BEHAVIORAL NEUROSCIENCE

Associative learning increases adult neurogenesis during a critical period

Megan L. Anderson,[1,]* Helene M. Sisti,[2,]* Daniel M. Curlik II[2] and Tracey J. Shors[2]

[1]Department of Neuroscience, Rutgers University, Piscataway, NJ 08854, USA
[2]Department of Psychology and Center for Collaborative Neuroscience, Rutgers University, Piscataway, NJ 08854, USA

Abstract

Learning increases the number of immature neurons that survive and mature in the adult hippocampus. One-week-old cells are more likely to survive in response to learning than cells in animals that are exposed to training but do not learn. Because neurogenesis is an ongoing and overlapping process, it is possible that learning differentially affects new cells as a function of their maturity. To address this issue, we examined the effects of associative learning on the survival of cells at different stages of development. Training did not alter the number of cells that were produced during the training experience. Cells that were 1–2 weeks of age at the time of training survived after learning but cells that were younger or older did not. In contrast, cells that were produced during training were less likely to survive than cells in untrained animals. Additionally, the number of cells that were generated after learning in trained animals was not different from the number in untrained animals. Finally, survival was not increased if the memory was expressed when the cells were about 1-week-old. Together, these results indicate that new neurons are rescued from death by initial acquisition, not the expression or reacquisition, of an associative memory and only during a critical period. Overall, these results suggest the presence of a feedback system, which controls how many new neurons become incorporated into the adult brain in response to learning.

Introduction

New neurons are produced in the adult hippocampus, a brain region involved in learning and some aspects of memory (Altman & Das, 1965; Squire, 1992; Cameron *et al.*, 1993; Gould *et al.*, 1999). Neurogenesis is an ongoing, dynamic process that is influenced by learning. Two types of learning that are known to enhance cell survival are associative learning of the trace eyeblink response and spatial learning during training with the Morris water maze. Indeed, cells that are 1-week-old at the time of training on either task are more likely to survive to become mature neurons (Gould *et al.*, 1999; Shors, 2009). Cells that are much older or younger at the time of spatial maze training were not more likely to survive (Epp *et al.*, 2007; Sisti *et al.*, 2007). However, the production of younger cells is affected by water maze training depending on the phase of learning (Döbrössy *et al.*, 2003; Ambrogini *et al.*, 2004b; Epp *et al.*, 2007). With regard to associative learning, we did not find a difference in the number of cells generated during training (Gould *et al.*, 1999). However, in that study, cells were labeled late in training after many animals had already learned. Therefore, it remains unclear whether trace conditioning increases the number of cells produced during training or only increases the number that survive thereafter. It is conceivable that the effects of trace conditioning on neurogenesis are different from those of spatial maze training. Such differences are not unprecedented. For example, ablation of neurogenesis impairs learning during trace conditioning but has no observable effect on spatial learning (Shors *et al.*, 2001, 2002).

A number of studies have shown that new neurons in the adult hippocampus respond to exposure of a previously learned experience (Kee *et al.*, 2007; Trouche *et al.*, 2009). From this, it has been proposed that the new cells are somehow used in aspects of relearning and memory retrieval and/or expression. However, it is unclear whether cell activity in response to memory events influences survival. Because of the growing interest in neurogenesis and its role in memory processes, it is important to differentiate the effects of associative learning from those of activation in response to memory expression on the survival of new neurons.

The critical period during which associative learning rescues new neurons from death has not been determined. The effects of learning vs. memory of the trace response have also not been determined. In the present study, we first used trace eyeblink conditioning to evaluate the effects of associative learning on cells at various stages in their development: (i) when they were being generated, (ii) when they were 1–2 weeks old or (iii) when they were already mature at > 3 weeks of age. Second, we examined the effects of associative learning vs. memory expression on the survival of cells generated in the adult hippocampus.

Materials and methods

Subjects

Adult male Sprague–Dawley rats were bred at Rutgers University in the Department of Psychology. After weaning, they were housed in groups of 2–3 in standard plastic 'shoebox' style cages (44.5 cm long by 21.59 cm wide by 23.32 cm high) with unlimited access to water and food. They were kept on a 12-h light–dark cycle, with lights on at 07:00 h. All handling and experiments were carried out in the light portion of the cycle. When the rats were > 60 days of age, they were housed alone. All experiments were conducted with full compliance with the rules and regulation specified by the PHS Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg ⁄ kg; Henry Schein, Indianapolis, IN, USA) and supplied with isoflurane inhalant (Baxter Healthcare, Deerfield, IL, USA) and oxygen during surgery. Under anesthesia, the head was shaven and a head stage with four electrodes was mounted to the skull. The electrodes were stainless steel wires (0.005 in.) implanted through and around the eyelid. Two electrodes were used to record electromyographic activity and to detect eyeblinks, and the other two delivered a periorbital stimulation to the eyelid, which elicits an eyeblink as the unconditioned stimulus (US). Rats were allowed to recover for a minimum of 3 days before training with the associative learning task.

Trace eyeblink conditioning

Rats were connected to a cable that allowed free movement within the conditioning chamber. Animals were first acclimated to the conditioning apparatus without the presentation of any stimuli for approximately 40 min (100 25-s trials). During the acclimation period, spontaneous blinks were recorded during a 500-ms period for each trial. After acclimation, rats were returned to their home cage. Training began 24 h after the initial acclimation period. Spontaneous eyeblinks were again recorded for 30 trials immediately before the first session of training with trace eyeblink conditioning.

During a trial, a 250-ms white noise conditioned stimulus (CS; 82 dB) was followed by a 500-ms trace interval, and then immediately thereafter by the US, a 100-ms stimulation of the eyelid (0.62–0.65 mA), which always elicits a behavioral response. The inter-trial interval was 25 ± 5 s. Eyeblinks were detected by changes in the eyelid electromyogram, which were recorded during the trace interval and compared with a 250-ms baseline recording before the onset of the CS. Eyeblinks that occurred during the trace interval, which were
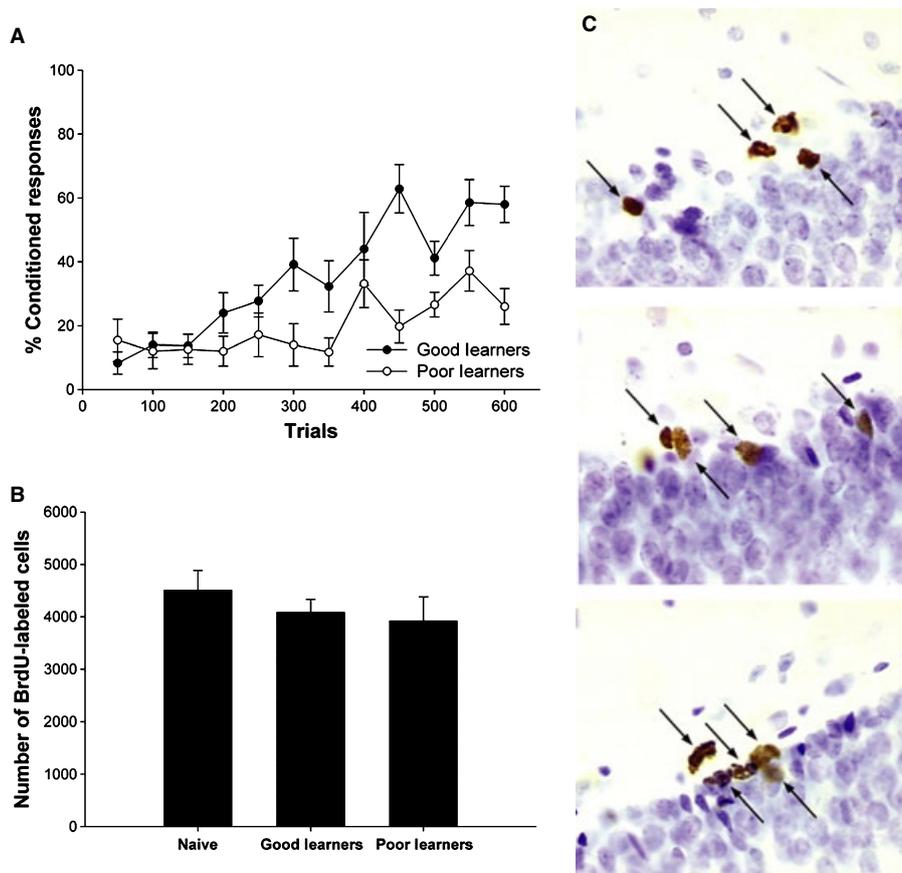

FIG. 1. Effects of trace eyeblink conditioning on production of newly generated cells. (A) Acquisition of trace eyeblink conditioned responses for good and poor learners. (B) Total number of BrdU-labeled cells when animals were trained 30 min and perfused 4 days after injection compared with naïve animals. There was no change in the number of cells labeled with BrdU during the training experience. (C) Photomicrographs at 1000 × of BrdU-positive cells (arrows) from a naïve animal (top panel), a good learner (middle panel) and a poor learner (bottom panel) from similar areas along the infrapyramidal DG blade in the dorsal hippocampus. Images represent the differences in the number of BrdU-labeled cells in response to training when compared with naïves.

larger than 0.5 mV, > 2.5 standard deviations above the mean of the baseline recording and > 10 ms in length were considered conditioned responses (CRs).

Experiment 1

To assess cell production and cell survival, all rats were injected once with bromodeoxyuridine (BrdU; 200 mg/kg, i.p.; Sigma, Atlanta, GA, USA) in physiological saline solution. BrdU can be incorporated into replicating DNA in the S phase of the cell cycle and thereby marks cells that are actively proliferating. BrdU is available in the brain for a period of approximately 2 h after a systemic injection (Cameron & McKay, 2001). Groups of rats were injected with BrdU and then trained with the associative learning task at three different time points. The first group ($n = 6$) was injected with BrdU and trained just 30 min later (see Fig. 2A). The second group ($n = 8$) was injected with BrdU and trained 1 week later. The third group ($n = 7$) was injected with BrdU and trained 3 weeks later. All of the rats that were trained were exposed with 150 trials each day (presented in 50-trial blocks) for four consecutive days for a total of 600 trials. These animals were perfused 4 weeks after the BrdU injection, with the exception of animals in one group, which were perfused 24 h after the last day of training (i.e. 4 days after the BrdU injection).

Animals that emitted CRs on 60% or more of the trials during a block of trials were considered to have learned. Only rats that reached this learning criterion were used in assessing the effect of learning on the number of BrdU-labeled cells that survived after 4 weeks. Another group of animals were not trained but injected with BrdU and perfused at the same time points. These naïve animals were randomly assigned to one of three groups and matched to the corresponding experimental group (30 min, $n = 7$; 1 week, $n = 8$; 3 weeks, $n = 8$).

A final group was injected with BrdU and trained 30 min later but perfused the day after training rather than 3 weeks later. These animals were split into 'good' ($n = 7$) or 'poor' ($n = 7$) learners based on whether the animal met the previously described learning criteria. Some animals were injected with BrdU and injected 4 days later, but not trained ($n = 9$). The BrdU-labeled cell counts from this group were used to assess the number of cells that were being generated during the training experience, as they were compared with the number of cells that survived the learning experience.

Experiment 2

To examine the effect of memory expression on cell survival, groups of rats were trained with either one or two identical phases of trace eyeblink conditioning (see Fig. 3A). All trained rats in experiment 2

were exposed to 800 trials of trace conditioning, with 200 trials each day for four days (presented in 100-trial blocks) to ensure that the animals learned and learned well. In addition, trained groups received one single injection of BrdU 1 week prior to the start of phase 2 of training. One group of animals (Trace/Trace, $n = 8$) was trained during both phase 1 and phase 2. An additional group of animals
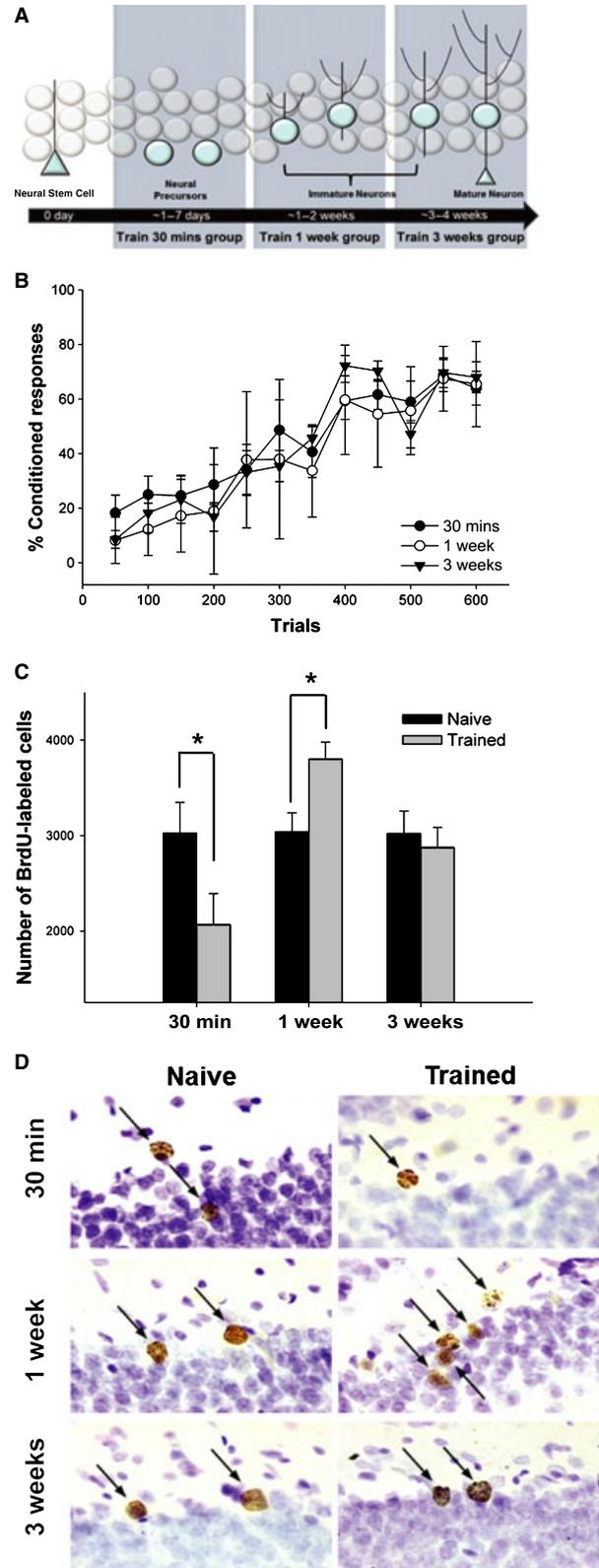

FIG. 2. Effects of trace eyeblink conditioning on survival of newly generated neurons. (A) Diagram illustrating the stage of development when BrdU-labeled cells were exposed to training. All animals were injected once with BrdU. They were then trained or not 30 min, 1 week or 3 weeks later. To assess the effects of learning on cell survival, animals were perfused 4 weeks after the BrdU injection. (B) Acquisition of the CR in animals trained 30 min, 1 week and 3 weeks after BrdU injection. (C) Total number of BrdU-labeled cells in the dentate gyrus in animals trained 30 min, 1 week or 3 weeks after injection compared with naïve animals. Trace conditioning increases the number of BrdU-labeled cells when rats are trained 1 week after injection, but decreases the number that survive when they are trained just 30 min after injection. (D) Photomicrographs at 1000 × of BrdU-positive cells (arrows) from each experimental condition. Cells are located along the infrapyramidal blade in the dorsal hippocampus. Images represent the relative differences in the number of BrdU-labeled cells in response to learning when compared with naïve animals. The granularity in some cells reflects the extended time interval between the BrdU injection and when the animals were perfused. *$P < 0.05$.
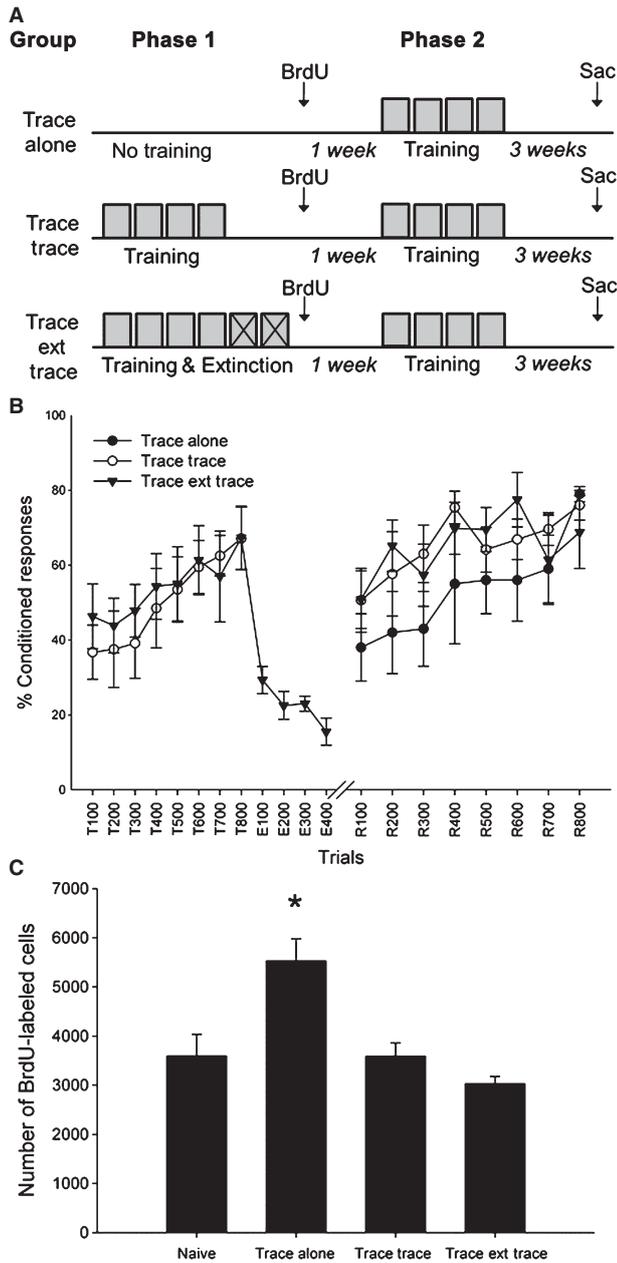
Fig. 3. Acquisition but not memory expression increases the number of surviving cells. (A) Schematic of the experimental design for experiment 2. One group (Trace Alone) of animals received no training during phase 1 and were given a BrdU injection seven days before phase 2 trace training. Another group of animals (Trace/Trace) received trace training during phase 1, before the BrdU injection, and were trained with trace conditioning again seven days after the BrdU injection. A third group of animals (Trace/Extinction/Trace) was treated similarly to animals in the Trace/Trace condition but received additional extinction trials following phase 1 before the BrdU injection. (B) Acquisition, extinction and reacquisition of the trace memory. Animals in the Trace/Extinction/Trace condition extinguished their responding to the CS but quickly reacquired the CR during retraining. Animals remembered the trace task more than one week after acquisition, regardless of extinction training. (C) Animals trained with only a single phase of trace conditioning possessed more BrdU-labeled cells than animals trained again after learning. *$P < 0.05$.

(Trace/Ext/Trace, $n = 6$) was trained during both phases, but these animals also received 2 days of 200 CS-alone extinction trials, which immediately followed phase 1 of training. Once the CR had been

extinguished, this group was injected with BrdU. They were then re-exposed to the paired stimuli 1 week later. Another group of animals (Trace Alone, $n = 5$) was only trained during phase 2. An additional group (Naïve, $n = 6$) remained experimentally naïve. All animals were killed 3 weeks after the BrdU injection. If initial acquisition but not reacquisition or memory expression increases the number of surviving cells, more cells will be present in the animals that were only trained 1 week after the BrdU injection. If reacquisition, or the expression, of the CR also increases cell survival, more BrdU cells will be present in the group that was trained again during phase 2 than in animals that were not trained.

As an additional control, a group was trained with trace conditioning and then injected with BrdU one week later (BrdU After Trace rats; $n = 6$). Another group was not trained but injected with BrdU at the same time point ($n = 13$). The two groups were killed seven days after the BrdU injection. The number of BrdU cells in these groups was used to determine whether the initial acquisition of the trace memory persistently alters the number of cells that are available to rescue during a second phase of training.

BrdU immunohistochemistry and quantification

All rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were extracted and kept in paraformaldehyde and later transferred to PBS until sectioning. A vibratome was used to cut coronal sections (40 μm) through one hemisphere of the entire dentate gyrus (DG). The slices were cut in 0.1 M PBS (pH 7.4). Every 12th slide was fixed onto a superfrost glass slide (Fisher, Suwanne, GA, USA) and allowed to air-dry. Once dry, slices were stained with peroxidase to visualize the cells that incorporated BrdU. In summary, the slices were pretreated with heated 0.1 M citric acid (pH 6.0). After rinsing with 0.1 M PBS, tissue was incubated in trypsin, followed by 2 M HCl with PBS rinses in between. Slides were kept overnight in primary mouse anti-BrdU (1 : 200; Becton-Dickinson, Franklin Lakes, NJ, USA) and 0.5% Tween-20 (Vector Laboratories, Burlingame, CA, USA). The next day, tissue was rinsed and incubated for 1 h in biotinylated anti-mouse antibody (1 : 200; Vector Labs), then in avidin–biotin–horseradish peroxidase (1 : 100, Vectastain ABC kit; Vector Labs), and lastly in diaminobenzidine (DAB SigmaFast tablets; Sigma) with PBS rinses in between. After rinsing one last time, slices were counterstained with 0.1% cresyl violet, dehydrated, cleared and then cover-slipped with Permount (Fisher Scientific, Fair Lawn, NJ, USA).

Quantitative analysis was performed blind to behavioral condition. Estimates of the total number of BrdU-labeled cells were determined using a modified unbiased stereology protocol (West *et al.*, 1991; Gould *et al.*, 1999). BrdU-labeled cell in the subgranular zone/granule cell layer and hilus on every 12th unilateral section throughout the entire rostro-caudal extent of the DG were counted at 1000 × on a Nikon Eclipse E400 light microscope, avoiding cells in the outermost focal plane. The number of cells was multiplied by 24 to obtain an estimate of the total number of BrdU-labeled cells in both hippocampi.

Results

Experiment 1

Learning does not increase the number of cells generated during training

The purpose of this experiment was to evaluate the effects of training and/or learning on the number of new cells generated in the adult

hippocampus. A repeated-measures ANOVA, with training session as the repeated measure, was used to analyse the learning data. Animals that were trained with paired stimuli emitted more CRs as the number of trials increased ($F_{11,132} = 11.41$, $P < 0.05$; Fig. 1A).

A one-way ANOVA was used to analyse the effect of training on the number of cells that were being produced during the training experience. Animals which began the training regime just 30 min after the BrdU injection had similar numbers of cells to animals that were untrained, provided that the cells were examined 1 day after training ended ($F_{2,20} = 0.68$, $P = 0.52$; Fig. 1B and C). Because cells are still dividing during this time period, at least very early in training as training began shortly after the BrdU injection, these data would suggest that training does not alter the number of cells that are generated or dividing during the training experience.

Animals that emitted 60% CRs in a trial-block were considered 'good learners' and were separated from those that did not and were considered 'poor learners'. As expected, the animals that learned to perform the CR had similar numbers of cells as animals that were trained but learned poorly or did not learn. Overall, these data suggest that neither training stimuli nor learning increases the number of cells that are being generated during the training experience.

Learning increases the number of cells that are 1 but not 3 weeks of age

The purpose of this experiment was to determine whether learning increases the number of cells that survive if they were exposed to the training experience when they were 30 min of age, 1 week of age or 3 weeks of age. Animals that were trained with paired stimuli emitted more CRs as the training trials increased ($F_{11,198} = 39.62$, $P < 0.05$; Fig. 2B). There was no difference in performance between groups because all animals were treated similarly up to this point in the experiment ($F_{22,198} = 0.83$, $P = 0.69$).

In previous studies, we found that trace conditioning only increases cell survival in animals that learn to perform the CR and learn it well (Dalla *et al.*, 2007; Waddell & Shors, 2008). Therefore, for this analysis, we only evaluated animals that learned to emit the CR. A two-way ANOVA (trained vs. naïve; time interval after BrdU injection) followed by Newman–Keuls *post-hoc* comparisons was used to analyse the effect of learning on cells that were at three stages of development. There was a significant interaction between training and developmental stage on the number of cells that remained in the hippocampus 3 weeks after training had ceased ($F_{2,38} = 6.25$, $P < 0.05$). The number of BrdU-labeled cells was increased in trained rats compared to the number in naïve rats when training occurred 1 week after BrdU injection ($P < 0.05$; Fig. 2C and D). In contrast, the number of cells that survived in animals that began training just 30 min after the injection was reduced ($P < 0.05$). The number of cells that were present in animals trained 3 weeks after the BrdU injection was similar to the number in those that were not trained but injected with BrdU and killed at the same time points ($P > 0.05$). Overall, these data indicate that learning increases the number of surviving cells when they are between 1 and 2 weeks of age but not when they are younger (30 min to 4 days) or older (3–4 weeks). They also indicate that the youngest cells are actually less likely to survive if they are generated during the training experience.

Experiment 2

Learning does not alter the number of cells generated after training

As expected, animals emitted more CRs across training trials and sessions. A repeated-measures ANOVA revealed a significant effect of session, indicating that the groups trained with paired stimuli acquired the CR over the course of training ($F_{7,35} = 19.97$, $P < 0.05$). There was no difference in the number of BrdU-labeled cells between the trained ($M = 5350.92$, $SE = 704.66$) and the untrained group ($M = 5080.00$, $SE = 458.51$; $t_{17} = 0.808$, $P > 0.05$). Therefore, successful acquisition of trace memory did not alter the number of cells present 1 week after the training experience had ceased. These data suggest that associative learning does not persistently increase the number of cells generated in the adult hippocampus.

Acquisition but not memory expression increases the number of surviving cells

In the second part of this experiment, we evaluated the effects of learning versus memory expression on the number of surviving neurons in the DG. During the first phase of training, animals were trained with 800 trials of trace conditioning. By the end of the first phase, most animals learned to accurately predict the occurrence of the US (Fig. 3B). The number of conditioned responses made by animals in the Trace/Trace condition increased over phase 1 ($F_{7,42} = 3.25$, $P < 0.01$). Similarly, the number of CRs made by animals in the Trace/Extinction/Trace condition increased during the paired stimuli portion of phase 1 ($F_{7,35} = 3.4$, $P < 0.05$). For the 2 days following acquisition of the task, these animals were further trained with 400 extinction trials during which the CS was presented alone. Animals during extinction training quickly ceased responding to the CS ($F_{3,15} = 8.05$, $P < 0.01$). Thus, at the end of the first phase, both groups of animals had learned the CS–US association, but one group had learned to suppress the CR. Animals that were trained during phase 1, responded with a CR on approximately 60% of the trials during the first trial-block during phase 2. Animals in the Trace/Trace condition responded similarly during the first trial block of phase 2 as during the last trial block of phase 1 ($F_{1,7} = 3.81$, $P > 0.05$). Animals in the Trace/Extinction/Trace conditions increased their responding significantly from the last trial block in the extinction phase to the first trial block in phase 2 ($F_{1,5} = 21.19$, $P < 0.01$). A third group of animals remained in their home cages during phase 1 and were only trained during phase 2 with paired stimuli 1 week after one injection of BrdU (Trace Alone rats). These animals also readily learned to accurately predict the US with a well-timed CR ($F_{3,12} = 3.80$, $P < 0.05$).

A one-way ANOVA revealed that there was a significant effect of training condition on the number of BrdU-labeled cells that survived ($F_{3,21} = 9.14$, $P < 0.01$; Fig. 3C). As in experiment 1, animals that received only a single phase of trace eyeblink conditioning (Trace Alone) when the cells were 1 week of age retained significantly more cells than the naïve controls ($P < 0.05$). Trace/Trace and Trace/Extinction/Trace animals both had a similar number of BrdU-labeled cells to naïve animals (both $P > 0.05$). Thus, expression of the memory alone did not increase the number of BrdU-positive cells. These data suggest that memory retrieval and/or expression does not increase the survival of newly generated neurons in the adult DG.

Discussion

Neuronal precursor cells in the DG proliferate for approximately 1 week after their birth, a process that produces thousands if not tens of thousands of new cells within days. However, within weeks, many of these new cells die (Cameron *et al.*, 1993; Cameron & McKay, 2001; Waddell & Shors, 2008). The cells that do survive extend axons, sprout dendritic spines and alter their response properties significantly (Ge *et al.*, 2006; Zhao *et al.*, 2006). Thus, these cells undergo

significant changes during the first few weeks after their birth. Neurons that are 1 week of age at the time of training are more likely to survive if learning is successful (Gould *et al.*, 1999; Waddell & Shors, 2008; Shors, 2009). However, the effects of trace eyeblink conditioning on cells of differing ages has not been determined. Here, we examined the effects of learning on cells at different stages in their maturity. We also examined the effects of memory expression alone on cell survival. The data provide confirmatory evidence as well as novel information. First, we also observed that cells that were about 1 week of age at the time of the learning experience tended to survive whereas those that were older did not. Second, animals that began training just 30 min after the BrdU injection did not possess any more cells after training than animals that were not trained. Because the animals that started training almost immediately after the BrdU injection were the number of BrdU-labeled cells was examined only a few days later, these data suggest that training does not significantly increase cell production. Even so, the number of those cells that survived 1 month later was significantly reduced when training began immediately after the BrdU injection. Thus, while cells that are 1–2 weeks of age are being rescued from death, those much younger are dying in significant numbers. It would appear that learning retains some neurons at the expense of those that are less mature. Together, these data provide a snapshot of how cells of different ages respond to learning.

Others have found that spatial training with the Morris water maze task increases cell survival but only during a critical period (Epp *et al.*, 2007). Cells which were approximately 1 week of age at the time of training were more likely to survive whereas those that were older or younger did not. Dupret *et al.* (2007) observed that spatial learning increases the number of apoptotic cells in the DG if the cells were 3–4 days old at the time of training. Thus, some cells that were exposed to spatial learning are more likely to die whereas others are more likely to survive. The authors suggested a mechanism or system which removes cells that were not selected for integration into the learning network (Dupret *et al.*, 2007). Our data reported here are generally consistent with such a system. Thus, it would appear that both trace eyeblink conditioning and spatial maze learning increase the survival of new neurons when they are about 1–2 weeks of age while inducing cell death for younger cells. This feedback system might be used to maintain an optimal number of newly generated neurons and ultimately would control the total number of granule cells in the DG.

The current data support the idea of a critical period during which new neurons in the adult hippocampus can be rescued from death by learning. Thus, some cells must possess a characteristic that makes them selectively sensitive to the positive effects of a learning experience. One might suggest NMDA receptor activation, as it is necessary for learning and implicated in the survival of new neurons (Tashiro *et al.*, 2006, 2007; Leuner *et al.*, 2007). However, it has been shown that these receptors do not contribute to cell activity until the cells are somewhat older (Ambrogini *et al.*, 2004a). It seems likely that the response to GABA release during the learning experience may be involved. GABA is excitatory very early in neurogenesis and can increase the extent of neuronal differentiation (Overstreet Wadiche *et al.*, 2005; Tonuka *et al.*, 2005). Another candidate is production of brain-derived neurotrophic factor, which is elevated during learning and increases neurogenesis (Kesslak *et al.*, 1998; Mizuno *et al.*, 2000; Scharfman *et al.*, 2005). That said, only a small percentage of cells express this neurotrophin's Trk receptor during the period when learning increases cell survival (Donovan *et al.*, 2008).

In a second set of experiments, we examined the effects of memory expression on the survival of cells that were not yet present at the time of the initial learning experience, as well as their response to reacquisition after the initial response was extinguished. Training and/or learning did not persistently increase the number of surviving cells, at least those generated during the week following the training experience. Moreover, cells generated after the initial learning and about 1 week of age were not rescued from death by expression of the memory alone or by reacquisition of a memory that had been behaviorally extinguished. Therefore, expressing the memory for a task that was learned before the cells were generated does not rescue an entirely new population of cells from death. These data are novel in that they dissociate the effect of learning versus that of memory on the survival of newly generated neurons in the adult hippocampus.

Learning typically occurs once but a memory can be expressed over and over again, admittedly with some new learning each time. It seemed unlikely that new cells would be rescued from death each time a memory is retrieved and, generally, the data presented here support this conclusion. Certainly, the cells that were initially rescued by learning might be reactivated during memory retrieval and this has been shown with respect to spatial learning. Trouche *et al.* (2009) found that cells rescued from death during spatial learning were responsive to a memory probe trial and to further training some weeks later. However, cells that are initially rescued from death during learning could respond to the memory experience without being required for recall of the memory. This must certainly be the case during trace conditioning because the hippocampus is not necessary for expression of the memory trace (Kim *et al.*, 1995; Takehara *et al.*, 2003). Conversely, expression of a spatial memory does depend on an intact hippocampus so it may affect survival differently than does associative learning (Riedel *et al.*, 1999). Regardless, the present data suggest that neither the neuronal activity associated with memory expression nor its rehearsal per se rescues new cells from death – cells that were not yet present at the time of the initial training experience. In general, the data presented here suggest a general system – perhaps a feedback system – that maintains a critical number of neurons in the adult hippocampus as a function of learning and memory.

Acknowledgements

Abbreviations

BrdU, bromodeoxyuridine; CR, conditioned response; CS, conditioned stimulus; DG, dentate gyrus; US, unconditioned stimulus.

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