

FINKELMEYER, A., NILSSON, J., HE, J., STEVENS, L., MALLER, J.J., MOSS, R.A., SMALL, S., GALLAGHER, P., COVENTRY, K., FERRIER, I.N. and MCALLISTER-WILLIAMS, R.H. 2016. Altered hippocampal function in major depression despite intact structure and resting perfusion. *Psychological medicine* [online], 46(10), pages 2157-2168. Available from: <https://doi.org/10.1017/S0033291716000702>

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2016

This article has been published in a revised form in Psychological medicine
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**Altered hippocampal function in major depression
despite intact structure and resting perfusion**

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A.F. is supported by Research Capability Funding from the Northumberland-Tyne-and-Wear
NHS Foundation Trust awarded to R.H.M.-W.

Word count: 4737

Abstract

Background: Hippocampal volume reductions in major depression have been frequently reported. However, evidence for functional abnormalities in the same region in depression has been less clear. We investigated hippocampal function in depression using functional magnetic resonance imaging (fMRI) and neuropsychological tasks tapping spatial memory function, with complementing measures of hippocampal volume and resting blood flow to aid interpretation.

Methods: Twenty patients with major depressive disorder (MDD) and a matched group of 20 healthy individuals participated. Participants underwent multimodal magnetic resonance imaging (MRI): fMRI during a spatial memory task, structural MRI and resting blood flow measurements of the hippocampal region using arterial spin labelling (ASL). An offline battery of neuropsychological tests, including several measures of spatial memory, was also completed.

Results: The fMRI analysis showed significant group differences in bilateral anterior regions of the hippocampus. While control participants showed task-dependent differences in blood oxygen level dependent (BOLD) signal, depressed patients did not. No group differences were detected with regard to hippocampal volume or resting blood flow. Patients showed reduced performance in several offline neuropsychological measures. All group differences were independent of differences in hippocampal volume and hippocampal blood flow.

Conclusions: Functional abnormalities of the hippocampus can be observed in patients with MDD even when the volume and resting perfusion in the same region appears normal. This

suggests that changes in hippocampal function can be observed independently of structural abnormalities of the hippocampus in depression.

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Introduction

Major depressive disorder (MDD) is associated with reduced hippocampal volumes (Arnone *et al.*, 2012, Campbell and Macqueen, 2004, Palazidou, 2012). Although there is evidence of reduced hippocampal volumes in first-episode, untreated patients (Cole *et al.*, 2011, Zou *et al.*, 2010) and in individuals at familial risk of depression (Amico *et al.*, 2011, Baare *et al.*, 2010, Chen *et al.*, 2010), meta-analyses suggest that volume reductions of the hippocampus are more pronounced with prolonged illness (McKinnon *et al.*, 2009), a higher number of depressive episodes (Videbech and Ravnkilde, 2004) and more severe depression (Arnone *et al.*, 2012, Vakili *et al.*, 2000). Depressed patients also show significant reductions in performance in a large range of cognitive domains (Burt *et al.*, 1995, Rock *et al.*, 2014), including memory and learning, some of which persist in the remitted state (Hasselbalch *et al.*, 2011). Given the prominent role of the hippocampus and surrounding brain structures in memory related functions (Burgess *et al.*, 2002, Lavenex and Banta Lavenex, 2013), structural changes in this region may therefore play a role at least in part in the cognitive dysfunction seen in MDD (Kaymak *et al.*, 2010, Trivedi and Greer, 2014).

Considering the large number of studies investigating hippocampal structure in MDD and the evidence for abnormalities in neuropsychological functions believed to involve the hippocampus, it is surprising that far fewer neuroimaging studies have investigated hippocampal function in this group. Functional magnetic resonance imaging (fMRI) studies of task-related hippocampal activations during memory tasks have been inconsistent showing both increased (Young *et al.*, 2014) and reduced activations (Fairhall *et al.*, 2010, Milne *et al.*, 2012) as well as no significant differences relative to healthy controls (Werner *et al.*, 2009). A magnetoencephalography (MEG) study using a spatial navigation task found

reduced theta band power in the right hippocampus and parahippocampal area in depressed patients suggesting reduced cortical activity in this region in a spatial task (Cornwell *et al.*, 2010). Earlier H₂(15)O positron emission tomography (PET) studies showed increased resting cerebral blood flow (CBF) in the hippocampus of depressed patients (Videbech *et al.*, 2002), but reduced blood flow increases during a verbal encoding task (Bremner *et al.*, 2004). A recent study of CBF using arterial spin labelling (ASL) also showed increased resting blood flow in the hippocampus in MDD (Lui *et al.*, 2009). The state of hippocampal function in depression is therefore not clear from the current evidence. Adding to this, the relationships between functional and structural abnormalities of the hippocampus in depressed patients remains largely unexplored. Differences in hippocampal function in depression could be a direct consequence of differences in hippocampal structure but may also emerge independently of such structural differences.

In the present study we investigated if hippocampal function, as measured with fMRI, is altered in depressed patients during performance of a spatial memory task. The task was designed to place specific demands on allocentric spatial memory, which is known to be dependent on the hippocampus (King *et al.*, 2002, Lee *et al.*, 2005). In the allocentric condition of this task, locations are remembered independently of an individual's location and orientation, which is in contrast to the egocentric condition in which locations are remembered relative to the position of the individual. We have previously demonstrated that the allocentric and egocentric conditions results in robust differences in blood-oxygen level dependent (BOLD) signal in the hippocampus in young healthy subjects, which motivates its use as a measure of hippocampal function in the present study (Nilsson *et al.*, 2013). A larger offline battery of neuropsychological tests was also completed to give a

complete picture of cognitive performance in the patient group relative to the control group.

To allow an exploration of the relationship between hippocampal function and structure, the hippocampi of all subjects were manually traced and were also considered in the analyses. Furthermore, arterial spin labelling (ASL) provided a measure of CBF at rest in the hippocampus to aid in the interpretation of group differences in BOLD signal. Specifically, group differences in BOLD signal could not only be due to differences in neural activity, but may also reflect changes in blood flow, blood volume or oxygen metabolism, all of which contribute to the BOLD signal (Buxton, 2012). Considering previous findings of increased hippocampal CBF in MDD (Lui *et al.*, 2009, Videbech *et al.*, 2002), the ASL measure was therefore included to explore whether potential group differences in BOLD signal could be accounted for by differences in resting CBF.

The present study therefore set out to answer the following main questions: 1) Do depressed patients exhibit abnormal hippocampal function relative to healthy controls, as evidenced by an altered BOLD signal in response to a spatial memory task that place demands on allocentric and egocentric memory systems? 2) Can such group differences in task-related BOLD signal be explained by differences in hippocampal volume or resting CBF?

Methods

Participants

A sample of 20 depressed patients and 20 healthy controls were recruited via their consultant psychiatrists or online advertising to participate in the study. Presence or absence of MDD diagnosis was confirmed via Mini International Neuropsychiatric Interview

(MINI) (Sheehan *et al.*, 1998). Patients were excluded if they had any other Axis 1 disorders other than anxiety, had previously received electroconvulsive therapy or had a change in psychiatric medication in the last four weeks. To take part, patients were required to have a score of 16 or above on the Hamilton Depression Scale (HAM-D)(Williams *et al.*, 2008).

Healthy controls were excluded if they or a first-degree relative had a history of psychiatric illness or they had a score of 5 or above on the HAM-D. This meant to ensure that controls were free of even mild depressive symptoms. Patients and healthy controls were excluded from the study if they were dependent on or abusing alcohol or other drugs in the past 12 months. Individuals with conditions contraindicative to MRI were excluded from the study. All participants were right-handed.

In both groups, depressed mood and anxiety were assessed using the Beck Depression Inventory (BDI)(Beck *et al.*, 1961) and the State and Trait Anxiety Inventory (STAI)(Spielberger, 1983). For patients, age of onset, number of episodes, illness duration and current medication regime were determined via retrospective self-report. Pre-morbid verbal IQ was determined using the National Adult Reading Test (NART)(Nelson and Willison, 1991). Table 1 shows patient and healthy control sample characteristics. Groups were matched in terms of age, sex and premorbid IQ.

The research was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent and the study was approved by the local Research Ethics Committee.

[TABLE 1 HERE]

Neuropsychological assessment

Participants completed a number of neuropsychological tasks encompassing a range of cognitive functions many of which are believed to engage the hippocampus. The battery included tasks of visuospatial and verbal memory: the object-relocation task (Kessels *et al.*, 1999), the Newcastle Spatial Memory Test, a computerized adaptation of a task previously described by other groups as “box task” (van Asselen *et al.*, 2005) or “executive golf” task (Feigenbaum *et al.*, 1996), the visual patterns test (Della Sala *et al.*, 1997), digit span, and the Rey Auditory Verbal Learning Test (Schmidt, 1996). Two additional tasks examined primarily executive function: the Stroop task (Golden and Freshwater, 2002) and the Trail Making Test (Tombaugh, 2004).

Spatial Memory fMRI task

For the fMRI scan participants performed a spatial memory task, which relied on spatial knowledge of a previously learned artificial environment which comprised a circular arena with seven spatial landmarks placed at equidistance on the walls. Prior to the scan session, participants were familiarized with this environment using a scale model. All participants were able to remember all landmark positions before entering the scanner.

During the scan, participants viewed computer renderings of the arena from a viewpoint above and slightly outside the arena (see Figure 1). Each trial consisted of three phases: encoding, delay and recall. In the encoding phase participants were shown the arena with a single pole marking the spatial location to be remembered. In the recall phase, participants were required to recall this location by making a forced choice between two marked locations. Crucially, participants were told that during the delay phase either the walls of the arena would rotate while their own position remained fixed or that their own position would rotate around the perimeter of the arena. Whereas the wall rotation forced

participants to rely on egocentric spatial relations to retrieve the target position, a change in their own position forced them to rely on allocentric spatial relations between the landmarks and the target. Since the rotations were not shown on the screen, participants were made aware of the rotation type via a verbal cue during the delay phase (“walls” for wall rotation or “you” for rotation of viewer location, see Figure 1). A third, control condition had identical trial structure and visuomotor demands but did not require participants to encode or recall spatial locations. Here, the empty arena was shown during the encoding phase and during the retrieval phase one of the two test locations was highlighted and participants simply had to press the button that corresponded to this highlighted location. Participants completed a total of 36 trials per condition (egocentric, allocentric, control) split into two runs. In addition, there were 36 baseline periods (9 seconds each) throughout the task, during which a fixation cross was presented. The length of each run was approximately 14 minutes.

[FIGURE 1 HERE]

Image acquisition and analysis

Scans took place on a 3T Achieva MR scanner (Philips Healthcare, Best, NL), using an 8-channel head coil as receiver. High-resolution T1 weighted anatomical images were acquired using a standard clinical 3D MPRAGE sequence (TR/TE=8.5/4.6ms, 320x320 matrix size, 225 slices, voxel size 0.8 mm isotropic). This was followed by two runs of the functional scan, using a gradient-echo echo-planar-imaging sequence (TE=30ms, TR=2600ms, flip angle=65°, voxel size 2.5mm x 2.5mm x 3.5mm, 40 axial slices, 325 volumes). Lastly, a set of flow-sensitive alternating inversion recovery (FAIR) ASL scans that used an improved inversion pulse (He and Blamire, 2010) were performed (TR=4000ms, TE=23ms, 4mm x 4mm

x 6mm voxel size, 64x64 matrix size, inversion time $TI=1700ms$, 40 tag-control pairs; additional inversion times were used for M_0 calculations), with four axial slices positioned along the length of the hippocampus.

Manual tracings of the hippocampus in both hemispheres were performed in Analyze 12.0 (Brain Imaging Resource, Mayo Clinic, MN) by an experienced tracer (JJM) who was blind to the status of the scans as patient or control. Tracings were performed on coronal slices and verified from axial and sagittal perspectives. The hippocampus was divided into an anterior region (including the body and head) and a posterior region (Maller *et al.*, 2007).

Hippocampal volumes were then calculated for the different subregions. To account for differences in head size, hippocampal volumes were divided by total intracranial volume which was determined from segmentation of the anatomical scan using SPM8 (Wellcome Centre for Neuroimaging, University College London, UK) in Matlab R2010b (The MathWorks Inc., Natick, MA, USA). Tracings also served as region-of-interest (ROI) definitions for the analysis of the fMRI and ASL scan data.

SPM8 was also used for the fMRI image analysis. Pre-processing included slice-time correction, realignment/unwarping and co-registration with the anatomical scan. First-level models were calculated in native space to allow for the extraction of contrast estimates from the individually traced hippocampus ROIs. They included two regressors for the encoding phase (encoding vs control), three regressors for the delay phase and three regressors for the recall phases (allocentric recall, egocentric recall, control). The regressors for egocentric and allocentric recall further included the trial specific reaction time as parametric modulator. All regressors were constructed as (short) boxcar functions of their respective event onsets and durations (3s encoding, 3.75s delay, 5s retrieval) convolved

with the canonical hemodynamic response function implemented in SPM8. Movement parameters were included as regressors of no interest. One patient had excessively moved during the fMRI scans (volume-to-volume motion exceeded half a voxel size) and was excluded from further fMRI data analysis.

The primary interest of the present paper was the difference between the allocentric and egocentric recall phase. Following the estimation of the first-level model, we therefore computed contrast images that subtracted the egocentric from the allocentric recall phase estimates. Average values for the hippocampus ROIs were then extracted from these Allocentric-versus-Egocentric (AvE) contrast images for statistical analysis. An additional whole-brain analysis for this contrast is reported in the supplementary material.

ASL images were first re-sliced to 2mm isotropic voxel size, then re-aligned to the first scan and manually co-registered with the anatomical scan using rigid-body transformations. All subsequent ASL processing was based on extracted signal levels from the hippocampus ROIs and from grey matter and white matter segments. The average tag-control signal level difference of the FAIR scan was used to calculate blood perfusion values according to a standard kinetic model (Buxton *et al.*, 1998). Additional parameters for these calculations (e.g. T1 of arterial blood) were based on published findings (Lu *et al.*, 2004, MacIntosh *et al.*, 2010, Roberts *et al.*, 1996).

Statistical analyses

Statistical analyses were performed in SPSS Statistics 21 (IBM, New York, NY, USA). Initial group comparisons of the AvE contrast, volumes and some neuropsychological measures were performed using two-tailed, independent sample t-tests, adjusted for unequal

variance if necessary. Neuropsychological measures that provided counts out of a fixed number of trials or items as a dependent measure were analyzed using Generalized Linear Models with a binary logistic, binomial distribution model. Because sex differences in CBF have previously been reported (Parkes *et al.*, 2004), any analysis involving regional CBF included sex as a covariate. Additional group comparisons included either (regional) hippocampal volumes (AvE contrast, neuropsychological measures) or regional CBF (AvE contrast) as covariates to eliminate their potential impact on the group differences.

Results

fMRI results

Table 2 shows the results of the extracted AvE contrast for each of the four hippocampus ROIs. There were significant group differences for the AvE contrast in the two anterior ROIs, with statistical trends in the same direction for the posterior regions (see Table 2). As shown in Figure 2, which shows BOLD signals of the two conditions relative to the control condition, this group difference appears to be driven by the control group. Compared to the control condition, controls showed reductions in BOLD signal in the allocentric condition in bilateral anterior regions (left: $p=.030$, right: $p=.001$) and right posterior ROI ($p=.006$), but not the left posterior ROI ($p=.467$), whereas no significant change from the control condition was observed in any ROI during the egocentric condition (all $p>.211$). The patient group did not show significant differences in either the egocentric or the allocentric condition relative to the control condition in any of the ROIs (all $p>.570$). A whole-brain analysis across both groups showed widespread BOLD signal differences in the AvE contrast, but no significant group differences (see supplementary material). In the control group, clusters of negative

AvE contrast can be found in bilateral anterior hippocampus at an uncorrected level of $p < .001$ (see supplementary material).

[TABLE 2 HERE]

[FIGURE 2 HERE]

To investigate if the group differences are specific to the hippocampus regions, we extracted contrast estimates from three additional ROIs that also showed negative BOLD signal during the allocentric condition and a negative AvE contrast (at least $p < 0.001$, uncorrected, in whole-brain analysis). These included a cluster in the posterior cingulate cortex (MNI: -8, -52, 30; 137 voxels), a cluster in the rostral anterior cingulate cortex (MNI: -6, 38, -6; 206 voxels) and a cluster in the medial prefrontal cortex (MNI: -2, 64, 10; 211 voxels). Comparing the AvE contrast in these regions between patients and controls showed no significant differences (all $p > 0.385$).

Behavioral performance

Test results of the neuropsychological tasks are shown in Table 3. Significant differences between the groups were seen in the majority of the tasks measuring verbal and spatial memory. In contrast to our earlier study in young healthy controls, in which performance in the egocentric condition of the fMRI task was near perfect (96% correct), 3 patients and 5 controls had near or sub-random task performance in this condition in the present study (less than 23 of 36 trials correct). This likely indicates non-compliance with or a misunderstanding of the instructions for this particular condition. We therefore excluded these participants from the analysis of behavioral performance of the fMRI task. As this may have also influenced the main fMRI findings, we repeated the above ROI analysis with these

participants excluded. This did not fundamentally alter the results presented above (see supplementary information). There were no significant group differences in the behavioral performance of the spatial memory fMRI task, but the otherwise expected task effects (see supplementary material for detailed results).

[TABLE 3 HERE]

Hippocampal volumes

The comparison of normalized hippocampal volumes showed no significant differences between patients and controls for any of the individual ROIs or for any combined volume (all $p > .274$). Detailed results are given in the supplementary material.

ASL results

Group had no significant effect on CBF in any of the hippocampal regions (all $p > 0.287$) or for the overall grey and white matter segments of the imaged slab. There were significant main effects of sex in all but one region (left posterior, $p = .105$), with females showing higher CBF than males. See supplementary material for detailed ASL results.

Controlling for volume or CBF

Including the respective regional volume as a covariate in the group comparisons of the AvE contrast did not alter the earlier findings strongly. The significant group effect in the two anterior ROIs (left: $p = .029$, right: $p = .023$) and the statistical trend in the right posterior ROI ($p = .096$) remained. Furthermore, the previous trend-level group difference in the left posterior ROI was no statistically significant ($p = .038$). Controlling for regional CBF and sex had little influence on the earlier AvE group effects although their p-values were generally

slightly increased (left anterior: $p=.048$, right anterior: $p=.074$, left posterior: $p=.138$, right posterior: $p=.083$). Previously significant group differences in the neuropsychological tasks remained significant when covarying for differences in the normalized volume of the entire hippocampus.

Discussion

The present study investigated hippocampal function and its relationship to hippocampal structure in major depression using multimodal MR imaging and neuropsychological testing. Our ROI analysis of the BOLD signal during the fMRI task showed that the contrast between allocentric and egocentric recall conditions was significantly different between the two groups in bilateral anterior hippocampus: Healthy controls showed a reduced BOLD signal during the allocentric condition relative to the egocentric condition. In contrast, in patients there was no reduction in BOLD signal in the allocentric compared to the egocentric condition in any of the ROIs, which was also not different from the control condition.

The reduction in BOLD signal in the allocentric condition relative to the egocentric condition in the control group is consistent with our previous findings in healthy young participants (Nilsson *et al.*, 2013). This demonstrates that the level of hippocampal engagement differs between these two conditions. This BOLD signal decrease may signify a task-related reduction in hippocampal neuronal activity in response to the allocentric condition, though we have previously discussed alternative interpretations (Nilsson *et al.*, 2013). Thus, whilst this negative-going BOLD signal is difficult to interpret, it is nonetheless evidence that the different task conditions result in differential activity in the anterior hippocampus in control subjects.

In contrast, depressed patients showed no change in BOLD signal from the control condition in either the allocentric or egocentric condition in any of the hippocampal regions.

Accordingly, this finding can be described as a lack of modulation of hippocampal activity in response to task demands in the patient group. Similar reduced modulations of hippocampal BOLD in response to task demands have been observed in previous studies in depression although they are often described as reduced increases in activity in the patient group (Bremner *et al.*, 2004, Fairhall *et al.*, 2010, Milne *et al.*, 2012). Whilst the relative nature of the BOLD signal prevents any conclusions regarding the absolute levels of neuronal activity, the current results demonstrate that the systematic, task-related differences in underlying hippocampal activity in healthy controls are absent in depressed patients.

To test the specificity of this finding to the hippocampus we investigated three other ROIs that also showed strong negative BOLD signal in the allocentric condition, both compared to control and the egocentric condition. These regions are frequently associated with the so-called default mode network (Raichle and Snyder, 2007), which shows reductions in BOLD signal during a wide variety of tasks and has been argued to be dysfunctional in depression (Dutta *et al.*, 2014). Analyses of these additional ROIs did not indicate a difference between patients and controls in the amount of reduction of BOLD signal during allocentric recall.

The observed group effect in the hippocampus therefore does not appear to be the reflection of a more generic alteration of default-mode network activity in MDD.

Furthermore, analyses that included hippocampal volumes or resting blood flow as a covariate did not fundamentally alter the results, indicating that the differential pattern of

BOLD signal in the patient group cannot be explained by volumetric differences or by alterations in hippocampal blood flow.

Beyond the hippocampus, the fMRI task recruits a large network of brain regions, including large parts of the parietal lobe, all of which contribute to the task (Nilsson *et al.*, 2013). At the whole brain level we did not observe any significant group differences in BOLD signal. While this could be due to the lower sensitivity of a whole-brain analysis, it suggests that patients did not use fundamentally different brain regions to perform the task. In line with such an argument, there were also no significant behavioral differences between the groups in any of the conditions of the fMRI task, but both groups showed large differences between the two conditions and (expected) increases in error rate and response time with rotation angle in the allocentric but not the egocentric condition. Thus, the observed group difference in hippocampal BOLD did not translate into differential performance in the task. This also suggests that group differences in BOLD signal were not due to other task-related factors (e.g. motivation, perceived difficulty). It is worth noting that the fMRI task was developed primarily to investigate differences in hippocampal function and not to detect behavioral differences.

In support of the limited sensitivity of the task for detecting behavioral group differences in the scanner, the patient group demonstrated impaired performance on a number of spatial and verbal memory tasks performed outside the scanner, which is consistent with previous research (Hinkelmann *et al.*, 2009, Ravnkilde *et al.*, 2002). One explanation for this discrepancy could be that compared to the fMRI task, the offline spatial measures required participants to remember a greater number of locations (“object-location binding”, “position-only memory”) and to remember locations over longer time periods (“between-

search errors"). It is conceivable that the higher cognitive load in the offline spatial memory measures resulted in an increased sensitivity for detecting group differences. Furthermore, it should be emphasized that the present study is not the first to demonstrate an altered modulation of hippocampal activity in MDD in the absence of behavioral group differences (Bremner *et al.*, 2004, Fairhall *et al.*, 2010).

Contrary to previous findings we did not observe decreases in hippocampal volume in the patient group. This is perhaps not surprising given that a recent meta-analysis on this topic has shown only small to medium effect sizes for hippocampal volume reductions in depression (effect size approx. -0.3) with significant heterogeneity across studies (Arnone *et al.*, 2012). It has also been suggested that pharmacological treatment may counteract hippocampal volume reductions in MDD (Arnone *et al.*, 2013). Since all but one patient of the current study were medicated this may have contributed to the fact that we did not find volume differences. Furthermore, it has been suggested that such reductions mostly occur in depressed patients who have been ill for some time or who have had multiple episodes (McKinnon *et al.*, 2009). While a number of our patients certainly fell into this category, there was considerable heterogeneity in terms of clinical characteristics and age in the present sample. This, combined with the relatively moderate sample size means that the power to detect small volume differences in the present study was likely insufficient.

It is also noteworthy that, similar to the previous neuroimaging studies of hippocampal function in depression (Bremner *et al.*, 2004, Fairhall *et al.*, 2010, Milne *et al.*, 2012), our group difference in BOLD signal was located in anterior regions of the hippocampus, whereas volumetric findings in depression tend to show differences in more posterior regions of the hippocampus (Cole *et al.*, 2010, Maller *et al.*, 2007, Maller *et al.*, 2012,

Neumeister *et al.*, 2005). It is possible that the different spatial resolutions of the anatomical and functional scans play a role in this apparent discrepancy. The narrow and elongated shape of the hippocampus may make the posterior part of the hippocampus more sensitive to detecting morphological changes in anatomical scans with high spatial resolution, whereas it would increase the influence of partial volume effects in lower resolution functional scans. If the same pathophysiological process that is responsible for the structural changes seen in other studies was also responsible for the functional changes seen in the current study, it may mean that functional changes can precede changes in structure, which is why we were able to detect the former but not the latter. Alternatively, it is possible that independent pathological processes are at work, with some responsible for volume changes in posterior parts of the hippocampus, while others are responsible for functional changes in anterior hippocampus. To reduce the potential influence of partial volume effects, replications with higher-resolution functional scans would be necessary. Such research should also involve a functional task that produces task-related BOLD changes in the posterior hippocampus.

The ASL based measure of hippocampal resting blood flow did not show differences between the two groups. In contrast, sex had a significant effect on CBF in nearly all investigated regions. Such differences between males and females have consistently been reported using both MR based (Liu *et al.*, 2012, Parkes *et al.*, 2004) and PET methodologies (Henriksen *et al.*, 2013). This suggests that our ASL methodology was sensitive enough to detect some group differences, but if any differences in CBF between our patient and control group existed, they were too small to be detected in our sample. The previous study showing hippocampal hyperperfusion in depression using PET (Videbech *et al.*, 2002)

studied a patient sample that was considerably different from the present study. Whereas patients in the PET study were all inpatients and described as “rather acutely depressed” (p. 38), our sample consisted of outpatients that tended to have been ill for at least some time. The PET study also showed that the differences were more pronounced in patients who were either un-medicated or had only received medication for less than 1 week (Videbech *et al.*, 2002). A more recent PET study that found increased resting blood flow in the left parahippocampal gyrus in a whole-brain analysis also only studied un-medicated patients (Monkul *et al.*, 2012). In our sample, apart from one un-medicated patient, all patients had been on stable medication for at least four weeks prior to the scan. It can be speculated that blood flow changes in the hippocampus are restricted to rather short periods at the beginning of an acute depressive episode and are reversed by medication. In support of this, a large study using ASL to measure CBF in acutely depressed patients found hippocampal hyperperfusion only in a group of non-treatment-refractory patients, both compared to treatment-refractory patients and to controls (Lui *et al.*, 2009). It was outside the scope of our study to determine any treatment response of our patients, thus our sample is likely a mix of treatment-refractory and non-refractory patients.

In summary, we have presented evidence for reduced modulation of hippocampal activity during a spatial memory task in depressed patients relative to healthy controls. This reduced modulation could not be accounted for by differences in hippocampal structure or resting cerebral blood flow in the same region, suggesting that volumetric or baseline hemodynamic differences are not underlying the functional differences. Whether or not these differences in hippocampal function are reflective of an early degenerative process that has yet to result in observable volumetric reductions can only be answered with

longitudinal investigations, which could also answer the question if and when such functional abnormalities may be reliably linked to behavioral impairments.

Final accepted author version

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Final accepted author version

Acknowledgements

We graciously acknowledge the help of the staff of the Newcastle Magnetic Resonance Centre, Newcastle University, in conducting this research.

Financial support

A.F. is supported by Research Capability Funding from the Northumberland-Tyne-and-Wear NHS Foundation Trust awarded to R.H.M.-W.

Conflict of interest

None of the authors report biomedical financial interests or potential conflicts of interest that are relevant to the subject matter of this manuscript.

Ethical standards

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.

Tables

Table 1: Participant characteristics

Table 2: Hippocampus-ROI fMRI results

Table 3: Results of neuropsychological testing

Final accepted author version

Figure legends

Figure 1: Task design and example trials for the allocentric and egocentric condition. Not shown is that the verbal cue during the delay was displayed on top of a scrambled version of the scene. In both examples the orange/darker dot marks the correct location and the white dot is the distractor. During “control” trials, no pole was shown at encoding and during recall one of the two colored dots was marked with a pole.

Figure 2: BOLD signal beta coefficients of the four hippocampus ROIs for patients and control participants for the allocentric (Allo) and egocentric (Ego) retrieval phase.

Figure 1

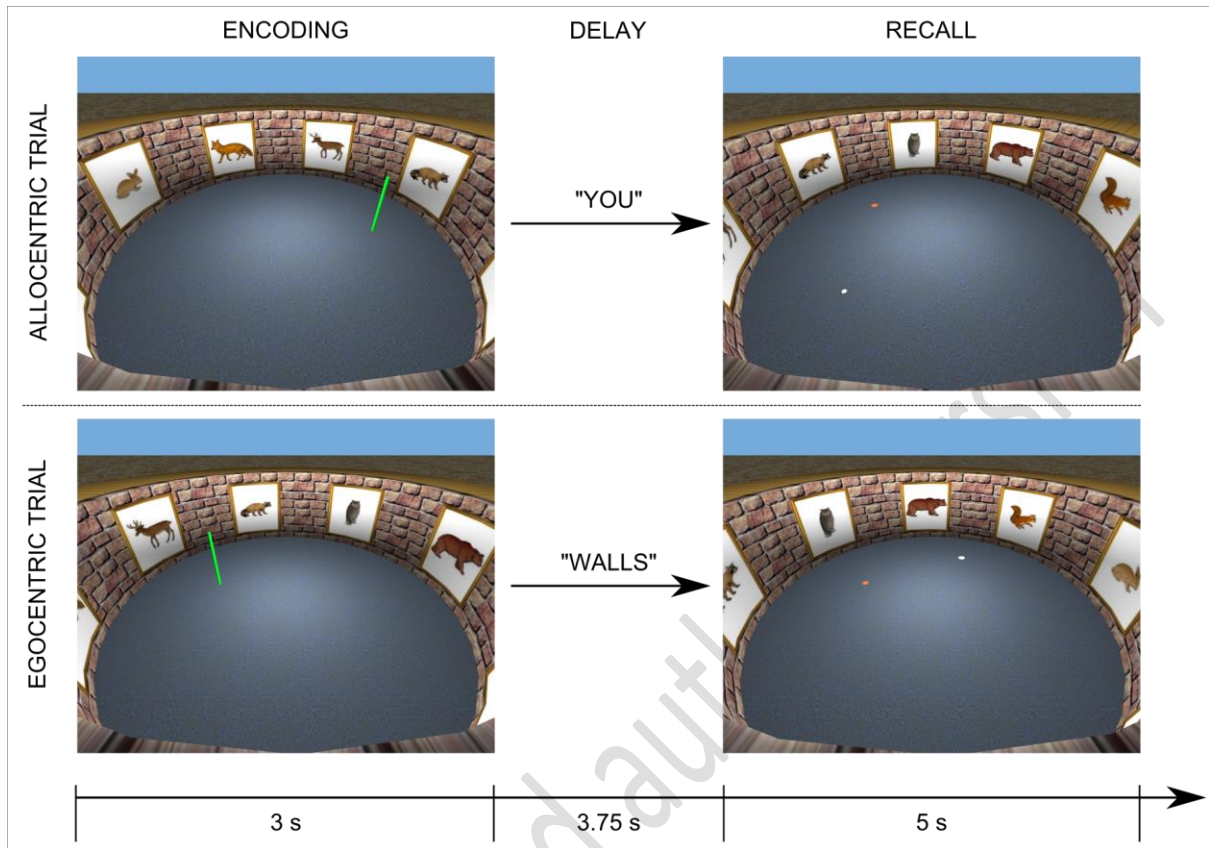


Figure 2

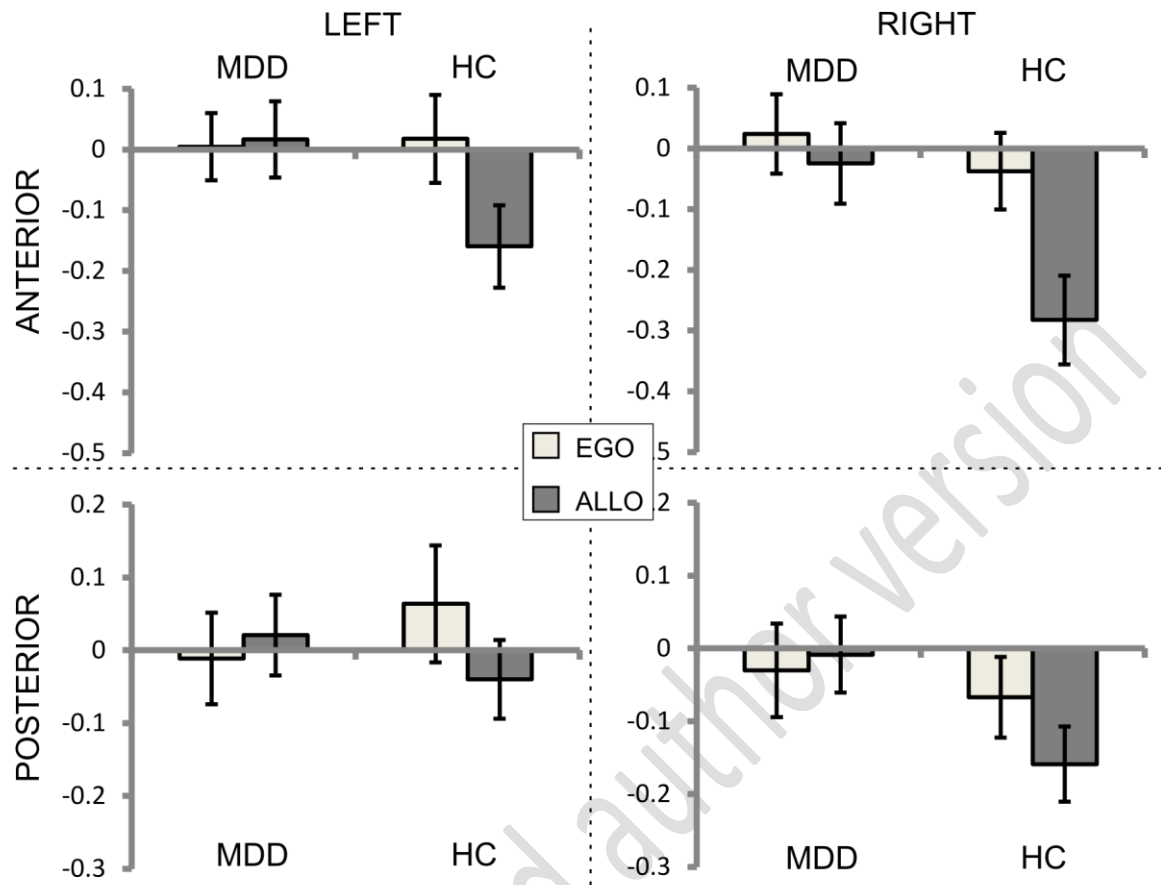


Table 1: Participant characteristics

	Patients	Controls	group difference
Sex (f/m)	10/10	12/8	chi ² =0.404 p=.525
Age (years)	45.3 (11.9)	42.6 (12.1)	t=0.725 p=.473
BDI	37.8 (11.6)	1.5 (2.3)	t=13.69 p<.001
State anxiety	47.2 (13.0)	30.2 (10.8)	t=4.450 p<.001
Trait anxiety	57.5 (13.7)	30.6 (10.0)	t=7.000 p<.001
verbal IQ	113.6 (11.4)	115.4 (6.4)	t=0.617 p=.541
HAM-D	23.5 (5.4)	0.15 (0.67)	t=19.24, p<.001
Age of onset (years)	28.3 (15.0)		
Illness duration (years)	10.4 (9.4)		
Number of episodes			
mean (standard deviation)	3.87 (3.49)		
median	3		
min/max	1/11		
previous hospitalizations (yes/no)	3/17		
Medication	none: 1 SSRI: 5 SSRI + other* : 2 SNRI: 2 SNRI + other* : 6 atypical AD: 1 TCA: 1 other: 2		

Means and standard deviations (in parentheses) of participant characteristics. SSRI=Selective Serotonin Reuptake Inhibitors, SNRI=Serotonin and Noradrenalin Reuptake Inhibitors, AD=Atypical antidepressants, TCA=Tricyclic antidepressants. *Other included Pregabalin, Lithium, Aripiprazole, Quetiapine.

Table 2: Region-of-interest fMRI results of AvE contrast

Hippocampus ROI	Patients (n=19)	Controls (n=20)	Condition effect	Group difference
Left anterior	0.016 (0.189)	-0.177 (0.312)	t=-1.893, p=.066	t=-2.325, p=.025
Left posterior	0.031 (0.251)	-0.104 (0.200)	t=-1.018, p=.315	t=-1.853, p=.072
Right anterior	-0.050 (0.257)	-0.245 (0.301)	t=-3.180, p=.003	t=-2.170, p=.036
Right posterior	0.024 (0.239)	-0.032 (0.177)	t=-1.019, p=.314	t=-1.728, p=.092

Means (and standard deviation) of the Allocentric-vs-Egocentric contrast of the retrieval phase. The condition effect column shows the result of a one-sample t-test of this contrast against 0 (across both groups). The group difference column shows the result of an independent-sample t-test for the difference between patients and healthy controls. This t-test did not assume equal variances. Bolded figures represent significant ($p < .05$) results.

Table 3: Results of neuropsychological testing

	Patients	Controls	group difference	Effect size (R ²)
Digit span				
forward span	6.35 (1.31)	7.40 (1.19)	t=2.657, p=.011*	0.157
backward span	4.85 (1.39)	5.50 (1.43)	t=1.458, p=.129	0.053
forward correct trials	7.80 (2.33)	9.75 (2.26)	$\chi^2=11.51$, p=.001*	0.253
backward correct trials	6.45 (2.16)	7.65 (2.41)	$\chi^2=4.104$, p=.043	0.098
Rey auditory verbal-learning test				
A1 (items correct)	5.95 (1.84)	6.60 (2.06)	$\chi^2=1.136$, p=.287	0.028
A5 (items correct)	10.7 (3.50)	12.8 (1.99)	$\chi^2=15.98$, p<.001*	0.340
A1 to A5 (items correct)	45.8 (12.1)	52.0 (9.51)	$\chi^2=21.67$, p<.001*	0.420
B (items correct)	5.63 (2.11)	6.00 (1.86)	$\chi^2=0.371$, p=.542	0.009
A6 (items correct)	9.68 (3.56)	11.6 (2.54)	$\chi^2=10.84$, p=.001*	0.241
A7 (items correct)	8.33 (4.13)	11.2 (3.17)	$\chi^2=20.66$, p<.001*	0.409
Recognition A (number of hits)	11.8 (2.52)	13.5 (1.87)	$\chi^2=12.32$, p<.001*	0.278
Recognition B (number of hits)	7.58 (3.44)	9.42 (2.09)	$\chi^2=8.704$, p=.003*	0.197
Spatial working memory				
2D between-search errors	228.4 (90.3)	168.0 (100.0)	t=2.005, p=.052	0.096
3D between-search errors	249.9 (114.1)	156.5 (103.5)	t=2.519, p=.016*	0.143
Object relocation task				
position-only memory (error)	207.3 (48.5)	169.2 (33.8)	t=2.882, p=.006*	0.179
object-location binding (#correct)	12.5 (4.26)	14.4 (3.86)	$\chi^2=8.121$, p=.004*	0.184
combined process (error)	386.1 (135.2)	342.0 (132.2)	t=1.043, p=.304	0.028
Visual patterns test				
maximum pattern size	9.05 (2.37)	9.80 (1.85)	t=1.114, p=.273	0.032
Stroop task				
word reading (seconds)	54.5 (17.5)	46.1 (7.9)	t=1.941, p=.063	0.090

color naming (seconds)	77.3 (21.0)	66.3 (11.7)	t=2.040, p=.050	0.099
incongruent words (seconds)	140.1 (45.2)	115.5 (38.6)	t=1.851, p=.072	0.083
Trail making test				
A (seconds)	35.4 (9.51)	27.6 (8.69)	t=2.696, p=.010*	0.161
B (seconds)	68.9 (26.6)	51.8 (17.0)	t=2.415, p=.021*	0.133
B-A (seconds)	33.4 (22.3)	24.2 (14.1)	t=1.572, p=.124	0.061

Means (and standard deviations) of neuropsychological task measures. Group differences were calculated with independent-samples t-tests, adjusted for unequal variance if necessary, or with Wald- χ^2 tests as part of a generalized linear model with logit link function if the measure represented a count and was limited by the task. Effect sizes represent coefficients of determination of ordinary-least-squares general linear models and their equivalent based on maximum likelihood for generalized linear models. *Denotes significant effects following correction for multiple-comparison using a false-discovery-rate of 5%.

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**Altered hippocampal function in major depression
despite intact structure and resting perfusion**

Supplementary Material

Contents

Control condition of spatial memory task	2
fMRI task behavioral results	2
Whole-brain fMRI analysis	4
Hippocampal volumes and blood flow results.....	7
fMRI ROI analysis in reduced sample.....	8

Control condition of spatial memory task

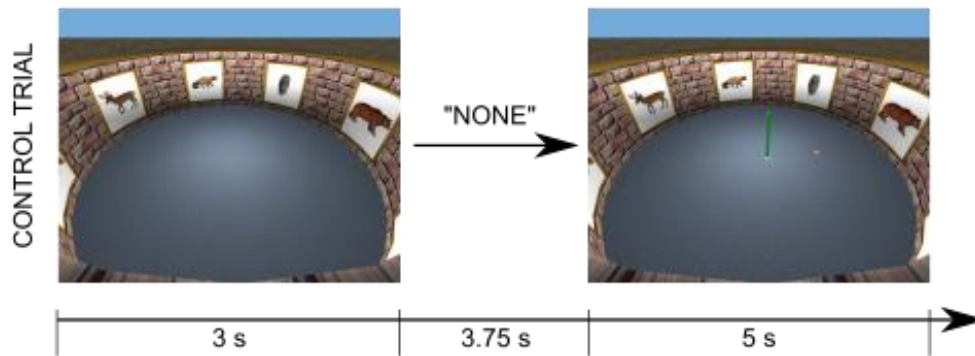


Figure S1: Trial structure of the control condition of the fMRI task. Only the empty arena with its landmarks was shown during encoding. During retrieval participants were instructed to press the button that corresponded to the disc that was indicated by the green pole marker.

fMRI task behavioral results

Table S1: Behavioral findings of fMRI task

Source	SS	df	MS	F	p
Errors					
<i>Within-subject effects</i>					
Condition (egocentric, allocentric)	1.395	1	1.395	97.004	<.001
Condition x group	0.027	1	0.027	1.860	.183
Error (condition)	.417	29	0.014		
Rotation angle (45, 90, 135 degrees)	0.519	2	0.259	22.388	<.001
Angle x group	0.000	2	0.000	0.004	.996
Error (angle)	0.672	58	0.012		
Condition x angle	0.379	2	0.189	13.296	<.001
Condition x angle x group	0.067	2	0.034	2.354	.104
Error (condition x angle)	0.826	58	0.014		
<i>Between-subject effects</i>					
Group	0.115	1	0.115	1.477	.234
Between	2.266	29	0.078		
Response Time					
<i>Within-subject effects</i>					
Condition (egocentric, allocentric)	34.702	1	34.702	63.550	<.001
Condition x Group	0.663	1	0.663	1.212	.280
Error (condition)	15.836	29	0.546		
Rotation angle (45, 90, 135 degrees)	2.339	2	1.169	16.705	<.001
Angle x group	0.211	2	0.105	1.506	.230
Error (angle)	4.060	58	0.070		
Condition x angle	0.572	2	0.286	3.690	0.031

Condition x angle x group	0.378	2	0.189	2.438	0.096
Error (condition x angle)	4.499	58	0.078		
<i>Between- subject effects</i>					
Group	0.977	1	0.977	0.779	.385
Between	36.378	29	1.254		

SS=Sum of square, df=degrees of freedom, MS=mean square; Results with p<.05 in bold.

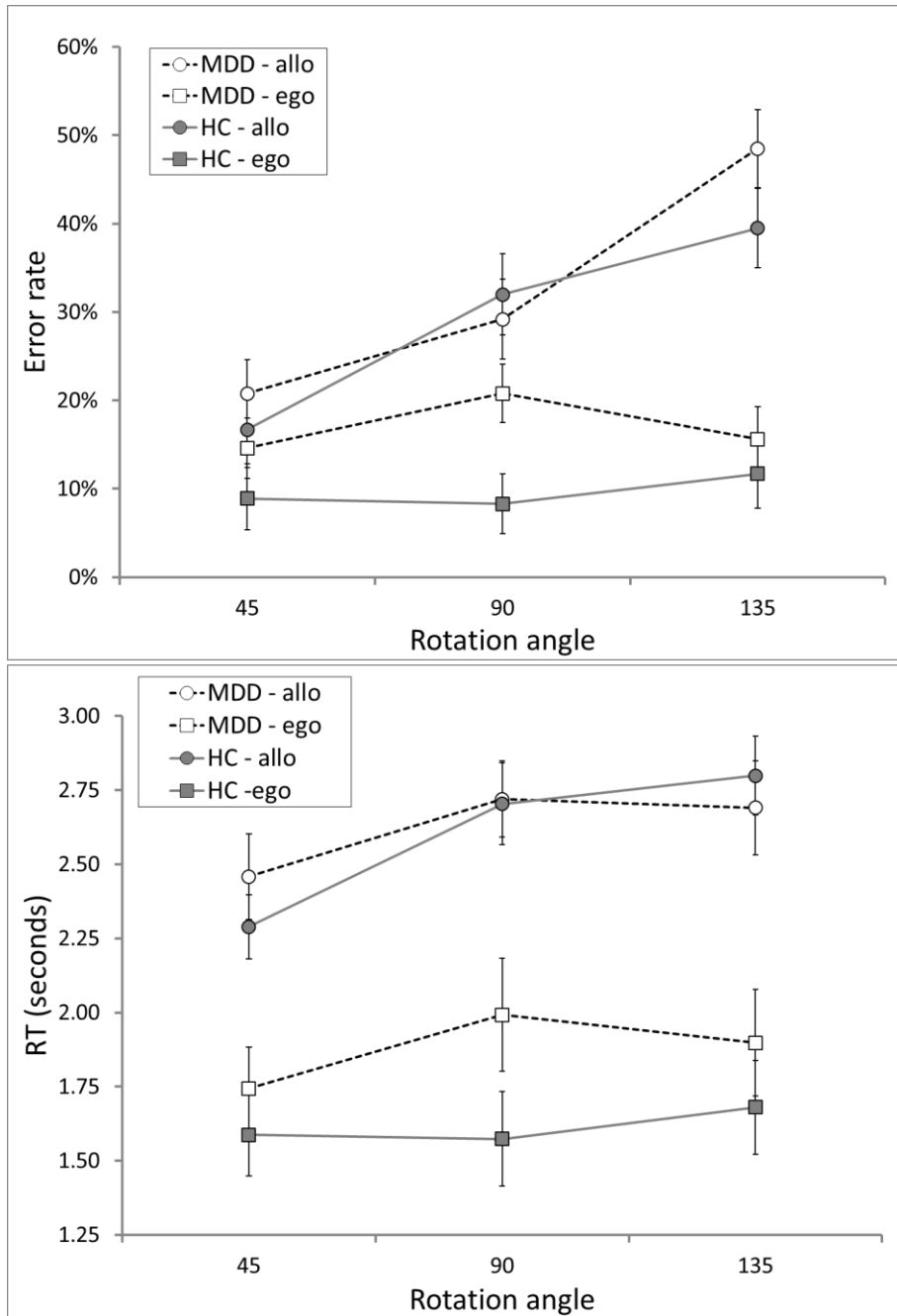


Figure S1: Behavioral findings of fMRI task (as presented in ANOVA results, table S1). Mean error rates (top) and mean reaction times (bottom). Error bars are +/- 1 standard error of mean.

Whole-brain fMRI analysis

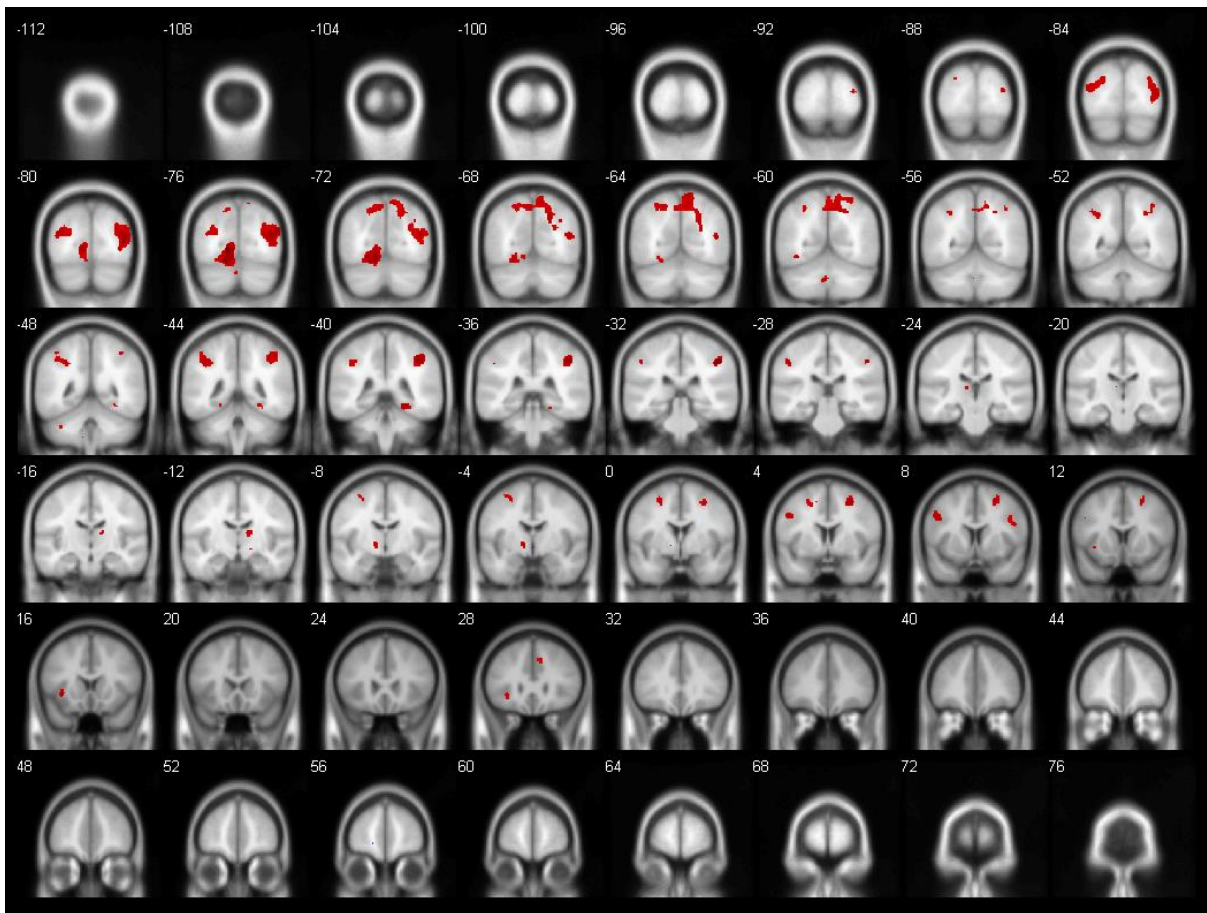


Figure S3: Results of whole-brain analysis of AvE contrast across the entire sample ($p < .05$, FWE-corrected). Areas in red show significant BOLD signal differences in the positive direction (i.e. allocentric condition > egocentric condition), whereas the area in blue show significant differences in the negative direction (allocentric < egocentric).

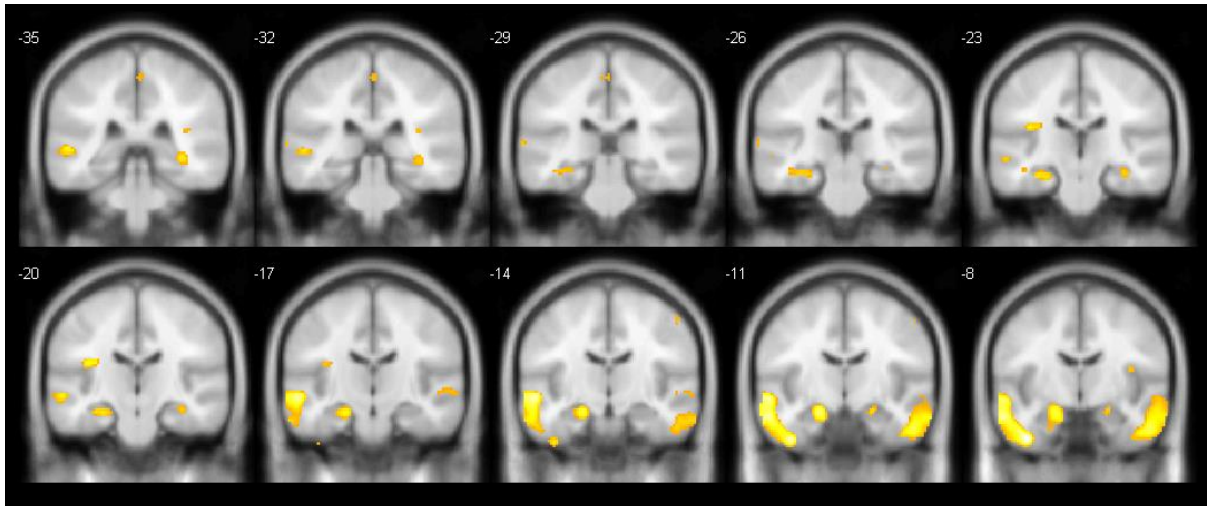


Figure S4: Pattern of findings from the whole-brain analysis of negative AvE contrast (Egocentric > Allocentric) in the healthy control group only ($p < .005$, uncorrected) in the hippocampal region. No hippocampal differences were seen at this level in patients.

Table S2: Whole-brain fMRI results

cluster size (voxels)	Peak location	Peak MNI coordinates (mm)	Peak T	Peak p-value (FWE)
positive AvE contrast (Allocentric > Egocentric)*				
609	L Lingual Gyrus	-12, -76, 0	7.207	<0.001
	L Lingual Gyrus	-12, -72, -10	6.860	0.001
2611	R Supramarginal Gyrus	46, -32, 42	7.119	<0.001
	R Inferior Parietal Lobule	40, -40, 46	6.595	0.002
	R Middle Occipital Gyrus	40, -78, 16	6.513	0.003
67	R Fusiform Gyrus	24, -40, -12	6.640	0.002
143	R Superior Frontal Gyrus	26, 4, 52	6.587	0.002
312	L Middle Occipital Gyrus	-38, -80, 20	6.374	0.004
	L Middle Occipital Gyrus	-28, -76, 20	5.880	0.016
	L Superior Occipital Gyrus	-24, -86, 28	5.832	0.018
91	L Precentral Gyrus	-48, 8, 34	6.372	0.004
29	L Insula	-34, 14, -2	6.293	0.005
45	L Globus Pallidus	-12, -4, 0	6.258	0.005
40	L Inferior Parietal Lobule	-50, -28, 42	6.226	0.006
93	L Mid Frontal Gyrus	-26, 0, 54	6.052	0.010
	L Precentral Gyrus	-30, -6, 58	5.751	0.022
	L Superior Frontal Gyrus	-14, 4, 52	5.614	0.031
40	R Thalamus	14, -12, 14	6.037	0.011
27	L Cerebellum Vermis	-4, -60, -36	5.959	0.013
38	R Inferior Frontal Operculum	48, 8, 24	5.756	0.022
	R Inferior Frontal Operculum	42, 8, 32	5.601	0.033
negative AvE contrast (Egocentric > Allocentric)* (no significant clusters)				
AvE contrast, controls > patients[†] (no significant clusters)				
AvE contrast, patients > controls[†] (no significant clusters)				

Results of whole-brain fMRI analysis *using $p < .05$ (FWE) at voxel level and extend-threshold of 20, [†] using $p < .001$ (uncorrected) and FWE-correction at cluster level. Only the three largest local peaks of clusters are reported. L=Left, R=Right.

Hippocampal volumes and blood flow results

Table S3: Hippocampal Volumes

	Patients		Controls		Controls vs Patients (%TICV, df=38)
	ml	%TICV	ml	%TICV	
L anterior ROI	3.154 (0.090)	0.2230 (0.0051)	2.947 (0.082)	0.2158 (0.0051)	t=-1.001, p=.323
L posterior ROI	0.371 (0.019)	0.0263 (0.0013)	0.347 (0.024)	0.0254 (0.0018)	t=-0.399, p=.692
R anterior ROI	2.873 (0.089)	0.2034 (0.0058)	2.715 (0.084)	0.1983 (0.0043)	t=-0.695, p=.492
R posterior ROI	0.375 (0.025)	0.0265 (0.0017)	0.373 (0.026)	0.0273 (0.0019)	t=0.333, p=.741
L combined	3.525 (0.042)	0.2493 (0.0235)	3.942 (0.038)	0.2412 (0.0225)	t=-1.110, p=.274
R combined	3.247 (0.043)	0.2299 (0.0275)	3.088 (0.036)	0.2257 (0.0161)	t=-0.593, p=.557
Total	6.772 (0.082)	0.4791 (0.0490)	6.382 (0.072)	0.4669 (0.0363)	t=-0.900, p=.374

Mean (standard deviations) hippocampal volumes in ml and as percentage of total intracranial volume (TICV). R=right, L=left, ROI=region of interest

Table S4: Regional cerebral blood flow results

region	Depressed (n=20)		Control (n=20)		Significance	
	males	females	males	females	group	sex
L anterior hippocampus	34.90 (8.20)	46.46 (13.3)	40.19 (12.2)	47.95 (11.1)	F=0.81, p=.360	F=7.18, p=.011
L posterior hippocampus	44.89 (17.8)	53.15 (14.7)	44.91 (13.2)	54.08 (15.3)	F=0.01, p=.929	F=2.77, p=.105
R anterior hippocampus	34.07 (9.91)	49.49 (14.7)	41.79 (13.4)	46.17 (10.3)	F=0.21, p=.649	F=6.29, p=.017
R posterior hippocampus	35.53 (11.2)	60.20 (17.5)	47.11 (16.3)	48.42 (17.9)	F=0.01, p=.985	F=5.13, p=.030
GM	28.94 (6.83)	45.17 (9.83)	34.59 (6.92)	42.65 (9.82)	F=0.17, p=.574	F=19.04, p<.001
WM	17.96 (4.50)	26.91 (7.45)	22.46 (4.22)	26.88 (6.09)	F=1.16, p=.287	F=12.99, p=.001

Means (and standard deviation) of regional CBF in ml/100g/min of the different brain regions. The last two columns show p-values for ANOVAs with group and sex as fixed factors. L=left, R=right, GM=gray matter, WM=white matter

fMRI ROI analysis in reduced sample

Table S5: Region-of-interest fMRI results of AvE contrast

Hippocampus ROI	Patients (n=16)	Controls (n=15)	Condition effect	Group difference
Left anterior	-0.017 (0.177)	-0.243 (0.335)	t=-2.46, p=.020	t=-2.316, p=.031
Left posterior	-0.008 (0.252)	-0.094 (0.218)	t=-1.17, p=.252	t=-1.016, p=.318
Right anterior	-0.069 (0.268)	-0.310 (0.313)	t=-3.23, p=.002	t=-2.291, p=.030
Right posterior	-0.001 (0.249)	-0.077 (0.190)	t=-0.92, p=.364	t=-0.992, p=.330

Means (and standard deviation) of the Allocentric-vs-Egocentric contrast of the retrieval phase. The condition effect column shows the result of a one-sample t-test of this contrast against 0 (across both groups). The group difference column shows the result of an independent-sample t-test for the difference between patients and healthy controls. This t-test did not assume equal variances. Bolded figures represent significant ($p < .05$) results.

Table S6: Participant characteristics (reduced sample)

	Patients	Controls	group difference
Sex (f/m)	7/9	7/8	$\chi^2=0.027$ p=.870
Age (years)	44.0 (12.9)	40.8 (12.0)	t=0.713 p=.481
BDI	38.6 (11.4)	1.6 (2.6)	t=12.32 p<.001
State anxiety	47.6 (13.8)	28.6 (9.3)	t=4.449 p<.001
Trait anxiety	59.0 (12.7)	29.9 (9.2)	t=7.188 p<.001
verbal IQ	112.3 (11.9)	114.1 (5.7)	t=0.557 p=.582
HAM-D	23.3 (5.4)	0.0 (0.0)	t=16.59, p<.001