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**Hippocampus and basal forebrain volumes modulate effects of anticholinergic treatment on delayed recall in healthy older adults**

# Hippocampus and basal forebrain volumes modulate effects of anticholinergic treatment on delayed recall in healthy older adults

Stefan J. Teipel[1,2], Davide Bruno[3], Michel J. Grothe[1], Jay Nierenberg[4,5], Nunzio Pomara[4,5]

[1]German Center for Neurodegenerative Diseases (DZNE) – Rostock/Greifswald, Rostock, Germany

[2]Department of Psychosomatic Medicine, University of Rostock, Rostock, Germany

[3]Department of Psychology, Liverpool Hope University, Liverpool, UK

[4]Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, USA

[5]Department of Psychiatry, School of Medicine, New York University, New York City, NY, USA

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**Corresponding Author:**
Stefan J. Teipel, M.D.
Department of Psychosomatic Medicine
University of Rostock,
and DZNE Rostock,
Gehlsheimer Str. 20,
18147 Rostock, Germany
Tel.: 01149-381-494-9470
Fax: 01149-381-494-9472
E-mail: stefan.teipel@med.uni-rostock.de

## Abstract

INTRODUCTION: Volumes of hippocampus and cholinergic basal forebrain are associated with delayed recall performance and may modulate the effect of a muscarinic receptor antagonist on delayed recall in healthy volunteers.

METHODS: We studied 15 older adults before and after oral administration of a single dose of 1mg or 2mg of the preferential M1 muscarinic receptor antagonist trihexyphenidyl (Artane™) or placebo in a double-blind randomized cross-over design. Hippocampus and basal forebrain volumes were measured using MRI.

RESULTS: We found a significant interaction between treatment and hippocampus volume and a trend level effect between treatment and anterior basal forebrain volume on task performance, with an attenuation of the association between volume size and performance with trihexyphenidyl.

DISCUSSION: These findings suggest a reduction of delayed recall performance with increasing doses of the muscarinic antagonist that is related to an uncoupling of the association of task performance with cholinergic basal forebrain and hippocampus volumes.

## Introduction

Administration of trihexyphenidyl hydrochloride (Artane™) and other muscarinic receptor antagonists has been reported to decrease delayed recall in healthy volunteers [1-3]. These findings are consistent with cholinergic transmission playing a key role in attention and memory tasks that require effort and concentration [4]. The main cholinergic input to the human cerebral cortex arises from the basal forebrain cholinergic nuclei [5]. The hippocampus is involved in the coherent representation of a memory, a requirement for successful retrieval [6]. The main cholinergic input to the hippocampus arises from the most anterior subnuclei of the cholinergic basal forebrain [7], termed Ch1 and Ch2 according to Mesulam's nomenclature [8].

In the present study, we examined whether the volumes of the anterior basal forebrain and hippocampus, as measured from structural MRI scans, modulate the effect of anticholinergic treatment with trihexyphenidyl on delayed recall performance in a group of 15 cognitively and physically healthy older adults. We expected that a higher volume would be associated with higher delayed recall performance and that this association would be reduced with higher doses of trihexyphenidyl.

## Participants and Methods

The study included 15 healthy elderly individuals (8 women), mean age was 66.9 (SD 3.7) years, ranging between 62 and 74 years, and mean education was 16.7 (SD 2.3) years. Individuals did not take medications known to affect cognitive functioning, such as neuroleptics or antidepressants, at least 2 weeks before beginning the study and had a negative urine toxicology screen. Further details of recruitment have been described before [9]. All subjects were only examined if they gave their written informed consent. The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

## Neuropsychological testing and Study Design

Participants were recruited as part of a study on the effects of APOE variants on the response to trihexyphenidyl [9]. Delayed recall (after 15 minutes) was tested using the Buschke Selective Reminding Test [10], administered before as well as 1, 2.5 and 5 hours after drug/placebo administration. Each subject participated in the 3-week, double-blind, randomized, placebo-controlled study, with sessions taking place one week apart: treatment conditions were placebo, 1.0mg and 2.0mg of trihexyphenidyl. To exclude training effects within and across treatment sessions, twelve parallel word lists were used, in a randomized rotational basis across sessions. The same list was used across subjects in each of the 12 assessment periods (treatment (3) X test time (4)).

## MRI data acquisition and analysis

The acquisition was performed on a 1.5 T Siemens Vision system (Erlangen, Germany) at the Nathan S. Kline Institute for Psychiatric Research, NY, USA. Images were acquired using a sagittal magnetization prepared rapid gradient-echo sequence [MPRAGE; repetition time (TR)/echo time (TE)=11.4/11.9 ms, 1 excitation (NEX), matrix=256 x 256, FOV=307 mm, 1.2mm$^3$ isotropic voxel, 172 slices, no gap].

MRI data processing followed procedures described previously for hippocampus [11] and basal forebrain [12] volumetry, implemented in SPM8 and the VBM8-toolbox in Matlab. Basal forebrain subregions [8] were determined according to a map from an *in cranio* post mortem MRI scan and histology of a single individual's brain, as previously described [12]. The total intracranial volume (TIV) was used in the statistical model to account for differences in head size, and was calculated as the sum of the total segmented gray matter, white matter and cerebrospinal fluid volumes in native space. We selected volumes of left and right hippocampus as well as of most anterior basal forebrain nuclei, Ch1 and Ch2 according to Mesulam's nomenclature [8], that provide the main cholinergic innervation of the hippocampus [7].

**Statistical analysis**

We determined the effect of treatment on delayed recall across subjects using a mixed effects model with subject-related random effects, controlling for age and sex. For time after drug intake (0 to 5 hours), we compared a linear with a second order polynomial term. The model fit was compared between the two nested models (first vs. second order polynomial term for time) using Akaike's information criterion (AIC) [13].

To test for an interaction between volumes of basal forebrain and hippocampus, respectively, and the drug effect, we selected performance at the time of peak drug effect (1 to 2.5 hours post ingestion [14]); we also tested for main effects of volume and drug, and controlled for total intracranial volume, sex and age in all analyses. Significance of parameters was determined using t-statistics with degrees of freedom determined according to the Satterthwaite approximation.

Analyses were performed with R, version 3.1.1, including the libraries "lme4" and "lmerTest", available at http://cran.r-project.org/web/packages.

**Results**

In the basic model across all time points, we found a significant linear effect of time on delayed recall performance ($t=-4.6$, $df=162$, $p<0.001$). There was no significant main effect of treatment ($t=-1.68$, $df=162$, $p=0.09$). The second order polynomial model improved the fit over the linear model (AIC=837.7 for the linear model, and AIC=813.1 for the polynomial model).

For hippocampus, considering the peak drug effect time points only, we detected a significant volume by treatment interaction (left and right hippocampus: $t=-2.1$, $df=72$, $p=0.04$; Figure 1) as well as a significant main effect of treatment ($t=-3.2$, $df=72$, $p=0.002$). With anterior basal forebrain volume, we observed a trend level significant interaction effect ($t=-1.8$, $df=72$, $p=0.074$; Figure 2), and a significant main effect of treatment ($t=-3.2$, $df=72$, $p=0.002$). AIC of nested models indicated moderately improved fit for the complex models compared with the basic model: basic model AIC = 423.4; full model with Ch1/2, AIC = 421.3; full model with left hippocampus, AIC = 422.3; full model with right hippocampus, AIC = 422.2.

- Figures 1 and 2 near here -

## Discussion

We found a decline in delayed recall performance in response to the muscarinic antagonist trihexyphenidyl, consistent with previous studies [1-3] and predictions. We used a mixed effects model to take blocking of observations within subjects and inter-individual variation in performance levels into account [15]. The time activity curve of the drug was best modeled by a second order polynomial consistent with the previously reported central pharmacodynamics of trihexyphenidyl [14]. Under placebo, hippocampal and anterior basal forebrain volumes were positively associated with delayed recall performance. The treatment-induced decline of performance was associated a reduced or even revers association between regional volume and task performance with a higher dose of the muscarinic receptor antagonist (Figures 1 and 2). The treatment-induced reductions of performance, compared with placebo, were more pronounced at higher volumes. This finding suggests the presence of a possible floor effect for performance at smaller volumes whereby performance levels only decrease slightly as a consequence of medication since cholinergic blocking is less effective when the system and its input areas in the hippocampus are already impaired.

The numerically similar effects for the hippocampus and the cholinergic system would indicate that cholinergic input towards the hippocampus from the anterior basal forebrain (the main source of cholinergic projections to the hippocampus [7]) only partially regulates hippocampus-related determinants of delayed recall performance [16]. Due to the limited number of participants in our sample, however, we did not formally test for such a potential mediation effect.

Contrary to our observations, it may be expected that the effect of anticholinergic treatment on task performance should be less pronounced with larger volumes rather than with smaller volumes, if the former indicate a higher number of neurons with viable M1 muscarinic receptors that could compensate for a partial block of receptors. This assumption would hold if variation in volume size were not linked as a state marker to performance but

mainly represented a trait marker of reserve capacity, and if the level of anticholinergic effect were just above the threshold necessary to elicit functional effects. Here, however, we examined a sample of older adults where a low dose of the drug already induced significant decline in performance. This suggests that variation in cholinergic system integrity serves at least partially as a state marker for functional performance, and at smaller volumes the blockade of muscarinic receptors occurs in an already impaired cholinergic system. To better resolve this question, one would need to study the interaction between anticholinergic treatment, and hippocampus and basal forebrain volumes in healthy young adults where variation in volume is expected not to be rate limiting for task performance.

Increased atrophy of the cholinergic basal forebrain and decline of cholinergic functioning over and above the effects of normal aging have been associated with cognitive decline and the development of dementia in neurodegenerative conditions such as Alzheimer's disease and Lewy body disorders [17]. Controlled anticholinergic stress tests have been proposed as prognostic markers for the development of dementia in the elderly [18, 19]. MRI-based measurements of the structural integrity of the cholinergic basal forebrain and its functionally relevant target areas may provide important neurobiological additional information to such pharmacological stress tests.

In summary, our findings, which should be replicated in an independent sample, suggest that anticholinergic treatment leads to a partial uncoupling of hippocampus and basal forebrain atrophy from delayed recall task performance, providing *in vivo* evidence that both structures functionally subserve delayed recall mediated by cholinergic input to the hippocampus.

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**Figure legends:**

**Figure 1: Association of delayed recall performance with left hippocampus volume at different levels of treatment**

Using the “effects” library in R, we computed the fitted values and standard errors for delayed recall under the model for the interaction term of treatment by (mean centered) volume with the values of the other predictors being fixed at typical values, i.e. for an interval scaled covariate at its mean, and for a factor at its proportional distribution in the data, as described in [20].

Treatment 1mg = 1 mg trihexyphenidyl

Treatment 2mg = 2mg trihexyphenidyl

**Figure 2: Association of delayed recall performance with anterior basal forebrain (Ch1/2) volume at different levels of treatment**

For legend see figure 1.

**Figure 1: Fit of delayed recall performance with left hippocampus volume at different levels of treatment**

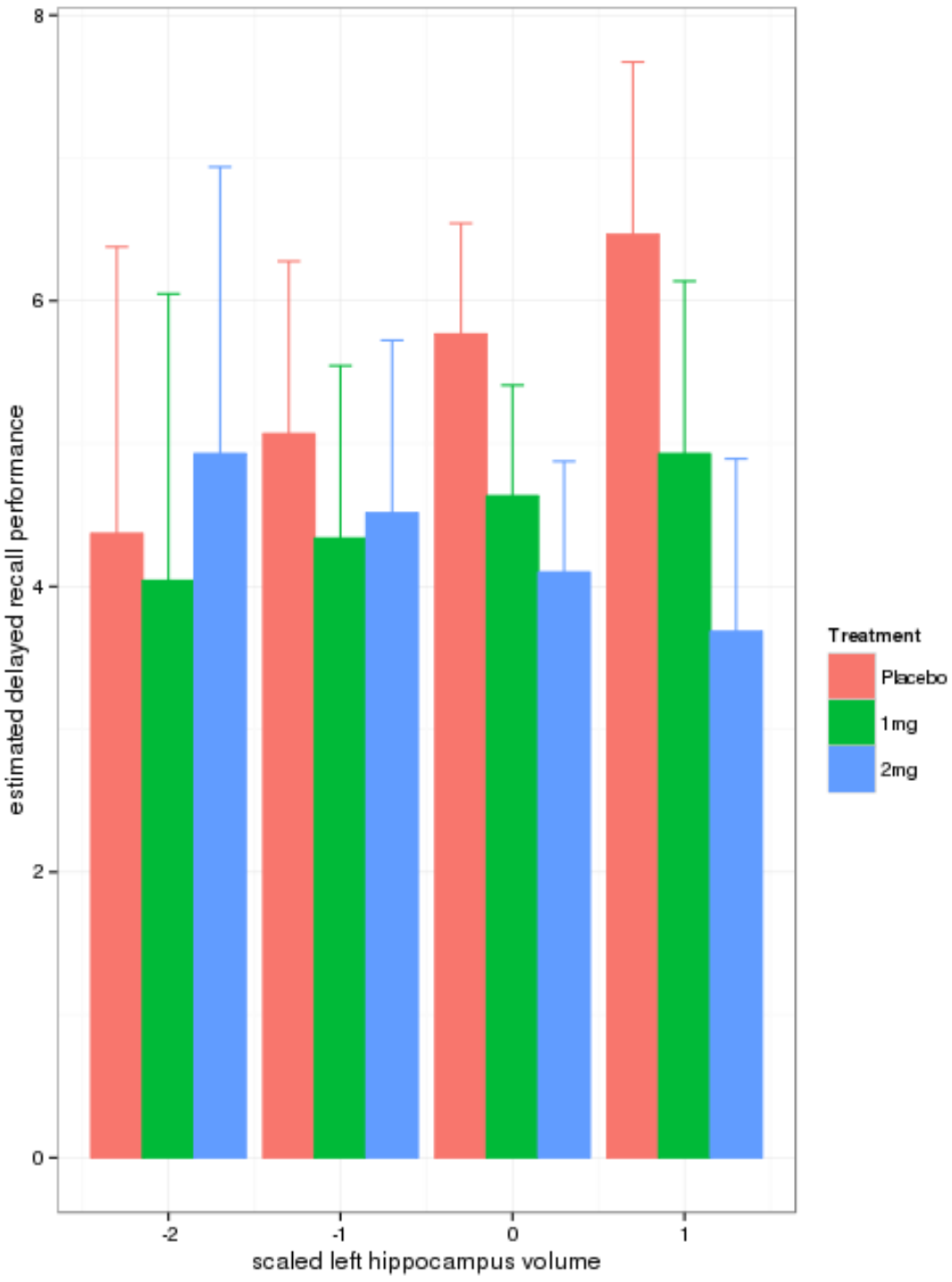

**Figure 2: Fit of delayed recall performance with anterior basal forebrain (Ch1/2) volume at different levels of treatment**