Induction of Fos and Arc is limited to neurons following behavioral testing

We first examined whether expression of immediate early genes such as Arc and c-fos is limited to neurons in the granule cell layer following behavioral testing. Mice (n=4) were trained in the water maze (6 trials per day for 5 days) and then given a series of 3 probe tests. Using stereological methods, we then evaluated whether Fos+ and Arc+ cells also expressed the mature neuronal marker, NeuN, in the dentate gyrus (Fig. S1). We identified a total of 114 Fos+ cells, and each of these was also NeuN+. Similarly, we identified a total of 121 Arc+ cells, and these were also all NeuN+. This indicates that Fos and Arc expression is limited to neurons following behavioral testing, and therefore all double-labeled cells identified in the dentate gyrus are neuronal.

Induction of Fos in the dentate gyrus following water maze training and testing

To examine whether Fos is selectively upregulated in the dentate gyrus following water maze training and testing two groups of mice were trained in the water maze. Mice in the training only group were trained in the water maze over three days. On the first two days they received six training trials (presented in 2 blocks of 3 trials). On the third day of training, mice received only the first block of three trials. We chose this abbreviated training schedule since in our previous experiments we found that escape latencies reached asymptote by the fourth day of training. Therefore, at this time point significant learning is still occurring and we avoid the high levels of stress typically associated with first day of training. The second group of mice was trained for 5 days (6 training trials per day), and then presented with a series of 3 probe tests one day following the completion of training. Both groups of mice were perfused 90 minutes following the completion of these behavioral procedures. Fos expression in the dentate gyrus induced by training (n=5) or probe testing (n=4) was then compared to levels in home cage control mice (n=5) or mice treated with the chemical convulsant, pentylenetetrazol (n=3) (PTZ; 50 mg/kg). Fos expression in dentate gyrus differed between groups (F(1,13) = 1871.7, P < 0.001) (Fig. S2). Both water maze training and water maze testing induced similar levels of Fos, suggesting that both encoding and retrieval activate a similar pool of neurons. These levels were higher than those in mice taken from their home cage, and much lower than those treated with PTZ. These results establish that Fos is selectively upregulated in the dentate gyrus following water maze training and testing.

Performance of mice across multiple probe tests

To maximize immediate early gene expression in the dentate gyrus, we used multiple probe tests to assess spatial memory. Analysis of performance across tests revealed that some extinction occurred. For example, in Experiment 1, while mice searched selectively in each of the probe tests (Ps < 0.01; paired t-tests), they spent more time in the target zone during the first two tests compared to the final test (F(2,46) = 12.53, P < 0.001; Fishers LSD test, Ps < 0.005) (Fig. S3). While it is possible that extinction effects might complicate the interpretation...
of our experiments, there are at least two reasons why this is unlikely to be the case. First, extinction involves the formation of a new inhibitory memory that competes with the original memory for control of behavior. In Experiment 1, all BrdU+ cells were the same age at the time of the probe tests and, therefore, would be equally likely to be recruited into a new extinction memory. However, we did not find this to be the case. The degree of overlap between the Fos+ and BrdU+ was sensitive to the delay between BrdU treatment and training. Second, blocking protein synthesis in the hippocampus does not disrupt extinction, and so it is likely that extinction of spatial learning depends primarily on extrahippocampal regions.

**Preferential recruitment of adult-generated neurons from the innermost layers of the dentate gyrus into spatial memory networks**

In Experiment 1, around 60% of all BrdU+ cells were located in the innermost fifth of the dentate gyrus, similar to previous reports. To examine whether this subpopulation is preferentially recruited into spatial memory networks we compared Fos expression in BrdU+ and NeuN+ cells within this subregion (Fig. S4). Fos expression was elevated in BrdU+ cells relative to NeuN+ cells only in groups of mice with longer delays between BrdU treatment and training (delay × cell type interaction: F(4,32) = 3.12, P < 0.05). In the 6 week group Fos expression was approximately 10 times higher in BrdU+ cells compared to NeuN+ cells (Fishers LSD test, P < 0.05). These analyses reveal that new neurons located in the innermost fifth of the dentate gyrus may play especially important roles in water maze memory.

**Vast majority of BrdU+ cells are neuronal 10 weeks following BrdU treatment**

In Experiment 1, there was a 10 week delay between BrdU treatment and spatial memory testing. To estimate the proportion of BrdU+ cells that are neurons at this time point, a separate group of mice (n=7) were treated with BrdU (50 mg/kg; 5 days, 2 injections per day) and then perfused 10 weeks later. Within the granule cell layer, around 90% of the BrdU+ cells expressed the mature neuronal marker NeuN. In contrast, less than 2% of the BrdU+ cells expressed the astrocytic marker, GFAP (Fig. S5). Since Fos and Arc expression is limited to neurons following behavioral testing (Fig. S1), our analyses may underestimate the recruitment of new neurons into spatial memory networks in the dentate gyrus in Experiment 1.

**Induction of Arc in BrdU+ cells**

Mice were trained in the water maze (5 days, 6 trials/day) either 1 week (n=11) or 6 weeks (n=8) after BrdU treatment (100 mg/kg; 5 days, 2 injections per day). Spatial memory was assessed one day following the completion of training (Fig. S6). In this retention test, both the 1 w (paired t-test: t(10) = 7.69, P < 0.001) and 6 w (paired t-test: t(7) = 13.23, P < 0.001) groups of mice searched selectively for the platform. Following this retention test, Arc levels were elevated in the BrdU+ cells relative to the NeuN+ population only in the 6 week group (delay × cell type interaction: F(1,14) = 20.67, P < 0.001; Fishers LSD test, P <
This experiment confirms that 6 week-old neurons are preferentially recruited into spatial memory networks in the dentate gyrus. In this same experiment we also estimated the proportion of BrdU+ cells expressing the mature neuronal marker, NeuN, the immature neuronal marker, doublecortin\(^9\), and the astrocytic marker, GFAP. As in all of our quantifications, only BrdU+ cells within the granule cell layer were analysed. In the 1 week group, 83.0 ± 6.2 % of BrdU+ cells were also NeuN+, 33.8 ± 13.7 % were doublecortin+ and only 1.6 ± 0.9 % were GFAP+. In the 6 week group, 89.2 ± 4.2 % of BrdU+ cells were also NeuN+, 7.5 ± 2.5 % were doublecortin+, and only 1.2 ± 1.2 % were GFAP+. These analyses indicate that within the granule cell layer the vast majority of BrdU+ cells are neuronal, even with the shorter survival times used in these experiments (2 weeks and 7 weeks respectively). However, because not all BrdU+ cells were NeuN+, our analyses may underestimate the recruitment of new neurons into spatial memory networks in this and other experiments.

1 week-old granule cells are not transiently recruited into spatial memory networks

To evaluate the possibility that 1 week-old neurons are transiently incorporated into spatial memory networks in the dentate gyrus, a group of mice (n=11) was trained in the water maze (6 trials per day for 5 days) one week following BrdU treatment (100 mg/kg; 5 days, two injections per day). These mice were then tested in a series of 3 probe tests one day following the completion of training (Fig. S7). In the probe test, mice searched selectively (paired t-test: t(10) = 4.24, \(P < 0.005\)). Following this probe test, robust numbers Fos+ and BrdU+ cells were identified in the dentate gyrus. However, no Fos+/BrdU+ cells were identified, suggesting that 1 week-old neurons are not transiently recruited into networks supporting spatial memory in the dentate gyrus.

References


