Evidence for a Virtual Human Analog of a Rodent Relational Memory Task: A Study of Aging and fMRI in Young Adults

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ABSTRACT: A radial maze concurrent spatial discrimination learning paradigm consisting of two stages was previously designed to assess the flexibility property of relational memory in mice, as a model of human declarative memory. Aged mice and young adult mice with damage to the hippocampus, learned accurately Stage 1 of the task which required them to learn a constant reward location in a specific set of arms (i.e., learning phase). In contrast, they were impaired relative to healthy young adult mice in a second stage when faced with rearrangements of the same arms (i.e., flexibility probes). This mnemonic inflexibility in Stage 2 is thought to derive from insufficient relational processing by the hippocampus during initial learning (Stage 1) which favors stimulus-response learning, a form of procedural learning. This was proposed as a model of the selective declarative and relational memory decline classically described in elderly people. As a first step to examine the validity of this model, we adapted this protocol to humans using a virtual radial-maze. (1) We showed that performance in the flexibility probes in young and older adults positively correlated with performance in a wayfinding task, suggesting that our paradigm assesses relational memory. (2) We demonstrated that older healthy participants displayed a deficit in the performance of the flexibility probes (Stage 2), similar to the one previously seen in aged mice. This was associated with a decline in the wayfinding task. (3) Our fMRI data in young adults confirmed that hippocampal activation during early discrimination learning in Stage 1 correlated with memory flexibility in Stage 2, whereas caudate nucleus activation in Stage 1 negatively correlated with subsequent flexibility. By enabling relational memory assessment in mice and humans, our radial-maze paradigm provides a valuable tool for translational research. © **2011 Wiley-Liss, Inc.**

KEY WORDS: relational representation; mnemonic flexibility; virtual radial-maze; basal ganglia; hippocampus

INTRODUCTION

Conscious memory of facts and events, i.e., declarative memory is affected by aging (Grady and Craik, 2000) as well as neuropsychiatric and neurodevelopmental disorders, such as schizophrenia (Boyer et al., 2007) and Alzheimer's disease (Gabrieli, 1996). Translational research attempts to develop reliable animal models of these neural alterations to study their underlying defective molecular events and to develop clinical therapies. This approach requires an interface between non-human animal experimentation and human studies with equivalent procedures to assess memory in humans and animals.

On the basis of the relational theory, a two-stage radial-maze concurrent spatial discrimination learning paradigm was initially developed to assess relational memory flexibility in mice (Marighetto et al., 1999). According to the relational memory theory (Eichenbaum, 2004), this flexibility relies on a relational representation mediated by the hippocampus and can be exemplified in the ability to compare and contrast information acquired from separate sources to guide inferential decision in novel situations (Cohen and Eichenbaum, 1993; Gross and Greene, 2007). In Stage 1, the mouse is presented with six pairs of arms on a radial-maze. One arm in each pair is always rewarded and the same pairs of arms are presented throughout the first stage of the task. In Stage 2, novel recombined pairs of arms are created and presented to assess memory flexibility. The ability to find the reward location when presented with recombined pairs requires choosing between separately acquired pieces of information, and is thought to rely on a relational representation of separate pair-experiences made during Stage 1.

It has been repeatedly observed that, despite having mastered the initial discrimination task (Stage 1), aged mice (Marighetto et al., 1999; Marighetto et al., 2000; Etchamendy et al., 2001; Touzani et al., 2003; Mingaud et al., 2008) and young adult mice with hippocampal lesions (Etchamendy et al., 2003) fail the flexibility probes in Stage 2. We further demonstrated that, although not essential for initial learning, spontaneous hippocampal activation during Stage 1 was crucial for memory flexibility in Stage 2 (Mingaud et al., 2007; Mingaud et al., 2008). Learning under temporary inactivation of the hippocampus in Stage 1 was associated with an over-activation of the dorso-lateral striatum, which is a critical memory structure for stimulus-response learning, a type of procedural learning (Packard et al., 1989; McDonald and

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White, 1994; Mingaud et al., 2007). Our radial-maze paradigm in mice enabled us to dissociate two forms of memory expression relying on distinct brain circuits. This dissociation has been proposed as a model of the classical human distinction between declarative memory and procedural memory systems.

In this study, we examined the validity of this mouse model in humans using virtual reality to create a parallel radial-maze protocol. First, healthy young and older participants were tested on our radial-maze relational task to determine if the presumed decrease in hippocampal function associated with normal aging impairs scores on flexibility probes (Grady and Craik, 2000). Second, performance of young and aged participants in the radial maze task were compared to their performance in the wayfinding task, where they were required to find the shortest route possible between two landmarks in a virtual town. The wayfinding task is an established spatial memory task that requires the formation of a cognitive map and is dependent on the hippocampus (Hartley et al., 2003; Maguire et al., 1998). Finally, we assessed our model with fMRI in young adults to test for hippocampal and prefrontal involvement in Stage 1 and 2 in flexible learners and striatal involvement in less flexible learners. Indeed, it has been repeatedly shown that the prefrontal cortex plays a major role in forming and conserving relational memory (Blumenfeld and Ranganath, 2006; Repovs and Baddeley, 2006). Furthermore, to successfully capture hippocampal activity during spatial memory formation, a contrasting fMRI control task was designed and validated.

METHODS

Behavioral Experiments

Research participants

To study the effects of age on memory flexibility in our radial maze protocol, we recruited 43 healthy young adult participants (22 women and 21 men; mean age: 25.1 yrs, standard deviation (SD) ± 4.11 yrs) and 61 older adults (27 men and 34 women). The older adults were high-functioning, community dwelling individuals with a mean age of 66.9 yrs (SD ± 7.9 yrs). All older participants scored 27 and above on the MMSE (mean MMSE score of 28.6, SD ± 1.62). The young and older adult groups were given the virtual concurrent spatial discrimination learning task.

Out of these participants, 30 healthy young adults (18 women and 12 men; mean age: 26.53 yrs, SD ± 4 yrs) and 25 older adults (12 women and 13 men; mean age: 63 yrs, SD ± 5.3 yrs) were also tested in the wayfinding task. For these two subgroups of participants, the order of task administration (virtual concurrent spatial discrimination learning task vs. wayfinding task) was counterbalanced across participants.

All volunteers were screened for a history of psychiatric and neurological disorders with a medical questionnaire. Participants were also excluded based on conditions that could influence cognitive performance, such as diabetes, heart disease, hypertension, and drug and alcohol abuse. Informed written consent was obtained from all research participants and the study was approved by our institutional research ethics committee.

Virtual tasks

A commercially available computer game (Unreal Tournament 2003; Epic Games, Raleigh, NC) was used to create the virtual environments. The virtual tasks were administered to participants sitting at a computer desk with a 17″ monitor.

Concurrent spatial discrimination learning task

For the virtual concurrent spatial discrimination learning task, participants navigated through a virtual environment that contained a 12-arm radial-maze with a central starting location. The maze was surrounded by an enriched landscape with mountains, trees, a desert, an oasis, etc. (Fig. 1). A large opaque sliding door located at the entrance of each arm controlled the access into the pair of arms that participants were to explore during a particular trial. At the end of each arm, there was a staircase leading to a pit where, in some of the arms, an object could be picked up. This discrimination task was a virtual adaptation of the task developed by Marighetto et al., (1999) for mice. It was comprised of two consecutive stages, which differed only in terms of the specific pairs of arms presented to the participant (Fig. 1).

Stage 1: learning phase. The 12 arms were combined into six invariant pairs of adjacent arms. Within each pair of arms (designated as pairs (1+2−), (3+4−), (5–6+), (7–8+), (9+10−), and (11+12−), as illustrated in Fig. 1), one arm (always the same one) contained an object at the bottom of the staircase (indicated here by a plus following the arm number) and the other never did (indicated by a minus following the arm number). Pairs (1/2), (3/4), (9/10), and (11/12) had an object on the left arm and pairs (5/6) and (7/8) had an object on the right. The relative locations of these rewards (unequal between right and left side) were such that all six pairs could be rearranged into a maximum number of pairs (four) of adjacent arms with opposing valence during Stage 2. The six pairs of arms were presented in turn to the participant according to a pseudorandom sequence and the participant was explicitly asked to progressively learn which arm contained an object within each pair. In Stage 1, a trial was defined as the presentation of all six pairs of arms.

Participants always started a trial from the center of the radial-maze, in front of two open arms. They had to choose one of the two arms, go down the staircase, and retrieve the object. Participants were then automatically brought back to the central platform in front of a novel pair of opened arms. Choice accuracy was measured by recording the percentage of positive arm choices (percent correct). A minimum of six trials was administered (i.e., the successive presentation of the six pairs was repeated for a minimum of six times). Training went on until participants reached a criterion of choice accuracy of

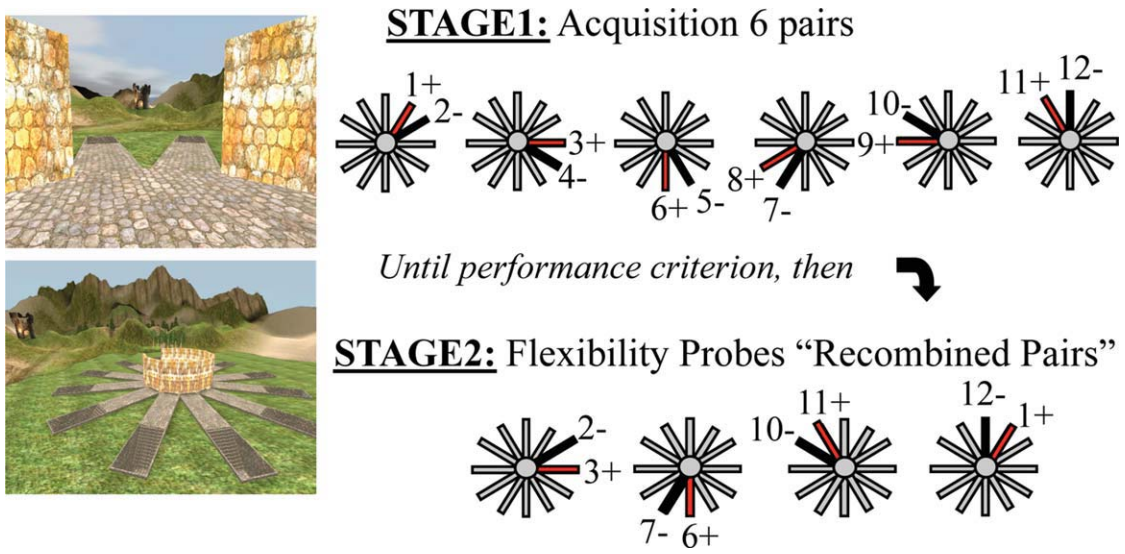

FIGURE 1. Two views of the virtual radial-maze and a schematic representation of the behavioral paradigm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

at least 92% (11/12 pairs correct over two trials) and at least 75% (18/24 pairs) over the last four trials.

Stage 2: flexibility probe test phase. Eight of the 12 arms located in the virtual environment used in Stage 1 were used in this phase. The reward contingency among the arms remained the same. In other words, the arms that contained objects in Stage 2 were the same as in Stage 1; however, their presentation was modified such that the arms were rearranged into novel pairs. There were four recombined pairs: (2–3+), (6+7−), (10–11+), and (12–1+), and these pairs were presented in a pseudorandom sequence. Two trials were administered, each consisting of the presentation of all four recombined pairs. In our task, to perform well on the probe trials, one needs to have acquired the precise spatial relationship between the target arms and the environmental landmarks; and the relationships between the six pairs of arms acquired separately. When the arms are rearranged during Stage 2 the perspective shifts, however the spatial relationships between the target arms and the environmental landmarks remain the same, allowing people to compute the locations of the target arms from a novel perspective. Learning spatial relationships to build a cognitive map are characterized by flexibility (O'Keefe and Nadel, 1978 p 89) and are dependent on the hippocampus (Morris et al., 1982). The inability to use knowledge acquired during Stage 1 is evidence of inflexibility to use this knowledge from a novel perspective. In other words, going down the incorrect arms in Stage 2 suggests that the task had been acquired in Stage 1 with stimulus-response relationships that are rigid and not effective when there is a shift in context (O'Keefe and Nadel, 1978). For example, during Stage 1, participants learn that arm 1 and arm 3 contain objects (positive arms) through repeated presentation of pairs 1+2− and 3+4−, they subsequently use this knowledge to choose the correct arm during Stage 2 when confronted with the rearranged pair 2–3+. Participants must compare and contrast knowledge relative to each initial pair to choose the adequate rewarded arm within the rearranged pair. Participants must also use a cognitive map in order to flexibly access their acquired spatial relationship between the target arm 3 and environmental landmarks in a novel situation (because it is now presented with arm 2 instead of arm 4).

Wayfinding in the virtual town

The wayfinding task is a typical relational memory task in which the participant has to find shortcuts in a virtual town. This protocol was modeled after the virtual town published by Hartley et al., (2003). The virtual town employed in this study, previously used in Etchamendy and Bohbot, 2007, is composed of different buildings, houses, and alleys, and includes eight distinct landmarks (e.g., shops, cinema). The landmarks are arranged in such a way that from each landmark, no other landmark is visible, thus preventing the use of a strategy based on sequential stimulus-response associations and encouraging the use of a mental cognitive map. A two-dimensional top-view map of the town was used to calculate "ideal paths" (i.e., most direct routes) between pairs of landmarks.

Acquisition. To learn the topography of the town, participants freely explored the virtual town for at least 20 min. Occasional verbal direction from the experimenter was necessary to ensure that (1) participants attended each landmark, (2) each location was visited more than once, and (3) all the roadways were fully explored.

Probe trials. To assess their spatial representation of the town, participants were placed in front of a particular landmark location and were instructed to navigate to another specific landmark taking the shortest path. Eight different probe trials were

designed in such a way that any two landmarks were paired only once during the experiment. In addition, each of the eight landmarks was used only once as a starting position and once as a destination. This prevented the development of familiar routes. At each probe trial, performance was measured by subtracting the path length taken by the participant from the ideal path length to end up with an error distance.

fMRI Experiment

Research participants

Twenty-three young healthy participants (9 men and 14 women; mean age 23.3 yrs, SD ± 3.3 yrs) were included in this study. The participants were right-handed and had no history of psychiatric or neurological disorders. Informed written consent was obtained from all participants and the study was approved by our institutional research ethics committee.

Experimental task

The task was identical to the concurrent spatial discrimination learning task, described in the behavioral experiment section.

Visuo-motor control task

The control task took place in a different environment comprised of a 12-arm radial-maze. The 12 arms were arranged into six pairs presented sequentially to participants. For this task, the position of the reward within each pair was completely randomized and changed from one trial to another. Participants were explicitly asked to randomly visit an arm within each pair in order to retrieve an object. The experimenter specified that the position of the object was totally randomized and varied across trials, that no rule predicted its position, and that the participants had nothing to learn. At the same time, participants were asked to count backward by increments of 3 from 1,000 to discourage rehearsal of learned information.

Pilot fMRI visuo-motor control task

To design an appropriate control task for virtual navigation fMRI tasks, a pilot study with eight young adult participants was conducted. Participants performed the same experimental and visuo-motor control task as described above except no counting was required during the control task. To examine the effectiveness of the control task, experimental trials of the first fMRI scan were contrasted against the first control trials (performed before any experimental trials) and experimental trials were contrasted against control trials performed after experimental trials.

fMRI acquisition data

The scanning session consisted of scans of a duration of 10 min each. The number of scans recorded varied across participants because it was a function of the number of trials needed to attain the criterion performance on Stage 1. In each scan, the participants performed alternate blocks of experimental and visuo-motor control tasks (see Fig. 2). During Stage 1 of the learning task, a trial (i.e., the successive presentation of the six pairs) alternated with the presentation of six pairs in the visuo-motor control task. This was repeated until participants reached the predetermined criterion performance. Stage 1 was followed by Stage 2, whereby participants were given two sets of four recombined pairs probe trials, each interleaved with two sequences of four pairs in the visuo-motor control task.

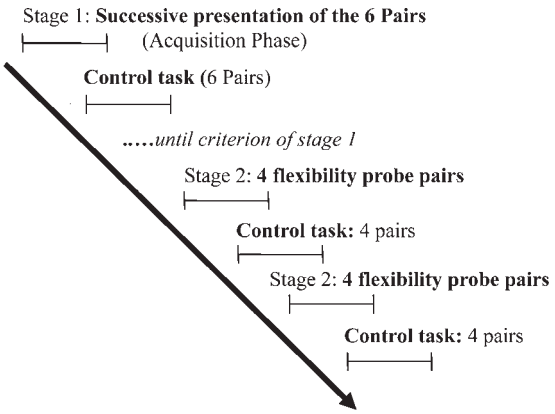

FIGURE 2. Experimental design of the fMRI scanning sessions during which participants engaged in the various stages of the Concurrent Spatial Discrimination Learning Task interspersed with a visuo-motor control task.

Because of the variability between participants in the time taken to perform the task, we used custom made software to record frame times, every keystroke made by the participant as well as the keystrokes made by the experimenter, which marked the transition from one task to another (Iaria et al., 2003). Recording the keystrokes of the experimenter allowed us to exclude frames acquired during the transitions between tasks from the analysis.

The magnetic resonance imaging (MRI) scans were obtained with a Siemens Sonata 1.5 T system (Siemens AG, Erlangen, Germany). For the anatomical images, a three-dimensional gradient echo acquisition was used to collect 80 contiguous 2 mm T1-weighted images in the sagittal plane. Each functional scan was acquired using 32 contiguous 4 mm axial slices positioned parallel to the hippocampus and covering the entire brain [64 × 64 matrix; echo time (TE), 50 ms.; number of frames, 200; time between measurements, 3 s; field of view, 256 mm]. BOLD signal images were spatially smoothed (6 mm Gaussian Kernel), corrected for motion, and linearly transformed into standard stereotaxic space (Talairach and Tournoux, 1988) using in-house software (Collins et al., 1994). Individual t-maps of the comparisons between experimental and control tasks in each scan, as well as group-averaged statistical images and correlation maps were obtained using the in-house FMRISTAT software package (available at http://www.bic.mni.mcgill.ca/users/keith/) (Worsley et al., 2002).

The t-statistic thresholds corrected for multiple comparisons for the whole brain volume were $t = 6.63$ ($P < 0.05$) and $t = 7.53$ ($P < 0.001$). On the basis of our a priori hypothesis, the hippocampus, caudate nucleus, and DLPFC were treated as

regions of interest. Therefore, uncorrected thresholds of $t = 1.96$ ($P < 0.05$), $t = 3.5$ ($P < 0.001$), and $t = 3.79$ ($P < 0.0005$) were used for these areas of predicted searches and correlation analyses.

RESULTS

Behavioral Experiment

Concurrent spatial discrimination learning task and effects of aging on behavioral flexibility

Aged participants needed significantly more trials (mean: 9.95; sem +/−0.55) to acquire the position of objects and to attain criterion performance during Stage 1 than young adult participants (mean: 7.48; sem ± 0.41) ($F = 10.8$, $P < 0.001$). However, as shown in Figure 3A, they progressively reached a mean percent of correct choices (96%) close to that of young volunteers (98%). These observations were confirmed by an analysis of variance (ANOVA) showing that performance progressively increased similarly in the two age groups (see Fig. 3A) across the last four trials before attaining criteria [effect of Trials: $F(3,102) = 100.46$, $P < 0.0001$; effect of Age: $F(1,102) = 12.57$, $P < 0.01$; interaction Age × Trials: $F(3,102) = 1.48$, $P > 0.05$]. These results support the conclusion that all young and older participants acquired the virtual concurrent spatial discrimination learning task.

When presented with recombined pairs during the flexibility probe test (Stage 2), young participants were efficient at choosing the appropriate arms for collecting the objects (mean performance: 83.4% correct, see Fig. 3B). In contrast, aged participants' performance dropped dramatically (mean performance: 64.1% correct responses) relative to younger adults, who were better able to flexibly use previously acquired knowledge. This result was confirmed by an ANOVA [effect of Stage: $F(1,102) = 83.21$, $P < 0.0001$; effect of Age: $F(1,102) = 22.52$, $P < 0.0001$; interaction Stage × Age: $F(1,102) = 13.66$, $P < 0.0005$], and is further depicted in Figure 3B.

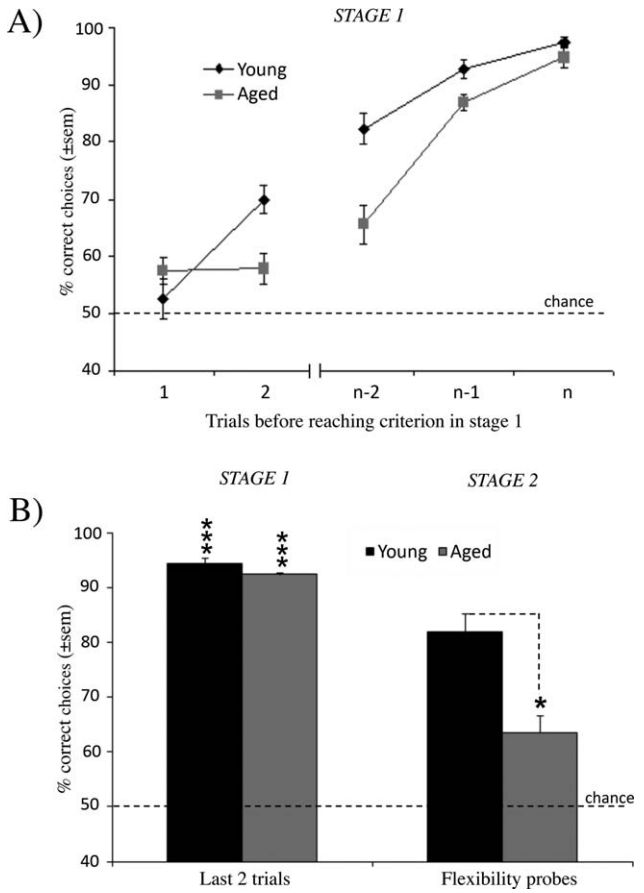

FIGURE 3. Data recorded in the behavioral experiment with older and young adults illustrating the effects of aging. A: Mean [± standard error of mean (SEM)] percentage of correct choices over the first two and last two trials before reaching criterion performance in the encoding phase (Stage 1) for the young adult group and for the older adults. At the end of training, their performance was 98% and 96% correct respectively during the last trial. B: Mean (± SEM) percentage of correct choices over the last two trials of the encoding phase (Stage 1) relative to the two sets of four flexibility probe trials for the young adult group and for the older adult group. ***: $P < 0.001$ vs. chance level (50%) and ***: $P < 0.0001$ vs. young group. The data show that the older adult group of participants was less flexible at using their knowledge acquired in Stage 1 than young adult participants during the flexibility probes (Stage 2); however, they reached the same level of discriminations in Stage 1.

Effects of aging on the Wayfinding task

The performance of young and older adult participants was compared on the wayfinding task. Older adults made significantly greater distance errors in finding the target location than younger adults ($t = -5.71$; $P < 0.0001$) (Fig. 4C).

Correlation between wayfinding in the virtual town and the concurrent spatial discrimination learning task

Performance of the young and older adult participants during the spatial concurrent discrimination learning task was compared to their wayfinding ability in the virtual town (Fig. 4A and B). In young adults, a strong and selective correlation was found between performance in the wayfinding task and their accuracy during the flexibility probes in the radial maze task ($r = -0.515$, $P < 0.005$). This means that young adults who used the shortest and most direct paths to find target landmarks in the virtual town also showed the best performance during the flexibility probes of our concurrent spatial discrimination learning task. This correlation is most likely understated since many participants scored 100% on the flexibility probe. Such a correlation was not observed when comparing wayfinding performance and accuracy at the end of Stage 1 ($r = -0.015$, $P > 0.05$), a stage that does not discriminate between flexible and inflexible learners. In other words, the correlation between the virtual town and concurrent spatial discrimination learning task was selective to the Stage 2 flexibility

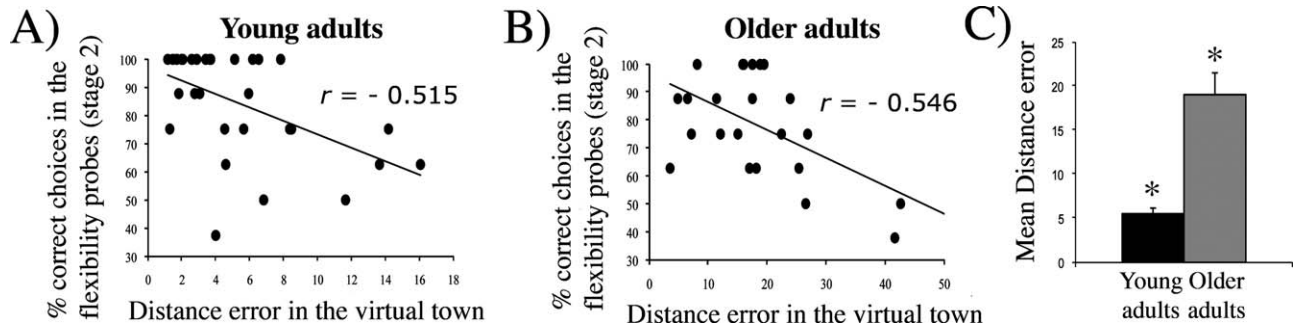

FIGURE 4. Performance on the Wayfinding task. A: Negative correlation between mean performance over the two flexibility probe trials and mean error on the probe trials of the virtual town (error calculated as distance traveled beyond the shortest route) in young adults. The data suggest that young adults making few wayfinding errors in the virtual town had the best performance in the flexibility probes of the radial-maze task ($r = -0.515$, $P < 0.005$). B: Negative correlation between mean performance over two flexibility probe trials and mean error on the probe trials of the virtual town (error calculated as distance traveled beyond the shortest route) in older adults. The data suggest that older adults making few wayfinding errors in the virtual town had the best performance in the flexibility probes of the radial-maze task ($r = -0.546$, $P < 0.01$). C: Mean (± SEM) distance error on the probe trials of the virtual town of young and older adults (*: $P < 0.001$). Older adults made significantly more mean distance errors than young adults demonstrating impairment in forming cognitive maps.

probes, which successfully discriminates the more flexible spatial learning from the inflexible stimulus-response learning.

Similar results were seen in the older adult participants. A strong negative correlation was observed between performance on the virtual town task and accuracy during the flexibility probe ($r = -0.546$; $P < 0.01$). Furthermore, no correlation was seen between virtual town performance and accuracy at the end of Stage 1 ($r = -0.117$, $P > 0.05$) as expected since Stage 1 can be solved using both flexible spatial learning and inflexible stimulus-response learning.

Overall, this indicates that participants who made more distance errors in the virtual town were also less flexible on the probes trials.

fMRI Experiment

Behavioral data

During the fMRI, all young adult participants learned the position of the objects within each of the six pairs of arms during Stage 1 and attained criterion performance with a mean of 7.56 trials. Their performance increased progressively over the last four trials (Repeated Measures ANOVA: $F(3,66) = 23.629$, $P < 0.001$). Their mean performance over the last two trials of the training was 96.2% of correct choice. In contrast, when confronted with recombined pairs during Stage 2, their accuracy dropped significantly to 60.7% ($F(1,20) = 46.3$, $P < 0.001$).

On the basis of the high variability between individuals observed during the flexibility probes, we dissociated two subgroups of participants qualified as flexible or inflexible according to their performance during the flexibility probes of Stage 2. Participants performing the flexibility probes at 80% correct choice and above were qualified as flexible ($n = 8$), those displaying performance below 80% of correct choice were qualified as inflexible ($n = 15$). Given that the probe (Stage 2) has 8 trials and the probability of a success on an individual trial is 0.5, 7 out of 8 successes were used as the cutoff to obtain a binomial probability of $P < 0.05$. The probability that someone will get 7 out of 8 trials correct by chance is less than 5%. The two groups acquired the initial concurrent discriminations over the last four trials of Stage 1, progressively and equally well [interaction Group × Trials: $F(6,60) = 0.86$, $P > 0.05$]. The flexible group ($n = 8$) performed above 89% correct in Stages 1 and 2. In contrast, the inflexible group ($n = 15$), which acquired the initial concurrent discriminations in Stage 1 correctly, are those who failed to transfer such knowledge during the flexibility probes in Stage 2 with a performance of ~ 44% correct. This finding was confirmed by an ANOVA indicating a different pattern of performance between the end of Stage 1 and the flexibility probes in Stage 2 by group (flexible vs. inflexible) [interaction Group × Stage ($F(1,20) = 16.1$, $P < 0.0001$].

fMRI data

We examined the brain regions involved in the performance at each stage of the task. First, the fMRI data were analyzed at the acquisition phase (Stage 1) for the entire participant pool (n = 23). Then, the same analysis was performed for the flexibility probes (Stage 2) and for the flexible and inflexible subgroups. In accordance with our a priori hypotheses, particular attention was focused on the BOLD signal in the hippocampus, the caudate nucleus, and prefrontal cortex during the statistical analyses.

First trial (6 pairs) of Stage 1. There was no statistical differences between the two subgroups of participants regarding the performance during the first trial of Stage 1 [Repeated Measures ANOVA: effect of Group: $F < 1$, $P > 0.05$]. The fMRI analysis on the entire participant pool showed that, in contrast to the control task, there was statistically increased BOLD signal in the right hippocampus in the first experimental trial of the acquisition phase (Stage 1) (Fig. 5A). Table 1 reports the t values and stereotaxic coordinates (Talairach and Tournoux, 1988) of the voxels of peak activation.

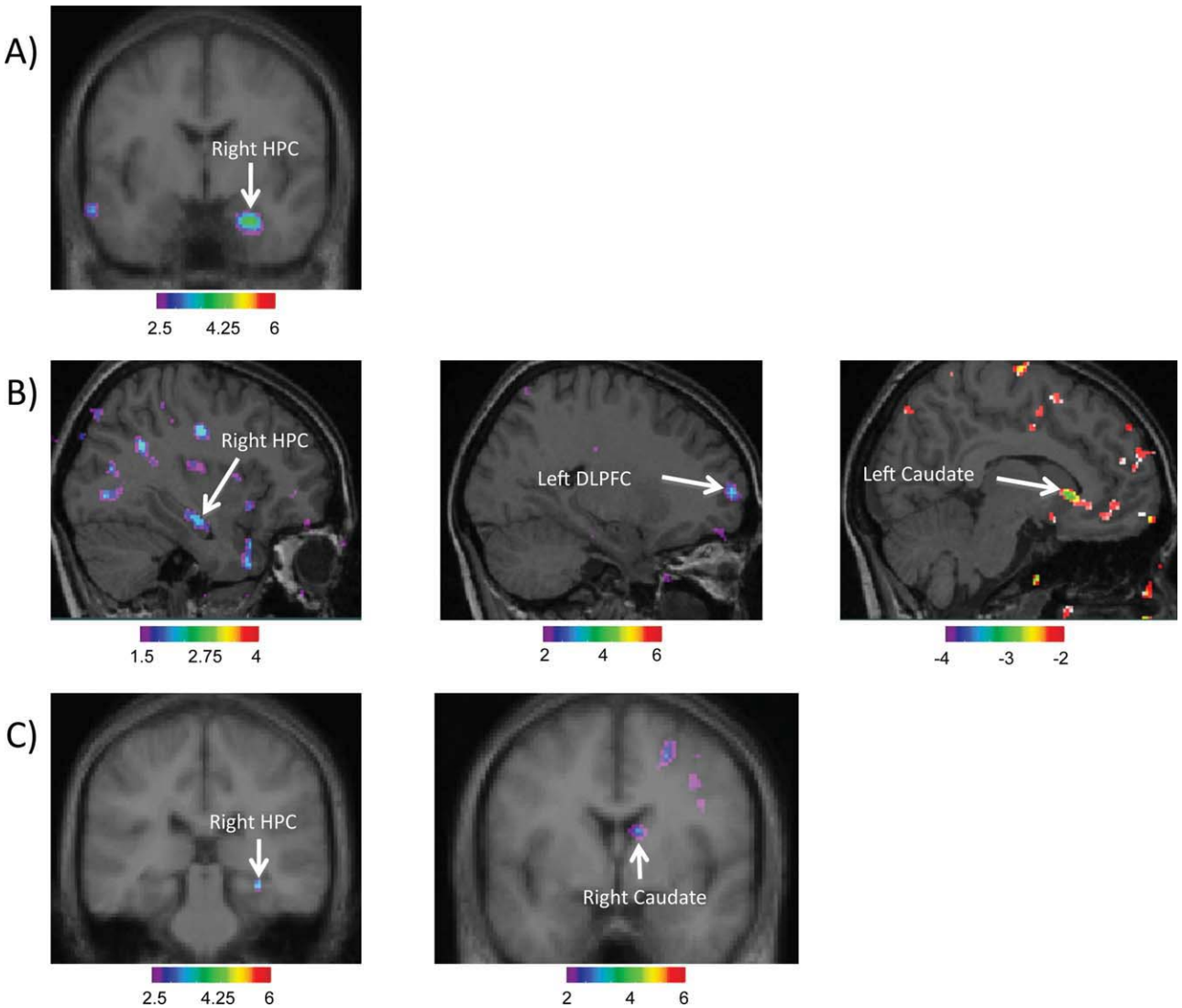

FIGURE 5. fMRI results, A: During the first trial (Stage 1) of the learning phase, activity in the right hippocampus ($x = 26.2$, $y = -6.2$, $z = -28.1$; $t = 4.41$, $P < 0.0005$) was observed in the whole group in contrast to corresponding control trials. B: Correlations between BOLD signal at time of initial acquisition of the task in Stage 1 (first trial minus corresponding control trials) and performance in subsequent flexibility probes trials (Stage 2) (Left) Positive correlation between the BOLD signal increases in the right hippocampus ($x = 33.4$, $y = -17.5$, $z = -16.1$; $t = 2.4$, $P < 0.05$) and the (Middle) left dorso-lateral prefrontal cortex (DLPFC; $x = -19.7$, $y = 62.0$, $z = 4.1$; $t = 3.28$, $P < 0.05$). (Right) Negative correlation between the BOLD increase in the left caudate nucleus ($x = -8.0$, $y = 19.7$, $z = -1.8$; $t = -3.09$, $P < 0.05$) C) Probe trials of Stage 2. (Left) Activity in the right hippocampus ($x = 32.2$, $y = -32.0$, $z = -13.6$; $t = 3.52$, $P < 0.001$) found in the flexible learners in contrast to the corresponding control trials. The t-maps are superimposed onto the anatomical average of all participants and displayed in the coronal plane. (Right) BOLD signal increases in the right caudate nucleus ($x = 10.2$, $y = -1.2$, $z = 15.8$; $t = 3.04$, $P < 0.05$) found in the inflexible learners in contrast to the corresponding control trials. The t-maps are superimposed onto the anatomical average of all participants and displayed in the coronal plane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

All Trials of Stage 1. Analysis of BOLD signals over all Stage 1 experimental trials showed significant left DLPFC ($t = 4.33$, $P < 0.0005$) activation in the participant group as a whole. Significant activation was also seen in the left fusiform gyrus and left middle occipital gyrus. Another peak was also noted in the left DLPFC ($t = 4.06$, $P < 0.0005$).

Activity in the right hippocampus ($t = 4.41$, $P < 0.0005$) was found in the whole group while performing the first trial during the encoding phase (Stage 1) in contrast to corresponding control trials. As expected from our a priori hypotheses, correlative analyses with subsequent performance during flexibility probes (Stage 2) on the entire group showed an increase of the BOLD signal in the right hippocampus ($t = 2.4$, $P < 0.05$) and left DLPFC ($t = 3.28$, $P < 0.05$) during the first trial of Stage 1 (Fig. 5B and Table 1). In addition, a negative correlation was observed between BOLD signal increases in the left caudate nucleus and flexibility performance during Stage 2 ($x = -8.0$, $y = 19.7$, $z = -1.8$, $t = -3.09$, $P < 0.05$). In summary, as per our initial predictions, we showed that activation of the hippocampus or caudate nucleus at the beginning of the initial

TABLE 1.

Brain Activity during the Concurrent Spatial Discrimination Learning Task Completed by Young Adults

[X, Y, Z]	Anatomical location of peak	*t* value
Brain activity common to all participants during the first experimental trial of Stage 1		
26.2, −6.2, −28.1	Right Hippocampus	4.41
Brain activity common to all the participants during all experimental trials of Stage 1		
−29.9, −55.0, −15.9	Left Fusiform Gyrus	8.08
−34.2, −85.0, 22.1	Left Middle Occipital Gyrus	6.61
−36.1, 52.0, 12.2	Left Middle Frontal Gyrus	4.33
−19.6, 21.0, 47.9	Left Superior Frontal Gyrus	4.06
Brain activity common to all the participants during all 8 tests of flexibility (Stage 2)		
45.9, 25.0, 34.3	Right Middle Frontal Gyrus	3.85
Brain activity found in the efficient and impaired groups during all 8 tests of flexibility (Stage 2)		
Flexible Group		
32.2, −32.0, −13.6	Right Hippocampus	3.52
Inflexible Group		
10.2, −1.2, 15.8	Right Caudate Nucleus	3.04
42.3, 6.0, 38.4	Right Inferior Frontal Gyrus	4.28
31.6, 8.0, 48.7	Right Middle Frontal Gyrus	3.94
Correlative analysis of brain activity and performance on flexibility probes during first experimental trial of Stage 1		
33.4, −17.5, −16.1	Right Hippocampus	2.4
−19.7, 62.0, 4.1	Left Dorsal Lateral Prefrontal Cortex	3.28
−8.0, 19.7, −1.8	Left Caudate Nucleus	−3.09

encoding phase is predictive of later flexible expression of memory during the probe tests.

Flexibility probe tests of Stage 2. Examining BOLD signals of the whole group during the probes revealed significant DLPFC activation ($t = 3.85$, $P < 0.001$). As described in the fMRI behavioral data section, flexible and inflexible learners showed significantly different performance during the flexibility probe trials ($\sim$ 89% and 44% of correct responses respectively). For this reason, we examined the probe fMRI data for each group independently (Table 1). As per our hypotheses, we focused on the BOLD signal in the hippocampus, the caudate nucleus, and the DLPFC. In the flexible group, we saw significant increase of BOLD signal in the right hippocampus ($t = 3.52$, $P < 0.001$) (Fig. 5C). In the impaired inflexible group, no activity was found in the hippocampus. Instead, we saw BOLD signal increases in the left caudate nucleus ($t = 3.04$, $P < 0.05$) during the flexibility probes in comparison with the control condition. In addition, we noted that this latter group showed significant BOLD signal peaks in the right DLPFC ($x = 42.3$, $y = 6.0$, $z = 38.4$; $t = 4.28$, $P < 0.0005$; $x = 31.6$, $y = 8.0$, $z = 48.7$; $t = 3.94$, $P < 0.0005$). In summary, Stage 2 flexibility probes showed hippocampal activation in the flexible group, and caudate nucleus and frontal activation in the inflexible group.

Hippocampus

Pilot fMRI visuo-motor control

When experimental trials involving the spatial memory experimental task were contrasted against control trials (without counting backwards) that followed, lower activation in the HPC was observed ($x = 30.5$, $y = -11.2$, $z = -26.2$, $t = -3.81$, $P < 0.0005$) (Fig. 6A) in the experimental trials compared with the control trials. In other words, there was more activity in the HPC during the control task than the experimental task. This effect was reversed to show greater activity in the HPC when the same experimental trials were contrasted against the very first control trials that preceded the spatial memory task ($x = 24.0$, $y = -31.0$, $z = -7.9$, $t = 2.49$, $P < 0.05$) (Fig. 6B). The fact that changing the control task is sufficient to reverse HPC activity observed during the experimental task required further investigation. Subjective reports indicated that during the control trials where no counting was required, participants took time to recollect what they did during the experimental trials. Specifically, they reported visualizing the landscape and recollected which of the two arms contained the target object. During the first control trials, however, participants could not rehearse what they learned during the experimental trial because the control trials preceded all experimental trials. Consequently, participants were asked to count backward during the control trials of the radial-maze presented here in order to prevent rehearsal of the spatial memory task.

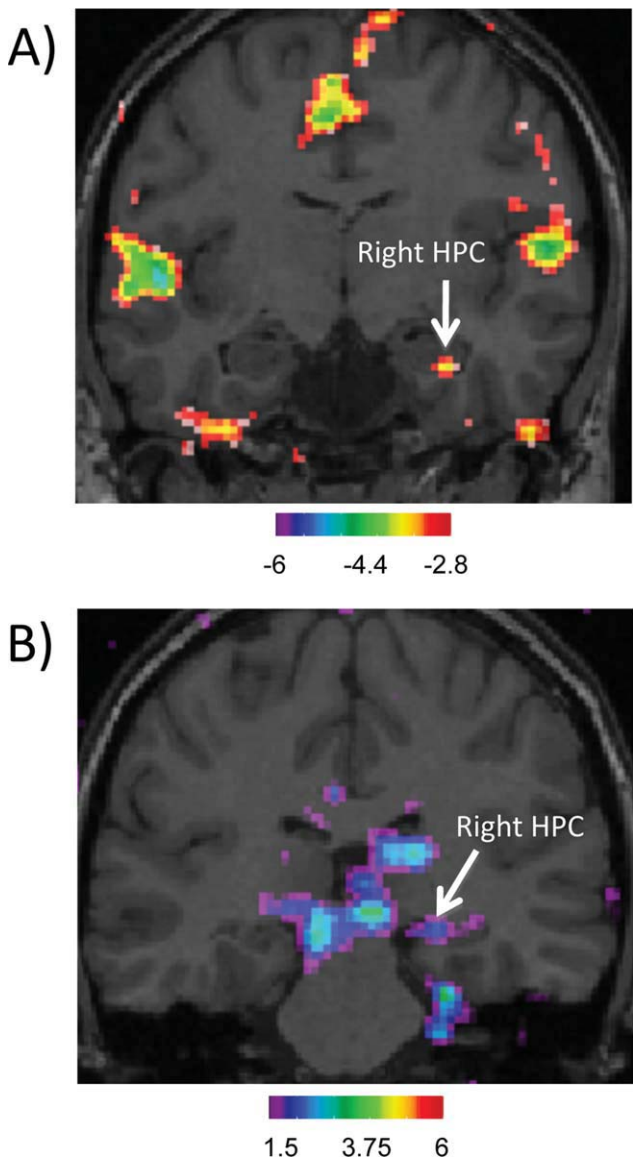

FIGURE 6. Pilot fMRI visuo-motor control task. Contrasts of experimental trials against control trials with no counting, A: Negative activation was observed in the right HPC ($x = 30.5$, $y = -11.2$, $z = -26.2$, $t = -3.81$, $P < 0.0005$) when experimental trials were contrasted against control trials that followed. B: Positive activation was observed in the right HPC ($x = 24.0$, $y = -31.0$, $z = -7.9$, $t = 2.49$, $P < 0.05$) when the same experimental trials were contrasted against the first control trials that were performed before all experimental trials. These results highlight the importance of the control task in determining either a positive or negative activation in hippocampus. Subjective reports indicated that during the control trials, participants took time to recollect what they did during the experimental trials. Specifically, they visualized the landscape and recollected which of the two arms contained the target object. As such, the control trials that followed the experimental trials involved spatial recollection of the experimental task, therefore acting as another experimental condition. Following this pilot study, a backwards counting task was added to the control task in order to prevent spatial memory rehearsal. This procedure was successful in eliminating hippocampal activity during the control task. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

The present findings show that the performance profile of humans parallels that of mice, to include flexible encoding, effects of age, and neurobiological substrates. First, the positive correlation between the performance in flexibility probes in Stage 2 and wayfinding ability in a virtual town supports the interpretation that both tests tax the same kind of mnemonic representation, namely a relational representation of items in the environment. Second, older adults exhibited a deficit of performance in the flexibility probe similar to the one previously demonstrated in aged mice (Marighetto et al., 1999). Furthermore, older adults displayed impairments on the wayfinding task. These results suggest a deficit in relational memory with aging. Finally, while all young volunteers similarly acquired the initial pair-discrimination task in the MRI, only a subset of individuals succeeded in the flexibility probes, whereas others performed at chance level and failed. The probe trials of the fMRI experiment showed a clear dissociation between the two subgroups. As per our predictions, those who were successful on the flexibility probes showed hippocampal activation, whereas those who were inflexible showed activation of the caudate nucleus of the striatum. In addition, success on the flexibility probes positively correlated with initial (first trial) hippocampal activation and negatively correlated with activation of the caudate nucleus on later trials. This dissociation, parallels a previous experiment in mice showing that temporary induced inactivation of the hippocampus during Stage 1 produced an over-activation of the striatum, sparing initial learning but resulting in a subsequent flexibility deficit in Stage 2 (Mingaud et al., 2007). Hence, our two-stage radial-maze discrimination learning paradigm enables us to assess, in both humans and mice, the flexibility of relational memory, shown by the capability to compare and contrast information originating from separate sources to make an informed choice decision in a novel situation. Altogether, current data in humans and previous findings in mice demonstrate that the flexibility of relational memory requires the functional integrity of the hippocampus, at the time of encoding and retrieval.

Our pilot fMRI study revealed the importance of the control task in determining activation of the hippocampus during our spatial memory experimental task. Young adult participants tested on the concurrent spatial discrimination learning task were initially scanned with the same control task as that reported in the current experiment, with the exception that they were not asked to count backward from 2000. Results showed decreased activity in the hippocampus during the experimental task compared with the control task. (Fig. 6A). Similar results have been reported by other studies in the literature (Stark and Squire, 2001; Shipman and Astur, 2008). Subjective reports indicated that the control trials that followed the experimental trials involved spatial recollection of the experimental task. Therefore, the control trials acted as another experimental condition that yielded even greater hippocampal activity than the experimental task, presumably because there was more time to engage in active formation of cognitive maps. Following this pilot study, a backward counting task was added to the

control task to prevent spatial memory rehearsal. This procedure successfully allowed hippocampal activity to be captured during the relational memory experimental task.

Convergent evidence from human neuroimaging (Kumaran and Maguire, 2006) and animal models (Frank et al., 2000; Wood et al., 2000; Gilbert and Kesner, 2006) identify the hippocampus as critical to the disambiguation of memory for overlapping sets of stimuli (White and McDonald, 2002; Lipton et al., 2007; Brown et al., 2010). Hence, the critical factor making our recombination task sensitive to the dysfunction of hippocampal relational processing might be the overlap between spatial attributes. Interestingly, Bayley et al. (2005) showed that after having successfully learnt a standard eight-pair object discrimination task, two amnesic patients exhibited normal performance in recombined pair trials. In that study objects could be investigated separately. The choice of whether to select a particular object depended on the stimulus properties of that object alone, and did not elicit the hippocamal processes of disambiguation.

All participants showed DLPFC activation throughout the learning phase of Stage 1, indicating that both groups used the DLPFC for encoding. In addition, although not statistically strong, we showed that initial activation of the hippocampus and the DLPFC positively correlated with subsequent success on the flexibility probes. Numerous neuroimaging studies in humans have shown that the magnitude of activation in the human hippocampus and DLPFC during encoding correlates with later indices of relational memory (Kirwan and Stark, 2004; Ranganath et al., 2004; Uncapher and Rugg, 2005; Blumenfeld and Ranganath, 2006; Staresina and Davachi, 2006). Our finding agrees with the current literature, stating that the DLPFC's role in organizing incoming information in short-term memory contributes to long-term memory formation (Petrides, 2000; Blumenfeld and Ranganath, 2006; Repovs and Baddeley, 2006; Murray and Ranganath, 2007). Observations in humans indicate that effective relational binding in working memory is central to long-term recollection (Olson et al., 2006; Staresina and Davachi, 2006).

Our findings are also in line with the current view indicating that the caudate nucleus is involved in the formation of rigid mnemonic stimulus-response representations, after repetition of the same stimuli. Caudate nucleus activation correlated with low performance in the flexibility probes. Previous fMRI studies showed that spatial learning and stimulus-response learning depend on the hippocampus and caudate nucleus memory systems, respectively (Hartley et al., 2003; Iaria et al., 2003; Bohbot et al., 2004, 2007; Voermans et al., 2004). Poldrack et al., (2001) revealed that the caudate nucleus is involved in a later phase of the training, when participants make faster and automatic classification responses. Here, activation of the caudate nucleus during the flexibility probes was associated with activation of the DLPFC. This converges with data showing strong anatomical connections between the DLPFC and the caudate nucleus (Alexander et al., 1986; Lawrence et al., 1998), and with data showing their functional interactions that sustain procedural or "habit" learning, i.e., rule learning (Packard and Knowlton, 2002; Seger and Cincotta, 2006).

In the fMRI experiment, over half of the participants did not show activity in the hippocampus during the flexibility probes and this was associated with a drastic deficit in performance. This phenomenon in half of the young volunteers executing the Concurrent spatial discrimination learning task in the fMRI contrasted with the participants who performed the task behaviorally. Participants who performed the task behaviorally displayed an above-chance performance in the flexibility probe. Hence, while initial learning could engage the hippocampal relational memory system in standard conditions of testing, over a half of healthy young adult participants tested in the scanner shifted towards preferential engagement of the caudate nucleus/procedural learning and memory system. This finding is interesting because the scanning environment is associated with stress, and was shown to elevate cortisol levels (Tessner et al., 2006). Previous studies in humans (Schwabe et al., 2007) and in mice (Kim et al., 2001, 2007) show that stress before learning facilitates stimulus-response learning at the expense of a more cognitive spatial learning.

In accordance with predictions based on our mouse studies, we showed that aged participants acquired initial discriminations (Stage 1) with extensive training but failed to resolve the flexibility probes. These results suggest that older adults acquired the virtual concurrent spatial discrimination learning task with the rigid stimulus-response strategy dependent on the caudate nucleus. Importantly, consistent with this assumption, older adults were significantly impaired at finding target landmarks in the virtual town, an indication of a hippocampal dysfunction. This is consistent with current findings showing that in rodents and in humans, aging is associated with a dominant use of stimulus-response and nonspatial strategies (Barnes et al., 1980; Moffat et al., 2007). Some elderly humans have impaired spatial memory (Driscoll et al., 2003; Meulenbroek et al., 2004; Iaria et al., 2009), and this has been linked to hippocampal atrophy (Raz, 1999; Driscoll et al., 2003). In addition, within the aged population, better spatial memory has been shown to correlate with increased hippocampal volume (Chen et al., 2010; Head and Isom, 2010). Further, memory in normal aging was previously associated with an over-activation of the caudate nucleus, instead of the activity typically seen in the hippocampus of young adults (Della-Maggiore et al., 2000; Gron et al., 2006). Our data suggest that both humans and mice tend to use caudate nucleus/striatal-dependent inflexible response learning strategies with age instead of the more flexible hippocampal-dependent spatial learning strategies used by young adults.

Our radial-maze paradigm enabled us to dissociate two forms of memory expression relying respectively on relational and procedural memory systems in humans as previously shown in mice. This paradigm may be a valuable tool in identifying cellular and molecular bases of relational memory and its dysfunctions, and in increasing the predictive validity of preclinical therapeutic studies.

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