

## **Associations between pro-inflammatory cytokines, learning and memory in late-life depression and healthy aging**

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**Running Header:** Inflammation and memory in late-life depression

**Key words:** Aging; Cognition; Inflammation; Late-life depression; Learning; Memory

**Key points:** 1) Inflammation may affect cognition in typical ageing and late-life depression; 2) Patients with late-life depression demonstrated a unique association between a persistent inflammatory marker and both learning and memory, whereas healthy older adults do not; 3) Persistent inflammation alone may not be sufficient to account for cognitive difficulties, but may interact with other factors.

**Sponsors:** This work was supported by the National Institutes of Health (AK, NIMH 7RO1 MH073989-04). Additional support was provided by the National Institutes of Health (GP, NIMH RO1 MH056528).

**Word count:** 3,725

## **Abstract**

**Objectives:** Pro-inflammatory cytokines may play a role in learning and memory difficulties and may be exacerbated in late-life depression (LLD), where pro-inflammatory markers are already elevated due to aging and age-related vascular risk. **Methods:** Learning and memory, and pro-inflammatory cytokines-Interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-6 (IL-6) were measured in 24 individuals with LLD and 34 healthy older adults (HOA). Hippocampal volumes were segmented using Freesurfer software.

**Results:** Pro-inflammatory cytokines were higher in LLD compared to HOA. Regression analyses demonstrated that educational level and right hippocampal volume significantly contributed to explaining the variance in learning. For memory performance, educational level, right hippocampal volume and a group-by-IL-6 interaction significantly contributed to the model. **Conclusions:** High levels of IL-6 impact cognition in LLD but not HOA. Results suggest that high levels of inflammation alone are not sufficient to account for cognitive difficulties, but may interact with other factors in at-risk populations like LLD, to contribute to memory difficulties.

## Introduction

Late-life depression (LLD) is a clinical diagnosis of major depressive disorder (MDD) occurring (or re-occurring) in late-life, typically after age 60-years (American Psychiatric Association, 2013). Recent research has focused on the mechanism leading to LLD (Alexopoulos et al., 2002). There is growing awareness that inflammatory processes interact with brain health and may be an additional mechanism by which cardiovascular risk becomes disease in LLD (Elderkin-Thompson et al., 2012; Joynt et al., 2003; Rosenblat et al., 2014), and impacts cognition (Charlton et al., 2014; Taylor et al., 2008). Inflammation is frequently elevated in aging (Bruunsgaard and Pedersen, 2003; Dinarello, 2006; Maggio et al., 2006) and is associated with cardiovascular risk (also common in aging), but the mechanism, and the way these factors (depression, cardiovascular risk, inflammation) interact and impact cognition is still debated (Joynt et al., 2003). The aim of the current study is to examine whether inflammatory markers are greater in LLD compared to healthy older adults (HOA) and the extent to which these markers are associated with the cognitive difficulties frequently observed in LLD.

It has been suggested that serotonin loss in aging may be a risk factor for developing LLD (Meltzer et al., 1998). In LLD, reduced serotonin already present in the disease, may be further exacerbated by release of inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ); cytokines known to negatively impact serotonin production and integrity (Linthorst et al., 1995a). Furthermore, the release of TNF- $\alpha$  as well as interleukin 1 $\beta$  (IL-1 $\beta$ ) is thought to induce synaptic pruning, leading to impaired neuroplasticity and structural brain changes that then negatively impact cognition (Rosenblat et al., 2014). During inflammation, some cytokines occur early and activate a more persistent inflammatory response. This cascade of events may be particularly damaging to the sensitive hippocampal region, resulting in difficulties in learning and memory (Elderkin-Thompson et al., 2012).

Older age is associated with increased levels of pro-inflammatory cytokines IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 (Baune et al., 2012; Bruunsgaard & Pedersen, 2003; Dinarello, 2006; Maggio et al., 2006). Inflammation has been associated with typical age-related cognitive changes (including learning and memory) and dementia (Holmes et al., 2009; Jordanova et al., 2007; Weaver et al., 2002). The mechanism for this association may be an age-related sensitization of microglia leading to a magnified neuroinflammatory response that impairs synaptic plasticity, and leads to hippocampal dysfunction resulting in memory deficits (Barrientos et al., 2010). However, not all studies show this pattern of increased inflammation in aging (Mooradian et al., 1991; Whooley et al., 2007). Although inconsistent findings may reflect between-cohort differences, authors also suggest that inflammatory markers must exceed a certain level (threshold) or occur with other factors, in order to be damaging (Penninx et al., 2003). Inflammation may have multiple, interacting effects on brain structure and function and it may be these factors in combination rather than age alone that lead to the poorest outcomes (Taylor et al., 2013).

Even higher levels of IL-1 $\beta$ , TNF- $\alpha$  and IL-6 are reported in LLD compared to HOA (Penninx et al., 2003; Thomas et al., 2005). A previous study by our group of LLD patients and HOA conducted a hierarchical regression analysis and found that IL-6 and depression status (LLD or HOA) contributed to the variance of learning and memory performance (Elderkin-Thompson et al., 2012). In the current study examining a different sample of participants, we will investigate the factors that contribute to learning and memory performance. We hypothesise that pro-inflammatory cytokines will be higher in individuals with LLD compared to HOA; and that levels of pro-inflammatory cytokines will be associated with learning and memory difficulties particularly among individuals with LLD.

## **Methods**

### *Participants and Procedures*

Data was collected in two large research studies investigating LLD and diabetes at the University of Illinois at Chicago (UIC). Individuals age 60 and older were recruited via community outreach (e.g., newspaper, radio, television advertisements) and relevant outpatient clinics within the School of Medicine. The study was approved by the UIC Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Participants underwent a preliminary telephone screen. Exclusion criteria consisted of current or past history of brain disorders (i.e., dementia, stroke, seizure, head injury, loss of consciousness, etc.), history of substance abuse or dependence, an Axis I psychiatric disorder diagnosis (other than MDD for the LLD group), psychotropic medication use and presence of metallic implants that preclude MRI. All study participants including LLD patients were free of any antidepressant medication for at least two weeks in order to study depressed mood in an untreated state.

After passing the telephone screen, participants received an evaluation including cognitive, i.e., Mini-Mental State Examination (MMSE; Folstein et al., 1975) and affective, i.e., Structured Clinical Interview for DSM-IV (SCID; Spitzer et al., 1992) screens for final inclusion. Screening measures were administered by a trained research assistant, and a board certified (AK) or board eligible (OA) psychiatrist completed the evaluation including the Hamilton Depression Rating Scale (HDRS; Hamilton, 1960). All raters were blind to telephone screen information. Participants attended two further visits to complete a neuropsychological assessment and MRI acquisition.

Final inclusion criteria for adults with LLD included a diagnosis of MDD on the SCID and a score  $\geq 15$  on the 17-item HDRS. HOA participants required an absence of depressive symptoms on the SCID and a score  $\leq 8$  on the HDRS. As individuals were assigned to groups based on the HDRS, this scale will not be included in the analyses. Participants

completed the Center for Epidemiological Studies Depression scale (CESD; Radloff, 1977) and the Geriatric Depression Scale (GDS; Yesavage, 1988).

All participants had an MMSE score  $\geq 24$ , scored within the normal range on the standardized neuropsychological assessment, and were native English speakers. History of stable (e.g., diabetes, hypertension) or remitted (e.g., cancer) medical illness was not an exclusionary factor. Weight in kilograms and height in centimeters were collected, and body mass index (BMI) was calculated, waist circumference and current smoking status were recorded. A non-fasting blood sample was obtained by venepuncture (see below for information on cytokine assays). Hemoglobin A1c (HA1c) was measured for all subjects, assessed at Alverno-PCL Laboratories (Hammond, IN). HA1c values have been documented to be minimally affected by normal food consumption (Langsted et al., 2008). Participants received an assessment of vascular risk using the Framingham Stroke Risk Profile (FSRP) score (Wolf et al., 1991).

155 individuals attended initial screening, of whom 85 participants were assessed for pro-inflammatory cytokines. A further 27 individuals were excluded from analysis: 11 had past substance abuse/dependence not disclosed at screening; five had English as a second language; five had contra-indicative comorbidities; two had sleep apnea; and four had other contra-indication due to medicine (n=2), recent neuropsychological assessment (n=1) or abnormal MRI (n=1). There were no differences between those with and without cytokine data in terms of highest education level, FSRP score, HA1c, or depression ratings (data not shown); those with cytokine data (Mean age=69.24, sd=7.58) were slightly older than those without (Mean age=66.65, sd=5.30;  $F=4.41$ ,  $p=.038$ ). Thirty-four individuals were classified as HOA (n=7, 20.6% with diabetes). Twenty-four individuals met criteria for LLD (n=9, 37% with diabetes). The rate of diabetes in Illinois for adults over 60 is 23.8% (Danaei et al., 2009); as the rate is higher than average in the LLD group, analysis will control for HA1c.

### *Cytokine Assays*

Levels of pro-inflammatory cytokines were determined in plasma/serum aliquots by enzyme-linked immunosorbent assay (ELISA) using commercially available Quantakine® kits (R & D Systems, Inc., Minneapolis, MN) for human IL-1 $\beta$ , TNF- $\alpha$  and IL-6. Briefly, 100 $\mu$ L of incubation buffer and 100 $\mu$ L of serum/plasma or standard is added to each well and incubated for 3 hours at room temperature (RT) on the orbital shaker. After washing wells 6 times with Wash Buffer, 200 $\mu$ L of Conjugate is added to each well, incubated for 2 hours at RT, washed using Wash Buffer as before, 50 $\mu$ L of Substrate Solution is added to each well and incubated for 60 minutes at RT. Following this, 50 $\mu$ L of Amplifier Solution is added to each well, incubate for 30 minutes at RT and 50 $\mu$ L of Stop Solution is added to each well. The optical density of each well is determined within 30 minutes using a microplate reader set to 490nm and wavelength correction is set to 650nm and the levels of cytokines are calculated. Standard curve was generated by plotting the mean absorbance for each standard and data points are linearized. The cytokines concentration in each sample was determined by reading it against the standard curve. Pro-inflammatory cytokine values were not normally distributed, therefore values were log transformed for use in the analysis.

### *Neuropsychological Assessment*

Learning and memory variables were the focus of this study. As part of a larger neuropsychological assessment, learning was measured using the California Verbal Learning Test-II (CVLT; Delis et al., 2000) immediate delay free recall (Trials 1-5), and immediate free recall measures from the Wechsler Memory Scale-III (WMS-III; Wechsler et al., 1998) Logical Memory I and Visual Reproduction I. Additionally, memory was measured using the CVLT long delay free recall (Delis et al., 2000), and long delay free recall measures from the WMS-III (Wechsler et al., 1998) Logical Memory II and Visual Reproduction II. Raw scores were transformed into z-scores using the mean and standard deviation of the whole sample. Z-scores were coded so high scores reflected good performance and were collated to produce a mean score for the domains of learning and

memory. Cronbach's alphas assessed how well the variables measured each latent construct. Values were considered good, indicating that each variable measured a unidimensional latent construct (learning,  $\alpha=.73$ ; memory,  $\alpha=.70$ ).

### *Neuroimaging Protocol*

Brain MRI were acquired on a Philips 3.0T Acheiva scanner using an 8-channel SENSE (Sensitivity Encoding) head coil (Philips Medical Systems, Best, The Netherlands).

Participants were positioned comfortably on the scanner bed, ear-plugs minimized noise discomfort, foam pads positioned the head and minimized movement. A high resolution three-dimensional T1-weighted image was acquired with a Magnetization Prepared Rapid Acquisition Gradient Echo sequence (FOV=240mm; 134 contiguous axial slices; TR/TE=8.4/3.9ms; flip angle=8°; voxel size=1.1X1.1X1.1mm) as part of a larger protocol.

### *Image Analysis*

The Freesurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) was used to segment T1-weighted volume scans. Processing included motion correction, removal of non-brain tissue, transformation into Talairach space, registration of image to an atlas and parcellation of the cerebral cortex into units based on gyral and sulcal structures (Destrieux et al., 2010). For the purposes of this analysis the volumes of the Left and Right hippocampi were extracted and expressed as a ratio of the Freesurfer calculated total brain volume.

### *Statistical Analyses*

Statistical analyses were performed in SPSS (version 22.0; IBM Corp., 2013). Group differences in demographics information and pro-inflammatory cytokines were examined using independent samples t-tests, Mann-Whitney U tests and Chi-Squared, as appropriate. Pearson product-moment correlations were performed to examine associations between age, vascular risk, pro-inflammatory cytokines, learning and memory, and hippocampal volumes. Regression (forward, stepwise) analyses examined factors that explained the



variance in learning and memory performance for the whole group, including group by inflammatory cytokine metrics.

## **Results**

### *Group Differences*

Levene's test for equality of variance was used to tests for homoscedasticity. The pro-inflammatory cytokines and three depression scales were not normally distributed and group differences will therefore be tested using non-parametric Mann-Whitney U tests. Other group differences were asses using independent samples t-tests and Chi-square for categorical variables.

There were no significant differences between the groups on age, sex, education, race, FSRP score, HA1c levels, number of diabetics or smokers in the group, body mass index, or waist circumference, see Table 1. For all pro-inflammatory cytokines, the LLD group demonstrated higher levels than the HOA, although this did not reach significance for TNF- $\alpha$ . No group differences were observed for left ( $t(46)=-1.60$ ,  $p=.117$ ) or right ( $t(46)=-.858$ ,  $p=.395$ ) hippocampal volumes or for learning or memory z-scores. As expected the LLD group demonstrated significantly higher scores on the CESD and GDS, see Table 1.

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Insert Table 1 approximately here

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### *Correlations*

Associations between FSRP, HA1c and pro-inflammatory cytokines were explored using non-parametric correlations (Spearman's rho). High vascular risk measured by the FSRP correlated significantly with IL-6 ( $r=.325$ ,  $p=.013$ ). No significant associations were observed

between FSRP and either IL-1 $\beta$  ( $r=-.027$ ,  $p=.843$ ) or TNF- $\alpha$  ( $r=-.004$ ,  $p=.974$ ). HA1c levels demonstrated a non-significant trend towards significance with IL-6 ( $r=.239$ ,  $p=.071$ ), but not with IL-1 $\beta$  ( $r=.033$ ,  $p=.808$ ) or TNF- $\alpha$  ( $r=.044$ ,  $p=.744$ ). Subsequent correlations were performed controlling for FSRP and HA1c as: i) FSRP and HA1c show some correlations with pro-inflammatory cytokines, ii) they are expected to have an impact on pro-inflammatory response, and iii) in the LLD sample the proportion of individuals with diabetes is higher than the Illinois state average (as previously stated).

Partial correlations between variables of interest were performed for the whole sample controlling for FSRP and HA1c, see Table 2. As some variables were not normally distributed z-scores were used in the analysis. Pro-inflammatory cytokines IL-1 $\beta$  and IL-6 correlated significantly with scores on depression scales. No significant correlations were observed in the relationship between pro-inflammatory cytokines and learning or memory scores, although a non-significant trend was observed between both learning and memory and IL-6. No significant correlations were observed with hippocampal volumes.

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Insert Table 2 approximately here

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#### *Regression analysis: explaining cognitive performance*

Separate forward stepwise regression analyses were performed with learning and memory as the dependent variables; included as independent variables were age, sex, FSRP, BMI, Education Level, GDS, HA1c, IL-1 $\beta$ , TNF- $\alpha$ , IL-6, left and right hippocampal volumes, group by cytokine interaction terms (Grp x IL-1 $\beta$ , Grp x TNF- $\alpha$ , Grp x IL-6). Z-scores were used in the analyses for variables that were not normally distributed (GDS, IL-1 $\beta$ , TNF- $\alpha$ , IL-6) and were used to calculate the interaction terms. See Table 3 for details and Figure 1 for visual representation of associations between IL-6 and learning and memory.

*Learning:* The model significantly explained the variance in learning (41.4%,  $F(2,34)=11.99$ ,  $p<.001$ ) with years of education (22.2%) and right hippocampal volume (19.2%) contributing to the model.

*Memory:* The model significantly explained the variance in memory (50.5%,  $F(3,33)=11.23$ ,  $p<.001$ ) with years of education (25.3%), right hippocampal volume (16.8%) and Grp x IL-6 (8.4%) contributing to the model. The Grp x IL-6 interaction demonstrated that high IL-6 is associated with poorer memory in the LLD group; whereas in the HOA group IL-6 levels are not associated with memory, see Figure 1.

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Insert Table 3 and Figure 1 approximately here

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## **Discussion**

Although previous studies have examined IL-1 $\beta$ , TNF- $\alpha$  and IL-6 and found higher levels in depressed patients (Penninx et al., 2003; Thomas et al., 2005), these markers have not always been examined within the same study. In this study we demonstrate higher levels of inflammatory markers (IL-1 $\beta$ , TNF- $\alpha$ , IL-6) in individuals with LLD compared to HOA.

Furthermore, results suggest that high IL-6 values are detrimental to memory performance in individuals with LLD, but not in HOA. Although the same pattern was observed for learning (Figure 1), the regression analysis did not reach significance.

It may be the impact of persistent inflammation *in combination* with the additional risk factor of LLD that accounts for memory performance, whereas high levels of IL-6 alone may be insufficient to explain memory performance in HOA. These results are in keeping with a recent hierarchical regression study by our group of LLD and HOA, in which IL-6 significantly explained the variance in similar measures of learning and memory for the whole sample, note that hippocampal volume was not measured (Elderkin-Thompson et al., 2012). It has

been suggested that high levels of IL-6 increase breakdown of serotonin and decrease serotonin production, by which mechanism neuroplasticity may be impaired, leading to structural brain abnormalities (i.e. reduction in hippocampal volume) thereby impacting cognitive function (Linthorst et al., 1995a).

Regression analyses also demonstrated that both right hippocampal volume and education contribute to explaining a large proportion of the variance in learning (41.2%) and memory (38.5%). The hippocampus is highly associated with learning and memory function across the lifespan (Cardenas et al., 2011). Furthermore, the right hippocampus has been associated primarily with memory retrieval (equivalent to memory in this study), although increased bilateral activation has also been noted in aging (Duverne et al., 2009). Among individuals with depression, verbal memory deficits are commonly associated with smaller hippocampal volumes across the lifespan and in LLD (Ballmaier et al., 2008) with some studies reporting bilateral hippocampal declines (Lloyd et al., 2004), although this association was not observed in the current study. Associations between cognitive functions and educational level are commonly observed, with education often used as a proxy for general ability (Richards and Sacker, 2003). When taken as a whole, results suggest that inflammation alone is not sufficient to “cause” difficulties in learning and memory. In fact, in LLD additional factors acting in combination may interact with inflammation to impact cognitive performance for example by affecting hippocampal serotonergic neurotransmission (Linthorst et al., 1995b).

In contrast to some previous studies, results in this study do not suggest a strong association between pro-inflammatory markers and cognition in HOA. Weaver et al. (2002) identified associations between being in the highest tertile for IL-6 levels at baseline and the bottom tertile (worst performance) of global cognitive decline scores seven-years later in HOA. Over a shorter follow-up period of three years, Jordanova et al. (2007) described associations between high IL-6 and decline in learning, using a similar learning measure to

that described in the current study. Jordanova et al. (2007) describe a British African-Caribbean population with high risk for cardiovascular disease, who may demonstrate greater risk compared to the HOA sample in the current study. Neither of the above studies examined cytokines as continuous variables which may suggest that there is some threshold of inflammatory response in HOA, beyond which IL-6 is associated with cognitive decline. Other studies that have identified associations between pro-inflammatory cytokines and cognitive decline have largely examined dementia states rather than HOA (Holmes et al., 2009). In a study of 300 community dwelling patients with Alzheimer's Disease, Holmes et al. (2009) found that high TNF- $\alpha$  at baseline, at six-month follow-up and recorded periods of illness, were all associated with increased rate of cognitive decline over six-months. Significantly, it was the combination of pro-inflammatory cytokines and presence of inflammatory events (periods of illness) that placed a patient at greatest risk of cognitive decline. Studies are beginning to provide further support for the notion that interactions between multiple factors are the best predictors of outcome. Bender et al. (2013) found that hippocampal sub-region volumes were negatively associated with age and memory performance in HOA, but *only* among participants with hypertension. There is also growing awareness that interactions between cardiovascular risk, genetic risk and psycho-social factors such as depression, may provide better explanations for outcomes than any individual variable (Joynt et al., 2003).

Significant correlations were observed between self-report endorsement of depressive symptoms (CESD, GDS) and inflammatory markers (IL-1 $\beta$  and IL-6) for the entire sample, even after controlling for vascular risk (FSRP and HA1c). These results are in keeping with the literature which suggests that pro-inflammatory makers may be a risk factor for the development and persistence of depressive symptoms across the lifespan (Joynt et al., 2003) and in aging (Penninx et al., 2003; Thomas et al., 2005). It is interesting to note that although TNF- $\alpha$  levels were higher in LLD compared to HOA, levels did not correlate with depressive symptoms; whereas IL-1 $\beta$  and IL-6 did correlate with depressive symptoms. This

pattern of results may reflect the mechanisms that lead to secretion of different cytokines in brain or a cumulative/threshold effect of inflammatory cytokines. It is worth noting that in the current data LLD and HOA did not differ on measures of learning and memory, or hippocampal volume. This may reflect the fact that the groups were matched on age, education and other health factors. Alternatively this could reflect some peculiarities of the LLD group, which were community dwelling and able to be medication free for the two week period required by this study. Although individuals in the LLD group all met criteria for MDD both on the SCID and the Psychiatrist administered HDRS, they may have been less severely depressed than samples in other studies.

Results should be interpreted within the confines of study limitations. Importantly, the sample size in this study was modest and the groups unequal (LLD, n=24; HOA, n=34), which precludes replication of analyses used in previous studies. Where pro-inflammatory cytokines have been described as categorical variables, results have found robust associations with longitudinal cognitive decline (Jordanova et al., 2007; Weaver et al., 2002) however the sample size in the current study was insufficient for this analysis. In addition, participants represented a proportion of the sample (not all participants agreed to the blood draw necessary for cytokine assays); however those with and without cytokine assays only differed on age (those with cytokine assays being slightly older). A single blood draw was taken when convenient (rather than at a prescribed time), therefore pro-inflammatory cytokines may be effected by diurnal variations. Future studies should control for these factors and potentially include multiple blood draws several weeks apart. Additional extraneous variables, such as current infection, obesity, physical activity, and health comorbidities may further influence results. Previous studies have suggested that a combination of current inflammatory markers and reported inflammatory events better predicts outcome than inflammatory markers alone (Holmes et al., 2009). A longitudinal study has demonstrated that different common infections are significantly associated with cognitive decline over five-year follow-up, beyond expected age-related change

(Nimgaonkar et al., 2016). Therefore more information about recent/current infections may both clarify risk and inform potential treatments. In this study we did not record self-report inflammation, and including a clinical and/or self-report infection screen could strengthen observed associations. The current study was also cross-sectional whereas longitudinal analyses could inform the direction of the associations described. Despite these limitations, significant results were observed in the current study.

## **Conclusion**

We identified higher levels of pro-inflammatory cytokines IL-1 $\beta$ , TNF- $\alpha$  and IL-6 in LLD compared to HOA, and significant associations between severity of depressive symptoms and inflammation. Regression analyses suggest a different effect of high levels of IL-6 on memory in HOA compared to individuals with LLD. In the presence of a persistent psychological condition such as depression, the addition of inflammation has a significant impact on cognitive abilities. Although high levels of pro-inflammatory markers alone may not be sufficient to “cause” cognitive difficulties, they may have a significant impact in already at-risk populations. Longitudinal studies are required to better understand the interaction between risk factors and the impact on brain and function.

**Acknowledgment:** This work was supported by the National Institutes of Health (AK, NIMH 7RO1 MH073989-04). Additional support was provided by the National Institutes of Health (GP, NIMH RO1 MH056528).

**Disclosure Statement:** The authors report no conflict of interest. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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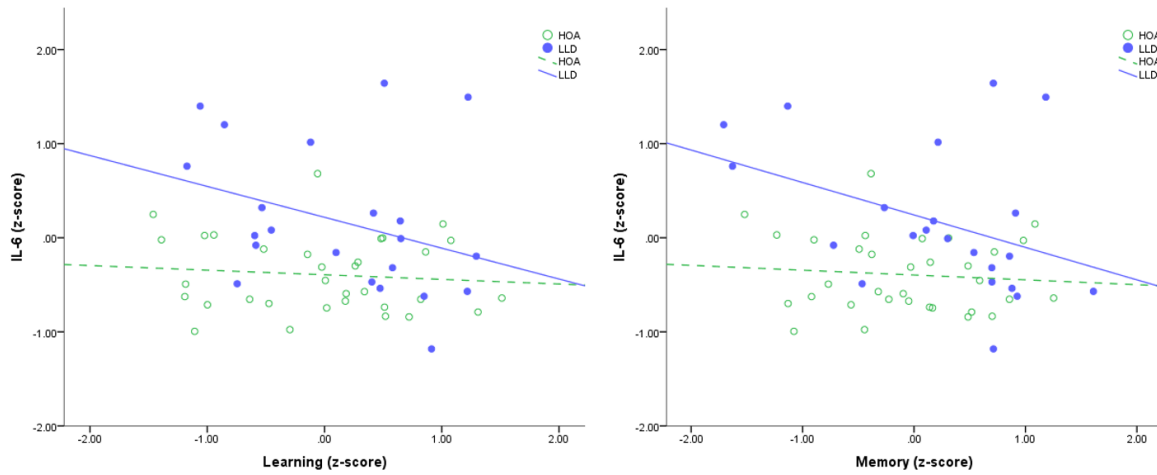
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## Figure Legends

Figure 1: Graphs showing associations between pro-inflammatory and cytokines depressive symptoms, by group.



**Table 1: Demographic Information by Group, mean (standard deviation) unless otherwise stated**

|  | HOA (n=34)               | LLD (n=34)                  | Group differences            | Effect size <sup>±</sup> |
|--|--------------------------|-----------------------------|------------------------------|--------------------------|
| <b>Demographics</b>                          |                          |                             |                              |                          |
| Age  | 70.15 (6.07)             | 67.21 (9.09)                | t(56)=1.48, p=.145           | d=-.380                  |
| Highest Educational Level (years)            | 16.41 (3.01)             | 15.92 (2.75)                | t(56)=.640, p=.525           | d=-.170                  |
| Sex (m,f), n                                 | 13,21                    | 8,16                        | X <sup>2</sup> =.146, p=.786 | -                        |
| Ethnicity (black, white, Hispanic, other), n | 8, 24, 0, 2              | 8, 14, 2, 0                 | X <sup>2</sup> =5.06, p=.168 | -                        |
| FSRP Total                                   | 11.50 (4.27)             | 11.25 (3.93)                | t(56)=.227, p=.821           | d=-.061                  |
| Diabetic (y,n), n                            | 7,27                     | 9,15                        | X <sup>2</sup> =2.01, p=.233 | -                        |
| HA1c   | 5.93 (.737)              | 6.31 (1.10)                 | t(56)=-1.58, p=.119          | d=.406                   |
| Body mass index                              | 26.99 (4.56)             | 29.23 (6.81)                | t(56)=-1.51, p=.138          | d=.387                   |
| Waist circumferences (cm)                    | 94.00 (13.93)            | 100.85 (19.27)              | t(56)=-.661, p=.525          | d=.407                   |
| Smoker (y,n) n                               | 5, 29                    | 3, 21                       | X <sup>2</sup> =.058, p=.801 | -                        |
| <b>Pro-inflammatory Cytokines</b>            |                          |                             |                              |                          |
| IL-1 $\beta$                                 | 1.52 (.699)              | 2.38 (1.03)                 | U(56)=180.5, p<.001          | d=.977                   |
| TNF- $\alpha$                                | 3.09 (1.40)              | 4.05 (2.16)                 | U(56)=325.5, p=.193          | d=.527                   |
| IL-6   | 1.24 (.443)              | 2.03 (1.22)                 | U(56)=201.5, p=.001          | d=.861                   |
| <b>Depression Scales</b>                     |                          |                             |                              |                          |
| HDRS   | 1.41 (1.83)<br>Range 0-6 | 18.96 (3.29)<br>Range 15-27 | U(56)=.00, p<.001            | d=6.59                   |
| CESD   | 5.85 (5.67)              | 31.67 (9.48)                | U(56)=9.0, p<.001            | d=3.31                   |
| GDS  | 2.10 (2.78)              | 18.86 (5.80)                | U(56)=4.0, p<.001            | d=3.69                   |
| <b>Cognitive Function</b>                    |                          |                             |                              |                          |
| Learning z-score                             | -.046 (.816)             | .065 (.807)                 | t(56)=-.510, p=.612          | d=.137                   |
| Memory z-score                               | -.096 (.717)             | .136 (.882)                 | t(56)=-1.11, p=.274          | d<.001                   |

FSRP=Framingham Stroke Risk Profile score; HA1c=Hemoglobin A1c; HDRS= Hamilton Depression Rating Scale; CESD=Center for Epidemiological Studies Depression scale; GDS=Geriatric Depression Scale; IL-1 $\beta$ =interleukin 1 $\beta$ ; TNF- $\alpha$ =tumor necrosis factor  $\alpha$ ; IL-6=interleukin 6

<sup>±</sup> Effect size= Cohen's d; Negative values indicate that LLD>HOA

**Table 2: Partial correlations between variables of interest for whole sample, controlling for FSRP and HA1c (z-scores used in analysis).**

|                                | Age                                | CESD                              | GDS                            | IL-1 $\beta$      | TNF- $\alpha$     | IL-6              | Learning                          | Memory           | Left Hippocampus                  |
|--------------------------------|------------------------------------|-----------------------------------|--------------------------------|-------------------|-------------------|-------------------|-----------------------------------|------------------|-----------------------------------|
| <b>CESD</b>                    | r=-.119<br>p=.472                  | -                                 | -                              | -                 | -                 | -                 | -                                 | -                | -                                 |
| <b>GDS</b>                     | r=-.145<br>p=.378                  | <b>r=.922</b><br><b>p&lt;.001</b> | -                              | -                 | -                 | -                 | -                                 | -                | -                                 |
| <b>IL-1<math>\beta</math></b>  | r=.064<br>p=.698                   | r=.272<br>p=.094                  | <b>r=.379</b><br><b>p=.017</b> | -                 | -                 | -                 | -                                 | -                | -                                 |
| <b>TNF-<math>\alpha</math></b> | r=.051<br>p=.757                   | r=.248<br>p=.128                  | r=.121<br>p=.461               | r=.022<br>p=.895  | -                 | -                 | -                                 | -                | -                                 |
| <b>IL-6</b>                    | r=.043<br>p=.794                   | <b>r=.411</b><br><b>p=.009</b>    | <b>r=.390</b><br><b>p=.014</b> | r=.025<br>p=.879  | r=-.171<br>p=.299 | -                 | -                                 | -                | -                                 |
| <b>Learning</b>                | r=-.278<br>p=.086                  | r=-.082<br>p=.618                 | r=-.134<br>p=.417              | r=-.227<br>p=.165 | r=.148<br>p=.368  | r=-.274<br>p=.094 | -                                 | -                | -                                 |
| <b>Memory</b>                  | r=-.275<br>p=.091                  | r=-.062<br>p=.708                 | r=-.078<br>p=.639              | r=-.172<br>p=.296 | r=.181<br>p=.270  | r=-.306<br>p=.058 | <b>r=.900</b><br><b>p&lt;.001</b> | -                | -                                 |
| <b>Left Hippocampus</b>        | <b>r=-.632</b><br><b>p&lt;.001</b> | r=.252<br>p=.122                  | r=.272<br>p=.094               | r=.005<br>p=.977  | r=.001<br>p=.997  | r=.016<br>p=.922  | r=.165<br>p=.316                  | r=.213<br>p=.193 | -                                 |
| <b>Right Hippocampus</b>       | <b>r=-.595</b><br><b>p&lt;.001</b> | r=.061<br>p=.714                  | r=.085<br>p=.606               | r=.016<br>p=.924  | r=-.152<br>p=.357 | r=-.046<br>p=.780 | r=.252<br>p=.121                  | r=.218<br>p=.183 | <b>r=.885</b><br><b>p&lt;.001</b> |

FSRP=Framingham Stroke Risk Profile score; HA1c=Hemoglobin A1c; CESD=Center for Epidemiological Studies Depression scale; GDS=Geriatric Depression Scale; IL-1 $\beta$ =interleukin 1 $\beta$ ; TNF- $\alpha$ =tumor necrosis factor  $\alpha$ ; IL-6=interleukin 6; Bold=significant at p<.05

**Table 3: Stepwise Forward Regression analysis for Learning and Memory: Statistics for variables contributing significantly to the model.**

|  | Standardised<br>Beta weight | Variance<br>explained (%) | Cumulative variance<br>explained (%) |
|--|-----------------------------|---------------------------|--------------------------------------|
| <b>Learning</b>  |                             |                           |                                      |
| Years of Education   | .550 (p<.001)               | 21.2%                     | 21.2%                                |
| Right Hippocampal Volume   | .458 (p=.001)               | 20.2%                     | 41.4%                                |
| <b>Model Statistics F=13.05, p&lt;.001</b>   |                             |                           |                                      |
| <i>(For reference and comparison to the Memory model below only: Grp X IL-6, Standardised Beta weight = -.232, p=.070)</i> |                             |                           |                                      |
| <b>Memory</b>  |                             |                           |                                      |
| Years of Education   | .501 (p<.001)               | 21.4%                     | 21.4%                                |
| Right Hippocampal Volume   | .439 (p=.001)               | 17.1%                     | 38.5%                                |
| Grp X IL-6   | -.264 (=0.043)              | 6.7%                      | 40.7%                                |
| <b>Model Statistics F=9.92, p&lt;.001</b>  |                             |                           |                                      |

Variables entered into stepwise regression as independent variables: age, FSRP, BMI, Highest Education Level, Gender, GDS, HA1c, IL-1 $\beta$ , TNF- $\alpha$ , IL-6, left and right hippocampal volumes, group by cytokine interaction terms (Grp x IL-1 $\beta$ , Grp x TNF- $\alpha$ , Grp x IL-6).

GDS=Geriatric Depression Scale; FSRP=Framingham Stroke Risk Profile score; IL-6=interleukin 6