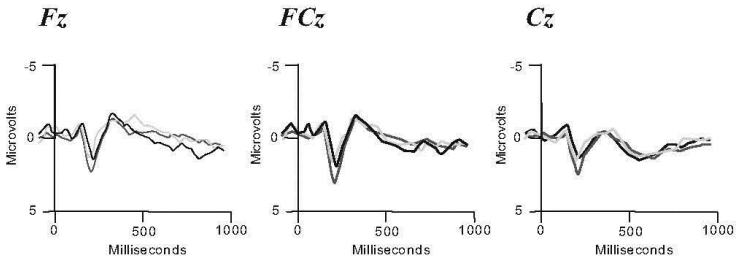

Figure 3. Grand average waveforms for the three groups across electrodes: Fz, FCz, and Cz (correct switches). The figure depicts the waveforms from electrodes that showed significant group differences in the P2 component. Ecstasy users are displayed in blue, polydrug users are displayed in black, and drug naive controls are displayed in lilac. The significant differences between ecstasy users and both control groups can be seen in FCz (200–250ms), the difference (approaching sig) between ecstasy users and the two control groups can be seen in Fz. Finally in Cz the difference between ecstasy users and polydrug users (approaching sig) can be observed.

users and polydrug controls in this component. This component is thought to reflect conflict monitoring and is also reported to be greater in trials with high conflict (Yeung & Cohen, 2006). Perhaps higher mean amplitude in this component would reflect inefficiency in processing this type of information.

Differences were also apparent in the P2 component, involved in early processing of stimuli. It was observed that ecstasy users displayed a significantly higher mean amplitude than both control groups at fronto-central site FCz. Further to this, the ecstasy users were approaching significance for higher mean amplitude than polydrug controls at central site CZ and approached significance for having a higher mean amplitude compared to both groups at frontal midline site Fz. Atypicalities at this early stage of processing in ecstasy users appear to provide evidence to suggest additional resources are being recruited as a compensatory mechanism. Perhaps additional recruitment of resources at this stage allowed for similar results behaviorally, despite a diminished P3 amplitude at a later stage of processing.

These results suggest evidence for an ecstasy/polydrug effect on the degradation of the executive function of mental set switching. Few previous studies have found cognitive atypicalities (Halpern et al., 2004); however those who have previously suggested no deficit in this executive function have neither had the purity of the cohort examined in the Halpern et al. (2004) study, nor perhaps equivalent lifetime dose. However in the follow up study (Halpern et al., 2011) using participants with minimal exposure to other drugs and a larger sample size, the effects of MDMA on switching were not evident, so perhaps of greater importance is the use of more sensitive measures of cognitive impairment such as EEG.

Limitations

Unlike the relatively "pure" MDMA user groups in the two studies by Halpen et al. (2004, 2011), the current ecstasy user group tended to take several other drugs—in particular cannabis. Although we attempted to control for this with the addition of a

Table 2
Mean Amplitudes Across Components for Each Electrode Measured

	PO7	PO3	O1	Oz	POz	PO8	PO4	O2	P7	P8
P3										
Ecstasy	1.25 (2.13)	2.59 (1.63)	0.68 (1.84)	0.40 (1.52)	2.67 (2.37)	1.63 (2.58)	2.24 (1.70)	0.37 (1.76)		
Polydrug	1.94 (2.05)	2.57 (1.54)	0.71 (1.90)	0.27 (1.72)	2.27 (0.94)	0.92 (3.11)	1.94 (1.77)	0.32 (2.82)		
Drug naive	2.03 (2.20)	3.56 (2.61)	2.37 (2.41)*	1.66 (2.33)	4.50 (3.26)	1.56 (3.49)	3.61 (2.75)	1.93 (2.72)		
N2										
Ecstasy	−2.56 (0.61)	−0.90 (0.46)	−1.58 (0.53)	−0.15 (0.48)	0.45 (0.60)	−0.66 (0.78)	−0.06 (0.60)	−0.48 (0.60)	−2.81 (0.46)*	−1.36 (0.63)
Polydrug	−2.08 (0.57)	−0.70 (0.44)	−1.17 (0.50)	0.47 (0.45)	0.81 (0.57)	−1.44 (0.74)	0.12 (0.57)	0.39 (0.57)	−1.88 (0.44)	−0.87 (0.60)
Drug naive	−0.60 (0.64)	0.05 (0.50)	0.20 (0.56)	0.70 (0.51)	1.09 (0.64)	−0.45 (0.82)	0.57 (0.64)	0.56 (0.63)	−0.66 (0.49)*	−0.13 (0.67)

	FC3	FC1	Fz	FC4	FC2	FCz	Cz
P2							
Ecstasy	−0.18 (4.22)	1.07 (1.38)	1.54 (1.63)	1.66 (1.33)	1.57 (1.24)	2.10 (1.45)	1.78 (1.24)
Polydrug	0.95 (1.18)	0.65 (1.34)	0.37 (1.84)	1.61 (1.64)	1.10 (1.69)	0.64 (1.43)	0.45 (2.34)
Drug naive	0.96 (2.30)	0.74 (1.86)	0.28 (1.12)	0.88 (1.53)	0.59 (1.29)	0.59 (1.71)	0.47 (1.63)

*$p < .05$.

polydrug control group, it was apparent that the ecstasy user group smoked significantly more cannabis and consumed significantly more cocaine than this group. Furthermore there were nine participants in the ecstasy user group who reported using ketamine in the last 30 days, compared to none in the polydrug group, this is potentially problematic for the interpretation of the current results given the association between ketamine use and executive function deficits in humans (for a review, see C. J. A. Morgan & Curran, 2006), and specifically that switching has been shown to be impaired in animals with ketamine exposure (Stoet & Snyder, 2006). Moreover, the polydrug user group also showed a diminished P3 response compared to drug naive controls in several sites, so perhaps it would be more accurate to call the observed effects "polydrug effects." With this in mind, it can also not be ruled out that premorbid factors do not predict drug use, and that such factors (e.g., differences in sensation seeking) contribute to the observed differences in the present study. In addition, the self-reporting of psychological state is potentially problematic and in future research, a structured psychiatric assessment may be more appropriate. Tobacco use was also not controlled for in the current study, there has been previous research to suggest that tobacco smoking has an effect on EEG measures (Ilan & Polich, 2001; Gilbert et al., 2004). In particular, abstinence from tobacco smoking in normal users of tobacco, has shown performance and activity decline that can last for up to 31 days (Gilbert et al., 2004). However, smokers were permitted to smoke tobacco on the day of testing so this is unlikely to have affected the results reported here. The quasi-experimental design used here also means that the current authors cannot state that the individual differences witnessed here are not the result of factors other than drug use, though we have attempted to control for many of these such as fluid intelligence, age, state mood, and residual intoxication of drugs. Residual intoxication of alcohol was self-report, but in future studies it would be advantageous to verify this with a breathalyzer to ensure no residual alcohol intoxication. Self-report measures for background drug use are also problematic, however because of the legal status of the drugs consumed, this remains the most appropriate measure of background drug use and is also the most commonly used in this area of research (Fox et al., 2001; Montgomery, Fisk, Newcombe, & Murphy, 2005; Montgomery, Fisk, Wareing, et al., 2005; Montgomery, Hatton, Fisk, Ogden, & Jansari, 2010). The purity of the tablets consumed by the current set of participants as well as the strength of the cannabis being consumed is questionable. However, Parrott (2004) reported that the purity of ecstasy tablets collected from amnesty bins in nightclubs in the United Kingdom is approaching 100%. However if this is not the case then this raises additional concerns over the magnitude of cognitive deficits incurred (Montgomery et al., 2010).

The present study provides evidence for differences in cognitive function in ecstasy/polydrug users. Given its association with resource allocation and higher level processing, electrophysiological differences in the P3 component suggest a deficit in the cognitive resources necessary for normal functioning in the set switching task. Furthermore atypical early processing of stimuli in ecstasy users is suggestive of compensatory mechanisms used to attenuate behavioral differences due to disturbances in normal processing. Moreover after controlling for cannabis-related effects, regression analysis suggests that set switching performance declines with increased use of ecstasy. The broader implications here suggest reduced cognitive flexibility with increased drug use. The inability to rapidly adjust behavior to suit environmental changes has consequences for real world situations, for example the ability to which ones job can be performed adequately. Furthermore, as this sample was relatively young, it would be interesting to observe persistent problems with cognitive flexibility in an ageing population with a history of heavy drug use.

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Received January 31, 2013
Revision received June 26, 2013
Accepted June 26, 2013 ■