Changing the rate and hippocampal dependence of trace eyeblink conditioning: Slow learning enhances survival of new neurons

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ABSTRACT

Trace eyeblink conditioning in which a conditioned stimulus and unconditioned stimulus are separated by a gap, is hippocampal dependent and can rescue new neurons in the adult dentate gyrus from death (e.g., Beylin et al., 2001; Gould et al., 1999). Tasks requiring more training trials for reliable expression of the conditioned response are most effective in enhancing survival of neurons (Waddell & Shors, 2008). To dissociate hippocampal dependence from acquisition rate, we facilitated hippocampal-dependent trace eyeblink conditioning in two ways: a shorter trace interval and signaling the intertrial interval with a post-US cue. Trace conditioning with a shorter trace interval (250 ms) requires an intact hippocampus, and acquisition is faster relative to rats trained with a 500 ms trace interval (e.g., Weiss et al., 1999). Using excitotoxic hippocampal lesions, we confirmed that eyeblink conditioning with the 250 or 500 ms trace interval is hippocampal dependent. However, training with the post-US cue was not hippocampal dependent. The majority of lesion rats in this condition reached criterion of conditioned responding. To determine whether hippocampal dependence is sufficient to rescue adult-generated neurons in the dentate gyrus, rats were injected with BrdU and trained in one of the three trace eyeblink arrangements one week later. Of these training procedures, only the 500 ms trace interval enhanced survival of new cells; acquisition of this task proceeded slowly relative to the 250 ms and post-US cue conditions. These data demonstrate that rate of acquisition and not hippocampal dependence determines the impact of learning on adult neurogenesis.

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1. Introduction

The dentate gyrus of the hippocampus produces thousands of new neurons each day (Altman & Das, 1965; Cameron & McKay, 2001; Cameron, Woolley, McEwen, & Gould, 1993; Hastings & Gould, 1999; Kaplan & Hinds, 1977; Markakis & Gage, 1999; van Praag et al., 2002). Many die within weeks, but survival can be enhanced by exposure to some learning tasks (e.g., Dayer, Ford, Cleaver, Yassaee, & Cameron, 2003; Döbrössy et al., 2003; Gould, Beylin, Tanapat, Reeves, & Shors, 1999). Using the eyeblink procedure, our laboratory has demonstrated a relationship between the rate of learning and the survival of new cells (Dalla, Papachristos, Whetstone, & Shors, 2009; Waddell & Shors, 2008). Tasks which require many trials are more effective in enhancing survival (Waddell & Shors, 2008). Similarly, animals that learn relatively slowly exhibit the largest increase in cell survival when the task is effective (Dalla et al., 2009; Döbrössy et al., 2003; Epp, Spritzer, & Galea, 2007;

Shors, 2009; Waddell & Shors, 2008). This pattern has also been noted in the hippocampal-dependent water maze procedure (Döbrössy et al., 2003; Epp et al., 2007).

The majority of learning procedures that enhance survival, such as trace eyeblink conditioning, also require an intact hippocampus for acquisition (Beylin et al., 2001; Gould et al., 1999; Leuner, Waddell, Gould, & Shors, 2006; Snyder, Hong, McDonald, & Wojtowicz, 2005; Waddell & Shors, 2008; Weiss, Bouwmeester, Power, & Disterhoft, 1999). Ablation of adult neurogenesis does not disrupt all tasks that require an intact hippocampus (Jaholkowski et al., 2009; Shors, Townsend, Zhao, Kozorovitskiy, & Gould, 2002) and hippocampal-dependent tasks do not always enhance survival (Epp et al., 2007; van Praag, Christie, Sejnowski, & Gage, 1999). We consistently observe enhanced survival following conditioning with a trace eyeblink procedure using a 500 ms gap between the CS and US. This task is both dependent on the hippocampus, and is slowly acquired relative to hippocampal-independent eyeblink conditioning tasks. The experiments presented here sought to facilitate trace conditioning while maintaining hippocampal dependence. To do this, we used two variants of trace eyeblink conditioning that produce faster conditioning than observed using

our typical 500 ms trace conditioning procedure. First, rats were trained with a shorter, but hippocampal-dependent trace interval (i.e., 250 ms; Weiss et al., 1999). Second, a cue was presented after delivery of the US to signal the onset of the intertrial interval (Bolles, Collier, Bouton, & Marlin, 1978; Mowrer & Lamoreaux, 1951). Both manipulations facilitated acquisition, and surprisingly, hippocampal-lesioned rats trained with the post-US cue did exhibit acquisition of the eyeblink CR. Permanent excitotoxic lesions of the hippocampus abolished acquisition of trace eyeblink conditioning using either the 500 ms or 250 ms trace interval as has been demonstrated (e.g., Walker & Steinmetz, 2008; Weiss et al., 1999). Only training with the 500 ms interval enhanced survival of adult-generated cells. Together, these results suggest only tasks requiring many trials, or are more difficult, enhance survival of adult-generated cells. Further, insertion of a post-US cue attenuated the lesion-induced deficit. Precisely how this cue attenuates the lesion deficit in trace conditioning is not known. We hypothesize that the post-US cue reduces fear to the context, and therefore facilitates a discrimination between the trace interval and intertrial interval (Bolles et al., 1978; Kaplan & Hearst, 1982; Mowrer & Lamoreaux, 1951).

2. Methods

2.1. Subjects

Rats were obtained from a breeding facility at Rutgers University. Male rats, between 60 and 70 days old at the time of BrdU administration or hippocampectomy were used. Rats were housed in groups of 3 until surgery. Following surgery, rats were housed alone in standard plastic 'shoebox' cages (44.5 cm long, 21.59 cm wide and 23.32 cm high). Rats had ad lib access to rat chow and water and were maintained on a 12 h light-dark cycle. All experiments were conducted with full compliance to the rules and regulations specified by the PHS Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg) and atropine (0.04 mg/kg). After being placed in the stereotaxic instrument, the scalp was cleaned with Betadine, and an incision was made. For hippocampal lesions, NMDA was infused at 6 sites per hemisphere for a total of 12 infusions made at the following coordinates relative to bregma: AP: −2.5 mm, ML: ±1.6, DV: −3.8; AP: −4.2 mm, ML: ±2.6 mm, DV: −3.1; AP: −5.3 mm, ML: ±5.0 mm, DV: −5.9 mm; AP: −5.3 mm, ML: ±4.2 mm, DV: −3.4 mm; AP: −5.8 mm, ML: ±4.6 mm, DV: −6.1 mm; AP: −6.0 mm, ML: ± 5.6 mm, DV: −4.1 mm. The DV coordinate was calculated relative to the surface of the brain. A concentration of 20 µg/µl was used and at each injection site, and 0.35 µl of NMDA was infused at a rate of 0.5 µl/min. Infusions were made using a gastight Hamilton syringe with an inner diameter of 0.13 mm and an outer diameter of 0.483 mm. For sham lesions, the syringe was lowered, but no infusion was conducted. In preparation for eyeblink conditioning, all rats with the exception of naïve controls in Experiment 2 were implanted with 4 eyelid electrodes (insulated stainless steel wire, 0.005 inch.) through the upper eyelid (orbicularis oculi muscle). Rats receiving hippocampal lesions or sham surgeries were given a minimum of 2 weeks recovery before eyeblink conditioning began due to the extent of the lesion. Rats used in Experiment 2 were given a minimum of 4 days recovery following placement of the eyeblink headstage before BrdU administration.

2.3. Trace eyeblink conditioning

For eyeblink conditioning, headstages were connected to a cable that allowed free movement within the conditioning chamber. Twenty-four hours prior to any behavioral manipulation, rats were acclimated to the conditioning apparatus for 1 h. During this acclimation period, spontaneous blink rates for each rat were recorded. The following day, rats began conditioning. Rats were placed in the eyeblink conditioning chambers and were trained with the trace eyeblink conditioning procedure for 200 trials a day for four consecutive days (800 trials total). The CS was an 83 dB, 250 ms white noise. The US was a 100 ms periorbital shock (0.65 mA). The CS and US were separated by either a 500 ms trace interval (Trace 500) or a 250 ms trace interval (Trace 250), in which no stimuli were delivered. For the Trace 500 Cue condition, the CS and US were presented as described, with the addition of a 100 ms key light illumination. The onset of the key light was 500 ms following the offset of the US. The intertrial interval (ITI) was 25 ± 5 s. To detect the occurrence of an eyeblink, the maximum EMG response occurring during a 250 ms prestimulus baseline recording period was added to four times its standard deviation. Responses that exceeded that value and were longer than 3 ms were considered eyeblinks. Eyeblinks were considered conditioned responses (CRs) if they began 250 ms prior to US onset. Eyeblink performance was calculated as the percentage of trials on which a CR was produced in response to a CS.

2.4. Histology

Following behavioral testing, rats were deeply anesthetized and perfused transcardially with 10% formalin. Brains were extracted and post-fixed in a 10% formalin solution with 30% sucrose. Coronal sections (45 µm) throughout the rostrocaudal extent of the hippocampus were collected. Every 5th section was mounted onto gelatin coated slides and stained with cresyl violet. Only rats with bilateral damage to the dorsal and ventral hippocampus were included in data analysis (Fig. 1). These criteria resulted in the following number of animals in each condition: Trace 250 Sham, n = 8; Trace 250 Lesion, n = 7; Trace 500 Sham, n = 8; Trace 500 Lesion, n = 7; Trace 500 Cue Sham, n = 8; Trace 500 Cue Lesion, n = 7.

2.5. BrdU injections and inclusion criteria

Rats were given a single intraperitoneal injection of BrdU in saline (200 mg/kg). Eyeblink conditioning began 7 days later. Rats were sacrificed 21 days after BrdU administration. Rats were dropped from analysis due to poor EMG signal in the eyeblink conditioning task (n = 2) and absence of BrdU labeling (n = 1). The remaining rats were distributed as follows: Naïve, n = 9; Trace 250, n = 7; Trace 500, n = 8; Trace 500 Cue, n = 7.

2.6. Immunohistochemistry

Rats were deeply anesthetized with sodium pentobarbital (200 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were dissected from the skulls and post-fixed for at least 2 days. Coronal sections (40 µm) throughout the entire rostrocaudal extent of the dentate gyrus were cut with a Vibratome from one hemisphere into a bath of 0.1 M PBS (pH 7.5). For BrdU peroxidase staining, a 1:12 series of sections were mounted onto glass slides, dried and pretreated by heating in 0.1 M citric acid (pH 6.0). Slides were then rinsed in PBS, incubated in trypsin for 10 min, denatured in 2 M HCl:PBS for 30 min, rinsed and incubated with mouse antibodies to BrdU (diluted 1:250 with 0.5% Tween-20; Vector Laboratories, Burlingame, CA). The next day, slides were rinsed, incubated with biotin-
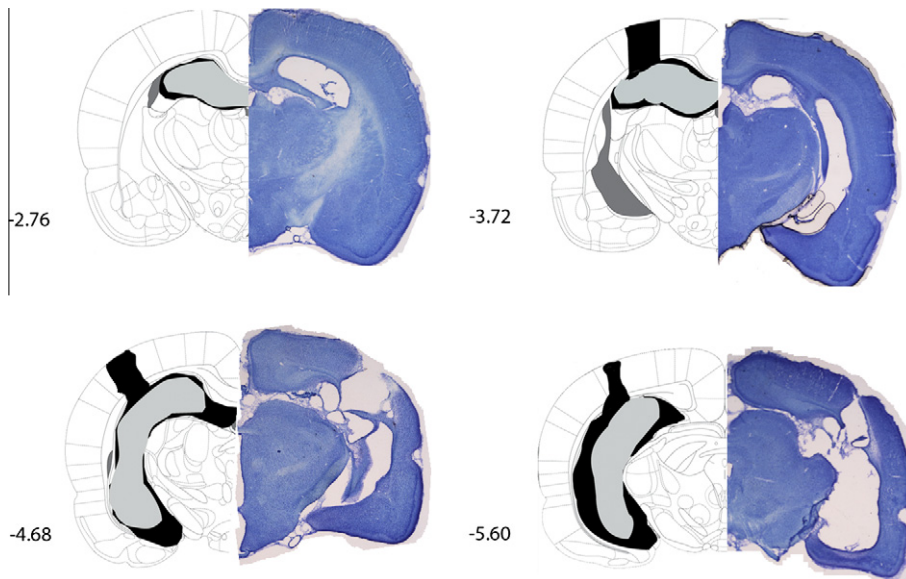
Fig. 1. Rats were given excitotoxic lesions of the entire hippocampus. The drawings (left hemisphere) represent the largest lesion (black) and smallest lesion (gray) of rats included in analysis for Experiment 1. Photographs of representative lesions comprise the right hemisphere. Rats were included if the majority of the dorsal and ventral hippocampus was lesioned. Rats with excessive neocortical damage were excluded. Coordinates are anterior/posterior from bregma as determined by Paxinos and Watson (2007).

ylated anti-mouse (1:200; Vector) for 60 min, rinsed, incubated with avidin–biotin complex, rinsed and reacted in 0.01% diaminobenzidine with 0.003% H_2O_2. Slides were counterstained with cresyl violet, dehydrated, cleared and coverslipped under Permount (Fisher Scientific, Fair Lawn, NJ).

2.7. Cell counting

Quantitative analysis was conducted blind to behavioral condition. For BrdU peroxidase, estimates of total numbers of BrdU-labeled cells were determined using an unbiased stereology protocol previously reported to successfully quantify BrdU labeling (Gould et al., 1999; West, Slomianka, & Gundersen, 1991). BrdU-labeled cells in the subgranular zone (SGZ) and granule cell layer (GCL) on every twelfth unilateral section throughout the entire rostrocaudal extent of the dentate gyrus were counted at 1000× (100× objective with a 10× ocular) on an Olympus BX-50 light microscope, avoiding cells in the outermost focal plane. The number of BrdU-labeled cells per dentate gyrus was then multiplied by 24 to obtain an estimate of the total number of BrdU-labeled cells throughout the rostrocaudal axis (Fig. 3).

3. Results: Experiment 1: hippocampal lesions abolish trace eyeblink conditioning and this is partially reversed with the addition of a post-US cue

The percentage of conditioned responses across trial blocks for each training and lesion condition are presented in Fig. 2. Each training condition is presented separately to ease inspection of the impact of hippocampal lesions. Repeated measures ANOVA across the 12 blocks of Trials with Training Condition and Lesion as between subjects factors revealed a significant effect of Trial, $F_{(11, 429)} = 42.17$, $p = .0001$. The Trial × Lesion interaction was significant, $F_{(11, 429)} = 14.46$, $p = .0001$. The Trial × Training Condition interaction was significant, $F_{(22, 429)} = 1.93$, $p = .007$. The Trial × Lesion × Training Condition interaction was also significant, $F_{(22, 429)} = 2.34$, $p = .001$. The main effect of Lesion was significant, $F_{(1, 39)} = 48.22$, $p = .0001$, as was the main effect of

Training Condition, $F_{(2, 39)} = 11.09$, $p = .0001$, and Lesion × Training Condition interaction, $F_{(2, 39)} = 9.63$, $p = .0001$.

To clarify the impact of hippocampal lesions in each training condition, each condition was analyzed separately, with Lesion as the between subjects factor. Repeated measures ANOVA across the 12 blocks of conditioning in the Trace 250 condition, presented in the left panel of Fig. 2, revealed a significant effect of Trial $(11, 143) = 8.68$, $p = .0001$, as well as a significant Trial × Lesion interaction, $F_{(11, 143)} = 7.56$, $p = .0001$. The main effect of Lesion was also significant, $F_{(1, 13)} = 104.28$, $p = .0001$. As expected based on previous findings, hippocampal lesions abolished conditioned responding in the Trace 250 stimulus arrangement (Walker & Steinmetz, 2008; Weiss et al., 1999). Analysis of the Trace 500 groups using repeated measures ANOVA across the 12 blocks of conditioning revealed a significant effect of Trial $F_{(11, 143)} = 12.47$, $p = .0001$, as well as a significant Trial × Lesion interaction, $F_{(11, 143)} = 9.53$, $p = .0001$. The main effect of lesion was significant, $F_{(1, 13)} = 4.29$, $p = .05$. As expected, rats in the lesion condition failed to achieve over 20% conditioned responding at any point during training. The impact of hippocampal lesions on acquisition of trace conditioning with insertion of the post-US cue was analyzed using repeated measures ANOVA across the 12 blocks of conditioning. This ANOVA revealed a significant effect of Trial $F_{(11, 143)} = 25.69$, $p = .0001$, as well as a significant Trial × Lesion interaction, $F_{(11, 143)} = 2.78$, $p = .003$. The main effect of Lesion was not significant, $F_{(1, 13)} = 3.78$, $p = .07$. As depicted in the right panel of Fig. 2, hippocampal lesions reduced asymptotic responding relative to sham operated controls. However, this group exhibited an increase in conditioned responding across trials similar to sham operated controls. Thus, insertion of the post-US cue attenuated the lesion-induced deficit. This result is surprising, as the post-US cue was a third discontiguous stimulus.

4. Experiment 2: rate of acquisition and not hippocampal dependence predicts the impact of learning on survival of adult-generated cells

Repeated measures ANOVA on the 12 blocks of conditioning presented in Fig. 4 revealed a significant effect of Trial
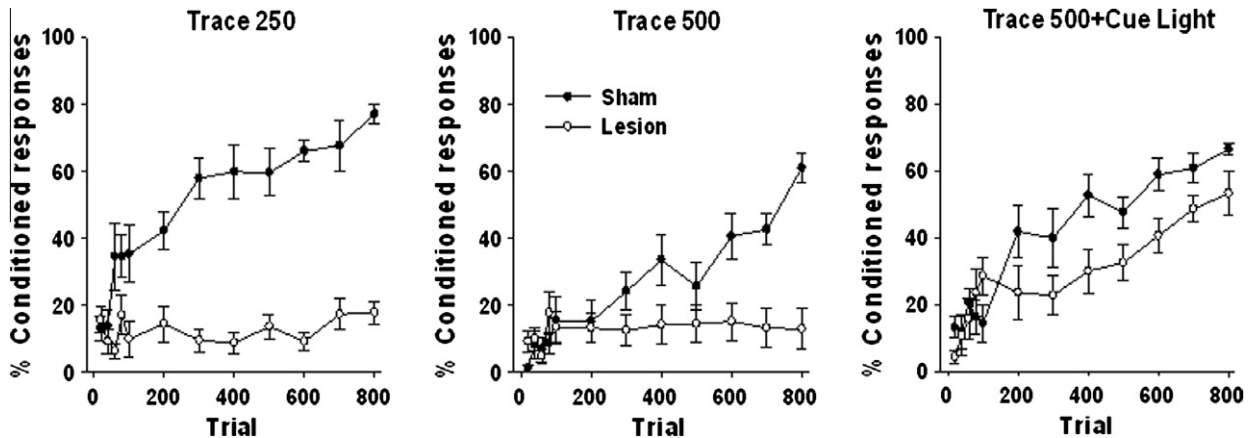
Fig. 2. Behavioral results from animals trained with the Trace 250, Trace 500 and Trace 500 Cue procedures after receiving excitoxic hippocampus lesions or sham surgery. Data are presented as a mean percentage of conditioned responses ±SEM with the first 100 trials presented in 20 trial blocks with the remaining 700 trials in 100 trial blocks.
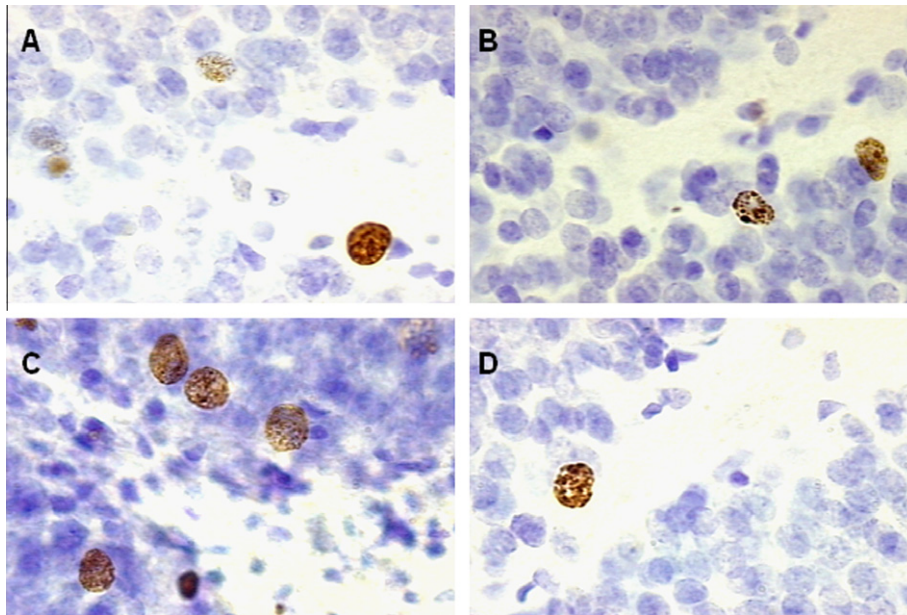

Fig. 3. BrdU-labeled cells in the dentate gyrus of the hippocampus from similar sections of a rat in the (A) 21 day Naïve condition, (B) Trace 250 condition, (C) Trace 500 condition and (D) Trace 500 Cue condition. Images were magnified 1000×.

$F(11, 209) = 26.30$, $p = .0001$. The Training Condition × Trial interaction was not significant $F(22, 209) < 1$, indicating that all three groups increased conditioned responding across trials. The main effect of Condition was significant, $F(2, 19) = 4.35$, $p = .028$, reflecting differences in the number of conditioned responses. Though all three groups reached the same level of asymptotic responding, rats in the Trace 500 condition expressed fewer conditioned responses across trial blocks compared to either Trace 250 or Trace 500 Cue.

Only rats in the Trace 500 condition exhibited an increase in BrdU + cells when sacrificed 21 days following BrdU administration (Fig. 3). This was confirmed with a one-way ANOVA, which confirmed a significant effect of Training Condition, $F(3, 27) = 9.75$, $p = .0001$. Multiple comparisons using Bonferoni's posthoc test confirmed that the number of BrdU + cells was significantly higher in the Trace 500 condition relative to Naïve, untrained controls ($p = .0001$). Trace 500 exhibited significantly more cells than Trace 250 ($p = .001$) and Trace CL ($p = .01$). Naïve, Trace 250 and Trace CL did not differ from one another ($p > .05$). Thus, hippocampal dependent training is not sufficient, as evidenced by the absence of a pro-survival effect in the Trace 250 con-

dition. Further, facilitation of acquisition of trace eyeblink conditioning with the post-US cue abolished the increase in survival. This pattern of results suggests that hippocampal dependence is not sufficient to rescue adult-generated hippocampal cells from death, but rather, only rats exhibiting slow acquisition of hippocampal-dependent learning tasks exhibit increased survival.

5. Discussion

The experiments presented here demonstrate that hippocampal dependence of eyeblink conditioning is not sufficient to reduce death of adult-generated hippocampal cells.

Rather, within the range of hippocampal-dependent tasks tested, learning rate predicts the effect of learning on survival of adult-generated cells (Waddell & Shors, 2008). Training with a 250 ms trace interval resulted in faster acquisition compared to training with the 500 ms trace interval, though both arrangements require the hippocampus (Beylin et al., 2001; Weiss et al., 1999).
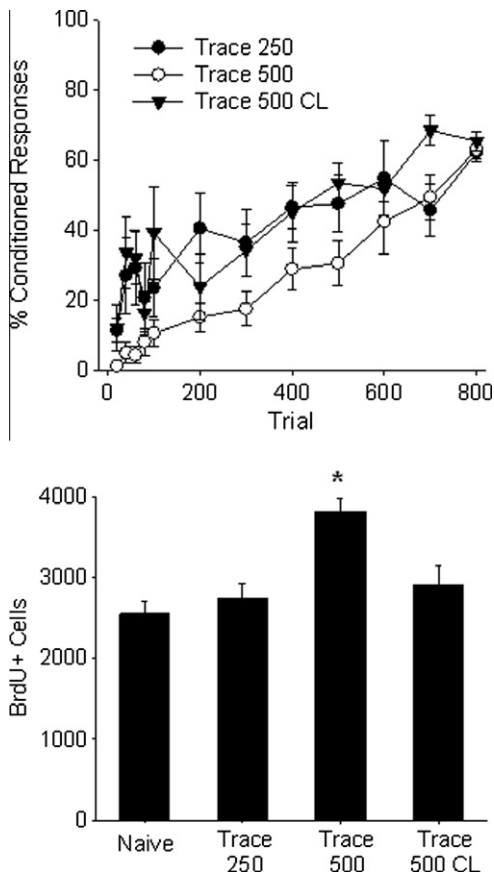
Fig. 4. (A) Behavioral results from animals trained with the Trace 250, Trace 500 and Trace 500 Cue procedures. Data are presented as a mean percentage of conditioned responses ±SEM with the first 100 trials presented in 20 trial blocks with the remaining 700 trials in 100 trial blocks. (B) Bars represent the mean number of BrdU-labeled cells in the SGZ and GCL of the dentate gyrus ±SEM of naïve animals and groups Trace 250, Trace 500 and Trace 500 Cue conditions.

Only training with the 500 ms trace interval increased survival. Surprisingly, addition of a third, discontiguous cue after delivery of the US facilitated the rate of acquisition and also rendered trace conditioning hippocampal-independent.

The strategy used to acquire trace conditioning is not clear. It has been proposed that the hippocampus is necessary to solve trace conditioning tasks because it is needed to detect the contingency between the discontiguous CS and US (Rodriguez & Levy, 2001). Other interpretations of hippocampal dependence of trace conditioning propose that the hippocampus is necessary to discriminate between the trace interval and the intertrial interval (Bolles et al., 1978; Kaplan & Hearst, 1982; Mowrer & Lamoreaux, 1951). In support of this, signaling the end of a trace trial with a different stimulus than that used as the CS can reverse a trace conditioning deficit induced by a very long trace interval in a conditioned suppression preparation (Bolles et al., 1978). The results presented here align with this second proposal, as addition of a third, discontiguous stimulus partially reversed the hippocampal-lesion-induced deficit and facilitated acquisition of trace eyeblink conditioning in intact animals. Rats with permanent excitotoxic lesions of the hippocampus exhibited a significant increase in well-timed conditioned responses as presentation of training trials with the post-US cue progressed.

Trace eyeblink conditioning and trace fear conditioning are particularly sensitive to hippocampal lesions (Bangasser, Waxler, Santallo, & Shors, 2006; Beylin et al., 2001; Kim, Clark, & Thompson, 1995; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Weiss et al., 1999). Hippocampal lesions retard trace eyeblink con-

ditioning even when the trace interval is extremely brief (i.e., 50 ms; Walker & Steinmetz, 2008). Recent evidence suggests that the hippocampus may be selectively necessary for aversive trace conditioning. Lesions of the hippocampus do not abolish acquisition of appetitive trace conditioning and this does not appear to be sensitive to parametric changes in CS and trace interval duration (Kyd, Pearce, Haselgrove, Amin, & Aggleton, 2007; Thibaudeau, Dore, & Goulet, 2009; Thibaudeau, Potvin, Allen, Dore, & Goulet, 2007). This selectivity of motivational system may implicate the role of fear and therefore, the role of the post-US cue as a safety signal. Post-shock cues can reduce the expression of fear and even abolish helplessness associated with unpredictable shock (Mineka, Cook, & Miller, 1984). Fear as indexed by freezing and ultrasonic vocalizations is high during early eyeblink conditioning trials and decreases as conditioning continues and the eyeblink CR emerges (Britton & Astheimer, 2004; Lee & Kim, 2004). It is possible that aversive trace conditioning relies on the hippocampus because the US elicits fear that accrues to the context as well as the CS (Britton & Astheimer, 2004). If the post-US cue is serving to reduce fear by signaling a period free of aversive stimulation, then the hippocampus is needed to distinguish between the reinforced and non-reinforced intervals comprising the experimental session. Further experiments are necessary to determine whether the post-US cue is acting to signal the intertrial interval.

Ablation of adult-generated hippocampal cells does not reliably disrupt hippocampal-dependent learning (e.g., Clelland et al., 2009; Jaholkowski et al., 2009; Shors et al., 2002). A recent report demonstrated that ablation of neurogenesis in the adult mouse only disrupted a spatial or visual discrimination task when the stimuli were very similar to each other (Clelland et al., 2009). When discrimination tasks were easy, in that the stimuli were very different, performance between irradiated and sham-irradiated mice did not differ (Clelland et al., 2009). This finding aligns well with the proposal that the dentate gyrus is involved in pattern separation and discrimination learning (e.g., Clelland et al., 2009; Kesner, Lee, & Gilbert, 2004; Lee & Kesner, 2004; Leutgeb, Leutgeb, Moser, & Moser, 2007; Rolls, 1996). The rate of learning also appears to predict the impact of water maze learning on survival of new cells (Gould et al., 1999; Greenough, Cohen, & Juraska, 1999; van Praag et al., 1999). Survival is increased by hippocampal-dependent water maze training only when animals require at least 2 days of training (Epp et al., 2007; Gould et al., 1999). Thus, learning rate appears to reliably predict the impact of learning on neurogenesis across preparations.

The mechanism through which learning reduces cell death of adult-generated cells is not known. Experience-dependent modulation of survival is critically dependent on the age of the cell (e.g., Döbrössy et al., 2003; Tronel et al., 2010). Because the pro-survival effect of learning directly relates to the rate of learning, we presume that new cells are modulated by local network activity when it is relatively protracted. Our laboratory consistently observes learning-dependent modulation of cell survival 1 week following BrdU administration (e.g., Dalla, Bangasser, Edgecomb, & Shors, 2006; Gould et al., 1999; Leuner et al., 2004, 2006; Waddell & Shors, 2008), a developmental phase at which adult-generated cells in the rat extend axons into CA3 (Hastings & Gould, 1999). Similarly, water maze training increases survival 6–10 days following BrdU administration (Epp et al., 2007; Kee, Teixeira, Wang, & Frankland, 2007; Tronel et al., 2010). Environmental enrichment also increases survival within 2 weeks of cell development in the mouse and the BrdU-labeled cells selectively exhibit markers of activity when re-exposed to the same environment (Tashiro, Makino, & Gage, 2007). A recent study found that spatial learning characterized by high cognitive demands facilitated maturation of adult-generated cells, and enhanced dendritic complexity of granule cells generated 1 week prior to training (Tronel et al., 2010). These findings suggest

experience modulates survival and cell development, and further suggest that immature cells rescued by enrichment or learning encode at least some aspects of the experience.

The contribution of new neurons to memory formation presumably relies in part upon the maturity of the cells at the time of learning. Adult-generated cell development parallels cell development in the neonate (Esposito et al., 2005; Ge et al., 2006; Overstreet Wadiche, Bromberg, Bensen, & Westbrook, 2005). Newly generated cells first exhibit tonic excitation in response to GABA followed by GABA-mediated synaptic inputs to glutamatergic-mediated synaptic inputs (Ben-Ari, Khazipov, Leinekugel, Caillard, & Gaiarsa, 1997; Ge et al., 2006; Tozuka, Fukuda, Namba, Seki, & Hisatsune, 2005). The rate of this gradual switch from depolarizing GABA to hyperpolarizing GABA likely differs between rats and mice (Snyder et al., 2009). Cell maturation in the mouse is approximately 2 weeks slower than that demonstrated in rats, as indexed by neuronal markers and activity-dependent immediate early gene expression (Snyder et al., 2009). Development of retroviral techniques have enabled visualization of adult-generated cells in vivo to analyze cell maturation in the mouse (e.g., Ge et al., 2006; Song et al., 2005; Zhao, Teng, Summers, Ming, & Gage, 2006), thus examining the more protracted cell maturation between rats and mice. It is notable that in the rat, newly generated neurons exhibit enhanced plasticity at an immature phase (Schmidt-Hieber, Jonas, & Bischofberger, 2004). Newly generated cells expressing PSANCAM, a marker known to be expressed in new cells for the first 1–3 weeks after mitosis, exhibit a lower threshold for induction of long-term potentiation (Schmidt-Hieber et al., 2004; Snyder et al., 2009). Because this period of PSANCAM expression parallels the window during which many newly generated cells die (Dayer et al., 2003), it is possible that associative learning induces plasticity and recruits new cells into existing circuitry. Learning a particularly difficult spatial task enhanced survival and promoted development of complex dendritic arbors in immature cells, facilitating incorporation into existing circuitry (Tronel et al., 2010). Further experiments are necessary to understand how new neurons might contribute to memory formation. Increased survival induced by learning may produce a memory that is more stable and less susceptible to interference or decay.

Individual differences in learning rate may reflect differences in motivational state. Hippocampal theta is a rhythm proposed to index alertness or attentiveness (see Berry and Seager (2001) for review). Rabbits exhibiting high theta ratio prior to eyeblink conditioning acquire the CR faster than rabbits exhibiting low theta activity (Berry & Thompson, 1978; Nokia, Penttonen, Korhonen, & Wikgren, 2008). In slow learners, theta increases as training progresses and the CR emerges (Berry, 1982; Berry & Seager, 2001; Nokia, Penttonen, Korhonen, & Wikgren, 2009). Eyeblink conditioning tasks may increase survival only to the degree the task maintains attention across training sessions. Thus, adult-generated neurons may serve to promote learning when learning is particularly challenging. In summary, these data clearly dissociate the effects of learning on neurogenesis from those that are dependent on the hippocampus for learning.

Acknowledgments

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