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Arrest of Adult Hippocampal Neurogenesis in Mice Impairs Single- But Not Multiple-Trial Contextual Fear Conditioning

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The role of adult hippocampal neurogenesis in contextual fear conditioning (CFC) is debated. Several studies demonstrated that blocking adult hippocampal neurogenesis in rodents impairs CFC, while several other studies failed to observe an impairment. We sought to determine whether different CFC methods vary in their sensitivity to the arrest of adult neurogenesis. Adult neurogenesis was arrested in mice using low-dose, targeted x-irradiation, and the effects of irradiation were assayed in conditioning procedures that varied in the use of a discrete conditioned stimulus, the number of trials administered, and the final level of conditioning produced. We demonstrate that irradiation impairs CFC in mice when a single-trial CFC procedure is used but not when multiple-trial procedures are used, regardless of the final level of contextual fear produced. In addition, we show that the irradiation-induced deficit in single-trial CFC can be rescued by providing preexposure to the conditioning context. These results indicate that adult hippocampal neurogenesis is required for CFC in mice only when brief training is provided.

Keywords: dentate gyrus, memory, learning, hippocampus, postnatal neurogenesis, contextual fear conditioning

The dentate gyrus (DG) is one of a small number of brain regions that retains the ability to generate neurons in adulthood. Adult-born neurons in the DG become granule cells that form functional synapses (Toni et al., 2008; Toni et al., 2007) and exhibit activity-related plasticity (Ge, Yang, Hsu, Ming, & Song, 2007; Schmidt-Hieber, Jonas, & Bischofberger, 2004; Wang, Scott, & Wojtowicz, 2000). There is a growing consensus that adult-born hippocampal neurons make functionally significant contributions to hippocampal physiology and behavior, based on evidence that these cells are activated in situations that evoke hippocampus-dependent learning (Kee, Teixeira, Wang, & Frankland, 2007; Ramirez-Amaya, Marrone, Gage, Worley, & Barnes, 2006), reports that arresting adult hippocampal neurogenesis impairs performance in some hippocampus-dependent behavioral tasks (e.g., Clelland et al., 2009; Saxe et al., 2006; Shors et al., 2001; Snyder, Hong, McDonald, & Wojtowicz, 2005; Zhang, Zou, He, Gage, & Evans, 2008), and neural network models positing plausible mechanisms through which adult-born neurons might contribute to learning and memory (Aimone, Wiles, & Gage, 2009; Becker, 2005; Becker, Macqueen, & Wojtowicz, 2009; Meltzer, Yabaluri, & Deisseroth, 2005; Wiskott, Rasch, & Kempermann, 2006). Despite the consensus that adult-born neurons are functionally significant, there is little or no agreement on which behavioral tasks are sensitive to the disruption of adult neurogenesis and on which underlying psychological processes are altered when adult neurogenesis is arrested.

The literature on contextual fear conditioning is characteristic of the disagreement in the field. Contextual fear conditioning (CFC) is a form of learning produced by pairing an aversive stimulus, such as a footshock, with a distinctive context. Several studies in mice and rats demonstrated that blocking adult hippocampal neurogenesis with low-dose irradiation (Hernandez-Rabaza et al., 2009; Ko et al., 2009; Saxe et al., 2006; Warner-Schmidt, Madsen, & Duman, 2008; Winocur, Wojtowicz, Sekeres, Snyder, & Wang, 2006; Wojtowicz, Askew, & Winocur, 2008) or inducible genetic systems (Imayoshi et al., 2008; Saxe et al., 2006) impairs CFC. When neurogenesis was arrested prior to conditioning in these studies, mice exhibited less conditioned fear of the shock-paired context than did control mice in a postconditioning test session. However, several other studies failed to observe any effect of arresting adult neurogenesis on CFC (Clark et al., 2008; Dupret et al., 2008; Pollak et al., 2008; Shors, Townsend, Zhao, Kozorovitskiy, & Gould, 2002; Zhang et al., 2008). Some of the discrepancies between studies may be attributable to differences in the ablation methods, which vary in the extent of the ablation, the cell types targeted, and the side effect profile. However, differences in the ablation method do not explain all of the inconsistencies, as conflicts exist even among studies using the same method. For instance, several studies reported that arresting neurogenesis with low-dose irradiation impaired CFC (Saxe et al., 2006; Warner-Schmidt et al., 2008; Winocur et al., 2006; Wojtowicz et al., 2008),

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whereas another study found no effect of irradiation (Clark et al., 2008).

Another critical variable is likely to be the CFC methodology, which has varied considerably in the neurogenesis literature. For instance, some studies (Farioli-Vecchioli et al., 2008; Imayoshi et al., 2008; Saxe et al., 2006; Warner-Schmidt et al., 2008; Winocur et al., 2006; Wojtowicz et al., 2008; Zhang et al., 2008) trained subjects with pairings between a discrete stimulus and a footshock (a procedure sometimes termed "background" context conditioning), while other studies (Clark et al., 2008; Hernandez-Rabaza et al., 2009; Ko et al., 2009) used context-shock pairings without a discrete cue (sometimes termed "foreground" context conditioning). Studies have also differed in the number of conditioning trials administered, the shock intensity, and the final level of conditioned fear produced.

We sought to determine whether different CFC methods vary in their sensitivity to the arrest of adult neurogenesis. We arrested adult neurogenesis using low-dose, targeted x-irradiation, and then assayed the effects of irradiation in conditioning procedures that varied in the use of a discrete conditioned stimulus, the number of trials administered, and the final level of conditioning produced. Irradiation was selected because it is a common method of arresting neurogenesis employed by several laboratories, produces a complete and permanent arrest of adult neurogenesis, and leaves other neurogenic niches intact when applied focally (Meshi et al., 2006; Santarelli et al., 2003). Although side effects of low-dose x-irradiation have been identified (e.g., Monje, Mizumatsu, Fike, & Palmer, 2002), behavioral or physiologic correlates of these side effects have not been detected (Wojtowicz, 2006). Moreover, in studies employing multiple ablation strategies, the behavioral effects of irradiation have been identical to those of inducible genetic strategies (Clelland et al., 2009; Saxe et al., 2006; Saxe et al., 2007).

Here, we demonstrate that irradiation impairs CFC in mice when a single-trial CFC procedure is used but not when multiple-trial procedures are used, regardless of the final level of contextual fear produced. In addition, we show that the irradiation-induced deficit in single-trial CFC can be rescued by providing preexposure to the conditioning context. These results indicate that adult neurogenesis is necessary in mice for CFC only when brief training is provided.

Method

Subjects

A total of 345 male 129S6/SvEvTac mice were purchased from Taconic (Germantown, NY) and arrived at 7–9 weeks of age. Mice were housed five per cage with *ad libitum* food and water. Irradiation was performed at 9–10 weeks of age and behavior testing occurred between 15 and 21 weeks of age.

Apparatus

Fear conditioning was conducted in chambers obtained from Med Associates (St. Albans, VT), with internal dimensions of approximately 20 cm wide × 16 cm deep × 20.5 cm high. The chambers had metal walls on each side, clear plastic front and back walls and ceilings, and stainless steel bars on the floor. A house light (CM1820 bulb, 28v, 100mA) mounted directly above the chamber provided illumination. Each chamber was located inside a larger, insulated, plastic cabinet that provided protection from outside light and noise. Each cabinet contained a ventilation fan that was operated during the sessions. A paper towel dabbed with mint solution was placed underneath the chamber floor. Mice were held outside the experimental room in their home cages prior to testing and transported to the conditioning apparatus individually in standard mouse cages. Chambers were cleaned with 70% ethanol between each set of mice.

The training and context test sessions were conducted with the conditioning chambers configured exactly as described above. For the tone test sessions (described below), the chambers and handling procedure were changed in several ways so fear of the tone CS could be assessed in the absence of contextual cues associated with shock. The floor and walls of the chamber were covered by plastic inserts; the chamber was scented with lemon; the ventilation fan was not operated; chambers were cleaned with a nonalcohol disinfectant between runs; the room lighting was altered; and mice were transported to the apparatus in a different type of cage.

The behavior of mice was recorded with digital video cameras mounted above the conditioning chamber. Video recordings were analyzed using FreezeFrame software from Actimetrics (Evanston, IL). This software assesses freezing by measuring changes in the intensity of each pixel between successive frames of the video file. Each file was examined individually, and statistical analysis of the pixel-change distribution was used to detect motion and distinguish it from background pixel noise. We have determined that the scores obtained with this software are highly correlated with the scores assigned by human observers.

To assess the unconditioned response to shock, we measured the distance traveled by mice during the shock. Videos of the shock response (filmed from above the conditioning chamber) were converted to a sequence of still frames using VirtualDub software (http://www.virtualdub.org/), and then imported into ImageJ (http://rsb.info.nih.gov/ij/) as a stack. The path of the mouse was traced manually using the segmented line tool, and the length of the path in centimeters was obtained using the "measure" function.

Procedure

Irradiation was performed as described previously (Meshi et al., 2006; Santarelli et al., 2003; Saxe et al., 2006). Briefly, mice were x-irradiated three times (5 Gy per dose) in the course of one week, for a cumulative dose of 15 Gy. Sham mice were treated identically but did not receive irradiation. Mice were anesthetized with ketamine and xylazine (105 mg/kg, 6 mg/kg IP), placed in a stereotaxic frame, and exposed to cranial irradiation. A lead shield covered the entire body but left unshielded a treatment field above the hippocampus.

Experiment 1: Tone-shock fear conditioning. The fear conditioning procedure took place over three consecutive days. On Day 1, mice (Sham $n = 15$, Irrad. $n = 15$) were placed in the conditioning chamber and received 3 pairings between a tone (20 s, 80 dB, 2 KHz) and a coterminating shock (1 s, 0.5 mA). The tones commenced at 120, 290, and 400 s after the start of the session.

The tone test was conducted on Day 2. Mice were placed into the altered conditioning chambers (described above), and the tone was presented twice for 20 s at 120 and 290 s into the session. No

shocks were administered. Freezing was scored for the 1 min prior to the first tone presentation (pretone freezing) and during each tone presentation (tone-elicited freezing).

On Day 3, mice were tested for conditioned fear of the training context. The testing procedure and context were identical to those used on Day 1, except the CS and shocks were not given. Mice were placed into the chambers for 3 min. The entire session was scored for freezing.

Experiment 2: Context-shock conditioning. The procedure was based on that of Wiltgen, Sanders, Anagnostaras, Sage, and Fanselow (2006). Mice receiving the delayed shock (Sham $n = 17$, Irrad. $n = 13$) were placed in the conditioning chambers and 3 min later received one shock (2 s, 0.75 mA). Mice were removed 15 s following the shock. Mice in the no-shock condition (Sham $n = 7$, Irrad. $n = 7$) were placed in the chamber for 197 s and no shocks were delivered. Mice in the immediate shock condition (Sham $n = 7$, Irrad. $n = 6$) received a shock 10 s after placement in the chamber and were removed 15 s after the shock ended. The next day all mice were returned to the conditioning chamber for 3 min for a test of context-elicited fear.

Experiment 3: Tone-shock fear conditioning with varying shock intensities. Conditioning was conducted as described in Experiment 1, except the shock intensity was varied between groups: 0.3 mA (Sham $n = 25$, Irrad. $n = 25$), 0.4 mA (Sham $n = 31$, Irrad. $n = 33$), or 0.7 mA (Sham $n = 13$, Irrad. $n = 12$).

Experiment 4: Effect of additional context exposure or shocks. One group of mice (Sham $n = 15$, Irrad. $n = 17$) received context-shock conditioning as described above (shock at 180 s, 2 s duration, 0.75 mA). A second group of mice (Sham $n = 24$, Irrad. $n = 24$) also received context-shock conditioning except they received two shocks commencing at 90 and 180 s after the start of the session. A third group of mice (Sham $n = 13$, Irrad. $n = 14$) received context-shock training as described above (one shock at 180 s) but were given preexposure to the conditioning context 24 h prior to conditioning. For the preexposure, mice were transported to the chambers as on the conditioning day, placed in the conditioning chamber for 197 s, and then returned to their home cages. To control for possible effects of context exposure on freezing, we included a fourth group of mice (Sham $n = 6$, Irrad. $= 6$) that was treated the same as the previous group except that no shock was delivered. All mice received a 3-min context test session 24 h after conditioning.

Doublecortin (DCX) immunohistochemistry was used to confirm the irradiation-induced arrest of neurogenesis. Mice ($N = 12$) were killed by transcardial perfusion following fear conditioning 6.5 or 12.5 weeks after irradiation. Immunohistochemistry was conducted as described previously (Meshi et al., 2006), and sections were counterstained with Harris hematoxylin.

Data Analysis

The primary measure of interest was percent time freezing. Freezing data from the tone test sessions were subjected to a 2 (Treatment: Irrad. v. Sham) $\times$ 2 (Period: Pre-Tone v. Tone) analysis of variance (ANOVA) with Period as a repeated measure. The freezing score for the tone period was a mean taken across the two 20-s tone presentations. Data from the context test sessions were initially subjected to 2 (Treatment) $\times$ 3 (Minute) ANOVA with Minute as a repeated measure, and with additional factors as described below. Although the effect of Minute reached significance in some analyses, it never interacted significantly with other variables. Therefore, to simplify reporting of the results, we removed Minute from the analyses reported below; the analyses were thus performed on mean freezing across Minutes 1 through 3. Interactions were probed using *t* tests with the Bonferroni correction. Alpha was set to .05 for all analyses.

Results

Experiment 1: No effect of irradiation on tone-shock fear conditioning. Immunohistochemical labeling of DCX was completely absent in the DG of irradiated mice at 6 and 12 weeks post irradiation (Figure 1), indicating that neurogenesis was halted for the duration of behavioral testing. We began by testing the effects of irradiation on CFC by using a tone-shock conditioning protocol. We previously reported that irradiation impairs CFC produced via multiple tone-shock pairings (Saxe et al., 2006). However, another recent study found no effect of arresting neurogenesis in this procedure (Zhang et al., 2008). We trained irradiated and sham mice as described by Saxe et al. (2006; Figure 2A): mice were given 3 tone-shock pairings, and then context- and tone-elicited fear were assayed separately. Nevertheless, both irradiated and sham mice acquired robust conditioned fear of the tone and context (Figure 2B and 2C). In the tone test session, freezing during the tones was significantly higher than during the pretone period [$F(1, 28) = 102.8$, $p < .001$]. There was no effect of irradiation [$F(1, 28) = 1.3$, $p = .267$] or of the interaction [$F(1, 28) = 1.84$, $p = .186$] on freezing during the tone test. Context-elicited freezing was also robust in both groups of mice. Although context- and tone-elicited freezing appeared to be slightly lower in irradiated mice, these effects did not approach statistical significance [$F(1, 28) < 1$].

Experiment 2: Irradiation impairs context fear conditioning produced via a single context-shock pairing. Next, we trained mice using a context-shock ("foreground") conditioning protocol (Figure 3A) that was reported to be highly sensitive to pretraining hippocampal lesions (Wiltgen et al., 2006) and to the arrest of adult hippocampal neurogenesis (Hernandez-Rabaza et al., 2009;

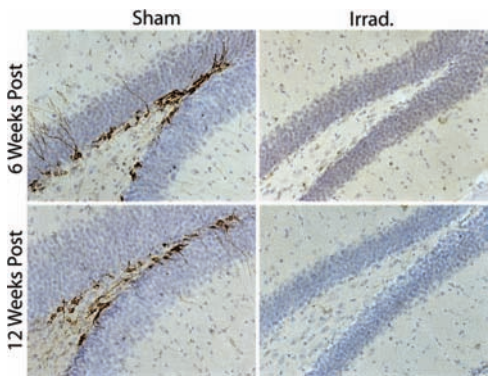

Figure 1. X-irradiation produces a complete and lasting arrest of adult hippocampal neurogenesis. Doublecortin (DCX) immunohistochemistry labels immature neurons lining the inner granule cell layer of sham mice. DCX-labeled cells are absent in irradiated mice, indicating that neurogenesis was ablated. Nuclei are counterstained in blue with Harris hematoxylin.

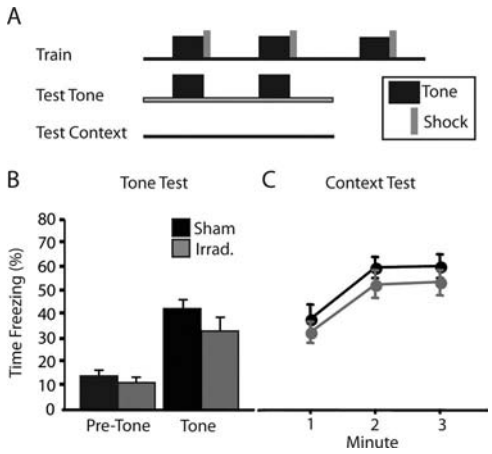

Figure 2. Arrest of hippocampal neurogenesis does not impair contextual fear conditioning (CFC) produced via tone-shock pairings. (A) Irradiated and sham mice were given three tone-shock pairings. Tone-elicited fear was assessed by presenting the tones in an alternate context. Context-elicited fear was tested by returning mice to the shock-paired context. Irradiation failed to affect freezing in the tone test session (B) or in the context test session (C).

Ko et al., 2009). Mice were placed in the conditioning chamber and given a single footshock 180 s later. Context-elicited fear was tested on the following day. We included two control groups: an immediate shock group, which received a footshock 10 s after being placed in the chamber; and a no-shock group, which was placed in the chamber for the same amount of time as the conditioned group but did not receive a footshock. The immediate shock condition was included to control for possible unconditioned effects of shock. An immediate shock typically does not produce fear conditioning, putatively because subjects do not have adequate time to acquire a mental representation of the context prior to receiving the shock (Fanselow, 1986; Landeira-Fernandez, DeCola, Kim, & Fanselow, 2006). As expected, both control groups showed very little freezing in the context test session (Figure 3B), indicating that exposure to an immediate shock or to the context alone was not sufficient to generate conditioned fear of the context. Because two control groups did not differ in their levels of context-elicited freezing [Effect of procedure: $F(1, 24) < 1$] or in their sensitivity to irradiation [Procedure $\times$ Treatment interaction: $F(1, 24) < 1$], the groups were combined into single control group in the following analyses.

To test for an effect of irradiation in this procedure, we subjected the context test data to 2 (Treatment) $\times$ 2 (Procedure: Control v. Conditioned) ANOVA, which yielded a significant interaction effect [$F(1, 58) = 5.1$, $p = .028$]. The interaction was probed by separately testing for effects of irradiation in the conditioned (shock at 180 s) and control groups. Among conditioned mice, irradiated mice exhibited significantly less context-elicited freezing than did sham mice [$F(1, 32) = 6.0$, $p = .020$]. Among control mice, there was no effect of irradiation [$F(1, 26) < 1$]. These results indicate that irradiation impairs CFC produced by a single context-shock pairing, and the impairment is not explained by unconditioned effects of context exposure or shock alone.

One advantage of the tone-shock training protocol over the context-shock protocol is that tone-elicited freezing can serve as an embedded positive control. That is, tone-elicited freezing can demonstrate that a deficit in context-elicited fear is not caused by changes in unconditioned processes such as shock sensitivity. Because the context-shock protocol does not offer such an embedded positive control, we explicitly assessed the unconditioned response to shock in this experiment. We measured the distance traveled by the mice during the shock, and used this information to calculate the mouse's velocity, which has been previously shown to be a measure of shock sensitivity (Wiltgen et al., 2006). As shown in Figure 3C, velocity was similar in all groups of mice. The data were subjected to a 2 (Treatment) $\times$ 2 (Shock Latency: 10s or 180s) ANOVA. There was no effect of Treatment [$F(1, 43) < 1$], Shock Latency [$F(1, 43) < 1$], or the interaction [$F(1, 43) = 1.2$, $p = .286$], indicating that differences in shock sensitivity do not explain the reduced context fear in irradiated mice or in mice receiving the immediate shock.

Experiment 3: Altering the shock intensity does not increase the sensitivity of tone-shock fear conditioning to irradiation. Our tone-shock conditioning procedure produced somewhat more conditioned fear than did our context-shock conditioning procedure. In this experiment, we asked whether the final level of context fear affects the sensitivity of tone-shock fear conditioning to irradiation. To manipulate the level of context fear, we used the same tone-shock conditioning procedure as in Experiment 1 but varied the shock intensity between subjects (0.3, 0.4, or 0.7 mA). As shown in Figure 4, shock intensity affected the overall levels of conditioned fear in the tone and context test sessions, but did not affect the sensitivity to irradiation. Data from the tone test sessions were subjected to a 2

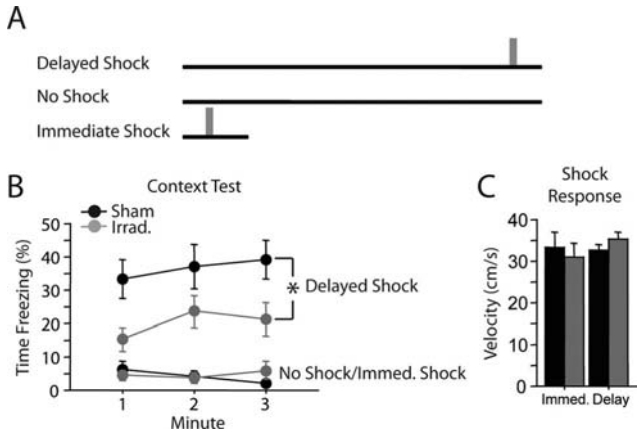

Figure 3. Irradiation impairs CFC produced via a single context-shock pairing. (A) Fear conditioning was produced by placing the mouse in the conditioning chamber and delivering one footshock 180 s later (Delayed shock). One control group received context exposure but no shock. The other control group received a footshock within 10 s of being placed in the chamber (Immed. shock). Mice were returned to the conditioning chamber on the following day to assess context-elicited fear. The two control groups exhibited negligible levels of freezing in the context test (B) and did not differ from each other, and, therefore, were combined as one control group. There was no effect of irradiation in the control groups, but among mice receiving the delayed shock, irradiated mice exhibited significantly less context-elicited fear than sham mice. Neither irradiation nor the shock latency (immediate vs. delayed) affected the unconditioned response to shock, operationalized as the mouse's velocity of locomotion during the shock (C). * $p < .01$.

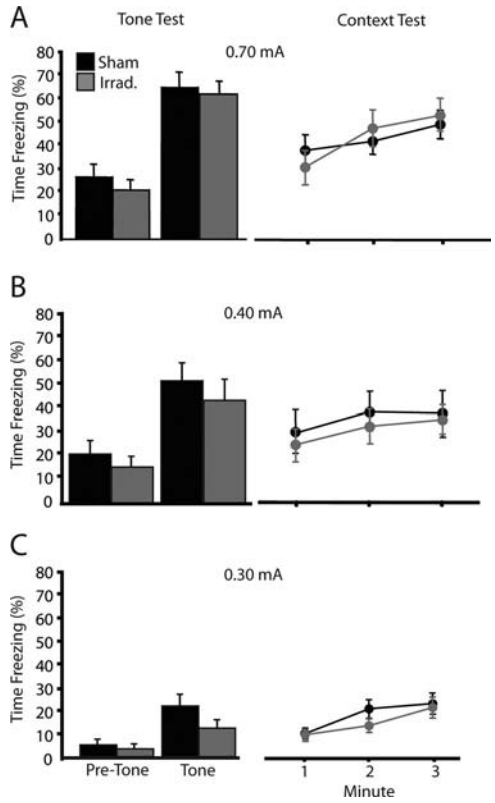

Figure 4. Manipulating the final level of conditioned fear via changes in shock intensity fails to increase the sensitivity of tone-shock fear conditioning to irradiation. The figures depict freezing during the tone and context test sessions for mice given tone-shock fear conditioning with shocks of 0.7, 0.4, or 0.3 mA. Shock intensity strongly influenced tone- and context-elicited freezing, and irradiation failed to affect tone- or context-elicited fear at any shock intensity level.

(Treatment) × 3 (Shock Intensity) × 2 (Period: Pre-Tone v. Tone) ANOVA with Period as a repeated measure. There was a significant effect of shock intensity [$F(2, 133) = 44.8$, $p < .001$] and a significant Intensity × Period interaction [$F(2, 133) = 17.3$, $p < .001$], indicating that the variation in shock intensity affected freezing in the tone test session. However, there was no main effect of irradiation treatment [$F(1, 133) = 3.2$, $p = .077$], and irradiation treatment did not interact with any other variable (F's < 1). Data from the context sessions were subjected to a 2 (Treatment) × 3 (Shock Intensity) ANOVA. Again, there was a significant effect of shock intensity [$F(1, 133) = 31.0$, $p < .001$], but no effect of irradiation [$F(1, 133) < 1$] or of the interaction [$F(2, 133) < 1$]. The results indicate that the tone-shock conditioning procedure is insensitive to irradiation across a broad range of conditioned fear levels.

Experiment 4: The irradiation-induced impairment in context-shock fear conditioning is rescued by context preexposure or by an additional shock. The differential sensitivity to irradiation of tone-shock versus context-shock conditioning might be explained by the different amounts of training provided in each procedure. In the tone-shock procedure, mice were given three tone-shock pairings, but in the context-shock procedure mice were given only a single context-shock pairing. If the amount of training affects the sensitivity to irradiation, the deficit in context-shock conditioning should be rescued by additional training. We hypothesized that additional training could be provided in two ways: through additional shocks or through additional preshock exposure to the conditioning context. In this experiment, we asked whether these manipulations would rescue the CFC deficit in irradiated mice.

Mice were given either (1) context-shock fear conditioning as described in Experiment 2 (shock at 180 s), (2) context-shock fear conditioning with two shocks rather than one shock (shocks at 90 and 180 s), or (3) context-shock fear conditioning (one shock) with preexposure to the conditioning chamber prior to conditioning (Figure 5A). Because activity tends to habituate over time in an environment, and the absence of activity can be confused with freezing, we included a control group that received the same amount of context exposure as Group 3, but with no shock.

Figure 5B shows mean percent time freezing in the context test sessions. Data were analyzed using planned comparisons (t tests), which were justified by the a priori predictions derived from the results of Experiments 1 through 3. The predictions were as follows: (1) irradiated mice will display less context-elicited freezing than sham mice after a single context-shock pairing; (2) additional context exposure or (3) an additional shock will abolish the difference between sham and irradiated mice; and (4) freezing levels will not differ between sham and irradiated mice given

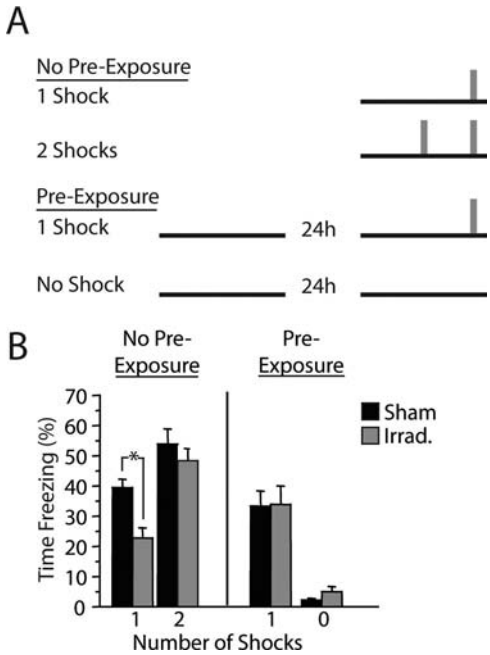

Figure 5. The irradiation-induced impairment in context-shock fear conditioning is rescued by context preexposure or by an additional shock. (A) Mice were given context-shock fear conditioning with one or two shocks, or with one shock plus preexposure to the conditioning context. A control group received context exposure but no shock. (B) Freezing during the context test session. Irradiation impaired CFC in mice given a single context-shock pairing but had no effect in mice that received two shocks or that received context preexposure. Freezing was minimal and unaffected by irradiation after context-alone exposure. * $p < .05$.

context-alone exposure. All four predictions were confirmed. Irradiated mice froze less than sham mice after a single context-shock pairing [$t(30) = 3.3$, $p = .003$]. However, the difference between irradiated and sham mice was abolished in the groups receiving context preexposure [$t(25) = 0.2$, $p = .735$] or an additional shock [$t(46) = 0.5$, $p = .594$]. Among mice given only context exposure, freezing levels were very low, and there was no effect of irradiation [$t(9) = 1.8$, $p = .103$].

Discussion

We found that CFC with a single context-shock pairing was sensitive to the arrest of neurogenesis via irradiation, whereas conditioning with multiple tone-shock pairings was not. The difference in sensitivity to irradiation was not related to differences in the final level of conditioned fear produced, as tone-shock fear conditioning was insensitive to irradiation even when the final level of conditioned fear was equated with that produced by a single context-shock pairing. The difference in sensitivity to irradiation was instead attributable to the number of conditioning trials or to the amount of context exposure. The irradiation-induced impairment in context-shock conditioning was rescued when mice were given an additional shock or preexposure to the conditioning context. These results suggest that, in mice, adult neurogenesis is necessary for CFC only when brief training is provided.

The learning that underlies CFC is typically thought to include two distinct processes: the acquisition of a mental representation of the context and of an association between the context representation and the unconditioned stimulus representation (e.g., Rudy, Huff, & Matus-Amat, 2004). Our finding that the irradiation-induced deficit in context conditioning could be rescued by extra context exposure would seem to argue that arresting adult hippocampal neurogenesis impedes the acquisition of the context representation. However, the rescue by an additional shock suggests that arresting neurogenesis impedes the acquisition of the context-shock association. It may be that both of these learning processes are impeded, but not altogether halted, by the arrest of neurogenesis, and the extra context exposure or additional shock permits normal levels of memory strength to accrue. An alternative explanation is that arresting neurogenesis impedes only the acquisition of the context representation, and this acquisition process is ameliorated by extra context exposure or by the emotional arousal produced by the additional shock. Arousing stimuli can promote acquisition and consolidation of weak memories that might otherwise be forgotten (Anderson, Wais, & Gabrieli, 2006; McGaugh, 2006). Multiple shocks presumably produce greater arousal than a single shock, and this greater arousal may further strengthen context memory and thereby alleviate the deficit in irradiated mice.

These results begin to explain discrepancies in the neurogenesis literature. In the mouse, single-trial CFC is often impaired by manipulations that interfere with hippocampal neurogenesis (Farioli-Vecchioli et al., 2008; Ko et al., 2009), whereas multiple-trial CFC is usually not impaired (Clark et al., 2008; Dupret et al., 2008; Pollak et al., 2008; Zhang et al., 2008). Yet there are exceptions to this generalization. Two mouse studies, including one of our own, reported that neurogenesis-arrested mice are impaired in multiple-trial CFC (Imayoshi et al., 2008; Saxe et al., 2006). If arresting neurogenesis impedes acquisition of CFC but does not altogether stop it, as our data suggest, study-to-study variability could originate from at least two sources. First, a residual deficit may be present even after multiple conditioning trials, and this deficit may be detected on occasion due to sampling variability. Second, acquisition speed is likely to vary according to the particulars of the conditioning protocol. We predict that multiple-trial CFC procedures will be sensitive to the arrest of neurogenesis under conditions that prolong acquisition, such as increased task difficulty. For instance, acquiring a mental representation of a complex context likely requires more time and/or trials than does acquiring a representation of a simple context. When the conditioning context is complex, arresting neurogenesis may thus impair context conditioning even when more than one conditioning trial is given. Consistent with this hypothesis, two studies (albeit in rats) reporting effects of irradiation on multiple-trial CFC used conditioning chambers with transparent walls permitting views of a complex surrounding environment (Winocur et al., 2006; Wojtowicz et al., 2008). Finally, mouse strains differ considerably in their rates of neuronal proliferation and survival (Kempermann, Kuhn, & Gage, 1997), cognitive ability, and behavioral performance (Crawley et al., 1997), and these factors are likely to modulate the behavioral effects of arresting neurogenesis (e.g., Holick, Lee, Hen, & Dulawa, 2008).

An alternative view is that multiple-trial CFC is usually insensitive to the arrest of neurogenesis because the hippocampus is not recruited in this version of the procedure. Indeed, pretraining lesions of the hippocampus often fail to affect CFC when multiple context-shock (Wiltgen et al., 2006) or tone-shock pairings are used (Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998; Maren, Aharonov, & Fanselow, 1997). However, these studies likely underestimate the role of the hippocampus in these learning paradigms, because when the hippocampus is lesioned prior to training, alternative learning systems can become engaged, masking the effect of the lesion. Posttraining lesions preclude these compensatory processes and reliably impair CFC (Anagnostaras, Maren, & Fanselow, 1999; Frankland et al., 1998; Maren et al., 1997). Furthermore, subtle pretraining manipulations (e.g., NMDA blockade; Bast, Zhang, & Feldon, 2003; Young, Bohenek, & Fanselow, 1994) can more strongly impair CFC than do gross lesions, which has prompted the hypothesis that the intact hippocampus actively inhibits contextual learning by extrahippocampal structures, namely the neocortex (Rudy et al., 2004). When the hippocampus is completely inactivated, this inhibitory function is compromised and extrahippocampal structures can compensate. More subtle hippocampal manipulations may fail to block the inhibition of compensatory processes, and, as a result, the hippocampal contribution to behavior is more plainly revealed. In summary, the hippocampus appears to be recruited in multiple-trial CFC, even though traditional hippocampal lesions sometimes fail to produce a behavioral effect.

The dissociation between single- and multiple-trial CFC procedures that we observed in the mouse does not appear to generalize to the rat. In the rat, arrest of hippocampal neurogenesis impairs both single- (Hernandez-Rabaza et al., 2009) and multiple-trial CFC (Hernandez-Rabaza et al., 2009; Snyder et al., 2009; Warner-Schmidt et al., 2008; Winocur et al., 2006; Wojtowicz et al., 2008). This apparent species difference may reflect species differences in the functional integration of adult-generated neurons. It appears that a considerably larger proportion of young, adult-generated neurons are activated by spatial learning in the rat than in the

mouse (Snyder et al., 2009), which suggests that young adult-generated neurons may have greater functional significance in rats than in mice. Based on this evidence, one might predict that adult-generated neurons are recruited under a broader range of conditions in the rat than in the mouse. If, as our data suggest, additional training precipitates a switch from neurogenesis-dependent to neurogenesis-independent processing mechanisms, then this switch may require more training in the rat than in the mouse.

The finding that adult hippocampal neurogenesis is necessary for single-trial CFC is consistent with research on the role of DG and CA3 in memory acquisition. CA3, the major target of DG granule cell projections, is thought to be specialized for rapid acquisition of spatial information. Blocking NMDA-mediated plasticity in CA3 impairs the acquisition of single-trial spatial learning, but does not impair multiple-trial learning (e.g., Nakazawa et al., 2003). Although blocking NMDA-mediated plasticity within DG does not impair single-trial acquisition of CFC, CFC acquired under this condition is less precise than in control animals, in that conditioned fear more readily generalizes to an alternate context (McHugh et al., 2007). This discrimination impairment is rescued with continued discrimination training, suggesting that the DG is particularly important for the rapid formation of precise spatial memories. Consistent with this hypothesis, it was recently shown that a DG-specific genetic manipulation is sufficient to accelerate acquisition of spatial information (Saab et al., 2009). Our data suggest that arresting neurogenesis impairs the DG-CA3 circuits that are involved in rapid memory acquisition.

Recent literature suggests several ways in which adult neurogenesis might contribute to mnemonic processes. One idea is that the addition of cells to the DG helps preserve the structure's ability to continually encode new memories without interfering with previous ones (Meltzer et al., 2005; Wiskott et al., 2006). According to this view, arresting neurogenesis might reduce the fidelity of new memories, because encoding would need to be mediated by cells whose plasticity is constrained to preserve older memories. An alternative view emphasizes the increased excitability and plasticity exhibited by young neurons relative to mature neurons. A characteristic feature of the DG is its sparse representation of input stimulation, meaning that relatively few DG granule cells become active in response to a given pattern of stimulation (e.g., Jung & McNaughton, 1993; Leutgeb, Leutgeb, Moser, & Moser, 2007). Highly excitable and plastic young neurons presumably reduce the sparseness of DG activity, increasing the degree to which different stimuli excite overlapping populations of cells. This decrease in sparseness may allow contemporaneous events to be integrated into temporally specific episodic memories (Aimone, Wiles, & Gage, 2006; Aimone et al., 2009). According to this view, arresting neurogenesis might impair the ability of the hippocampus to bind together multiple percepts (e.g., contextual stimuli, footshock) into a single episodic memory.

A third hypothesis is based on the view that the DG acts a gate that limits or slows hippocampal activation by cortical input stimulation (Hsu, 2007). The DG gate tends to favor persistent or repeated patterns of stimulation, in that brief stimuli are halted at the level of the DG, whereas prolonged or repeated stimuli activate the DG as well as downstream hippocampal subfields (e.g., Iijima et al., 1996). It is possible that arresting neurogenesis, and thereby removing a population of highly excitable young granule neurons, further slows the gating of cortical inputs, meaning that even more sustained patterns of stimulation are required before hippocampal processing mechanisms become engaged. Thus, in the absence of neurogenesis, very brief training episodes, such as a single context-shock pairing, may fail to recruit hippocampal memory networks. More sustained training episodes, such as multiple tone-shock pairings or extended exposure to a context, open the DG gate and initiate plasticity in CA3, a subfield believed to mediate rapid encoding of spatial memories (Nakazawa et al., 2003; Steele & Morris, 1999).

In summary, we found that arresting adult hippocampal neurogenesis via targeted, low-dose irradiation impairs CFC in mice when a single-trial conditioning procedure is used but not when multiple-trial procedures are used. The irradiation-induced deficit in single-trial CFC can be rescued by providing preexposure to the conditioning context. The data indicate that the contribution of adult neurogenesis to CFC in mice is revealed only when brief training is provided, suggesting that adult-generated hippocampal neurons may be integral for rapid acquisition of context memories.

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