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Importance of proactive treatment of depression in Lewy body dementias: The impact on hippocampal neurogenesis and cognition in a postmortem study.

Short title

Impact of SSRIs on neurogenesis, depression and cognition in DLB/PDD

Key words

Dementia, neurogenesis, depression, selective serotonin reuptake inhibitors, acetylcholinesterase inhibitors, cognition, hippocampus, postmortem

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Abstract

OBJECTIVE: To examine the impact of selective serotonin reuptake inhibitors (SSRIs) and depression on neurogenesis and cognition in dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD).

METHODS: Late-stage progenitor cells were quantified in the subgranular zone (SGZ) of the hippocampal dentate gyrus of DLB/PDD patients (n=41) and controls without dementia (n=15) and compared between treatment groups (unmedicated, selective serotonin reuptake inhibitors (SSRIs), acetyl cholinesterase inhibitors (AChEIs), combined SSRIs and AChEIs).

RESULTS: DLB/PDD patients had more doublecortin- positive cells in the SGZ compared to controls. The doublecortin- positive cell count was higher in the SGZ of patients treated with SSRIs and correlated to higher cognitive scores.

CONCLUSION: SSRI treatment was associated with increased hippocampal neurogenesis and preservation of cognition in DLB/PDD patients.

1. Introduction

Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) are synucleinopathies characterized by a progressive dementia syndrome and prominent psychiatric symptoms, usually associated with parkinsonism and typically dominated by attention, visuospatial and executive dysfunction and relatively preserved memory [1,2]. DLB accounts for 10-20% of people with late onset dementia [2] and PDD eventually develops in the majority of older patients with Parkinson's disease (PD) [3]. Current treatment strategies are limited to symptomatic approaches with AChEIs, conferring significant but limited benefits in cognitive function and neuropsychiatric symptoms in both PDD and DLB [4,5]. Depression is one of the main neuropsychiatric disorders associated with cognitive decline [6], has been established as a risk factor for dementia [7,8] and is frequent and challenging in neurodegenerative diseases including PD [9], DLB [1,10] and Alzheimer's disease (AD) [11].

Impaired neurogenesis is increasingly recognized as a key feature in aging and neurodegeneration. Age-related alterations in neurogenesis have been reported in postmortem human brain [12,13] and to a greater extent in a range of neurodegenerative conditions including AD, DLB and PD [14-19]. It has therefore been hypothesized that neurogenic dysfunction may contribute to cognitive impairment or may even represent a novel treatment target. Human autopsy studies have compared key markers of adult neurogenesis in the neurogenic niches in patients with synuclein disorders and age-matched controls. These studies report a significant decrease in early stage progenitor markers, Musashi1 and Nestin, but a significant increase in the cell proliferation marker proliferating cell nuclear antigen (PCNA) and the later stage neuronal precursor marker DCX in people with DLB [16-18]. However, no study to date has examined the relationship between altered neurogenesis and the rate of cognitive decline and/or the impact of pharmacological treatments upon

neurogenesis in DLB/PDD. These are both vital steps in determining the potential clinical significance of neurogenesis as a treatment target in these dementias.

A key potential factor in the relationship between neurogenesis, cognition and treatment response is the link between depression and neurogenesis. This is particularly pertinent due to the commonality of depression in people with DLB and PDD [20,21]. Post-mortem studies comparing hippocampal neurogenesis between people with depression and age-matched controls have reported conflicting results [22,23]. There is consistent evidence showing a decrease in the volume of the hippocampal dentate gyrus (DG), the main adult neurogenic niche, in patients with depression or anxiety [24,25], including a meta-analysis comparing 32 MRI studies [26]as well as supporting data showing that hippocampal volume is less affected in patients treated with anti-depressants [27].There is no evidence to show that hippocampal volume is lowered as a direct result of impaired neurogenesis alone, and other factors such as cell soma size, dendritic complexity and glial cell size and number could contribute to this phenomenon in depression. Evidence also suggests that there is a lower number of neural progenitor cells and mitotic cells in the DG of older depressed patients compared to controls [22]. Taken together, these data therefore suggest a potential link between depression and reduced neurogenesis and/or reduced survival of neural progenitors.

Rodent studies have clearly demonstrated that SSRI administration, the most frequently used class of antidepressant treatment, significantly increases neurogenesis [28-30]. Comparable findings have been observed in *in vitro* studies using human hippocampal progenitor cells [31]. Mechanisms that have been postulated as drivers of this effect include serotonergic mediation of neurogenesis, activation of the glucocorticoid receptor and an impact of key trophic factors such as BDNF[31-34]. It is therefore possible that SSRI administration may contribute to increased neurogenesis in people with depression or anxiety.

This study sought to elucidate the relationships between neurogenesis, cognition and SSRI treatment response in DLB and PDD. A better understanding of this association could have considerable implications for the treatment of depression in these conditions. This work also represents a novel avenue in identifying drivers of disease progression and treatment targets in clinically defined groups within the overall dementia patient group.

2. Methods

2.1 Study design

This study utilized post-mortem analysis and scrutiny of linked clinical records to explore four main aims.

1.To verify the increase in late stage progenitor cells in people with DLB/PDD via expression of the neuronal precursor marker DCX in the SGZ of the dentate gyrus.

2.To determine the relationship between neurogenesis and cognitive decline in DLB/PDD patients.

3.To examine the relationship between depression and neurogenesis in DLB/PDD.

4.To examine the impact of SSRIs on neurogenesis in these patients.

2.2 Subjects, diagnosis and assessments

Paraffin-embedded human autopsy hippocampal tissue at the level of the geniculate nucleus from PDD and DLB patients (n = 41) and age-matched controls (n = 15) was obtained from the Newcastle (U.K.) Brain Tissue Resource, with consent from next of kin, and University Hospital, Stavanger (Norway) with consent from patients and in accordance with the approval of the Joint Ethics in Medical Research, University of Bergen and the Medical Research Council (MRC). Neuropathological assessment and diagnosis followed the international consensus criteria for DLB [1]. Patients meeting the consensus pathological criteria for DLB, but with Parkinson's disease for more than one year prior to the onset of dementia were diagnosed as PDD. Brain tissue used in this study was selected to match confounding factors

such as age and fixation time of tissue. Control cases were neurologically normal, with only mild age-associated neuropathological changes and no history of psychiatric disease. The neuropathological features in these subjects were not of sufficient severity to be considered associated with dementia (e.g., neurofibrillary tangle Braak stages below IV [35]). Controls were also not medicated with SSRIs during life. No MMSE assessments were carried out on control subjects.

Depression was assessed in the DLB/PDD patients using serial scores from a standardized assessment instrument. The main assessments used were the Montgomery–Åsberg Depression Rating Scale (MADRS) where scores above 13 were indicative of, at least, mild depression [36] and Cornell Scale for Depression in Dementia (CSDD), where scores above nine were indicative of probable depression [36]. The information from these serial evaluations was converted into a binary variable of depression over the course of the dementia or no depression.

Cognitive data were collected from MMSE examinations for DLB/PDD patients. Final MMSE scores, measured at least within two years prior to death, were available for 39 out of 41 DLB/PDD patients. The average rate of annual cognitive decline per patient was determined by the number of MMSE points lost over the number of years with dementia from the maximum MMSE score: [(30 – MMSE score closest to death) / years with dementia] and was available for 35 out of the 41 DLB/PDD patients. Administration of SSRIs and AChEIs was assessed based on the medication notes of the last three consecutive years prior to death.

2.3 Immunohistochemistry

Paraffin-embedded coronal human hippocampal brain sections were sectioned at 7µm thickness. Sections were de-paraffinized, rehydrated and microwave-treated in citrate buffer, pH 6.0 for six minutes using a pressure cooker to expose epitopes. The endogenous peroxidase activity was quenched for 20 minutes in 3% H_2O_2 in tris-buffered saline. Sections

were immunostained for doublecortin (DCX; sc-8066, Santa Cruz Biotechnology (Dallas, USA), 1:200) for 48 hours at 4°C. After subsequent washes, sections were incubated with the appropriate secondary HRP-conjugated antibody (PI-9500, Vector Laboratories (UK), 1:250) for two hours at room temperature. After washes, sections were developed with diaminobenzidine (DAB) substrate (Vector Laboratories (UK). Counterstaining was performed with haematoxylin. Adjacent sections were incubated in the absence of the primary or secondary antibodies in order to determine non-specific antibody binding, and were devoid of immunoreactivity (data not shown). All immunohistochemistry was performed blind to clinical and pathological diagnosis.

2.4 Cell counting

Cell counting was performed using an E800 Eclipse, Nikon microscope (Nikon, UK) and the NIS Elements software version 3.0 (Nikon, UK). The extent of DG immunostaining per case depended exclusively on the amount of tissue supplied by the brain banks. Since, in most cases, this corresponded to a single section of DG, the length of DG within the section was measured using low magnification and software tools. Cells immunopositive for DCX were then counted throughout the entire DG and absolute number expressed per mm of length of DG. All cell measurements were carried out blind to clinical and pathological diagnosis.

2.5 Statistical analysis

Statistical analyses were performed using SPSS version 22.0 software (IBM) and GraphPad Prism Version 7.0 (GraphPad Software). Postmortem delay (PMD) was found to be significantly correlated with DCX values in the SGZ by Spearman's rank correlation (Spearman's rho, ρ=0.321, p=0.016) and a significant predictor of DCX levels in the SGZ via regression analysis (R=0.340, p=0.010). PMD was therefore controlled for in statistical analyses, as described below. Two of the DLB/PDD cases had missing PMD values. These values were therefore imputed from the mean. No other demographic factors correlated to DCX immunoreactivity in the SGZ.

An independent samples Mann-Whitney U test was performed to determine alteration in DCX levels in the SGZ in DLB/PDD patients compared to controls. PMD did not differ significantly between dementia patients and controls (Mann-Whitney U, p=0.410) and was therefore not included as a co-variate in this test. One-way analysis of covariance (ANCOVA) was conducted to compare endogenous hippocampal neurogenic immunoreactivity in the different treatment groups, including PMD as a covariate. Partial correlation analysis was performed to determine the association between the MMSE score closest to death and average rate of annual decline in MMSE score, and the number of DCX-immunopositive cells, controlling for PMD and duration of dementia in years.

An independent samples student's t-test was performed to compare mean number of DCX positive cells in the depressed compared to non-depressed dementia patients. PMD did not differ significantly between depressed and non-depressed patients (Mann-Whitney U, p=0.545) and was therefore not included as a co-variate in this test.

Data are presented as mean ± standard deviation of the mean (S.D). Statistical significance was considered as $p \leq 0.05$.

3. Results

3.1 Cohort characteristics

The mean age at death across the whole cohort was 80.11 ± 6.24 years (80.47 ± 8.48 years in controls and 79.98 ± 5.32 years in DLB/PDD) and genders were equally represented (48% males, 52% females).

In the dementia patients, mean duration of dementia was 4.6 ± 2.9 years, and mean MMSE score closest to death was 11.9 ± 7.8 (for details see Table 1). Figure 1 illustrates DCX-positive cells within the hippocampal SGZ, as detected by DAB immunohistochemistry. Consecutive sections from cases exhibiting no DCX-positive cells were subsequently stained for other markers to assess antigenicity of the tissue (data not shown).

3.2 Frequency of DCX-positivity in sub-granular zone of DLB/PDD patients

A significantly higher number of DCX-positive cells per mm length was measured in the SGZ of the hippocampal DG in DLB/PDD patients (n=41) compared to age-matched controls (n=15) (Mann-Whitney U test, W=240.0, Z=-3.47, p = 0.001; Figure 2A). A significantly higher DCX immunoreactivity was also observed in the SGZ of those DLB/PDD patients who were not treated with SSRIs or AChEIs (n=13) compared to age matched controls (n=15) (Mann-Whitney U test, W=168.0, Z=-2.29, p = 0.022; Figure 2A) suggesting that this increase is independent of a treatment effect.

3.3 Correlation of neurogenesis and cognitive decline in DLB/PDD

DCX levels in the SGZ at post-mortem were significantly correlated with higher MMSE scores closest to death (n = 39, r = 0.44, p = 0.006; Figure 2B), and a slower rate of cognitive decline (n = 35, r = -0.396, p = 0.023; Figure 2C). These results remained consistent in the SSRI treatment group (+/- AChEIs) with DCX levels being significantly correlated to both MMSE scores closest to death (n = 14, r = 0.615, p = 0.033) and to a slower rate of cognitive decline (n = 14, r = -0.640, p = 0.025). Similar correlation analyses did not yield statistically significant results for the AChEI treatment group (+/- SSRIs) (Table 2). Table 2 further lists partial correlation analyses between DCX SGZ levels and MMSE scores for the individual treatment groups, however, since n numbers are relatively low in these sub-groups one must use caution in interpreting this data.

3.4 Association of SSRI treatment and DCX levels in DLB/PDD

In order to evaluate whether treatment with SSRIs or AChEIs was associated with altered hippocampal neurogenesis, the mean number of DCX-positive cells in the SGZ of the hippocampal DG across the different treatment groups was evaluated (Figure 2D).

One-way ANCOVA analysis (independent variable: treatment type (controls, no treatment, AChEIs, SSRIs, AChEIs + SSRIs), covariate: PMD) indicated a significant treatment effect on

the number of neural progenitors in the SGZ (F=7.540, df = 4, 50, p=<0.001). Post-hoc analysis suggested that the strongest influence on DCX-positive cell number was the SSRI treatment (SSRI: n = 6 compared to controls: n=15, p= <0.001and compared tountreated DLB/PDD: n=13, p = 0.001). DCX levels were also significantly higher in patients undergoing SSRI treatment compared to patients treated with AChEIs only (n= 12, p = 0.002). The patients treated with both SSRIs and AChEIs (n = 10) did not exhibit a significant change in DCX immunoreactivity compared to the other DLB/PDD treatment groups but exhibited higher DCX levels in the SGZ than controls (p<0.001)

As an additional sensitivity analysis within the dementia group, the one-way ANCOVA analysis was repeated with all patients receiving SSRIs (+/- AChEIs) combined into one group (n=16). This group also showed greater DCX levels compared to the group receiving no SSRI treatment (One-way ANCOVA, independent variable: antidepressant treatment (no treatment, AChEIs, SSRIs +/- AChEIs), covariate: PMD, F=5.520, df=2,37, p=0.008). Post-hoc analysis suggested that SSRI treatment (n=16) is associated with greater DCX levels both when compared to no treatment (n=13) (p=0.023) and when compared to AChEIs-only treatment (n=12) (p=0.024).

3.5 Impact of SSRI treatment in DLB/PDD in the context of clinical depression

Standardized depression assessments were available for 35 out of 41 DLB/PDD patients, 16 of whom had presented a history of clinical depression during the course of the dementia. There was no difference in mean DCX levels in the SGZ of depressed (n=16) and non-depressed dementia patients (n=19) (independent samples student's t-test, t=-0.105, df=33, p=0.917). DCX immunoreactivity in the SGZ of DLB/PDD patients with depression was significantly different between patients receiving SSRI, AChEIs or no treatment (one-way ANCOVA, independent variable: treatment type (no treatment, AChEIs, SSRIs, AChEIs + SSRIs), covariate: PMD, F=4.918, df=3, 11, p=0.021) but no such treatment-related

differences were observed in DLB/PDD patients without a history of depression (one way ANCOVA, F=1.557, df=3, 14, p=0.244).

4. Discussion

The study has examined the inter-relationships between neurogenesis, cognition and treatment of depression in people with DLB/PDD. The findings suggest an association between preserved cognition and increased neurogenesis in DLB/PDD patients, and highlight a significant increase in neurogenesis in patients treated with SSRIs. The impact of SSRIs on neurogenesis was largely seen in patients with concurrent depression. This study builds on previously published studies, particularly one report of a greater number of late stage neural progenitors in the hippocampus in patients with DLB/PDD compared to age-matched controls [16], which was further increased by treatment with SSRIs. The results highlight endogenous neurogenesis as an important and clinically relevant phenomenon in DLB/PDD patients, and suggest neurogenesis as a treatment target. Of particular note, the work also emphasizes that proactive treatment of concurrent depression may be particularly important in these individuals.

Treatment with SSRIs has been shown to increase neurogenesis *in vivo* in animals [37], including non-human primates [38]. The elevation in hippocampal neural progenitor cells may be a neural compensatory mechanism to the degenerative process, which can be further augmented by SSRI treatment. This raises the possibility that other more potent regulators of neurogenesis, such as erythropoietin, retinoids and cannabinoids may be novel candidates for the treatment of DLB/PDD patients [39-42].

The cholinergic system has also been implicated in hippocampal neurogenesis, and postmortem studies have shown that cholinergic pathology may be involved in reducing neurogenesis in AD [14,18]. Our results suggest that the number of DCX-positive cells in the

hippocampus is greater following treatment with SSRIs than treatment with AChEIs in DLB/PDD patients.

The results of this study demonstrated that the higher SGZ DCX expression following antidepressant treatment was especially significant in the depressed group. Depression occurs in approximately 30% of DLB/PDD patients [43] and there is currently a considerable gap in the evidence base relating to the treatment of depression in these individuals. SSRIs and other antidepressants are also commonly used to treat symptoms such as agitation and sleep disturbance in dementia. The results of the current study emphasize the importance of proactive treatment of depression in people with DLB/PDD, but also highlight the need for treatment trials clarifying the impact of antidepressant treatment on cognition, function, disease progression and neurogenesis.

Although this is a large prospectively studied cohort for a post-mortem study, there are some limitations to power for statistical analysis of sub-group comparisons. Different institutions from which the tissue was obtained used different rating scales for measuring depression, restricting analysis to the use of a binary variable. It is also important to consider potential confounding factors which may have influenced the relationship between neurogenesis and cognitive decline when interpreting the data. Since duration of dementia impacts cognitive decline, the number of years with dementia was controlled for in the partial correlation analyses investigating the association between DCX immunoreactivity and MMSE score closest to death. In addition, there was also a significant relationship between higher DCX levels and the rate of cognitive decline. These findings therefore suggest that increased neurogenesis in the DG is associated with preserved cognition.

It is important to note that the main focus of this preliminary study was neurogenesis in the DG which is considered the main and the most extensively studied neurogenic niche in the adult mammalian brain. Recent evidence suggests that in the human brain, neuronal precursors originating in the additional mammalian neurogenic niche, the subventricular

zone (SVZ; located in the lateral ventricles), might be contributing to new neurons in the striatum [44], a brain region greatly implicated in the parkinsonian syndrome associated with DLB and PDD. An investigation of the potential relationship between neurogenesis in the SVZ and DLB/PDD would, therefore, be of great interest.

The mechanisms by which newly generated neurons following SSRI treatment have an impact on cognition and general brain function are still a question of debate in the field. Studies suggest that newly generate granule cells in animals exhibit greater synaptic plasticity than older ones and are preferentially integrate into circuits where they might be acting as circuit modulators [45,46]. Therefore these albeit few neurons, newly generated in certain regions of the adult brain, might be playing an important role in both stabilizing and altering the plastic brain network – this role might be especially crucial in cases of severe pathological alterations, as in dementia.

Due to the fact that the findings outlined above arise from a post-mortem study and not a randomized controlled trial, we are cautious in interpreting our data and emphasise that future work is required to further validate these results. Samples of DG analysed relied on the generosity of wax sections supplied by brain banks, most of which were no more than a few individual sections per case study. DCX immunopositivity was therefore normalized to length of the DG within each section. Future studies using more quantitative techniques such as stereology could better investigate expression of neurogenic markers in relation to total neuronal cell count and offer further insight into the clinical relevance of such findings. In this study, DLB and PDD cases were grouped together as one Lewy body dementia cohort to investigate effects of antidepressant treatment on neurogenesis and cognition. Whilst the one-year rule is still routinely clinically applied to differentiate between the two dementia forms, several studies have suggested differences in amyloid load and Lewy body cortical load [47-51] which could also have an impact on the rate of neurogenesis. It would, therefore, be of interest to take this into consideration in future studies investigating neurogenesis in DLB/PDD. Nevertheless, our study is the first illustrating that hippocampal neurogenesis may

be a clinically relevant phenomenon in DLB/PDD patients, correlating with the progression of cognitive impairment and enhanced by pharmacological treatment with SSRIs.

Overall, although preliminary, this study suggests the serotonergic system may be a novel therapeutic target to maintain cognitive and neurogenic function in DLB and PDD patients. The results also emphasize the importance of proactive and effective treatment of depression in people with DLB/PDD.

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Table/Figure Legends

Table 1: Demographics of DLB/PDD patients treated and not treated with AChEIs or SSRIs and controls

Value represents mean ± standard deviation of the mean of listed criteria in each group of patients, unless otherwise stated. No statistical difference was observed in age at death, gender, post mortem delay and duration of dementia. Abbreviations: PMD = Post mortem delay, MMSE = Mini mental state examination, DLB=Dementia with Lewy bodies, PDD=Parkinson's disease with dementia; AChEIs=Acetylcholinesterase inhibitors; SSRIs=Selective Serotonin Reuptake Inhibitors

		Age at death (years)	**Gender (% female)**	**PMD (hours)**	**Duration of dementia (years)**	**MMSE closest to death**
Control (n = 15)		80.47 ± 8.48 (n=15)	67 (n=15)	34.267 ± 17.32 (n=15)	n/a	n/a
DLB/PDD (n = 41)	Untreated	80.92 ± 5.72 (n=13)	77 (n=13)	36.308 ± 25.51 (n=13)	4.269 ± 2.91 (n=13)	11.38 ± 9.15 (n=13)
	AChEIs	78.67 ± 4.42 (n=12)	17 (n=12)	46.500 ± 37.53 (n=12)	4.500 ± 2.15 (n=12)	12.83 ± 7.43 (n=12)
	SSRIs	80.33 ± 5.82 (n=6)	50 (n=6)	42.932 ± 20.85 (n=6)	3.167 ± 2.64 (n=6)	14.83 ± 8.40 (n=6)

SSRIs + AChEIs	80.10 ± 5.99 (n=10)	40 (n=10)	49.959 ± 21.57 (n=10)	6.056 ± 3.71 (n=9)	9.25 ± 6.02 (n=8)

Table 2: Partial correlation analysis of MMSE cognitive scores and DCX levels in the SGZ in DLB/PDD patients

Since this is a partial correlation analysis controlling for two co-variates (Years of dementia duration and Post-mortem interval) the degrees of freedom (df) represent n-4. Abbreviations: MMSE = Mini mental state examination, AChEIs=Acetylcholinesterase inhibitors; SSRIs=Selective Serotonin Reuptake Inhibitors, df = degrees of freedom.

	Untreated	AChEIs	SSRIs	AChEIs + SSRIs	AChEIs + (AChEIs + SSRIs)	SSRIs + (AChEIs + SSRIs)
MMSE closest to death	r=0.475, p=0.140, df =9	r=0.273, p=0.445 df =8	r=0.846, p=0.154, df=2	r=0.766, p=0.076, df=4	r=0.372, p=0.129, df=16	r=0.615, p=0.033, df=10
MMSE rate of decline	r=-0.911, p=0.004, df = 5	r=0.462, p=0.179 df=8	r=-0.945, p=0.055, df=2	r=-0.792, p=0.060, df=4	r=-0.065, p=0.799, df=16	r=-0.640, p=0.025, df=10

Figure 1: Representative image of doublecortin positive staining (brown) with diaminobenzidine (DAB) in the human hippocampal dentate gyrus. Counterstaining performed with Haematoxylin (purple). H=hilus. Scale bar 50µm and 25µm respectively.

Figure 2

(A) Scatter plot of doublecortin (DCX) levels in the SGZ in control and DLB/PDD patients, (a=Mann-Whitney U test, W=240.0, Z=-3.47, p = 0.001) (B) Partial correlation plot between MMSE scores closest to death and DCX levels in the SGZ in DLB/PDD patients. (C) Partial correlation plot between annual decline of MMSE points per year of dementia and DCX levels in the SGZ of DLB/PDD patients. (D) Scatter plot of the estimated marginal means calculated by ANCOVA of DCX levels in the SGZ in DLB/PDD patients in different

treatment groups (a=SSRIs compared to controls p = <0.001, b=SSRIs compared to untreated DLB/PDD p=0.001, c=SSRIs compared to AChEIs, p=0.002, d=AChEIs compared to controls p <0.001). Abbreviations: DCX=doublecortin, SGZ=subgranular zone, PMD=postmortem delay, DLB=Dementia with Lewy bodies, PDD=Parkinson's disease with dementia, MMSE=Mini-Mental State Examination; AChEIs=Acetylcholinesterase inhibitors; SSRIs=Selective Serotonin Reuptake Inhibitors. Bars in A indicate mean ± standard deviation. Bars in D indicate mean ± standard error.

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