Brief Communication

Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity

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Producing and maintaining distinct (orthogonal) neural representations for similar events is critical to avoiding interference in long-term memory. Recently, our laboratory provided the first evidence for separation-like signals in the human CA3/dentate. Here, we extended this by parametrically varying the change in input (similarity) while monitoring CA1 and CA3/dentate for separation and completion-like signals using high-resolution fMRI. In the CA1, activity varied in a graded fashion in response to increases in the change in input. In contrast, the CA3/dentate showed a stepwise transfer function that was highly sensitive to small changes in input.

[Supplemental material is available for this article.]

Computational models of memory suggest a dynamic balance between two processes: *pattern separation* (making similar memories distinct by orthogonalizing the neural representations) and *pattern completion* (reestablishing a past pattern of activity in response to partial or degraded input) (Marr 1971; Treves and Rolls 1994; McClelland et al. 1995; O'Reilly and Norman 2002; Norman and O'Reilly 2003). The dentate gyrus (DG), with its sparse coding granule cells, is the hypothesized source of the separation signal, which should also be observable immediately downstream in CA3 via strong mossy fibers (Treves et al. 2008). Rodent electrophysiology has shown that place fields in CA3 are more likely to fully remap than CA1 across similar environments (separation) (Leutgeb et al. 2004; Kesner 2009). Likewise, immediate early gene (IEG) expression in CA3 in response to environmental change is discontinuous, whereas the response profile of CA1 varies incrementally as change in input increases (Vazdarjanova and Guzowski 2004).

Recently, we observed separation-like activity in the human hippocampus, most strongly in the CA3/DG (Bakker et al. 2008) using high-resolution BOLD fMRI. Participants viewed novel and repeated images as well as similar lures. We selected repetition-sensitive voxels and hypothesized that in areas exhibiting pattern separation, lures would have activity similar to that of first presentations. In contrast, areas exhibiting pattern completion would show lure activity similar to that of repetitions. We observed separation-like activity in CA3/DG and completion-like activity in CA1 (Fig. 1A).

The current study extends our previous findings by incrementally varying the change in input to test whether CA1 and CA3/DG have different transfer functions (Guzowski et al. 2004; Leutgeb et al. 2007; Leutgeb 2008; Kumaran and Maguire 2009). Small changes in input should be sufficient to cause separation-like activity in CA3/DG as was seen by the remapping of place cells in the rodent CA3 across highly similar environments (Leutgeb et al. 2004). Thus, we predict that for small changes in the stimulus, CA3/DG activity should be similar to that of first presentations. In contrast, CA1 activity should vary continuously with change in the input rather than showing an abrupt step function. Thus, CA3/DG and CA1 should have different levels of activity for small changes in input, but should converge at large changes in input, which should drive separation in both regions.

We parametrically varied change in input by using pairs of lures (Fig. 2) that had been separately evaluated for their mnemonic similarity (see Supplemental material). Eighteen participants (six males, 12 females; mean age = 20.8, SD = 2.8) performed an incidental encoding task indicating, via button press, whether images were of indoor or outdoor objects (2 sec each, 0.5 sec ISI). No memory test was administered. Images could be exact repetitions of previously shown images (repetitions), new images (first presentations), or images similar to those previously shown (lures).

A 3T Philips scanner was used to acquire high-resolution (1.5 mm isotropic) fMRI data of the hippocampus (see Kirwan et al. 2007; Supplemental material) and a whole-brain 0.75-mm isotropic MPRAGE structural scan. Data analysis was done using the Analysis for Functional NeuroImages (AFNI) software (Cox 1996).

Four vectors of interest were specified: (1) First presentations of subsequent repetitions or lures, (2) repetitions of previous items, (3) high-similarity lures, and (4) low-similarity lures (see Supplemental material). Foil items presented only once served as an implicit, nonzero baseline condition against which all estimates of each trial type were compared. The vectors were used to individually model each participant's functional data using a deconvolution approach based on multiple linear regressions (Ward 2001). The sum of the resultant fit coefficients over the expected hemodynamic response (~3–12 sec after trial onset) was taken as the model's estimate of the response to each trial

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Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.1971110.

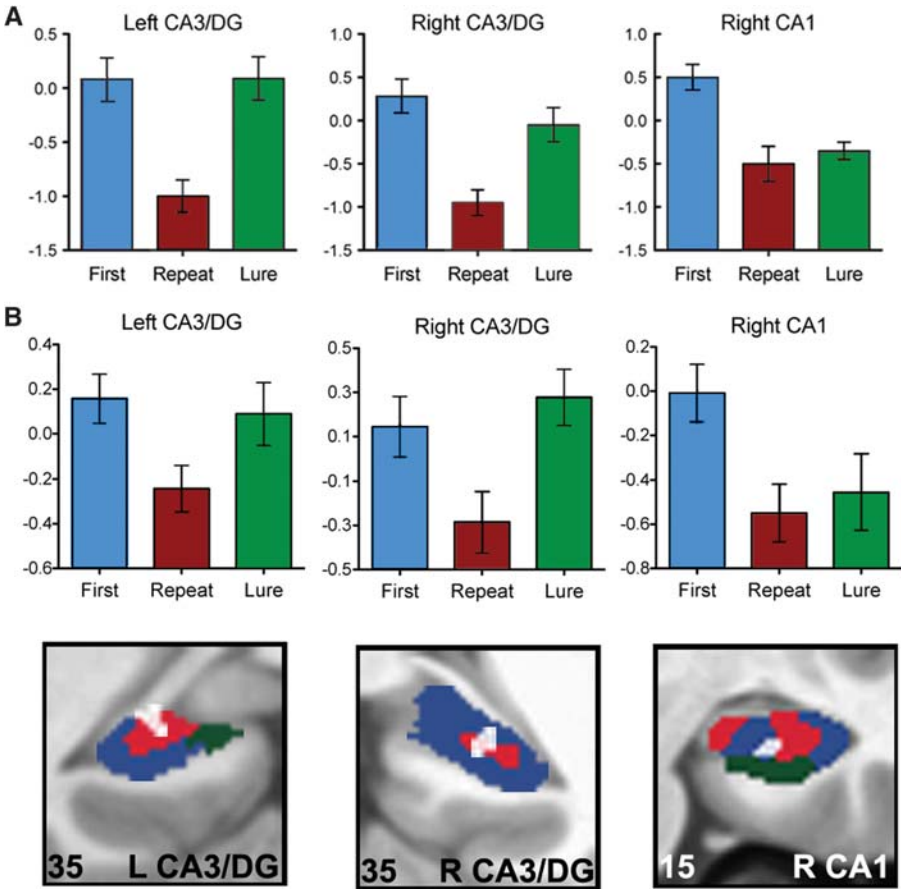

Figure 1. Mean activity (summed beta coefficients) of each trial condition from regions of interest in Bakker et al. (2008) (*A*) and the current study (*B*) as described in the text. A model segmentation of hippocampal subfields is overlaid on each brain slice to indicate the location of the subiculum (green), CA1 (blue), and CA3/DG (red). Regions of activity within the hippocampus are shown in white and labeled within each slice in the *bottom-right* corner. Distance of each slice from the anterior commissure ($y = 0$ in Talairach coordinates) is indicated for each slice in the *bottom-left* corner.

type. Cross-participant alignment was performed using Region of Interest-Advanced Normalization Tools (ROI-ANTS) (Avants et al. 2008; Klein et al. 2009; Yassa et al. 2010) based on regional alignment methods developed in our laboratory (Miller et al. 2005; Yassa and Stark 2009). For details on these methods see Supplemental material and Supplemental Table 1.

Group analysis began with a *t*-test to identify repetition-sensitive voxels (first vs. repeat condition: $P < 0.05$, 20 contiguous voxels), using our alignment model to localize activity to specific subregions within the hippocampus. The choice of a somewhat liberal statistical threshold for voxel selection reduces voxel selection biases for this initial filtering step (Baker et al. 2007). While repetition-sensitive voxels were found throughout the MTL (CA1, CA3/DG, subiculum, entorhinal, perirhinal, and parahippocampal cortices), we focus here on the CA1 and CA3/DG to address the specific hypotheses about their transfer functions. Following voxel selection and classification, mean beta coefficients for each trial type of interest, each region, and each participant were calculated. Completion-like activity was defined as activity during lures being significantly different from the activity of first presentations, but not significantly different from the activity of repetitions. Separation-like activity was defined as activity during lures being significantly different from the activity of repetitions, but not significantly different from the activity of first presentations.

Consistent with Bakker et al. (2008), we found regions in right CA1 that were consistent with pattern completion and regions in bilateral CA3/DG that were consistent with pattern separation (Fig. 1B). Three regions in both CA1 and CA3/DG differed between first presentations and repetitions. Within right CA1, two regions were consistent with completion-like activity as defined above (first vs. lures, $t_{(17)} = 2.27$, $P < 0.05$ and $t_{(17)} = 3.54$, $P < 0.01$; repetitions vs. lures, $t_{(17)} = 0.52$, n.s. and $t_{17} = 1.00$, n.s.) ambiguous region in left CA1 had lure activity that did not differ from first presentations or repetitions (first vs. lures, $t_{(17)} = 1.48$; repetitions vs. lures, $t_{(17)} = 2.01$). Within bilateral CA3/DG, two regions were consistent with separation-like activity as defined above (first vs. lures, left $t_{(17)} = 0.60$, n.s. and right $t_{(17)} = 0.83$, n.s.; repetitions vs. lures, left $t_{(17)} = 2.21$, $P < 0.05$ and right $t_{(17)} = 3.31$, $P < 0.01$). One ambiguous region in the right CA3/DG had lure activity that was significantly different from both first presentations and repetitions (first vs. lures, $t_{(17)} = 2.43$, $P < 0.05$; repetitions vs. lures, $t_{(17)} = 2.33$, $P < 0.05$).

We next asked whether CA1 and CA3/DG responded differently to the amount of change in input (mnemonic similarity). We split the lure stimuli at the median of their mnemonic similarity (see Supplemental material) and analyzed these two sets of

Figure 2. Examples of stimuli and their lures. Stimuli with high mnemonic similarity are shown at the *far left* and stimuli with low mnemonic similarity are shown at the *far right*.

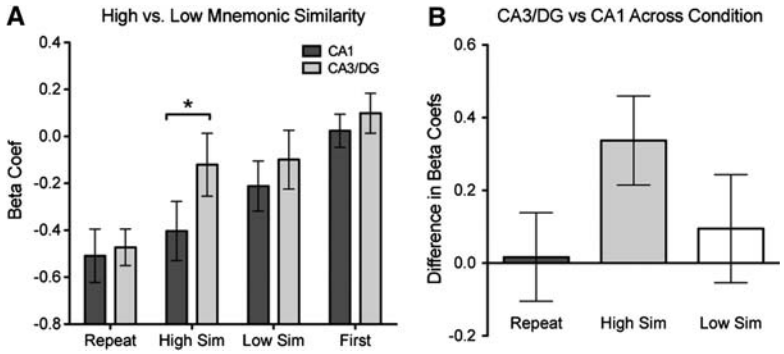

Figure 3. (*A*) Lures split into high mnemonic similarity and low mnemonic similarity. Average CA3/DG activity for high similarity lures was significantly larger than average CA1 activity. There was no difference between CA3/DG and CA1 for low similarity lures. (*B*) A one-way ANOVA conducted on the difference between the beta coefficients of CA3/DG and CA1 revealed a significant main effect across conditions.

lures separately. A clear difference in the transfer function in CA1 and CA3/DG emerged (averaging across all regions described above) (Fig. 3A). CA1 responded in a seemingly graded fashion to changes in input from none (repetition) to small (high similarity) to moderate (low similarity) to large (first). In contrast, even with a small change in the input, the activity level of CA3/DG increased markedly. For high similarity lures, activity in the CA3/DG was significantly higher than activity in CA1 ($t_{(17)} = 2.22$, $P < 0.05$; Fig. 3A). There was no regional difference in activity for low similarity lures ($t_{(17)} = 0.80$) or for first presentations ($t_{(17)} = 0.89$). These results are consistent with our prediction that we should see a difference between CA1 and CA3/DG at small changes in input, but not at larger changes where activity in both regions should converge.

To further investigate the specific hypothesis that there is a difference between the transfer functions of CA1 and CA3/DG, the difference in the activity of CA3/DG and CA1 was calculated (Fig. 3B). First presentations were not included as these are akin to the baseline and necessarily near zero. A one-way ANOVA revealed a significant main effect across the remaining conditions ($F_{(2,16)} = 3.61$, $P < 0.05$; one outlier ~4 SD removed), supporting the hypothesis that CA3/DG and CA1 have different transfer functions as change in input begins to increase. As change in input continues to increase (similarity decreases), we expect a gradual convergence of CA3/DG and CA1 activity as CA1 "remaps," albeit more incrementally. Evidence for this convergence is seen in the large change in CA3/DG-minus-CA1 activity from high-similarity lures to first presentations ($t_{(17)} = 2.30$, $P < 0.05$) and a more moderate change in CA3/DG-minus-CA1 activity from high-similarity lures to low-similarity lures ($t_{(17)} = 1.90$, $P = 0.08$).

Finally, we asked how small of a change in input was required to elicit this remapping-like signal in CA3/DG. We are limited in terms of our signal-to-noise and the need to have sufficient numbers of trials in each bin for stable data analyses. While the data showed signs of reduced reliability when lure trials were further separated into four bins, a significant difference remained between CA1 and CA3/DG in the highest-similarity lure bin ($t_{(17)} = 2.21$, $P < 0.05$). However, there was no reliable difference in the level of activity between CA1 and CA3/DG in lure bins 2, 3, and 4 (Supplemental Fig. 2). Thus, CA3/DG was sensitive to even very small changes in the input. In contrast, the amount of BOLD activity in CA1 varied incrementally as change in input increased.

We hypothesized that BOLD activity in the CA1 would reveal a graded transfer function as changes in input increased from none (repetitions) to small (high-similarity lures) to moderate (low-similarity lures) to large (first presentations). However, in CA3/DG, we expected that for even relatively small changes in the stimulus, activity would be similar to that of first presentations. For larger changes in the input, we did not expect to see a significant difference between the activity in CA1 and CA3/DG since the pattern of activity in both regions should converge toward separation. Our results were consistent with these predictions and with the animal literature, which has shown a linear transfer function in the CA1 that gradually encodes incremental changes in input (Leutgeb et al. 2005; Wills et al. 2005; Colgin et al. 2010) and has shown that with large differences between environments, place fields in both CA3 and CA1 remapped to new environments (Leutgeb et al. 2004).

We should note that in the rodent, there are some reports of greater separation in CA1 than in CA3 with small changes in input (for review, see Guzowski et al. 2004). While it is possible that the amount changed here was insufficient to see this reversal, this may not explain the results as the changes here appear to be quite small. It is quite possible though that the nature of the change in input is not comparable or that the combination of CA3 and DG in our data make it such that we would not be sensitive to a smaller separation signal in CA3 than in CA1. Along the same lines, there are also reports of the CA3 appearing to have more of a linear transfer function akin to the CA1 (Leutgeb et al. 2005, 2007; Leutgeb and Leutgeb 2007) than the stepwise function seen in Leutgeb et al. (2004) and Vazdarjanova and Guzowski (2004). It is unclear as to what the cause of this discrepancy might be, however, the processes of pattern separation and completion are dynamic functions and may depend heavily on the input feeding into the hippocampus.

Our high-resolution fMRI is currently unable to reliably separate CA3 from DG, so we must treat this region as CA3/DG. As the CA3 receives very strong "detonator" input from the DG along the mossy fibers (Urban et al. 2001), separation-like activity should be observable in both. However, this does lead to a potential mix of signals in our results. Future advancements in imaging technology may allow us to separate the two and better test potentially dissociable roles of the DG and CA3 in orthogonalization (e.g., Leutgeb et al. 2007).

One interesting observation from our data is that the BOLD activity in CA3/DG during lures does not match participant responses on lure trials in the behavioral task described in the Supplemental material. The behavioral data indicated a graded shift from completion-like responses toward separation-like responses (Supplemental Fig. 1B), much like the activity pattern observed in CA1, while the BOLD activity in CA3/DG showed a transition, especially in items with high overlap (lure bin 1) (Supplemental Fig. 2; Myers and Scharfman 2009). One possible reason for this incongruence is that in the present fMRI experiment there is no overt memory task. Participants only indicated if each image was an indoor or an outdoor item. We purposefully chose an incidental task to match the experimental design of our previous work (Bakker et al. 2008), as well as the incidental nature of random foraging tasks typically used in rodents. This design has an advantage over explicit recognition memory designs, as it is a more process-pure approach to engaging separation processes. An explicit memory test may cause subjects to participate in a

"recall to reject" strategy in which they recall a previous item (pattern complete) in order to reject a lure item (pattern separate). An incidental task minimizes this strategy by removing task instructions that could bias participants toward it. This concern aside, when an explicit task is present, both CA3/DG and CA1 can exhibit similar separation-like activity (Kirwan and Stark 2007). It is also important to note that overt task performance is subject to the individual participants' decision-making criteria, so that even if separation signals are elicited by the hippocampus, in some cases this may not be enough to trigger overall behavioral discrimination.

It is possible that when the parametric scale of mnemonically similar stimuli was created (see Supplemental material) participants were unable to perceptually distinguish similar items or that their responses were driven by recognition confidence. To test these hypotheses we ran two control experiments. The first evaluated whether participant confidence influenced behavior. The second evaluated whether or not lure stimuli were perceptually distinguishable. We found that participant confidence was not a significant confound as participants showed high rates of "confident" or "highly confident" responses across conditions and trial types (Supplemental Table 2). They were equally confident when they correctly identified a lure as similar to a previous item as they were when they incorrectly identified a lure as an exact repetition of a previous item. Second, we found that participants were able to distinguish between similar lure items during a perceptual similarity/working memory task (see Supplemental material), suggesting that their inability to pattern separate highly similar lures was not entirely a consequence of being unable to perceive the differences, but rather predominantly due to a failure in mnemonic pattern separation.

In conclusion, in addition to replicating our previous work (Bakker et al. 2008), the current study extended these findings by evaluating regional differences in BOLD activity across conditions and as a function of change in input. The results support the hypothesis that CA1 and CA3/DG have distinct pattern separation related transfer functions. CA3/DG is sensitive to small changes in input and is able to flexibly shift its representation in a stepwise-like manner, whereas CA1 is more resistant to small changes and responds incrementally. These data fit the predictions from the computational models (for review, see Treves and Rolls 1994; Norman and O'Reilly 2003) and are consistent with the rodent electrophysiology and IEG literature (e.g., Guzowski et al. 2004; Kesner 2009).

Acknowledgments

This work was supported by grants from the NIH (R03 AG032015) and the NSF (BCS 0544959), and by UC Irvine. We thank Samantha Rutledge, Megan Deaton, Tania Gutierrez, Marlo Asis, Gianna O'Hara, and Greg Sanchez for their assistance with data collection.

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Received August 4, 2010; accepted in revised form October 10, 2010.

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Learn. Mem. 2011, **18:**
Access the most recent version at doi:10.1101/lm.1971111