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Research Report

Probing the neural correlates of associative memory formation: A parametrically analyzed event-related functional MRI study

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ABSTRACT

The medial temporal lobe (MTL) is crucial for declarative memory formation, but the function of its subcomponents in associative memory formation remains controversial. Most functional imaging studies on this topic are based on a stepwise approach comparing a condition with and one without associative encoding. Extending this approach we applied additionally a parametric analysis by varying the amount of associative memory formation. We found a hippocampal subsequent memory effect of almost similar magnitude regardless of the amount of associations formed. By contrast, subsequent memory effects in rhinal and parahippocampal cortices were parametrically and positively modulated by the amount of associations formed. Our results indicate that the parahippocampal region supports associative memory formation as tested here and the hippocampus adds a general mnemonic operation. This pattern of results might suggest a new interpretation. Instead of having either a fixed division of labor between the hippocampus (associative memory formation) and the rhinal cortex (non-associative memory formation) or a functionally unitary MTL system, in which all substructures are contributing to memory formation in a similar way, *we propose that the location where associations are formed within the MTL depends on* the kind of associations bound: If visual single-dimension associations, as used here, can already be integrated within the parahippocampal region, the hippocampus might add a general purpose mnemonic operation only. In contrast, if associations have to be formed across widely distributed neocortical representations, the hippocampus may provide a binding operation in order to establish a coherent memory.

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1. Introduction

After an era in which lesion studies have identified the anatomical structures of the declarative memory system, which enables us to consciously retrieve previously encountered information (Tulving, 1983), functional imaging studies have begun to delineate the neural processes underlying declarative memory formation at the brain system level. In particular, fMRI studies implementing event-related designs have identified regions that are involved in successful formation of new memories by post-hoc sorting of stimuli based on whether they are subsequently remembered or forgotten (Sanquist et al., 1980; Paller et al., 1987; Brewer et al., 1998; Wagner et al., 1998; Fernández and Tendolkar, 2001 for review).

Declarative memory allows discriminating previously encountered stimuli from novel by means of recollection and familiarity: While recollection involves recognition with retrieval of contextual information, familiarity refers to a feeling that a stimulus has been encountered previously without contextual retrieval (Mandler, 1980; O'Reilly and Norman, 2002; Rugg and Yonelinas, 2003). It is well established that the medial temporal lobe (MTL) and in particular the hippocampus is crucial for declarative memory (Eichenbaum and Cohen, 2001). However, it is unclear whether all substructures of the MTL do critically support memory formation with and without contextual information in a unitary way or whether there is a functional dissociation. Here, different mediotemporal subcomponents such as the hippocampus and the parahippocampal gyrus, which is covered anteriorly with rhinal and posteriorly with parahippocampal cortices (Lavenex and Amaral, 2000) are of particular interest. For clarification purposes, we will henceforth use the term rhinal cortex when referring to the anterior parahippocampal gyrus and parahippocampal cortex when referring to the posterior parahippocampal gyrus and neighboring structures like the fusiform gyrus, which are covered in part by parahippocampal cortex (Amaral and Insausti, 1990).

Recent fMRI research has been motivated by behavioral data from patient studies either supporting (for example Vargha-Khadem et al., 1997; Mayes et al., 2002; Yonelinas et al., 2002; Turriziani et al., 2004) or contradicting (for example Manns et al., 2003; Stark and Squire, 2003; Wixted and Squire, 2004; Wais et al., 2006) a functional dissociation within the MTL with respect to associative and non-associative declarative memories. One line of studies found evidence for a functional dissociation within the MTL probing formation of memories with and without contextual information in the form of an associative learning task (Sperling et al., 2003) or a source memory paradigm (Small et al., 2001; Davachi and Wagner, 2002; Davachi et al., 2003; Ranganath et al., 2004), even for emotional content (Kensinger and Schacter, 2006). Taken together, these results suggest that the hippocampus and the parahippocampal cortex have a specific role in associative memory formation (Aggleton and Brown, 1999; Eichenbaum and Cohen, 2001; O'Reilly and Norman, 2002), while the rhinal cortex seems to have a specific role in encoding item information that can subsequently be remembered on the basis of familiarity (Davachi et al., 2003; Ranganath et al., 2004; Weis et al., 2004a).

Another line of research suggests that declarative memory formation related to both subsequent recollection and familiarity-based recognition depend on integrated processing within the MTL (for review see Squire et al., 2004). For example, investigating encoding and retrieval of novel face–name pairs, Kirwan and Stark (2004) showed that there was a greater activity during associative memory formation not only in the hippocampus but also in the rhinal and parahippocampal cortices. Moreover, the hippocampus was involved in item memory formation as well. The use of a source memory paradigm (Gold et al., 2006), comparable to those mentioned before (Small et al., 2001; Davachi and Wagner, 2002; Davachi et al., 2003; Ranganath et al., 2004), also supported a unified set of operations during memory formation with and without context. Finally, studies using a verbal associative encoding task (Jackson and Schacter, 2004) or testing subsequent recollection and familiarity-based recognition in the form of a Remember/Know paradigm (Eldridge et al., 2005) revealed no clear-cut functional dissociation between hippocampus and rhinal cortex.

What is the reason for these discrepant results? Certainly, a full double-dissociation is difficult to achieve in a highly interconnected, hierarchical or at least serially structured system like the MTL, where all substructures are participating in memory formation (Fernández et al., 1999). This may in particular play a role during encoding, because the qualitative difference at retrieval between familiarity-based recognition and recollection might be based on a quantitative difference in associative memory formation at encoding. Indeed, evidence is emerging that, under certain circumstances, the rhinal cortex, next to mediating item memory formation, may also serve associative memory (Aminoff et al., in press). The rhinal cortex seems to be particularly involved when subjects form "intra-item associations", the binding of a concrete feature (for example color) with an item (for example object) (Staresina and Davachi, 2006). Moreover, all studies mentioned before (except for Staresina and Davachi, 2006) were based on an experimental approach that compares a condition with more associative encoding and one with none or at least less associative, but single item encoding. A significant difference in brain activation between these two conditions has been interpreted as related to associative encoding and a difference in brain activity between the single item condition and subsequent misses has been related to single item memory formation. But this stepwise approach is partly based on null findings if it is used to fully dissociate brain regions involved in either single item or associative memory formation.

A parametric approach over several levels of associative encoding should be an alternative to this stepwise approach, because a parametric approach provides a somewhat closer brain-function correlation than a simple contrast and depends less on certain null findings (Staresina and Davachi, 2006). Since the particular role of different MTL subcomponents in associative memory formation is not entirely clarified yet, we set out to identify brain regions, particularly within the MTL, whose activity is related to associative memory formation by correlating regional brain activity with the amount of associative information stored successfully in declarative memory. The question at hand is whether the hippocampus has a selective role in associative memory formation or whether all

MTL substructures mentioned (i.e., hippocampus, rhinal cortex and parahippocampal cortex) show activity correlated with the amount of successful associative memory formation. To answer this question, we followed a similar approach as used by Staresina and Davachi (2006) using the association between a concrete feature (color) with photographs depicting either landscapes or buildings in a source memory experiment. The amount of associative information was manipulated by using different colors as a more global level of source and different shades of these colors as a detailed feature along the same associative dimension. This design allowed us to use a classical stepwise approach (subsequent associative memory versus subsequent item memory and subsequent item memory versus subsequent misses). Additionally, this design enabled a parametric approach correlating monotonically increasing brain responses with increasing associative memory formation. For that approach, we assumed that trials associated subsequently with mere item recognition contain the smallest amount of contextual memory formation, trials in which subsequent global source judgments were successful consist of an intermediate amount of contextual information, and trials in which subsequently both source judgments were accomplished contain the largest amount of contextual information.

2. Results

2.1. Behavioral data

For the purpose of our fMRI data analysis a considerable proportion of misses is required. Thus, we designed the study in a way to obtain a recognition memory performance clearly above chance level but with enough misses.

A Kolmogornov–Smirnov Test did not show a significant deviation of the data from Gaussian distribution, so that our data met the requirement to be further analyzed with *t*-tests. Overall recognition was high (80.5%). The chance level for a correct judgment of *"2 source hits"* and *"1 source hits"* was calculated based on the relative distribution within overall hits and is 16,6% and 33% respectively (i.e., for all pictures that were recognized the chance was one third to remember one source and one sixth to recollect both sources). Indeed did the accuracy of *"2 source hits"* ($t_{19}=8.68$, $p<0.001$) and *"1 source hits"* ($t_{19}=6.62$, $p<0.001$) differ from these thresholds. As expected, there was a better performance for *"1 source hits"* in comparison with *"2 source hits"* ($t_{19}=24.74$, $p<0.001$). For absolute percentage means of all critical conditions see additionally Table 1.

As is also evident from Table 1, statistical comparison of the reaction times revealed that *"correct rejections"* were significantly faster than *"1 source hits"* ($t_{19}=2.56$, $p<0.05$), *"item hits"* ($t_{19}=4.72$, $p<0.001$), *"misses"* ($t_{19}=4.68$, $p<0.001$) and *"false alarms"* (355 ms; $t_{19}=4.07$, $p<0.005$), but did not differ from those to *"2 source hits"*. Likewise, the reaction times for *"2 source hits"* were reliably faster than *"1 source hits"* ($t_{19}=2.14$, $p<0.05$) and *"misses"* ($t_{19}=3.27$, $p<0.005$), but did not differ from *"false alarms"*. There was no significant difference between responses to *"1 source hits"* compared with *"item hits"*, *"misses"* or *"false alarms"* (all $p>0.09$).

2.2. Functional imaging data

Note that we report only corrected *p*-values together with x/y/z coordinates and corresponding Brodmann's areas as indicated by the SPM anatomy toolbox (Eickhoff et al., 2005; Amunts et al., 2000) and the Talairach atlas (Talairach and Tournoux, 1988).

2.2.1. Overall subsequent memory effect

Following previous fMRI studies, investigating declarative memory formation, we first conducted a whole brain analysis contrasting all trials associated with successful subsequent retrieval (i.e., *"overall hits"*) against trials that were subsequently not successfully retrieved (i.e., *"misses"*).

As is evident from Fig. 1, there were activations in the MTL, the prefrontal cortex and posterior brain regions. Most relevant with respect to our experimental question was the activation in the left and right parahippocampal cortex (BA 37; [−30,−50,−7]; $p<0.001$ and BA 31 [26,−38,−9]; $p<0.001$). The biggest cluster occurred bilaterally in posterior regions in the right middle occipital gyrus (BA 19; [30,−88, 14]; $p<0.001$). These activations together with activations of left middle frontal gyrus (BA 6; [−42, 2, 33]; $p<0.01$) and the right superior parietal gyrus (BA 7; [28,−66, 55]; $p<0.05$) replicated earlier findings of a study with a similar experimental setup (Weis et al., 2004b), but do not relate to the questions, which we aimed to address here, and henceforth will not be commented on any further.

2.2.2. Stepwise analysis within the MTL

Here, we investigated whether there were subsequent memory effects related to source (e.g., *"2 source hits"+"1 source hits">"misses"*) and item (e.g., *"item hits">"misses"*)

Table 1 – Memory performance and reaction times

	Old items					New items		
	Uncertain	Misses	2 Source hits	1 Source hits	Item hits	Uncertain	Correct rejection	False alarms
%[a]	6.2±2.6	13.4±3	23.6±5	27.2±4.2	29.6±7.4	6.7±2.1	72.8±7.4	20.5±4.5
#[b]	15±6	32±7	57±12	65±14	71±18	8±3	87±9	25±5
ms[c]	2100±440	2076±434	1943±411	2003±434	2061±474	2122±484	1828±381	1948±355

[a] Mean percentage of trials (±standard deviation).
[b] Mean number of trials (±standard deviation).
[c] Mean reaction times (±standard deviation).

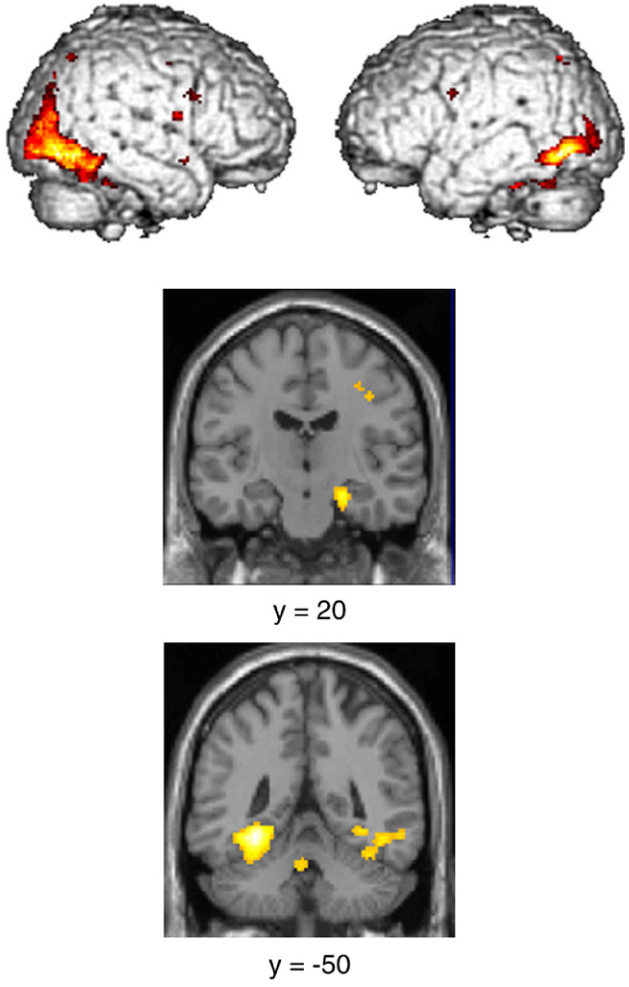

Fig. 1 – Regions activated more during encoding for later remembered than later forgotten items (subsequent memory effect). The global activation maps are shown overlaid onto a canonical brain and examples of local maxima (as indicated in the Results section) are shown superimposed on selected coronal slices of the mean high-resolution T1-weighted volume. All activated clusters were significant at the cluster level ($p_{(corrected)}<0.05$).

memory formation. Indeed, there was a reliable subsequent source memory effect in the left hippocampus ([−34,−30,−9]; $p<0.05$), left parahippocampal cortex (BA 19; [−30,−50,−7]; $p<0.001$) and right rhinal cortex (BA 35; [22,−20,−13]; $p<0.05$). However, a subsequent item memory effect was also found in a similar location of the left hippocampus ([−32,−28,−9]; $p<0.01$), left parahippocampal cortex (BA 37; [−30,−50,−7]; $p<0.005$) and right rhinal cortex (BA 35; [22,−20,−13]; $p<0.05$). A direct comparison between subsequent source memory formation as opposed to subsequent item memory formation (e.g., *"2 source hits"+"1 source hits">"item hits"*) revealed a larger activation for source memory formation in the parahippocampal cortex (BA 37; [−30,−50,−7]; $p<0.001$) and rhinal cortex (BA 28; [22,−20,−13]; $p<0.01$). Hence, the stepwise analysis revealed within the MTL a quantitative difference between activities related to source and item memory formation.

2.2.3. *Parametric analysis within the MTL*

A subsequent parametric analysis was conducted in order to see whether the significant subsequent memory effects for all conditions across all MTL subcomponents on one side and the larger activations for subsequent source memory could be explained by a gradually increasing activation due to different amounts of associative information across *"2 source hits"*, *"1 source hits"* and *"item hits"*. Assuming that *"item hits"* trials are accompanied with the least associative memory formation and *"2 source hits"* trials with the most, we weighted the three trial types accordingly: *"2 source hits"*=3; *"1 source hits"*=2; *"item hits"*=1 while comparing them with subsequent misses. Within the MTL, this analysis revealed reliable results in the left parahippocampal cortex/fusiform gyrus (BA 37; [−30,−50,−7]; $p<0.001$) and the left rhinal cortex (BA 34; [−16,−12,−19]; $p<0.01$) as well as the right rhinal cortex (BA 28; [22,−20,−13]; $p<0.001$). A subsequent pairwise analysis revealed that this effect was due to significant differences between *"2 source hits">"1 source hits"* and *"2 source hits">"item hits"*. Though visual inspection of the parameter estimates in Figs. 2A–C shows a larger activation for *"1 source hits"* as opposed to *"item hits"*, this effect did not reach the threshold of significance chosen.

2.2.4. *Summary of fMRI data analysis within the MTL*

Taken together, we found significant subsequent memory effects for source and item information in rhinal and parahippocampal cortices as well as the hippocampus. While there was no evidence for a quantitative difference between each of the subsequent memory effects depending on the amount of associative information encoded in the hippocampus (see Fig. 2D), activity in the rhinal and parahippocampal cortices, positively correlated with the amount of associations formed during the study phase (Figs. 2A–C).

3. Discussion

Recent research has focused on the division of labor within the MTL during declarative memory formations and retrieval. The present study used a traditional stepwise approach and a parametric approach with different levels of associative information in order to identify mediotemporal brain regions mediating associative memory formation.

First of all, our results are in line with previous studies on memory formation (Small et al., 2001; Davachi and Wagner, 2002; Davachi et al., 2003; Sperling et al., 2003; Jackson and Schacter, 2004; Kirwan and Stark, 2004; Ranganath et al., 2004; Weis et al., 2004b; Kensinger and Schacter, 2006) showing mediotemporal and left prefrontal activations. Though involvement of the prefrontal cortex was not the main focus of our study, we shortly want to discuss why the overall brain analysis revealed a smaller activity of left frontal regions compared to the other studies mentioned above. Firstly, the study was optimized for scanning MTL subregions opting for a short TE. This resulted in less sensitivity in brain areas not affected by susceptibility artifacts including the prefrontal cortex. Moreover, the orientation task during encoding was rather shallow as compared to the ones used in other studies. Note, however, that we replicated our previous findings within

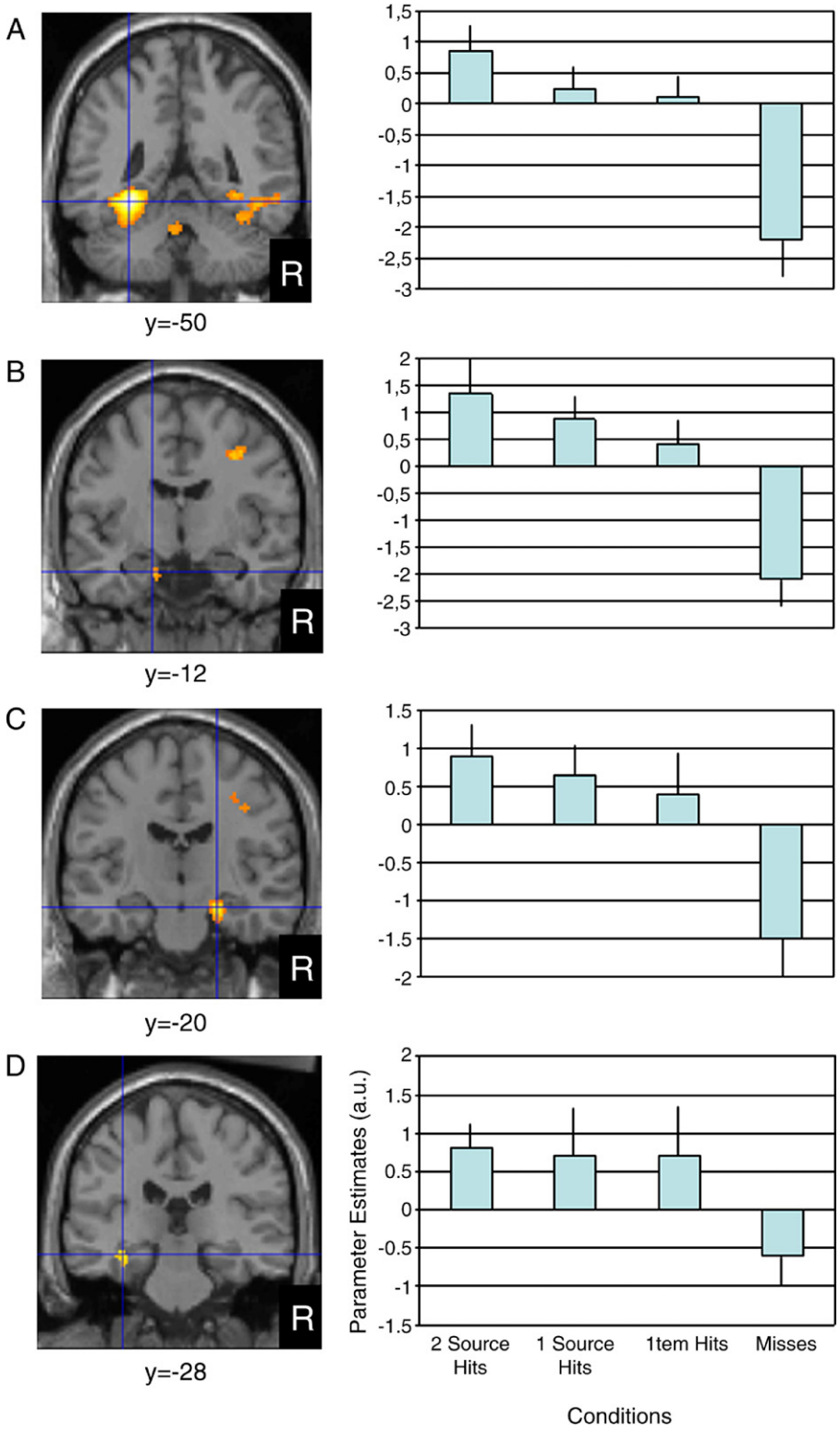

Fig. 2 – Summary of the stepwise and parametric analyses. Specific activations ($p_{(corrected)} < 0.05$) and corresponding contrast estimates derived from the local maxima are shown superimposed onto selected coronal slices of the mean high-resolution T1-weighted volume. Graded subsequent memory effects are shown in the left parahippocampal cortex (A), left (B) and right rhinal cortex (C). In contrast, there was no evidence for a quantitative difference between the subsequent memory effects for source and item memory in the left hippocampus (D).

the left prefrontal cortex using the same stimuli and the same orientation task (Weis et al., 2004b).

Turning to our main focus, the MTL, our results at first glance support recent fMRI studies assigning all mediotemporal substructures to both item and associative memory formation (Kirwan and Stark, 2004; Gold et al., 2006) and appears to stand in contrast to studies (Sperling et al., 2003; Davachi et al., 2003; Ranganath et al., 2004) assigning item memory formation to the parahippocampal gyrus (particularly rhinal cortices) and associative memory formation to the hippocampus. Here we will discuss why, instead of these two opposite interpretations, our data suggest a third interpretation.

A first interpretation is derived from the process dissociation theory, whereby MTL regions either support source or item memory formation (Sperling et al., 2003; Davachi et al., 2003; Ranganath et al., 2004; Kensinger and Schacter, 2006). Following this line of argument, the gradual increase of the parahippocampal and in particular the rhinal subsequent memory effect from the *"item hits"* condition over the *"one source hits"* to the *"two source hits"* condition has to be related to the fact that more and more single item features have to be encoded in order to form successfully more and more associations across the three conditions. Hence, the positive correlation of rhinal and parahippocampal activity with the associative conditions reflects simply the increasing demand for item feature encoding. The hippocampal effect reflects associative memory formation, which in our study might be already necessary for the single item condition, because our stimuli are complex, real-world pictures with numerous details already engaging some kind of associative memory formation. However, within this line of thought, one would expect a parametric activity increase in the hippocampus with the increasing amount of associations formed. But we did not find such an effect, so that we have to consider other mechanisms accounting for our results.

A second explanation might try to interpret our results in line with studies describing the MTL as a unitary system involved in both item and associative memory formation (see Squire et al., 2004 for review). Here, our data are in line with a recent fMRI study (Kirwan and Stark, 2004) that also found hippocampal activation in trials where single components were subsequently remembered without any association (see also Kirchhoff et al., 2000; Eldridge et al., 2005). Likewise Gold et al. (2006) found hippocampal and parahippocampal activation for subsequently remembered information regardless of a correct or incorrect source judgment. This finding was further supported by conducting source memory tests in memory impaired patients with damage thought to be limited to the hippocampus, in which these patients were similarly impaired on item and source memory tests. Hence, there is also evidence for the idea that the hippocampus plays a crucial role in single item memory formation. However, to acknowledge the different result patterns in the hippocampus and the rhinal cortices observed in our study, we would need to assume that these MTL subregions have some kind of different physiological properties with demand-dependant activity changes in the rhinal cortices and an all-or-none activity pattern in the hippocampus. Yet, there is to our knowledge no evidence for such a difference in physiological properties reported. Thus, also this interpretation appears not to be very likely.

We will leave these two rather contradicting explanations for a third, suggesting that the location within the MTL, where associations are formed, is defined by the kind of associations necessary to be established during the encoding task. Following this line of reasoning leads to the idea that different but coherent visual features (for example a complex real-world picture and its color), whose associations have been probed in this study, are already bound and integrated in late stages of the ventral visual stream (Taylor et al., 2006), which includes the parahippocampal gyrus with parahippocampal and rhinal cortices (for review see Bussey et al., 2005). In that sense our findings are in line with a study by Staresina and Davachi (2006), which compared the neural mechanisms that support later free recall and their relationship to subsequent associative recognition tested in a source memory procedure similar to the one applied here. Elucidating the successful associative binding of a concrete item feature (color) with the item (object representation as a word) itself, they also found an engagement of rhinal cortices in associative memory formation. The authors suggested that this experimental manipulation tested for a specific form of associative memory formation (namely intra-item) whereas other studies demanded the recovery of more abstract information not inherent to the item information (namely inter-item) as for example the experimental task in which the item was encountered (Davachi et al., 2003). Hence, for example the study by Ranganath et al. (2004), which used words dyed either in red or green and did not find rhinal activation related to associative binding, could be explained by the fact that the color could not be integrated within the meaning of the word but rather presented unrelated contextual information. This finding was recently replicated and extended, when Uncapher and colleagues found a hippocampal but no rhinal effect, when memory formation for color–word and location–word associations was probed (2006). Hence, the literature described here is in line with our idea that the rhinal cortex is involved in associative memory formation if within-domain integration is accomplished locally.

Moreover, there is more support for a role of the rhinal cortex in associative memory formation. Firstly, there is neuroanatomical evidence for associative connections not only within the hippocampus (for reviews, see Eichenbaum and Cohen, 2001; Brown and Aggleton, 2001), but also within the rhinal cortex (for review, see Lavenex and Amaral, 2000). Secondly, animal data using electrophysiological (Sakai and Miyashita, 1991; Higuchi and Miyashita, 1996) and lesion techniques (Burwell et al., 2004) also report an engagement of rhinal cortex in forming associations. Turning to the neuroimaging literature, Law et al. (2005) found that activation in parahippocampal and rhinal cortices increased with the gradual acquisition of arbitrary associations. Recently, Aminoff et al. (in press) used a new learning paradigm requiring new associations between meaningless visual patterns to show that spatial associations were mainly formed in parahippocampal cortex while nonspatial contexts activated the rhinal cortex. Hence, there is evidence at least supporting our idea that the gradually increasing activation in posterior and anterior parahippocampal gyrus found here can be related to

the gradually increasing associative encoding of item features that can be integrated at an earlier level than the hippocampus. Whether the type of binding is more "intra-item" than "inter-item" has to be determined in future studies. Moreover, it is important to elucidate in how far the more associative role of the parahippocampal gyrus and the rhinal cortex in particular during memory formation corresponds to its involvement during declarative memory retrieval, where it seems to mediate mainly non-associative recognition that can also be achieved on a feeling of familiarity (for example Henson et al., 2003, 2005; Weis et al., 2004a; Montaldi et al., 2006; Fernández and Tendolkar, 2006 for review).

However, though the richness of details in our stimuli probing item memory formation allowed us to gradually manipulate associative memory formation, it is not suited to address the question, whether the hippocampus mediates mere item memory formation only or not. By contrast, the more global activation of the hippocampus across conditions with different amounts of associative memory formation (together with the gradual activation of the rhinal and parahippocampal cortices to the very same conditions) suggests that the hippocampus performs a general purpose process exclusively related to associative memory formation. Thus, its activity does not increase with the number of associations formed when the rhinal cortex has already formed within-domain associations. Our data speak therefore for a more complex relationship between certain mediotemporal operations on one hand and associative memory formation on the other hand. For instance, when associations cannot be formed in the inferior temporal cortex, because the features to be bound are represented in widely distributed brain areas (object/location or face/name), the hippocampus has to add a binding operation in order to establish a coherent memory trace. By these means, it is not surprising that every study investigating associative memory formation with previously unrelated information (for example Sperling et al., 2003; Davachi et al., 2003; Jackson and Schacter, 2004; Kirwan and Stark, 2004; Ranganath et al., 2004; Gold et al., 2006; Uncapher et al., 2006) resulted in hippocampal activation.

However, as these studies did not systematically study the amount of association, it is not clear whether this would result in a graded hippocampal activation that is comparable to what we found in rhinal and parahippocampal cortex in our study. To our knowledge, only Staresina and Davachi (2006) used a parametric approach modeling a linear increase across subsequent item recognition, subsequent source recognition and free recall and found a gradual increase of hippocampal activity (next to other regions not relevant for this discussion). But given that there is no straightforward linear relationship between associative memory formation used for subsequent source recognition as opposed to free recall, the absence of a gradually increasing hippocampal activity in our study is not really contradicted by their findings.

4. Conclusion

Our data suggest that the MTL operates as a rather integrated system (Lavenex and Amaral, 2000; Suzuki and Eichenbaum 2000; O'Reilly and Norman, 2002), where the division of labor among the substructures is not absolute (Squire et al., 2004). We propose that the location, where associations are formed within the MTL, depends on their characteristics defined by the location of the feature representation in the neocortex. If associations can be formed neocortically by integration, the hippocampus provides a general purpose mnemonic operation during memory formation and if associations have to be formed across widely distributed neocortical representations, the hippocampus has to provide a binding operation in order to establish a coherent memory trace. Although our results can be interpreted in this way, this conclusion requires further explicit testing. Finally, it is important to remark, that our findings can only speak to memory formation and thus are not susceptible to studies of declarative memory retrieval.

5. Experimental procedure

5.1. *Participants*

Twenty healthy subjects (10 females) aged between 18 and 36 (average=25.1 years) participated in the experiment, which was performed following the guidelines provided by the local medical ethics committee, after giving informed consent. As indexed by an inclusion-interview, none of the subjects used any medication, had a history of drug abuse, head trauma, severe medical illness or color blindness. All subjects were right-handed as indexed by the Edinburgh Handedness Index (Oldfield, 1971).

5.2. *Stimuli*

We selected 360 stimuli out of a larger set of color photographs used in previous experiments showing large spatial layouts of natural landscapes with or without buildings (Weis et al., 2004a,b; Takashima et al., 2006). Photographs were similar in terms of overall visual complexity, brightness and contrast. First, all photographs were transformed in plain gray-scale pictures. Thereafter, for providing a global level of study context photographs were transformed into green-scale (hue 70) and red-scale (hue 255). Different shades of green or red were used to provide a detailed study context (color saturation: 100, 175 and 250; see Fig. 3). Pictures were randomly divided into five sets of 36 pictures with buildings and 36 pictures without buildings. Each photograph was equally often used as study item and as lure item for the recognition memory test across subjects. Moreover, each photograph was almost equally often used in each of the six different color/shade options across subjects.

5.3. *Experimental design*

The experiment consisted of 5 cycles, each containing a study and a test phase. MRI was acquired during both study and test, but only results related to the study phase will be reported here. During each study phase, 48 photographs of either landscapes with or without any buildings and colored in all six color/shade combinations equally often, were shown together with 24 randomly intermixed null-events (black

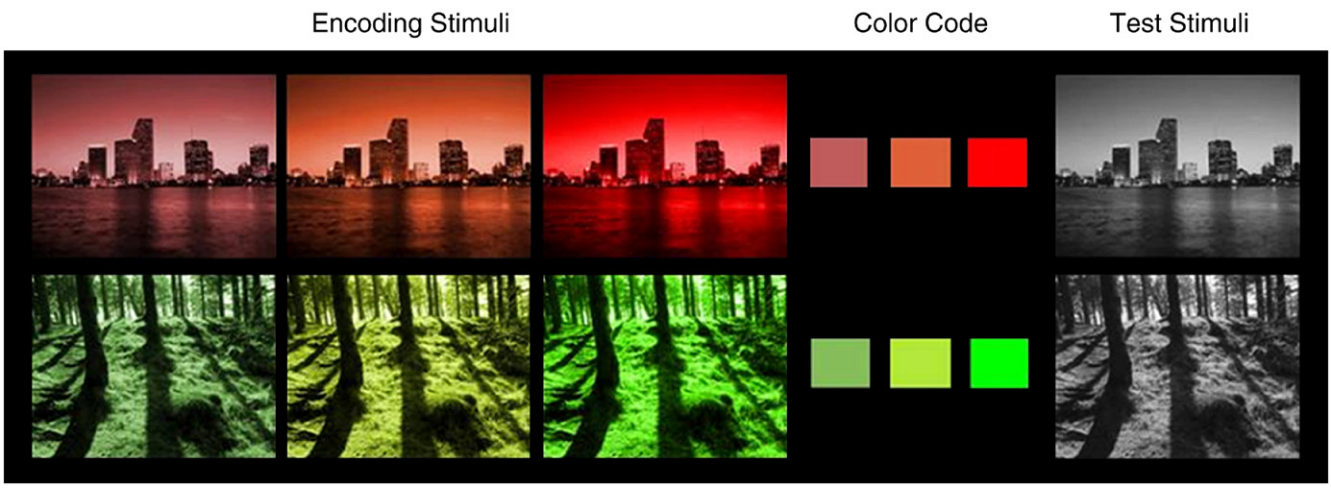

Fig. 3 – Example stimuli presented during study (colored) and test (gray-scaled). For details see Experimental procedures section.

screen with a central fixation cross for 2000 ms). Each photograph was displayed on the center of the screen for 1400 ms. Next, a fixation cross was displayed for a jittered interval lasting 2400 ms to 3700 ms. Finally, a picture with either three red or three green squares (depending on the color of the antecedent picture) was displayed for 1200 ms. During each test phase, the 48 photographs, which were studied during encoding, were presented gray-scaled randomly intermixed with 24 new, previously unstudied, gray-scaled photographs of the same categories. Photographs were again displayed for 1400 ms, followed by a jittered fixation period lasting between 2400 ms and 3700 ms. In addition, 36 null-events were randomly intermixed.

At study, subjects were instructed to encode the photographs firstly by judging covertly whether a building was visible or not and secondly indicating by a button press the color that the pictures were dyed in. During retrieval, the subjects were required to indicate by a button press whether the displayed photograph was either not seen before ("new") or was seen at study and previously presented in red ("old-previously red") or green ("old-previously green"). After a "new"-judgment, no further action was required for this particular item. If the subject judged the photograph to be "old, previously red" or "old, previously green", three red respectively green color-cues with the three different shades were shown, and subjects were required to decide, in which shade the photograph had been presented during the study phase (see Fig. 3). In keeping with previous studies (Weis et al., 2004a,b), for old/new- and shade-decisions, subjects could make use of an "uncertain"-response, in order to avoid a contamination by guesses. The use of the left or right hand for button presses was counterbalanced across subjects. Before scanning, a practice version was given to the participant in the MR scanner, to ensure that the subject was familiar with the entire procedure inside the scanner.

In summary, our experimental manipulation allowed separation of encoding trials into four different trials with regard to subsequent memory: (1) items that were subsequently recognized as being old and for which the correct color and shade was recollected ("*2 source hits*"), (2) items that were subsequently recognized as being old and for which only the correct color was recollected ("*1 source hits*"), (3) items that were subsequently recognized as being old without correct source judgment ("*item hits*"), and (4) items that were subsequently misclassified as new ("*misses*"). Additionally, we combined trial types 1, 2, and 3 together as "*overall hits*" when confirming a rather general subsequent memory effect.

5.4. *Magnetic resonance imaging data acquisition*

During MRI scanning, we acquired with ascending slice acquisition a T2*-weighted echo-planar imaging sequence (Sonata 1.5 T, Siemens, Erlangen, Germany; 33 axial slices; volume repetition time (TR), 2.29 s; echo time (TE), 30 ms; 90° flip angle; slice matrix, 64×64; slice thickness, 3.0 mm; slice gap, 0.5 mm; field of view, 224 mm). For structural MRI, we acquired a T1-weighted MP-RAGE sequence (176 sagittal slices; volume TR, 2250 ms; TE, 3.93 ms; 15° flip angle; slice matrix, 256×256; slice thickness, 1.0 mm; no gap; field of view, 256 mm).

5.5. *Data analysis*

Image pre-processing and statistical analysis was performed using SPM2 (Friston et al., 1995, www.fil.ion.ucl.ac.uk/spm/spm2). After discarding the first five volumes in every run to allow for T1 equilibration effects, the functional EPI-BOLD images were realigned and the subject-mean was co-registered with the corresponding structural MR images using mutual information optimization. Volumes were subsequently slice-time corrected and then normalized to the stereotactic anatomical MNI space. Data were smoothed with an isotropic 3D Gaussian kernel (8 mm FWHM). Functional MRI data were analyzed statistically using the general linear model. The explanatory variables were temporally convolved with the canonical hemodynamic response function provided by SPM2. In addition, the linear model included the subject effects and a temporal high-pass filter to account for various low-frequency effects.

For statistical analyses, relevant contrast parameter images were generated for each subject and these were subsequently subjected to a second-level random effects analysis. In this whole brain analysis, results were initially threshold at $p=0.001$ (uncorrected) and subsequently the cluster-size statistics were used as the test statistics and only clusters significant at $p\leq 0.05$ (corrected for multiple non-independent comparisons) are reported. This analysis was extended by a region of interest (ROI) analysis of the MTL. Given our regional specific hypothesis regarding involvement of the MTL, an ROI was defined separately for the left and right MTL that included the hippocampus, parahippocampal gyrus covering rhinal and parahippocampal cortices as well as fusiform gyrus, because it is also partly covered by parahippocampal cortex using the WFU Pick Atlas toolbox for SPM, which provides a method for generating ROI masks based on the Talairach Daemon database (Maldjian et al., 2003). In these ROI analyses, local maximum test statistics were employed and all reported p-values were corrected for multiple non-independent comparisons based on the family-wise error correction (Friston et al., 1996).

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