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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 weak changes derived from increasingly similar 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 (1, 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. 1, A to C and 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-irradiation, 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 arms, 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 (un-rewarded) locations and an additional correct (re-warded) 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

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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 a naive cohort of IR ($n = 10$) and sham ($n = 9$) mice on a challenging hippocampus-dependent spatial learning task and a two-choice spatial discrimination (pattern separation) task in the mouse touch screen (Fig. 2) (16). The mouse touch screen is useful in that all trials are directed by the mouse through an initiation process, and all testing is independent of the experimenter. The testing apparatus (Fig. 2B) consisted of a standard modular chamber fitted with an infrared

touch screen, a pellet dispenser, a receptacle with light illumination and head entry detectors, and a tone generator. Mice were pretrained through several iterative stages to nose-touch stimuli on the screen to obtain a reward (20). Mice were then trained on a paired associates learning (PAL) object-in-place task (21) that tests the ability to correctly associate three objects (flower, plane, and spider) with their correct spatial locations on the screen (left, middle, and right, respectively) (Fig. 2C). Mice were only rewarded when they identified the correct object in its correct location during a choice between two objects: one object in its correct spatial location and one object in one of two incorrect locations. Mice were given 36 trials per day plus correction trials over 55 days. Both IR and sham mice learned the task at the same rate (repeated measures ANOVA, $F_{53,742} = 0.18$, $P = 0.671$) (Fig. 2D). The demonstration that the performance of IR mice was not different from that of sham mice on this hippocampus-dependent spatial task indicates that mice without neurogenesis are still capable of acquiring, at a normal rate, a complex task involving spatial information on the touch screen.

These IR and sham mice were next tested for spatial discrimination ability in the touch screen

with the use of a hippocampus-dependent two-choice spatial discrimination paradigm (22) (Fig. 2E). Briefly, mice were required to choose the correct spatial location between two illuminated 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. Lit 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.

Given concerns regarding potential off-target effects due to focal x-irradiation, despite the long recovery period, we employed a second independent method to knock down neurogenesis, with the use of lentiviral expression of dominant negative Wnt (dnWnt) protein, and then tested these mice on the same RAM protocol (Fig. 3 and fig. S5A) used in the first experiment described above. We used the previously described lentiviral vectors expressing cytomegalovirus-driven dnWnt followed by an internal ribosomal entry site (IRES)–green fluorescent protein (GFP) from the same vector or a control vector expressing only GFP (GFPcon) (16, 23).

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: $F_{1,24} = 4.51$, $P = 0.044$;
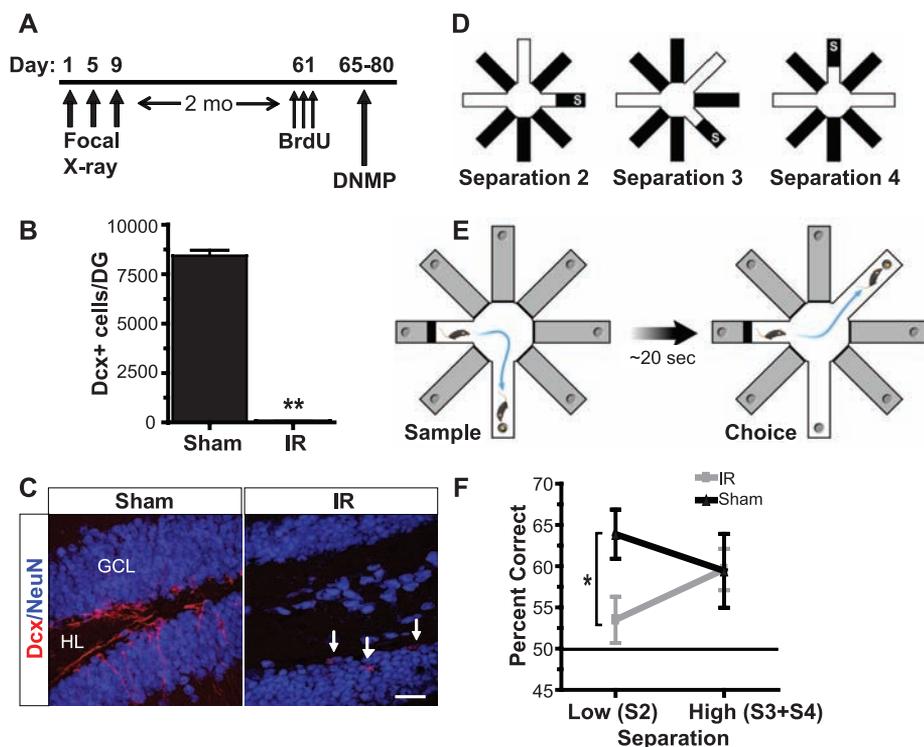

Fig. 1. Mice with ablated neurogenesis due to focal x-irradiation show impaired spatial memory for similar, but not distinct, spatial locations in the radial arm maze. (**A**) Mice were irradiated 2 months before behavioral testing. (**B** and **C**) Irradiation significantly reduced the total numbers of immature Dcx+ cells in IR mice [white arrows at right in (C)] compared with sham controls [(C), left] [independent samples t test, $t(17) = 29.82$, $P < 0.001$]. GCL, granule cell layer; HL, hilus. (**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 in the DNMP task. The horizontal black line represents chance. Error bars indicate SEM. Scale bars, 25 μm. **$P < 0.01$; *$P < 0.05$.

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 were then tested on a two-choice spatial discrimination task in which they had to respond to the correct location [e.g., left illuminated box of left screen in (E)] until a criterion of seven out of eight consecutive correct touches was recorded before reversing to the previously incorrect location [e.g., right illuminated box of left screen in (E)]. Mice were tested on either the low (S2, left screen) or high (S4, right screen) separation, as depicted in (E) during each testing day. (**F**) IR mice exhibited significantly impaired performance at low (S2) but not high (S4) separations during acquisition of this task, consistent with a pattern separation deficit similar to that observed in the first experiment (Fig. 1). (**G** and **H**) Irradiation significantly reduced the total numbers of immature Dcx+ cells in IR mice [right in (H)] compared with sham controls [left in (H)] [independent samples t test, $t(17) = 18.14$, $P < 0.001$]. Error bars indicate SEM. Scale bars, 50 μm. **$P < 0.01$; *$P < 0.05$.
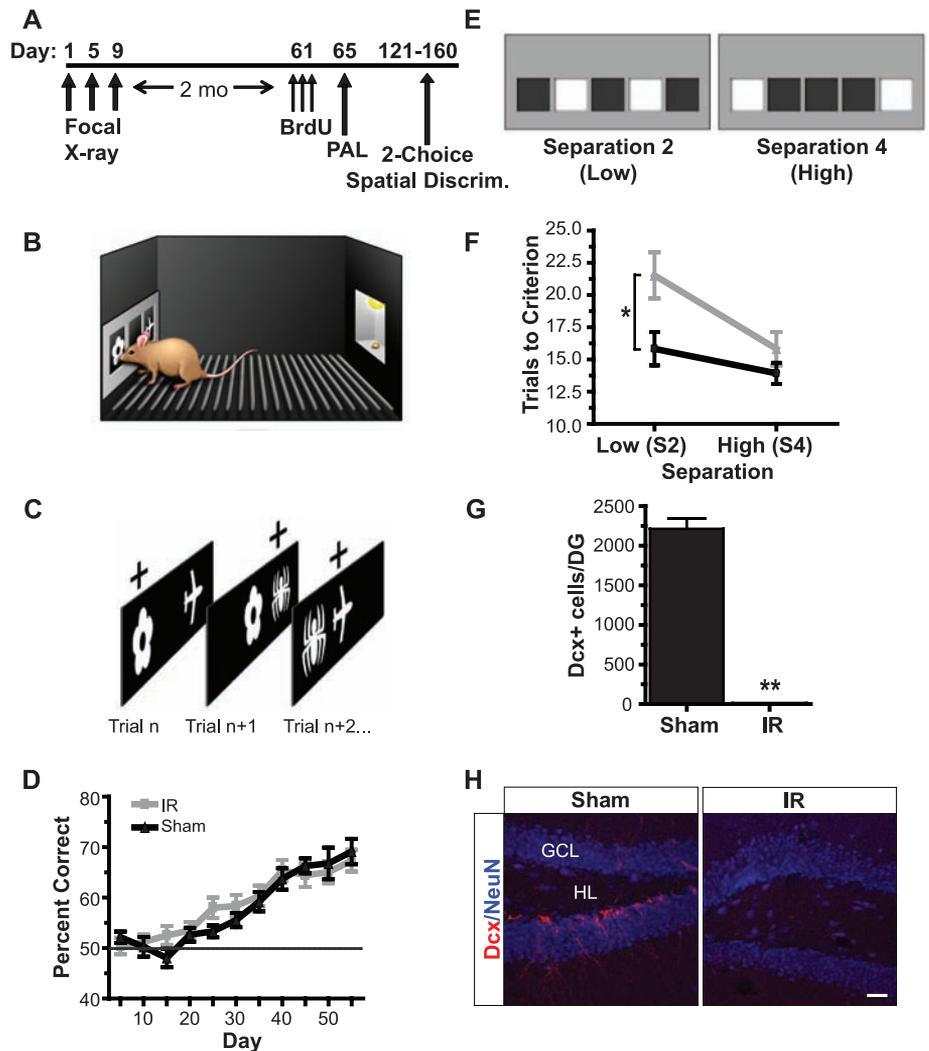

Bonferroni corrected t tests: S2: $t(24) = 3.02$, $P = 0.006$; S3 and S4: $t(24) = 0.46$, $P = 0.926$] (Fig. 3C). Thus, mice with decreased neurogenesis due to expression of the dnWnt protein are impaired at spatial pattern separation or the ability to correctly distinguish rewarded from non-rewarded 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 at 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

Fig. 3. Mice with decreased 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: $t(24) = 3.47$, $P = 0.002$]. Single channel images depicting Dcx+ cells are shown below triple channel images in (B). (**C**) Pattern separation was tested using a DNMP as in Fig. 1. dnWnt mice were impaired at low (S2) but not high (S3 and S4) separations in the DNMP task. The horizontal black line represents chance. Error bars indicate SEM. Scale bars, 50 μm. **$P < 0.01$; *$P < 0.05$.
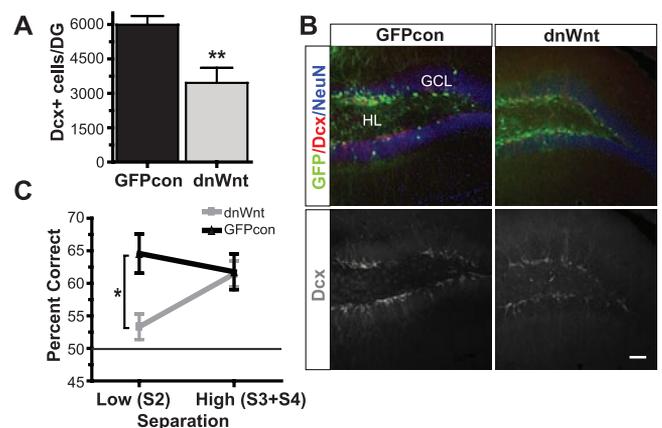
from spatially distinct inputs (comparable to the large separations used in this study). In addition, it has been suggested that recruitment of independent cell populations in the CA3 alone, presumably via direct input from the entorhinal cortex (*25*), may be sufficient to disambiguate memories for more distinct spatial inputs (*1*, *26*, *27*) or make associations between objects and space (*28*). In this

context, it is interesting to note that the impairments after ablation of neurogenesis described here are parameter-sensitive (i.e., specific to conditions with a high premium on pattern separation), which may help to explain the variable and sometimes contradictory results from other neurogenesis-ablation studies in which this parameter was not explicitly considered (*18*, *24*, *29*, *30*).

dnWNT mice with a ~50% decrease in neurogenesis had a similar pattern of impairment to that seen in the IR group in which neurogenesis was almost completely ablated, suggesting that there may be a critical threshold for the amount of neurogenesis that is behaviorally relevant. A level-dependent requirement of adult neurogenesis for hippocampus-dependent learning has also been reported in rats (*24*). In addition, our RAM task may be sufficiently challenging that even partial manipulation of newborn neuron numbers is adequate to impair performance.

The DG has been shown to be important for pattern separation, and our results show that adult neurogenesis appears to be important for the ability of the DG to perform that function optimally. It remains to be investigated whether immature neurons contribute to pattern separation directly or whether they contribute in more complex ways to a circuit necessary for normal DG function, as suggested by recent modeling studies (*31*), and whether the function of immature neurons is distinct from that of mature granule cells.

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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 in which it interacted with MHC class I molecules. IRAP deficiency compromised cross-presentation in vitro and in vivo but did not affect endogenous presentation. We propose the existence of two pathways for proteasome-dependent cross-presentation in which final peptide trimming 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 proteasome complexes is frequently followed by N-terminal peptide trimming, which can occur in the cytosol and by 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 SLYNTVATL (*4*, *5*). IRAP is a ubiquitous 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-

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