A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation

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The dentate gyrus (DG) of the mammalian hippocampus is hypothesized to mediate pattern separation—the formation of distinct and orthogonal representations of mnemonic information—and also undergoes neurogenesis throughout life. How neurogenesis contributes to hippocampal function is largely unknown. Using adult mice in which hippocampal neurogenesis was ablated, we found specific impairments in spatial discrimination with two behavioral assays: (i) a spatial navigation radial arm maze task and (ii) a spatial, but non-navigable, task in the mouse touch screen. Mice with ablated neurogenesis were impaired when stimuli were presented with little spatial separation, but not when stimuli were more widely separated in space. Thus, newborn neurons may be necessary for normal pattern separation function in the DG of adult mice.

The dentate gyrus (DG) is thought to contribute to spatial or episodic memory by functioning as a pattern separator (1–3). Pattern separation is the formation of distinct representations of similar inputs (4). At the cellular level, pattern separation is achieved through the dispersion of cortical inputs from the entorhinal cortex onto a greater number of dentate granule cells (DGCs) with small place fields. By virtue of low firing rates (5) and sparse connectivity between DGCs and CA3 pyramidal cells (6), DGCs are particularly adapted to maintain and transmit orthogonalized information. This ability to pattern separate, or to differentially encode small or interfering inputs, is particularly important for the accuracy of memory encoding. Similarly, at the behavioral level, the ability to form and use memories derived from very similar stimuli that are closely presented in space and/or time depends on the ability to pattern separate incoming, and often complex, information (7, 8). Lesions of the complete DG circuitry result in impaired pattern separation—dependent memory (7–9).

The DG is also one of two sites where neurogenesis is ongoing throughout life (10). Adult-born neurons integrate into DG circuitry (11–13) and are thought to play a role in learning and memory (11, 14, 15), but their contribution to hippocampal function remains unclear, in part because of the limited availability of behavioral assays probing this question. We used low-dose x-irradiation (16) to focally ablate neurogenesis in the hippocampus of 8-week-old adult female C57Bl/6 mice (17, 18), while sparing the rest of the brain, including the subventricular zone (Figs. 1A, C to 2, A, G, and H, and Figs. S1, A and B, S2, S3, and S4). To confirm that newborn neurons had been persistently ablated as well as to examine the extent of inflammation in the hippocampus after a 2-month recovery period post-ablation, we analyzed the brains of irradiated (IR) and sham test mice (n = 5) that were killed the day behavioral testing commenced. IR test mice did not show differences in microglia numbers or morphology compared to sham controls (fig. S1, C and D), but they did show a statistically significant reduction in total numbers of both immature neurons and proliferating cells in the hippocampus (fig. S2, A to E).

Two months after irradiation, IR (n = 10) and sham (n = 9) mice were tested in a delayed nonmatching to place (DNMP) radial arm maze (RAM) task that we developed to test spatial pattern separation—dependent memory (Fig. 1). As we had hypothesized that deficits resulting from a knock-down of neurogenesis might be subtle, we purposely designed a challenging spatial task by using a large eight-arm RAM and ensuring the use of external spatial cues in forming spatial memories while eliminating odor as a facilitatory intramaze cue. The difficulty of this task was reflected in lower performance levels by sham mice compared with other RAM tasks (19). Mice were tested for the ability to select, from a choice of two, the arm location that had not been presented in a previous sample phase (DNMP) (Fig. 1E). During the sample phase, all arms except a start arm and the sample arm were blocked off. The mouse was permitted to visit the sample arm and retrieve a food pellet reward. To eliminate the ability of mice to use odor as a facilitatory intramaze cue, the RAM apparatus was rotated on wheels between sample and choice presentations, so that the locations of the start and sample arms, but not the arms themselves, were held constant during each trial. The rotation took ~20 s. During the choice phase, arms in the start and sample (unrewarded) locations and an additional correct (rewarded) location were open. Correct arms varied in distance from the sample arm by a spatial separation of two, three, or four arms (Fig. 1D). Mice that entered the correct (rewarded) arm were considered to have made correct choices. Mice that made incorrect choices (i.e., entered the sample/unrewarded arm) were allowed to self-correct. Mice went through four trials (sample plus choice phases) per day of pseudorandomly presented combinations of start plus sample plus correct arms for 15 consecutive days (60 trials total, 20 trials of each spatial separation) (16).

We analyzed pattern separation—dependent memory by testing whether mice could differentiate between locations that were presented closely in space (separation 2 (S2)) versus those that were...
more highly separated (S3 and S4). IR mice were selectively impaired at low separations (S2) but not at high separations (S3 and S4) [significant group × separation interaction, repeated measures analysis of variance (ANOVA): $F_{1,17} = 4.57, P = 0.047$; Bonferroni corrected $t$ tests: $S2: t(17) = 2.55, P = 0.021; S3+S4: t(17) = 0.03, P = 0.974$] (Fig. 1F). These results suggest that adult hippocampal neurogenesis was not required to perform the task in which sample and correct arms were presented with a high degree of spatial separation (S3 and S4) but was required to correctly discriminate between choice and sample arms when presented in close spatial proximity.

To further examine whether loss of adult hippocampal neurogenesis results in global hippocampal deficits or specific pattern separation memory deficits, we tested naive cohorts of IR mice with ablated neurogenesis due to focal x-irradiation show impaired spatial memory for similar, but not distinct, spatial locations in the radial arm maze. (A) Mice were irradiated 2 months before behavioral testing. (B and C) Irradiation significantly reduced the total numbers of immature Dcx+ cells in DG regions. (D) Pattern separation was tested using a DNMP protocol in the RAM by varying the distance between sample and correct arms: S2, low; S3 and S4, high (S, start arm). (E) Each trial consisted of a sample phase (left) and a choice phase (right). The mouse had to nonmatch to the new location. (F) IR mice were impaired at low (S2) but not high (S3 and S4) separations during acquisition (correct spatial location between two illuminated choice boxes in two out of five possible locations until a criterion (seven out of eight consecutive touches) was reached. Once the criterion was met, the correct and incorrect locations automatically switched. Similar to the DNMP task in the RAM, pattern separation was tested by varying the distance between choice locations. Hit choice boxes were either far apart [separated by three unlit spaces (high separation, S4; Fig. 2E)] or close together [separated by one unlit space (low separation, S2; Fig. 2E)]. Spatial separations were held constant during each testing session per day but were varied across testing days.

In agreement with our findings using the RAM, IR mice were significantly impaired at low (S2) but not high (S4) separations during acquisition [significant group × separation interaction, repeated measures ANOVA, average trial to criterion: $F_{1,17} = 6.04, P = 0.025$; Bonferroni corrected $t$ tests: $S2: t(17) = 2.54, P = 0.020$; S4: $t(17) = 0.63, P = 0.540$] (Fig. 2F). Ablating neurogenesis with the use of focal x-irradiation induces impairments consistent with a deficit in pattern separation in two independent tasks carried out in two very different testing situations. This impairment appears to be specific, as IR mice were capable of learning difficult object-place associations (PAL) at the same rate and to the same performance level as sham mice. Furthermore, the spatial memory deficits observed were similar in both the navigable RAM and non-navigable touch screen.

Inhibition of Wnt signaling locally in the DG reduces the number of newborn neurons without affecting progenitor proliferation in other brain regions (23, 24). Eight-week-old C57Bl/6 female mice received bilateral stereotaxic injections of 1 μl of either dnWnt-expressing lentivirus ($n = 16$) or GFPcon lentivirus ($n = 15$) into the DG, resulting in a significant reduction in proliferating cells and neurogenesis [Fig. 3, A and B, and fig. S5]. Behavioral testing in the RAM commenced 2 months after viral injection. Similar to the pattern separation deficit observed in IR mice, dnWnt mice were impaired at low (S2) but not high (S3 and S4) separations compared with GFPcon mice [significant group × separation interaction, repeated measures ANOVA: $F_{1,24} = 4.51, P = 0.044$; $t(24) = 2.11, P = 0.044$; $t(24) = 2.55, P = 0.021$; $t(24) = 0.03, P = 0.974$] (Fig. 2G).
Fig. 2. Mice with ablated neurogenesis due to focal x-irradiation show impaired spatial discrimination for similar but not distinct spatial locations, but not impaired associative object-in-place memory, in the mouse touch screen. Mice were irradiated 2 months before behavioral testing as in (A). After pretraining for 7 to 10 days in which mice learned to nose-touch stimuli on the infrared touch screen (B) to obtain a reward, mice were trained on an associative object-in-place task (PAL) (C). For example, as in the left panel of (C), mice had to choose a flower at left as a correct association over the incorrect association of a plane at right to obtain a reward. (D) IR mice learned the PAL task at the same rate as sham controls (horizontal black line represents chance). (E) Mice with ablated neurogenesis due to targeted lentiviral expression of dnWnt show impaired spatial memory for similar, but not distinct, spatial locations in the radial arm maze in a similar pattern to that seen in irradiated mice. (A and B) dnWnt expression [right in (B)] significantly reduced the total numbers of immature Dcx+ cells in dnWnt mice (A) compared with GFP controls [left in (B)] [independent samples t test: t(24) = 3.47, P = 0.002]. Thus, mice with decreased neurogenesis due to expression of the dnWnt protein are impaired at spatial pattern separation or the ability to correctly distinguish rewarded from nonrewarded spatial locations only when stimuli to be discriminated are presented closely in space.

This study provides experimental evidence of a role for newborn neurons in the adult DG in spatial discrimination, consistent with a role in spatial pattern separation. We used two independent strategies to ablate neurogenesis, and the observed deficits were similar in two very distinct testing contexts. Mice with ablated neurogenesis showed a selective impairment specific to memory performance depending on pattern separation but were not impaired in the hippocampus-dependent PAL task, indicating that mice were able to learn complex associations in which space was a component.

Previous studies involving rodent lesions of either the dorsal hippocampus (22) or the DG (7–9) suggest that regions outside of the DG are responsible for disambiguating memories derived from spatially distinct inputs (comparable to the large separations used in this study). In addition, it has been suggested that recruitment of independent cell populations in the CA3 alone, presumably via direct input from the entorhinal cortex (25), may be sufficient to disambiguate memories for more distinct spatial inputs (1, 26, 27) or make associations between objects and space (28). In this case, the CA3 might be responsible for “remembering” when objects were present in a specific spatial location, whereas the DG might “remember” when those objects were present at more distinct positions in space.
IRAP Identifies an Endosomal Compartment Required for MHC Class I Cross-Presentation

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Major histocompatibility complex (MHC) class I molecules present peptides, produced through cytosolic proteasomal degradation of cellular proteins, to cytotoxic T lymphocytes. In dendritic cells, the peptides can also be derived from internalized antigens through a process known as cross-presentation. The cellular compartments involved in cross-presentation remain poorly defined. We found a role for peptide trimming by insulin-regulated aminopeptidase (IRAP) in cross-presentation. In human dendritic cells, IRAP was localized to a Rab14+ endosomal storage compartment. Cross-presentation involves IRAP in endosomes and involves the related aminopeptidases in the endoplasmic reticulum.

Peptide ligands for MHC class I molecules are produced by intracellular proteases (1). Initial antigen degradation by cytosolic proteasomes is frequently followed by N-terminal peptide trimming, which can occur in the cytosol and by the endoplasmic reticulum (ER) aminopeptidases (ERAPs) (2). Peptides are transported into the ER by the transporter associated with antigen processing (TAP) for loading of newly synthesized MHC class I molecules. Loading of MHC class I molecules with internalized, cross-presented antigens in dendritic cells (DCs) is thought to play an important role in priming of CD8+ T cell responses to pathogens and tumors, as well as in immune tolerance to self.

While screening crude microsome lysates for peptidases involved in N-terminal trimming of human leukocyte antigen (HLA) class I ligands, we identified insulin-regulated aminopeptidase (IRAP). IRAP was detected as an interferon γ (IFN-γ)–induced activity trimming a fluorogenic Leu–aminomethyl coumarin (AMC) substrate in anion exchange chromatography (Fig. 1A) (3). The peak containing IRAP also trimmed a precursor of the HLA–A2–restricted epitope SLNYTVATIL (4, 5). IRAP is a ubiquitin-zinc-dependent aminopeptidase closely related to ERAP1 and ERAP2 (6). IRAP localizes to regulated endosomal storage compartments in adipocytes and muscle cells together with the glucose transporter Glut4; these compartments are termed Glut4 storage vesicles (GSVs) (7). Signaling through the insulin or immunoglobulin E (IgE) receptors induces rapid translocation of ~50% of IRAP to the cell surface (7, 8). The function of the compartment storing IRAP in other cell types, such as DCs, remains unknown.

To evaluate whether IRAP qualifies as a trimming aminopeptidase, we tested its sub-

References and Notes
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Materials and Methods
Figs. S1 to S5
References
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