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Basal Forebrain Mediated Increase in Brain CRF is Associated with Increased Cholinergic Tone and Depression

Basal Forebrain Mediated Increase in Brain CRF is Associated with Increased Cholinergic Tone and Depression

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Abstract

In-vivo investigation of the volume of the cholinergic basal forebrain and of the hippocampus and their relationships to stress-sensitive biomarkers in cerebrospinal fluid (CSF) may offer a novel approach for improving our understanding of the pathophysiology of Major Depressive Disorder (MDD). Methods: In this study, we investigated the relationship between the volume of two brain structures, the Basal Forebrain Cholinergic Nuclei (BFCN) and hippocampus and the level of CSF Corticotropin-Releasing Factor (CRF) in older participants diagnosed with and without MDD. A standardized clinical evaluation was conducted to assign participants into one of two groups (Depressed: n = 28, Healthy controls: n =19), then CSF CRF levels and morphologic data were collected. Results: Results showed a positive correlation between levels of CRF and BFCN volume in participants with MDD, but not in healthy controls where there was a negative trend. Regression analyses showed that after controlling for the main effects of diagnosis and CRF, the interaction between CRF and MDD diagnosis was a significant predictor of BFCN volume (p = 0.003), but not of hippocampal volume. Conclusions: These results suggest that MDD may be characterized by cholinergic system and CRF abnormalities.

Keywords: depression; CSF CRF levels; MRI indices; hippocampus; basal forebrain cholinergic system

Highlights

- Major depression is a devastating disease that generates significant suffering and cost.
- After controlling for the main effects of diagnosis and Cerebrospinal Fluid Corticotropin-Releasing Factor (CSF CRF), the interaction between CSF CRF and major depression disorder diagnosis was a significant predictor of basal forebrain cholinergic nuclei volume, but not of hippocampal volume.
- By investigating both markers concurrently in participants with and without depression, we describe the extent to which the interaction between CSF CRF levels and depression diagnosis is associated with the volume of the forebrain cholinergic nuclei and of the hippocampus.
- These results contribute to our understanding of the role of brain's stress axis in depression.

1. Introduction

Cholinergic innervation of the cerebral cortex, the hippocampus, amygdala, and olfactory bulb originates from the basal forebrain area, an area of the brain characterized as a network of cholinergic neurons distinguishable by four cell groups (Mesulam et al., 1983a, 1983b). The basal forebrain is comprised of (1) the septal area which provides a projection into the hippocampus with 10% of its neurons presenting as cholinergic; (2) the diagonal band nucleus, comprised of ~70% cholinergic neurons; (3) the horizontal nucleus of the diagonal band, with ~1% cholinergic neurons, and finally, (4) the nucleus basalis of Meynert, comprised of ~90% cholinergic neurons. The basal forebrain is associated with complex behaviors, like wakefulness (Anaclet et al., 2015) and learning (Abe et al., 1998; Grothe et al., 2016; Ray et al., 2015) depending on widespread cortical innervation originating in the basal forebrain.

Lines of evidence support the relation between the cholinergic system and depression via degeneration of Basal Forebrain Cholinergic Nuclei (BFCN). Pharmacologically, cholinergic drugs show an effect on depressive state, as centrally-acting cholinergic medications can induce depressive symptoms, while anticholinergic medications can induce antidepressant effects (Janowsky et al., 1983). Depressed patients show a hypersensitivity to cholinergic challenge (Fritze, 1993), particularly with Rapid Eye Movement induction (Sitaram et al., 1980). Cholinergic abnormalities occurs in depression (Mesulam, 2004) and in-vivo molecular imaging shows decreased levels of acetylcholinesterase (AChE) in the cerebral cortex, typically interpreted as cortical cholinergic denervation due to basal forebrain/nucleus basalis atrophy (Bohnen et al., 2007), thereby supporting the hypothesis that depression may be related to cholinergic hypofunction.

The deficits in basal forebrain signaling may be associated with abnormalities in the brain's stress response system, such that the interaction between glucocorticoids and the cholinergic transmitter system contributes to BFCN degeneration (Geerings & Gerritsen, 2017; *cf*, Paul et al., 2015). Corticotropin-releasing factor (CRF), a 41 amino acid peptide, plays a key role in mediating adaptive biological and behavioral functions as part of the human stress response (Bremner et al., 1997; Ottenweller et al., 1989), and it can be readily measured in the Cerebrospinal Fluid (CSF). CRF has also been implicated in the pathophysiology of depression (Nemeroff et al., 1984; Owens et al., 1993). Accumulating evidence over the past 30 years indicates an association between CRF and MDD. Direct assessment of CRF in human central nervous system compartments has been limited to CSF and post-mortem brain examination, but CSF levels in MDD has been suggested as a diagnostic and treatment marker for MDD due to the significantly increased levels of CRF in depressed patients' CSF (Kasckow et al., 2001; Keck & Holsboer, 2001; Owens et al., 2000) and normalization of CRF levels in conjunction with depressive symptom relief (De Bellis et al., 1993; Heuser et al., 1998). Reviews of the literature indicate that increased CSF CRF levels have been observed in depressed patients who also exhibit a suppressed adrenocorticotropic hormone response to intravenously administered CRF (*cf*, Arborelius et al., 1999).

The hippocampus is involved in the regulation of stress hormones and with inhibition of the adrenocortical stress response (Sapolsky et al., 1984). Hippocampal volume is one of the best established structural surrogate measures for HPA axis dysfunction in stress (Sapolsky et al., 1996). Examining the extent to which basal forebrain and hippocampus volume loss may be predicted by depression and by stress exposure may support phenotyping of MDD subtypes that are characterized by HPA axis dysfunction and associated cholinergic system abnormalities.

The aim of this study was to investigate the association between the effects of depression and the level of CSF CRF on hippocampus and BFCN volumes in older participants. This offered a unique opportunity to test MRI indices with CRF in an understudied population. To investigate the role of the cholinergic hypothesis in depression and associated morphological characteristics, we predicted that increased levels of CRF would be associated with decreased volumes of both BFCN and the hippocampus in depressed participants, but that there would be no observed associations in healthy participants. Second, we predicted that the interaction between CSF CRF levels and depression diagnosis would be associated with BFCN and hippocampal volume separately.

2. Methods

2.1. Participants

Adults living in the US were recruited from the Memory Education and Research Initiative (Reichert et al., 2015) as part of a study on late-life MDD (see Pomara et al., 2012 for study protocol). This study was approved by the institutional review boards at the Nathan Kline Institute for Psychiatric Research and at the New York University Langone Medical Center. One hundred and thirty three participants completed the baseline evaluation, 51 completed the optional lumbar puncture procedure, three showed evidence in their MRI scans of confluent deep or periventricular white matter hyperintensities, defined as one or more hyperintense lesions measured at least 10mm in any direction, one had a Mental State Examination (MMSE) score below 28, creating a total of 47 participants. The final sample included 28 participants diagnosed with MDD and 19 healthy comparison participants. Data on age, education, depression, MMSE, and cognitive measures is located in Table 1. The majority of the 28 depressed participants (*n* =

21, 75%) reported a history of depression. Nine participants in the MDD group were likely to be in remission, as they endorsed a HAMD score (at screening) of 7 or lower. Sixteen of the 28 participants were on antidepressants.

2.2. Procedure

The procedures are described in detail by Pomara et al. (2012). Briefly, cognitively intact elderly adults were enrolled into a study consisting of four visits, each approximately one week apart. The first visit was used to explain study procedures and obtain informed consent. Data was obtained on participants' medical and psychiatric histories and their vital signs. An assessment of the presence or absence of a diagnosis was performed by a board-certified psychiatrist (NP) who used the Structured Clinical Interview for DSM-IV Axis I disorders (First et al., 1995). The severity of current depressive symptoms was evaluated using the 21-item Hamilton Depression Rating Scale (HAM-D), and global cognitive status was evaluated using the MMSE (Folstein et al., 1975). A subset of participants offered a collateral source to obtain information regarding the presence of dementia (n = 33) using the Clinical Dementia Scale (Morris, 1993). Finally, blood was drawn for apolipoprotein E genotyping. During the second visit, participants underwent an MRI scan of the head. During the third visit, they underwent a comprehensive neuropsychological assessment (Pomara et al., 2012), and in the fourth visit, a lumbar puncture on the participants was performed by a neuroradiologist under guided fluoroscopy to obtain a CSF sample for analysis.

2.3. Measures

2.3.1. Structured clinical interview for the DSM-IV Axis I disorders

Diagnosis was evaluated by a board-certified psychiatrist (NP) using the Structured Clinical Interview for the DSM-IV Axis I Disorders (First et al., 1995). This semi-structured interview collects information that permits the interviewer to discern the presence of lifetime and current psychiatric diagnoses.

2.3.2. Hamilton rating scale for depression

Depressive symptoms were evaluated using the Hamilton Depression Rating Scale (HAM-D, Hamilton, 1967). This is a 21-item clinician-administered scale, which produces a total score that reflects the severity of depression. Lower scores (0-7, 8-13) reflect normal range and mild depression, respectively. Middling scores (14-19) reflect moderate severity and higher scores (20-52) reflect severe to very severe depression. Inter-rater reliability coefficients have been reported as $\geq$ .84 (Schwab, Bialow, Clemmons, & Holzer, 1967).

2.3.3. Clinical dementia rating scale

A clinician administered the Clinical Dementia Rating Scale (Morris, 1993) to the participants, and when available, to their collateral sources. This measure uses a five-point scale to assess six domains of cognitive and functional performance applied to dementia. The domains include Memory, Orientation, Judgment and Problem-solving, Community Affairs, Home and Hobbies, and Personal Care. The participants screened (n = 33) had a score of 0, which reflects no cognitive impairment (Morris, 1993).

2.3.4. Mini mental state examination

A clinician administered the Mini Mental State Examination (MMSE, Folstein et al., 1975) to ensure normative cognitive function. The MMSE is an 11-item measure that examines five areas of cognitive function: orientation, registration, attention and calculation, recall, and language. A lower score represents higher severity of cognitive impairment. Specifically, a score of 0-17 suggests severe cognitive impairment, 18-23 suggests mild impairment, and 24-30 indicates no impairment.

2.3.5. CSF CRF assay

The CRF levels in the CSF were measured using a CRF ELISA kit (YK132, Human CRF ELISA, Yanaihara Institute Inc. Shizuoka, Japan) according to the manufacturer's instructions. Briefly, 50 μl conjugate buffer was added in each well. Then 50 μl of standard (0,0.039, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5 ng/ml) or CSF samples (MDD or controls) were added to the wells and incubated at 4°C overnight. After incubation, each well was aspirated and washed with wash buffer (200 μl). Next, 100 μl of SA-HRP solution was added into each well and incubated for 1 hour at room temperature. The wells were washed 4 times, incubated with 100μl of substrate (TMB solution) for 30min at room temperature, and terminated by the stop solution (100 μl). The optical density was determined within 10min using a microplate reader (FLUOstar, Omego, BMG Labtech) set at 450 *nm*. All samples were tested in duplicate. The mean absorbance values of the standard curve were calculated to determine the value of the samples by using the average absorbance reading of each sample.

2.3.6. MRI assessment

MRI acquisition

As per the protocol in Bruno et al., (2015), the MRI acquisition was performed on a 1.5 T Siemens Vision system at the Nathan Kline Institute. All images were acquired using a sagittal magnetization prepared rapid gradient-echo sequence [IMPRAGE; repetition time (TR)/echo time (TE)=11.4/11.9 *ms*, 1 excitation, (NEX), matrix=256 x 256, FOV=307 *mm*, 1.2 mm^3 isotropic voxel, 172 slices, no gap]. For evaluation of white matter hyperintensities, we used a fluid attenuated inversion recover sequence [FLAIR: TR/TE=9000/199 *ms*, inversion time = 2400 *ms*, NEXT=1, matrix 256 x 256, FOV=240 *mm*, slice thickness= 4 *mm*, 1 *mm* gap].

MRI preprocessing and analysis

Briefly, MPRAGE images were segmented into gray matter, white matter, and cerebrospinal fluid, and high-dimensionally registered to Montreal Neurological Institute (MNI) standard space, using a fully automated segmentation routine and the DARTEL algorithm for diffeomorphic nonlinear inter-subject registration, both available in the VBM8 toolbox. Warping parameters were applied to individual gray matter maps and voxel values were modulated to account for the volumetric differences introduced by the high-dimensional warps.

Individual volumes of the hippocampus and the BFCN were extracted automatically from the warped grey matter segments by summing up the modulated voxel values within respective regions-of-interest masks in template space. The hippocampus mask was obtained by direct manual delineation of the hippocampus in the MNI standard space template following a recently developed international consensus protocol for manual hippocampus segmentation on MRI (Frisoni et al., 2015; http:// www.hippocampal-protocol.net/SOPs/index.php). The BFCN mask was based on an MNI standard space stereotactic map of cholinergic nuclei in the basal

forebrain. This map was created using combined post-mortem MRI and histologic delineation of the BFCN in an autopsy brain, as described in detail previously (Kilimann et al., 2014). 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.

2.4. Statistical analyses

Demographic and clinical characteristics were summarized. An ANCOVA, controlling for TIV, was conducted to compare absolute volume of hippocampus and of BFCN. Two partial correlations were conducted to evaluate the strength and direction of the relationship between CSF CRF levels and BFCN volume, one in the depressed and one in the control group, controlling for TIV and age in both. Then, two additional correlations were conducted to test the strength and direction of the relationship between CSF CRF levels and hippocampal volume in the depressed and healthy group, controlling for TIV and age in each. Then, we correlated depression severity in the depressed sample with hippocampal volume and with BFCN, controlling for TIV. Following these tests, we conducted two forced-entry multiple regression analyses, replacing missing values with average scores. Using the total sample, the first regression aimed to test the extent to which the interaction between CSF CRF levels and depression diagnosis predicted the volume of BFCN, controlling for the influence of TIV, CRF, and age. The second regression aimed to test the extent to which the interaction between CSF CRF levels and depression diagnosis predicted the volume of the hippocampus, again, controlling for the influence of TIV, CRF, and age. We used BFCN and hippocampal volume data from the MR images as the dependent variables and Z scores for CRF, TIV, age and

diagnosis as the independent variables. Preliminary analyses were performed to ensure that there was no violation of the assumption of normality, linearity, multicollinearity, and homoscedasticity.

All tests were two-tailed, and statistical significance was established at an alpha of 0.05. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL).

3. Results

3.1. Descriptives

The sample was primarily male (n = 25, 56.8%, females n = 22), in their mid-to late sixties (M = 67.3 years, SD = 6.35y), and college educated (M = 16.6, SD = 2.7). The sample was cognitively intact per the MMSE (M = 29.7, SD = 0.6). Participants in the depressed group were either currently depressed or partially remitted, 21 participants reported recurrent depression, and 15 were taking antidepressants. A comparison of the depressed (n = 28) group vs. the healthy comparison group (n = 19) showed a significant difference on depressive severity per the HAM-D ($t(1,45) = 8.02$, $p < 0.001$). Specifically, the depressed group had a significantly higher severity of current depressive symptoms (M = 14.9, SD = 8.8) relative to healthy controls (M = 1.2, SD = 1.9). No group differences were observed by age ($t(1,45) = 0.835$, $p = 0.41$), number of women ($\chi^2(1, 45) = 2.4$, $p = 0.12$), and years of education ($t(1,44) = 0.274$, $p = 0.79$). Demographic and clinical information by group is described in Table 1 and in Pomara et al., (2012).

3.2. Analyses of Covariance and tests of association

Analyses of covariance revealed no differences of the absolute values of BFCN volume by group, controlling for TIV, $F(1,44)=2.508$, $p=0.120$, Healthy = 548.67 (SD = 68.48); MDD = 585.77 (SD = 79.10). Similarly, no group difference emerged with hippocampal volume, $F(1,44)=0.032$, $p=0.859$, Healthy = 7036.24 (SD = 836.00); MDD = 7071.43 (SD = 550.91). Absolute values of TIV by group are similar (Healthy = 1346.54 (SD = 164.64); MDD = 1366.28 (SD = 139.19).

Within the depressed group, a positive correlation was observed between CSF CRF and BFCN volume, when controlling for TIV and age, $r = 0.481$, $p = 0.013$, $R^2 = 0.231$. Among healthy controls, in contrast, no analogous significant correlation was found, $r = -0.295$, $p = 0.250$, $R^2 = 0.063$.

Within the depressed group, no significant correlation was observed between CRF and hippocampal volume, when controlling for TIV and age, $r = 0.320$, $p = 0.111$, $R^2 = 0.102$. Among healthy controls, no significant correlation was found, $r = -0.096$, $p = 0.715$, $R^2 = 0.009$.

Depression severity (HAMD) at the screening assessment in the depressed sample did not correlate with either hippocampal volume ($r = 0.251$, $p = 0.206$) or BFCN ($r = 0.077$, $p = 0.702$) when controlling for TIV. Figure 1 provides a scatterplot of the distribution of associations.

INSERT FIGURE 1 HERE

3.3. Regression models

In the first regression, age, sex, MDD status, TIV, and CRF were entered in Step 1. Results explained 27.2% of the variance in BFCN volume, $F(5, 41) = 3.069$, $p = 0.019$. The interaction term (CRF × MDD) was entered in Step 2, significantly increasing the total variance

explained by the model to 42.2%, $F(1, 40) = 10.313$, $p = 0.003$, thereby explaining an additional 15% of the variance in BFCN volume. These results reflect a positive interaction between CRF and MDD, which can be interpreted to mean that more CRF in MDD reflects higher BF volume.

In the second regression, age, sex, MDD status, TIV, and CRF were entered in Step 1. Results explained 59.9% of the variance in hippocampal volume, $F(5, 41) = 10.413$, $p < 0.001$. The interaction term (CRF × MDD) was entered in Step 2 and did not significantly increase the total variance explained, $F(1, 40) = 2.69$, $p = 0.109$, adding only 3% to the variance explained in hippocampal volume. The highest VIF score was 1.118, which is low and would suggest that multicollinearity did not play a role. Tables 1 and 2 outline the results.

INSERT TABLES 1 TO 3 HERE

4. Discussion

Our results showed a positive association between CRF and BFCN volume among depressed participants, but not among healthy controls. This was confirmed by both partial correlations and the significant interaction between CSF CRF levels and MDD diagnosis and BFCN volume. These results suggest there may be a cholinergic associated increase in brain CRF that may potentially contribute to the association between increased cholinergic tone and depression. Larger BFCN volumes may be interpreted as reflecting an increase of cholinergic activity. Though the smaller volumes, suggesting hypofunction, is a common interpretation of volumetric studies, the bigger volumes, suggesting hyperactivity, is not as widely accepted. In a study by Butler et al. (2013), basal forebrain septal nuclei volumes were found to be bigger in participants with temporal lobe epilepsy, which was also interpreted to be reflective of

dysfunctional hyperactivity. Animal studies also suggest that septal cholinergic neurons are resistant to seizure-related neuronal loss, and that nerve growth factor released from the hippocampus and transferred to septal nuclei may increase the number of cholinergic neurons, thereby increasing regional volume.

After the covariates were entered, which accounted for more than 40% of the variance, the level of CRF by depression status explained an additional 15% of the variance in BFCN volume. The participants' age was controlled for as prior research shows that decreasing size of cholinergic neurons in the basal forebrain are correlated with increasing age (Mesulam, Mufson, Rogers, 1987). In contrast, the combination of the covariates in the hippocampal regression predicted almost 60% of the hippocampal volume, to which the CRF × MDD interaction term contributed a very modest three percent, offering limited information in the prediction of hippocampal volume. Hippocampal volume changes in MDD have provided conflicting results though most studies have reported smaller volumes in MDD with increased neuronal loss in patients and preclinical models (Booij, 2015; Gerritsen et al., 2011; Wisse, 2015). One study that included 636 participants (81% male) found decreased hippocampal volume in MDD (Gerritsen et al., 2011), however this decrease was not explained by HPA axis activity. Other studies demonstrated smaller hippocampal volumes in samples comprised of mostly or all women (Booij, 2015, Elbejjani, 2015). The sample size of the current study may not have been sufficient to detect a loss of hippocampal volume, and it would be of interest to conduct a larger study with men and women to test for main effects.

CSF CRF is hypothesized to originate from hypothalamic neurons and/or extra-hypothalamic CRF-neurons in the BFCN; however the anatomical origin of CRF effects in MDD has not been identified. Preclinical studies may help to identify brain regions that contribute to

CSF CRF levels. The observed elevation in CSF CRF associated with depression has been shown to be state-dependent (Banki et al., 1987; Geracioti et al., 1997; Heuser et al., 1998; Nemeroff et al., 1984; Nemeroff et al., 1991). In these studies, antidepressants (which reduce CSF CRF) were discontinued prior to lumbar puncture. In our study, patients who were taking antidepressants were allowed to continue on these medications. Also, the CSF elevations may be prominent in melancholic depression (De Jong & Roy, 1990) of which only a few of our patients met this criterion. In De Jong et al., (1990) CSF CRF did not correlate with depressive severity, but only with certain items. Future research may address the possibility that cognitive and affective dimensions are related to CSF CRF.

To the extent that these results can be replicated, our results would be consistent with the research that links increased cholinergic tone to MDD. For example, Williams et al. (2013) collected 41 coronal blocks within the nucleus basalis of individuals, aged 41-60 years, diagnosed with either major depressive disorder (n=11, of which 7 died of suicide or asphyxiation), schizophrenia (n=13) or with age-matched controls (n=16). Examining cell density and neuroarchitecture, results indicated a larger nucleus basalis oval neuron soma in the combined clinical groups ($p = 0.038$), with no significant differences between controls and schizophrenia and major depression disorder separately. In depression, there was a trend towards reduced oligiodendrocyte density ($p = 0.065$) in nucleus basalis. Notably, the ratio of gemistocytic to fibrillary astrocytes was highest among depressed participants (39.9%) relative to schizophrenia (18.1 %) and controls (7.9 %). These results suggest glial cell abnormalities in the nucleus basalis among depressed individuals, which may be reflected in increased volumes of this region, and if replicated, may be useful in further characterizing the neuropathology of depression.

To the extent that the anticholinergic effects of antidepressant treatment and other medications influenced cholinergic basal forebrain volume, they might have contributed to the positive correlation between CRF levels and BFCN volume. We are unable to fully test the influence of medications as the small sample size constrains the opportunity to statistically control for MDD participants who might have been taking medications with central anticholinergic effects. However, we found that cerebrospinal anticholinergic activity (CSF AA) determined using a radioreceptor method (Mulsant et al. 2003; Tune & Coyle, 1980) originally developed for serum, but successfully also employed for determining CSF AA (Watne et al. 2014) showed no difference between the MDD and controls. Also, CSF AA showed no correlation with BFCN and with hippocampal volume. Therefore, it is unlikely that this factor contributed to our results.

The following limitations of the study should be observed. First, the sample size was lower than preferred, given the combination of the covariates in the regression. Second, there was heterogeneity of the clinical severity with some participants reporting that they were highly likely or very likely to be remitted. Third, the study design was cross-sectional, which constrains our ability to test the specificity of the cholinergic hypothesis of depression. Fourth, though education is correlated with hippocampal volume (Janowitz et al. 2014) and may need to be statistically controlled (Brown et al., 2012) we did not conduct this in our sample as we found no group difference in educational levels. Fifth, the results may apply only to late-life onset depression given the sample characteristics, thus these results may not apply to individuals under age 50y. Finally, the use of antidepressants has the potential to influence hippocampal volume in participants with depression (*cf.* Malykhin & Coupland, 2015), and thus consideration of the effects of medication should be considered in terms of effect on hippocampal volume.

Further investigation is needed to test the cholinergic hypothesis of depression, beginning with an investigation of the specific nature of the two-way interaction of depression and CRF to more usefully describe the deeper phenotype of depression, with an unmedicated sample across the lifespan. Finally, future work may benefit from a closer evaluation of the clinical characteristics and their relation to the BFCN to identify the pathophysiology of MDD. Also, it should be noted that in BFCN, besides the cholinergic neurons, GABAergic and glutamatergic neurons also constitute this structure (Chen et al., 2016; Hassani et al., 2009). The possible role of GABAergic and glutamatergic neurons within the BFCN as possible mediators of its relationship to CRF in MDD needs to be determined. Determining the relationship between CRF levels with ACh, GABA and glutamate in CSF may help to clarify the underlying mechanisms of the cholinergic regulation in BFCN associated with CRF in MDD.

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Table 1. *Demographic and Memory Characteristics of Study Participants by MDD Diagnosis*

Characteristic	Comparison Group (N=19)	MDD Group (N=28)	p values (t tests)
Age (years)	68.1 ± 7.3	66.5 ± 5.4	0.41
Education (years)[a]	16.7 ± 2.7	16.5 ± 2.7	0.79
21-item HAM-D	1.2 ± 1.9	14.9 ± 8.8	**<0.001**
MMSE	29.5 ± 0.5	29.8 ± 0.6	0.13
Total recall rating	64.4 ± 12.3	64.9 ± 13.9	0.91
Delayed recall rating	8.5 ± 2.8	9.5 ± 2.5	0.22
			p values (χ^2)
Females (n)	12 (63%)	10 (36%)	0.12

Table 2

Regression Analysis of Basal Forebrain Volume

Variable	*β*	*t*	sig	zero-order	partial
Sex	0.316	1.978	0.055	0.206	0.298
CRF	0.130	1.046	0.302	0.218	0.163
TIV	0.013	0.089	0.930	0.209	0.014
Age	-0.337	-2.528	0.016*	-0.338	-0.371
MDD Status	-0.154	-1.219	0.230	-0.241	-0.189
CRF × MDD	-0.405	-3.211	0.003*	-0.431	-0.453

Legend. CRF = Corticotropin-Releasing Factor, TIV = Total Intracranial Volume, MDD Status = Major Depressive Disorder.

1 = coded as control, 0 = MDD-status or equivalent.

*$p < 0.05$ **$p < 0.01$

Table 3

Regression Analysis of Hippocampal Volume

Variable	*β*	*t*	sig	zero-order	partial
Sex	0.221	1.638	0.109	-0.208	0.166
CRF	0.031	0.295	0.770	0.135	0.030
TIV	0.575	4.619	0.000	0.675	0.469
Age	-0.305	-2.714	0.100	-0.208	-0.276
MDD Status	0.073	0.686	0.497	-0.026	-0.070
CRF × MDD	-0.174	-1.639	0.109	-0.271	-0.166

Legend. CRF = Corticotropin-Releasing Factor, TIV = Total Intracranial Volume,

MDD Status = Major Depressive Disorder.

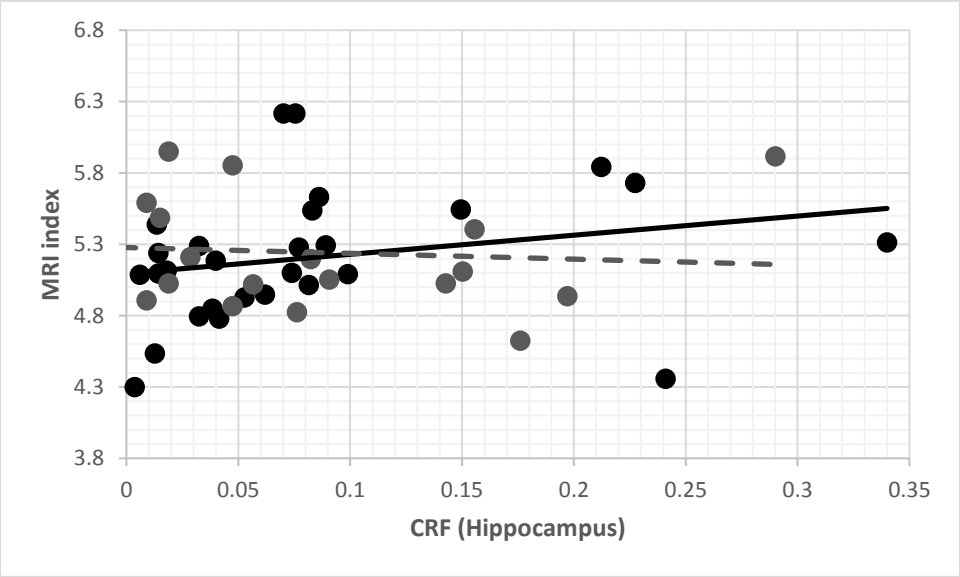
1 = coded as control, 0 = MDD-status or equivalent.

*$p < 0.05$ ** $p < 0.01$

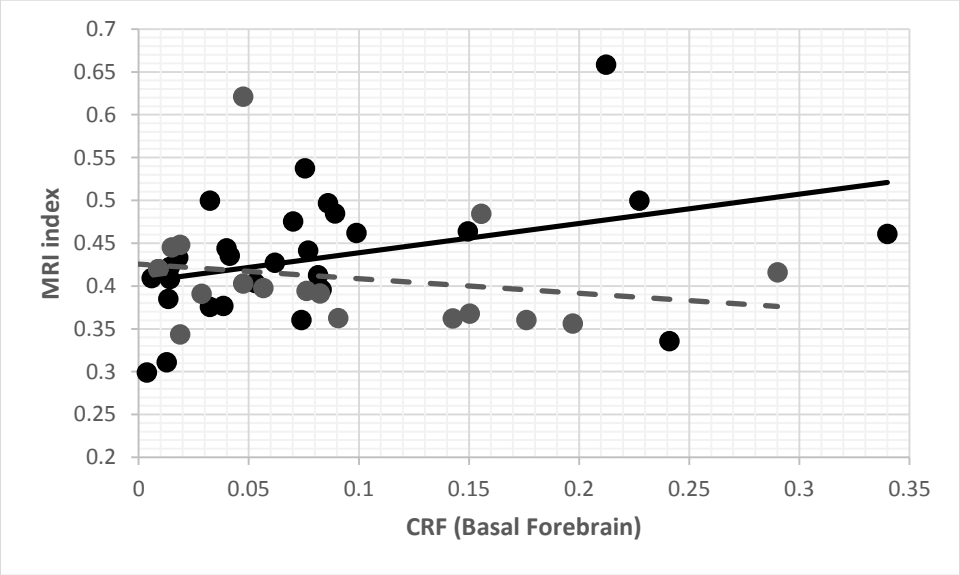
Figure 1

Distribution of the Association of MRI Index and CRF of Hippocampal Volume (A) and Basal Forebrain (B) between Depressed and Healthy Groups.

A



B



Legend. CRF = Corticotropin-Releasing Factor. MRI Index = Magnetic Resonance Imaging Index; Black/full line = depressed subjects; Gray/dashed line = control depressed subjects.