

Inhibition of Neurogenesis Interferes With Hippocampus-Dependent Memory Function

Gordon Winocur,^{1,2,3*} J. Martin Wojtowicz,^{4*} Melanie Sekeres,¹
Jason S. Snyder,⁴ and Sabrina Wang⁴

ABSTRACT: Rats treated with low dose irradiation, to inhibit adult hippocampal neurogenesis, and control rats were administered a non-matching-to-sample (NMTS) task, which measured conditional rule learning and memory for specific events, and a test of fear conditioning in which a discrete CS was paired with an aversive US in a complex environment. Irradiated rats were impaired on the NMTS task when the intervals between sample and test trials were relatively long, and in associating the shock-induced fear with contextual cues in the fear conditioning task. Irradiated rats were not impaired in learning the basic NMTS rule or in performing that task when the intervals between the sample and test trials were short. Nor were there group differences in conditioning the fear response to the CS in the fear conditioning task. The results, which extend the range of hippocampus-dependent tasks that can be said to be vulnerable to the effects of neurogenesis suppression, support the hypothesis that new hippocampal cells generated in adulthood participate in a broad range of hippocampal functions.

© 2006 Wiley-Liss, Inc.

KEY WORDS: NMTS; context fear-conditioning; learning and memory; adult neurogenesis; dentate gyrus

INTRODUCTION

Contrary to the traditional view that neurogenesis in the mammalian brain ceases during prenatal development, it is now well established that the hippocampus continues to produce neurons well into late adulthood in several species, including humans (Altman and Das, 1965; Eriksson et al., 1998; Gross, 2002). Moreover, converging evidence from anatomical (Hastings and Gould, 1999), electrophysiological (Wang et al., 2000; Snyder et al., 2001; van Praag et al., 2002; Schmidt-Hieber et al., 2004), and histochemical labeling (Cameron and McKay, 2001) studies indicates that the newly generated cells become integrated into the circuitry of the hippocampal system. There remains, however, considerable controversy over the functional significance of these new cells.

The hippocampus is critical to the process of forming and recovering certain types of memory (Milner, 1974; Squire et al., 2004), and so it follows that adult-generated hippocampal cells are somehow involved in the learning and memory functions of this structure. There is support for this view. Gould and her colleagues (Gould et al., 1999; Leuner et al., 2004) found that training adult rats on two hippocampus-dependent tasks—trace conditioning and spatial maze learning—resulted in a dramatic increase in the number of newly generated neurons in the dentate gyrus (DG) of the hippocampus (but see van Praag et al., 1999a; Snyder et al., 2005). In other experiments, exposure to an enriched environment (Kempermann et al., 2002) or running activity (van Praag et al., 1999a) increased hippocampal neurogenesis and also led to improved performance on a water-maze test of spatial memory; a test that is considered to be highly sensitive to hippocampal impairment. However, investigations of the effects of inhibiting hippocampal neurogenesis on performance in hippocampus-dependent tasks have not produced consistent results. For example, Shors et al., 2002 found that suppression of newly generated hippocampal cells significantly reduced trace eye-blink conditioning, but not context fear conditioning. With respect to spatial learning and memory, several investigators have reported that neurogenesis depletion does not affect spatial navigation learning in a water maze (Shors et al., 2002; Madsen et al., 2003; Snyder et al., 2005), but does disrupt long-term spatial memory on similar tasks (Madsen et al., 2003; Snyder et al., 2005). Further research is clearly needed to identify the behavioral role of adult-generated hippocampal cells and establish their relationship to functions normally controlled by the hippocampus.

In the present study, rats treated with low-dose irradiation, to inhibit adult hippocampal neurogenesis, were tested on a nonspatial, nonmatching-to-sample (NMTS) task, in a pool filled with opaque liquid. The NMTS task consists of a series of paired sample and test trials. For the sample trials, a distinctive stimulus cued the location of an invisible platform, where the rat could escape the liquid. In the subsequent test trial, the same stimulus was presented along with a different stimulus and the rat must swim to the new stimulus to find the platform. Following NMTS learning, a delayed condition (DNMTS) was introduced between the sample and test trials, and testing was

¹ Department of Psychology, Trent University, Peterborough, Ontario; ² Rotman Research Institute, Baycrest Centre, Toronto, Ontario; ³ Departments of Psychology and Psychiatry; ⁴ Department of Physiology, University of Toronto, Toronto, Ontario

Grant sponsor: Natural Sciences and Engineering Research Council of Canada; Grant number: OGP-8181; Grant sponsors: CIHR and Heart and Stroke Foundation of Canada; Grant numbers: IAP 66553 and T-5411.

*Correspondence to: Dr. Gordon Winocur, Rotman Research Institute, Baycrest Centre, 3560 Bathurst St., Toronto, ON, Canada M5E 2A1. E-mail: gwinocur@rotman-baycrest.on.ca (or) J. Martin Wojtowicz, Department of Physiology, University of Toronto, Toronto, ON, Canada. E-mail: martin.wojtowicz@utoronto.ca

Accepted for publication 1 November 2005

DOI 10.1002/hipo.20163

Published online 12 January 2006 in Wiley InterScience (www.interscience.wiley.com).

continued for an additional 10 days. NMTS rule-learning, which incorporates conditional and working memory components, is highly sensitive to frontal-lobe dysfunction (Moscovitch and Winocur, 1995), but is not typically affected by hippocampal lesions (Zola-Morgan and Squire, 1985; Aggleton et al., 1986). However, by increasing the interval between sample and test trials, the task puts increased demands on hippocampus-controlled memory function (Winocur, 1992). Thus, the task, designed in this way, yields dissociable learning and memory functions, related respectively to the frontal lobes and hippocampus.

In addition, the irradiated and control rats were administered a test of context fear conditioning, as measured by freezing behavior. Context fear conditioning is considered sensitive to hippocampal dysfunction (Kim and Fanselow, 1992; Anagnostaras et al., 2001), although interestingly, Shors et al. (2002) found that neurogenesis depletion did not affect performance on this task. In their study, fear conditioning was conducted in an enclosed chamber, which provided a distinctive and relatively homogeneous contextual environment. The intention in the present study was to test fear conditioning in a more complex environment. Accordingly, fear conditioning was administered in a chamber with clear Plexiglas walls, located in the center of a room that contained a variety of contextual cues as part of the background. To determine whether adult-generated hippocampal cells are equally involved in memory recovery at short and long delays, fear conditioning was tested 24 h and several weeks following training.

MATERIALS AND METHODS

Subjects

Forty male Long-Evans rats, ~4 months old, and weighing 250 g at the beginning of the study, were obtained from the Charles River Laboratories in St. Constant, Quebec, and served as subjects. The rats were shipped directly from the breeder to the Ontario Veterinary College, University Guelph, where they underwent irradiation or a control procedure (see later). A few days later, they were transferred to Trent University, where they were housed in individual cages with food and water, available at all times. The rats were maintained on a 12 h light–dark schedule, with lights on between 8:00 PM and 8:00 AM. All rats participated in fear conditioning and NMTS testing. One control rat died shortly after arriving at Trent, and did not participate in any testing. Consequently, data were reported for 20 irradiated rats and 19 control rats.

Throughout the study, which was approved by the Trent University Animal Care Committee, the rats were examined regularly by a veterinarian. The rats were generally in good health, although there was some loss of weight in the irradiated group.

Irradiation

Under sodium pentobarbital anesthesia, 20 rats received 7.5–10 Gy irradiation to the head (1 Gray = 1Gy = 100 rads).

Gamma irradiation was delivered over a 10-min period, on two consecutive days, using a Theratron 780-C (AECL Medical, MDS Nordion Co., Chalk River, Ontario, Canada). Two doses of 10 Gy were chosen, based on its ability to reduce proliferation of subgranular zone precursor cells by 78%, without any noticeable side effects, except for a small net weight loss, at 2 months after irradiation, (Snyder et al., 2005; Wang et al., 2005). Control rats were also transported to the irradiation facility and anesthetized, but did not receive radiation. The present study was done in two batches. In Batch 1 (10 controls and 10 irradiated), we used 10 Gy irradiation whereas, in Batch 2 (10 controls and 10 irradiated) we used a reduced dose of 7.5 Gy. The behavioral outcomes of the two batches were identical, and so we combined both groups in the behavioral analysis. The irradiation at 7.5 Gy produced similar effect on cell proliferation to 10 Gy dose (see Immunohistochemistry section). As reported previously, the irradiated animals weighed slightly less than the controls at the end of the study i.e., 2.5 months after irradiation. The initial weights of the control and irradiated groups (7.5 Gy and 10 Gy combined) were 248 ± 5 (SE) and 251.7 ± 2 g, and the final weights at the time of perfusion were 481 ± 11 and 440 ± 12 g, respectively. Thus, both groups gained a significant amount of weight, but the irradiated group gained less. This interaction between irradiation and weight was significant (2-way ANOVA, $F_{1,74} = 6.69$, $P = 0.012$). These effects are in line with the previous studies (Snyder et al., 2005; Wang et al., 2005).

BrdU Injection Paradigm

Approximately 1 week after the completion of behavioral testing, the rats were injected once intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU; Sigma, Oakville, Ontario, Canada; 200 mg/kg) so as to label the DNA of cells in S-phase of mitosis. This dosage ensures that the majority of cells actively dividing in the subgranular zone at the time of the injection will be labeled with BrdU. One week following injection, rats were euthanized with a lethal dose of sodium pentobarbital, and perfused using a phosphate buffered saline (PBS) flush, followed by 100 ml of 4% paraformaldehyde. Brains were refrigerated and fixed overnight in 4% paraformaldehyde solution, and then transferred to PBS with sodium azide.

Immunohistochemistry

The effects of irradiation were verified using standard immunohistochemical techniques. Eight right hippocampi from Batch 1 (10 Gy) and five from Batch 2 (7.5 Gy), selected at random, were sectioned at 30 μ m with a vibratome, and 12 sections were selected from each hippocampus, according to the random, systematic sampling method, followed by McDonald and Wojtowicz (2005). All antibodies and other reagents, as well as the immunohistochemical procedures, were described recently (McDonald and Wojtowicz, 2005).

Fear Conditioning

Approximately 4 weeks after irradiation treatment, irradiated and control rats underwent fear conditioning. Conditioning

and testing took place in a chamber (50 × 40 × 18 cm), located in the center of a standard testing room, which was different than the one in which the rats were housed (see later). The chamber, which was placed on a table 3 m above the floor, had clear Plexiglas walls and ceiling, with holes, to allow for ventilation. The floor consisted of metal bars, spaced 1.3 cm apart, that were connected to a shock generator (TechServe; Model 452A).

Each rat received one fear conditioning trial that began with the rat being placed in the chamber and allowed to explore freely for 5 min. Sixty seconds before the beginning of the trial, a preshock measure of freezing was obtained. Following Anagnostaras et al. (2001), freezing was defined by an immobilized crouching response, in which the only detectable movement was the rat's breathing. The time spent freezing during that period was manually recorded using a stopwatch.

Fear conditioning consisted of 10 CS (tone (2000 Hz; 80–90 dB)–US (1.5 mA; 1 s)) pairings, with a variable interval between each pair, that ranged between 10 and 120 s. In each pairing, the US coincided with the termination of the tone. After the last shock, the rat remained in the chamber for 60 s, during which time the total amount of freezing was recorded. The rat was then returned to the home cage.

Half the rats in each group were tested following a short, 24-h delay (Irradiated: $N = 10$; Control: $N = 10$) and the rest after a long, 28-day delay (Irradiated: $N = 10$; Control: $N = 9$) delay. All rats were tested initially in a context (CXT)-only condition and a CS + CXT condition. There was no US during testing.

Testing consisted of a single trial, in which the rat was returned to the chamber for 8 min and, in the absence of the CS (CXT-only), the amount of freezing was recorded. The rat was then returned to its home cage. Approximately 1 h later, the rat was returned to the chamber for CS + CXT testing. The rat was placed in the chamber, and the freezing time was recorded for 60 s. The CS was then sounded for 8 min, during which time the rat's freezing was recorded. After the CS was terminated, the rat's freezing was recorded for an additional 60 s, after which it was returned to its home cage.

NMTS Learning

The NMTS part of the study began the day after short-delay fear-conditioning testing, and was completed before fear conditioning was tested at the long delay. Twenty irradiated rats and 19 controls participated in NMTS and DNMTS (see below) testing.

NMTS and DNMTS testing were conducted in a circular pool (122 cm in diameter), located in the center of a room (360 × 360 cm). The room, which was different than the one in which the rats were trained and tested in the fear conditioning task, was illuminated by overhead fluorescent lights. The pool was filled with water, rendered opaque by diluted, nontoxic white tempera paint, and maintained at room temperature (21°C). Standard lab furniture (e.g., a rack of cages, testing equipment, a stool, and a cabinet) was distributed around

the room, and several pictures were mounted on the walls. Throughout the testing, the water was cleaned daily and changed every 2 days.

An inverted flower pot (24 cm high) with a white surface, that served as a platform (10 cm in diameter), was situated a few centimeters below the surface of the water. The stimuli for the sample and test trials were black and white cylinders (30 cm long and 3 cm in diameter), suspended 20 cm above the surface of the water. The position of the cylinders was controlled manually by the experimenter, through a system of pulleys, weights, and wires that ran inconspicuously outside the perimeter of the pool and along the ceiling. The water maze was divided into six equal zones. The dividing lines between the zones were invisible, but the experimenter became expert at identifying the borders, through extensive prior practice.

The NMTS task consisted of a series of paired sample and test trials. At the beginning of each sample trial, the black or white cylinder was suspended directly above the submerged platform. Both cylinders were present during the subsequent test trial, but the cylinder that was not present during the preceding sample trial was suspended over the platform and cued its location. Thus, if on a given sample trial, the black cylinder cued the platform, and then on the succeeding test trial, the white cylinder cued the platform. The black and white cylinders were selected as sample stimuli for each pair of trials, according to a semirandom schedule, which ensured that each cylinder was the sample stimulus on 50% of the trials over this phase of the experiment. For the sample trials, the location of the sample cylinder was randomly determined. For each test trial, the platform was moved to another zone, with the nonsample cylinder located directly above it. The sample stimulus was also moved to a different zone. The positions of the two stimuli were determined randomly with the proviso that the two stimuli were never located in the same zone. The location of the submerged platform and the locations of the two stimuli were changed after each sample and test trial, according to a random schedule, so as to eliminate the use of spatial cues.

At the beginning of each sample trial, the rat was placed in the pool in a location determined semirandomly, facing the wall of the pool, and allowed to swim to the submerged platform under the sample cylinder. The qualification was that the rat was never placed in a zone that contained the suspended cylinder. The rat remained on the platform for 20 s. On rare occasions at the beginning of training, when a rat would fail to find the platform within 120 s, it was picked up and placed on the platform for 20 s. The rat was then removed and placed under a heat lamp while the platform was moved, and the cylinders put in position for the test trial. The organization of the cylinders and platform took about 20 s. The rat was then placed again in the pool and allowed to swim to the submerged platform or until 120 s had elapsed.

In either case, the rat was allowed 20 s on the platform, before being returned to a holding cage under a heat lamp, to await the next pair of trials. Ten daily sessions, each consisting of five pairs of sample and test trials, were administered.

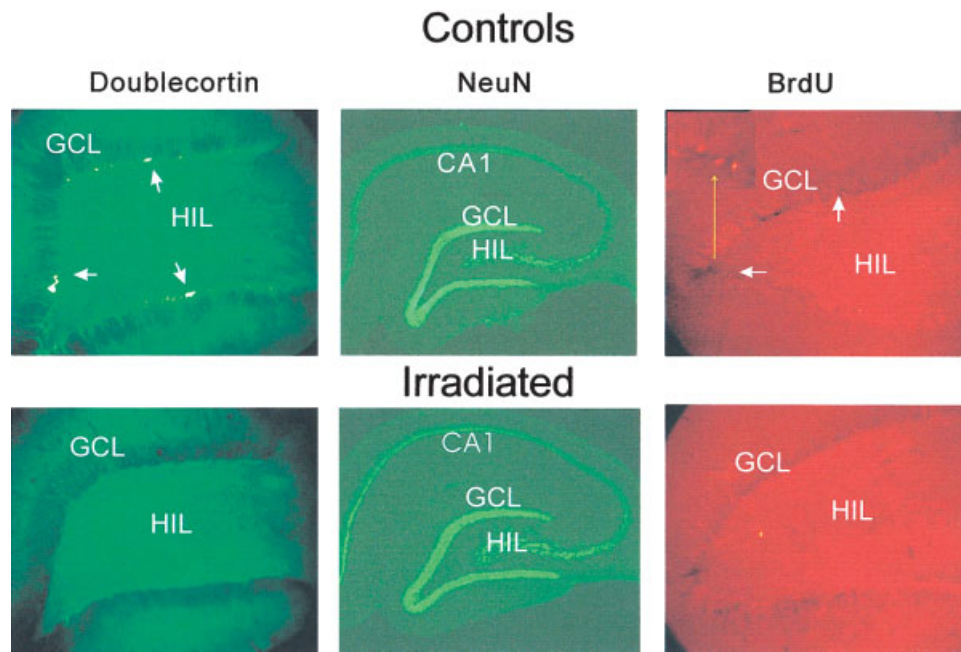


FIGURE 1. Comparison of fluorescent, immunohistochemical labeling for mature neurons (NeuN), proliferating cells (BrdU), and immature neurons (Doublecortin) in control and irradiated rats. Hilus (HIL), granule cell layer (GCL) of the DG, and CA1 field are indicated. Doublecortin+, young neurons (arrows) were almost completely depleted. In the NeuN panel, note that irradiation

did not change the appearance of cell layers in any of the hippocampal fields. BrdU+ cells were also virtually absent in the irradiated animals. Arrows in the BrdU panel point at labeled cells in the subgranular zone. One cluster is shown at higher magnification in the inset. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Delayed NMTS

The day after the completion of NMTS training, rats were administered 10 additional daily sessions. Each session consisted of four paired trials, with intervals of 0, 60, 120, or 240 s between the sample and test trials. (Note: The intervals do not include the 20 s required for repositioning the cylinders and platform.) The order of the sample-test trial intervals varied each day, according to a random schedule, with each interval occurring once each day.

RESULTS

Immunohistochemical Measures of Neurogenesis

Effects of cranial irradiation on neurogenesis were verified by immunohistochemical measurements at the end of the experiment i.e., ~2 months after the irradiation. Two measures of neurogenesis were employed in this study. The total number of BrdU+ cells and the total number of doublecortin+ cells in the subgranular zone/granule cell layer. In Batch 1 (10 Gy irradiation), there were $1,023 \pm 205$ (SE) BrdU+ cells per DG in controls, and 223 ± 53 (SE) in the irradiated animals. In Batch 2 (7.5 Gy), the corresponding numbers were 886 ± 191 and 202 ± 72 . Two-way ANOVA confirmed a significant effect of irradiation in both batches ($F_{1,24} = 24.9$, $P < 0.001$), but with no interaction ($F_{1,27} = 0.15$, $P = 0.07$). The total number of doublecortin+ cells (cells with a young neuronal phenotype) was $11,842 \pm$

$1,148$ (SE) in the controls, and 397 ± 152 (SE) in the irradiated animals of Batch 1 and $12,463 \pm 1,071$ (SE) and $1,277 \pm 169$ (SE) in the corresponding groups of Batch 2 (Fig. 1). Thus, the effects of irradiation at both doses were significant ($F_{1,24} = 200.1$, $P < 0.001$), but there was no significant interaction between treatment and batches ($F_{1,27} = 0.026$, $P = 0.87$). The effects of irradiation were selective for the proliferating and young cells. The volume of DG occupied by mature NeuN+ neurons in irradiated animals appeared to be slightly reduced (both batches combined), but the difference did not reach significance ($1.38 \text{ mm}^3 \pm 0.1$ (SE) in controls and $1.15 \text{ mm}^3 \pm 0.069$ (SE), in irradiated animals ($P = 0.072$, $t = 1.89$, 24 d.f.)). The volume of CA1 was $0.52 \text{ mm}^3 \pm 0.06$ (SE) and $0.42 \text{ mm}^3 \pm 0.05$ (SE) in controls and irradiated, respectively. This difference was not significant ($P = 0.18$, $t = 1.38$, 24 d.f.; Fig. 1).

In summary, the immunohistochemical data suggest that even at the lower dose of 7.5 Gy the irradiation produced a severe loss (90%) of newly produced neurons and ~80% reduction of all proliferating cells in the subgranular zone, but no detectable effects on the mature neurons. These results are in line with previous data (Snyder et al., 2005; Wang et al., 2005).

Nonmatching-To-Sample Task

There were no performance differences between the irradiated and control groups on the sample trials of the NMTS and DNMTS tasks. Consequently, data are presented only for the

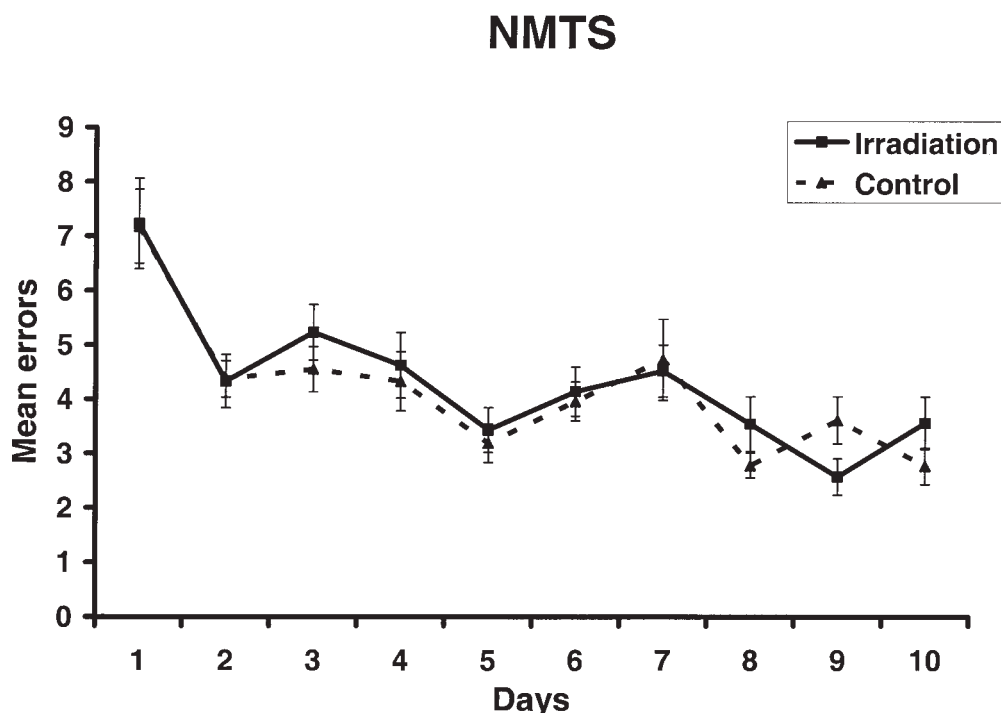


FIGURE 2. Mean error scores for Irradiated and Control Groups over 10 days of NMTS testing (Error bars denote SEM). The results indicate no differences between groups in learning the NMTS rule.

test trials. As well, the latency and error data were highly correlated on both tasks and led to the same conclusions. Thus, for presentation purposes, only the error data are reported here.

Error scores for the irradiated and control groups, over the 10 days of NMTS learning, are presented in Figure 2. As can be seen from the figure, performance of both groups was very similar across the entire training period. Analysis of variance (ANOVA), applied to the data, yielded a highly significant effect of days, $F_{9,333} = 15.48$, $P < 0.0001$, but no effect of Group, $F_{1,37} < 1$.

Delayed NMTS

An overall ANOVA, with Group as a between-group factor and interval and days as within-group factors, was conducted on the entire data set. This analysis yielded two significant two-way interactions—Group \times Interval, $F_{3,111} = 9.27$, $P < 0.0001$ and Days \times Interval, $F_{27,999} = 15.97$, $P < 0.0004$, and a three-way Group \times Days \times Interval interaction that just failed to reach statistical significance, $F_{27,999} = 10.63$, $P = 0.056$. To further investigate these interactions, we examined simple effects at each delay. For these analyses, error terms were pooled across the four delay conditions.

The mean number of errors, at each interval, were averaged over each of the 10 test days for the two groups and are presented in Figure 2. There were no differences between groups at the 0 s, $F_{1,37} < 1$, and 60 s, $F_{1,37} < 1$, interval. However, the Irradiation Group consistently made more errors than the Control Group at the 120 s, $F_{1,37} = 4.47$, $P = 0.04$, and 240 s, $F_{1,37} = 13.22$, $P = 0.0002$, intervals (Fig. 3).

The overall Group \times Days interaction was not significant, $F_{9,999} < 1$, but there was a significant Days effect, $F_{9,999} = 4.27$, $P < 0.0001$. The latter effect was due mainly to the groups' performance at the 120 and 240 s. delays, where both groups improved following an initial decline in performance from their asymptotic levels at the end of NMTS training. This was confirmed by ANOVA, which revealed significant effects of Days at the 120 s, $F_{9,333} = 2.66$, $P = 0.001$, and 240 s, $F_{9,333} = 3.555$, $P = 0.0003$, delays.

Fear Conditioning

The amount of time spent freezing by the Irradiation and Control Groups when tested at the short and long delays in the CXT-only and CS + CXT conditions are presented in Figure 4A,B, respectively. An overall ANOVA was conducted on the fear conditioning data, with Group and Delay as between-group factors and Cue Condition as a within-group factor. This analysis yielded a significant Group \times Cue Condition interaction, $F_{1,35} = 20.54$, $P = 0.0001$, and significant Group, $F_{1,35} = 16.40$, $P = 0.0003$, and Cue Condition, $F_{1,35} = 38.36$, $P < 0.00001$. Delay was not a significant factor in main or interaction effects.

We then examined the simple effect of Group in each cue condition. As can be seen in Figure 4A, overall, in the CXT-only condition, the Irradiation Group spent less time freezing than the Control group, $F_{1,35} = 33.96$, $P < 0.0001$. This difference was seen at both short, $t_{18} = 6.13$, $P < 0.0001$, and long, $t_{17} = 2.96$, $P = 0.009$, delays. The Group \times Delay interaction was not statistically significant, $F_{1,35} = 2.51$, $P =$

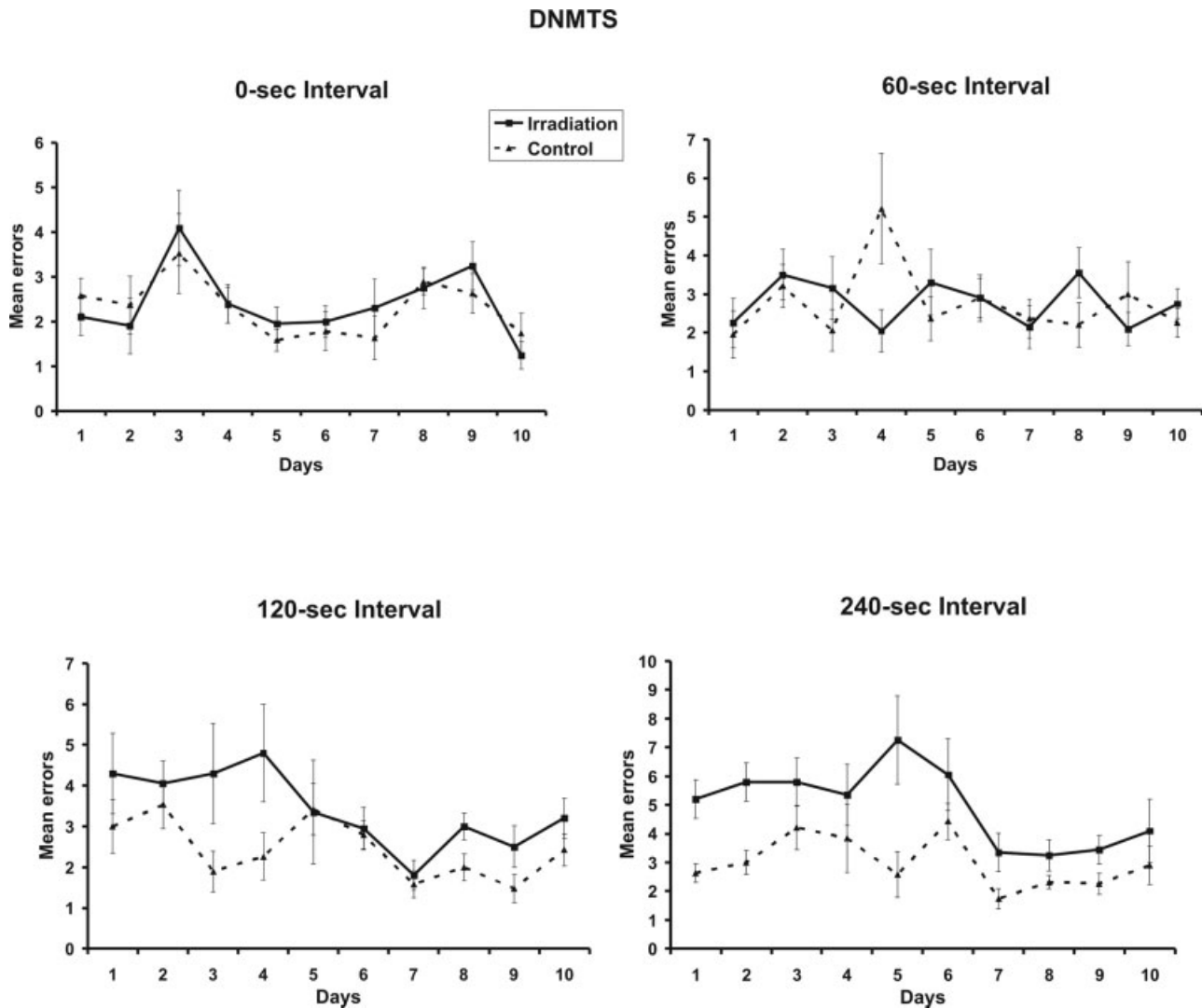


FIGURE 3. Mean error scores for Irradiated and Control Groups at all sample-test trial intervals of the DNMTS test (Error bars denote SEM). The results indicate impaired performance in the Irradiated Group at 120 and 240 s intervals.

0.12. In the CS + CXT condition (Fig. 4B), there were no significant main or interaction effects, all P 's > 0.29.

DISCUSSION

In this experiment, we compared the performance of rats treated with cranial irradiation to inhibit production of new brain cells, and control rats, on two tests of learning and memory—a NMTS task that measured conditional rule learning and memory for episodic events, and a test of fear conditioning, in which a discrete CS was paired with an aversive US in a complex environmental context. Interpretation of the results depends critically on the assumption that irradiation specifically depleted young neurons that were less than 4 weeks old. This is because the behavioral tests began 4 weeks after the irradiation

and the blockade of neurogenesis was irreversible. In previous studies using the same irradiation procedure, we have shown that the procedure had no effects on synaptic transmission or long-term potentiation attributed to the mature neurons in DG, but caused a selective reduction of the plasticity due to the immature neurons (Snyder et al., 2001, 2005, Wang et al., 2005). Additional measurements of the CA1 field and the DG in the irradiated vs. intact rats, using a mature neuronal marker NeuN, showed no significant decrease in sizes of these two hippocampal regions. Although there was a nearly significant reduction in the volume of DG by 17% at >2 months after the irradiation ($P = 0.072$, see Results), this may have been due to reduced neuronal recruitment rather than a direct effect on the mature neurons. Thus, according to all evidence available so far, the irradiation had a selective effect on neurogenesis. Nonspecific effects of irradiation on other brain regions are quite unlikely, in view of the lack of effects on non-

Context Fear Conditioning

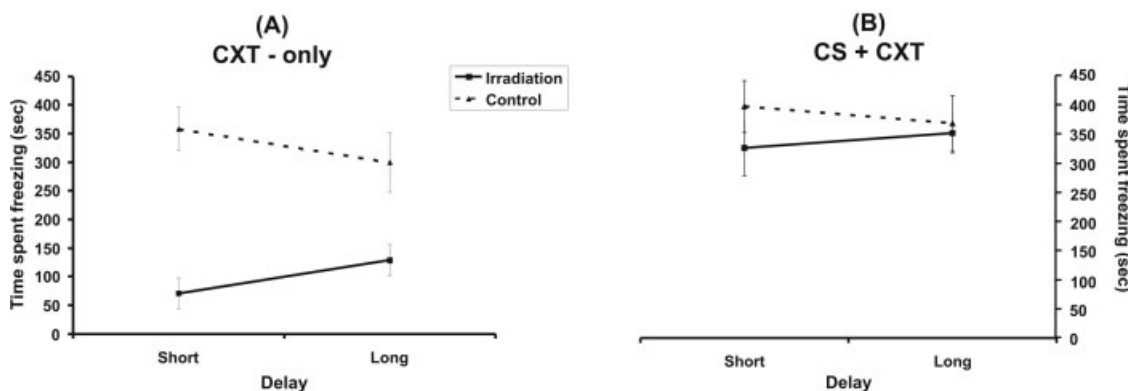


FIGURE 4. Amount of time spent freezing by the Irradiation and Control Groups when tested at the short and long delays in the CXT-only and CS + CXT conditions (Error bars de-

note SEM). The results indicate impaired context fear conditioning in the Irradiated Group when tested at short and long delays.

hippocampal tasks (see later and also a topical review by J.M. Wojtowicz in this issue).

Rats receiving irradiation were impaired in the NMTS task when the interval between the sample and test trials exceeded 60 s, and in associating the shock-induced fear with contextual cues in the fear conditioning task. Irradiated rats were not impaired in learning the basic NMTS rule or in performing that task when the interval between the sample and test trials was relatively short. Nor were there differences between irradiated and control groups in conditioning the fear response to the CS in the fear conditioning task. Thus, the suppression of adult neurogenesis interfered with the ability of rats to recall events at long delays, and in performing the type of relational learning that is necessary to form contextual associations. The pattern to note in these results is that the cognitive processes affected by irradiation are also disrupted by lesions to the hippocampus, while those processes that remained intact are considered to be independent of hippocampal control (Kim and Fanselow, 1992; Winocur, 1991, 1992).

The present results extend the range of tasks that can be said to be vulnerable to the effects of neurogenesis suppression and, importantly, provide additional evidence that the generation of new cells in the adult brain is particularly important for hippocampal function. It is widely held that neurogenesis provides a mechanism for hippocampal plasticity, in which new neurons participate in the formation of new memories. But, do adult-generated cells contribute to all hippocampus-dependent tasks? Shors et al. (2002) found that neurogenesis suppression impaired trace eyeblink conditioning, but not context-fear conditioning or spatial navigation, and argued that newly formed cells play a specific role in learning associations between temporally separated events. By comparison, Snyder et al. (2005) have argued that cells produced during hippocampal neurogenesis are involved in spatial memory and, specifically, in the long-term recall of learned spatial responses.

The present data provide clear evidence that the behavioral significance of adult-generated hippocampal cells extends beyond spatial memory. The NMTS task used in this experiment is a nonspatial, working memory task that also depends on the ability to recall a particular stimulus from the immediately preceding trial. The hippocampus is needed in such a task when the retention interval exceeds the limit that can be mediated by extra-hippocampal structures. The results of NMTS testing also argue against the hypothesis that the importance of adult-generated cells is restricted to learning associations between temporally displaced events. While irradiated rats were impaired on the DNMTS task at long intervals, the task is not one in which events separated in time must be integrated to establish a new response. Once the conditional NMTS rule has been learned, all that is required to perform successfully on the test trial is that the animal recalls the stimulus that was present on the sample trial, and then, following the rule, chooses the other one.

It could be argued that the fear conditioning results are consistent with the temporal displacement hypothesis. In all likelihood, given the nature of the task, successful conditioning to context required that the animal form associations between multiple environmental cues in creating a representation of the context in which conditioning occurred. This process necessarily involves the integration over time of cues located throughout the environment. Significantly, in the Shors et al. (2002) study, where neurogenesis depletion did not affect context-fear conditioning, the context was simpler, highly distinctive, and less demanding in terms of cue integration. Under such conditions, hippocampal damage may not impair conditioning to context (Winocur et al., 1987; Winocur, 1997). The impaired context fear conditioning observed in the irradiated rats in the present study could be attributed to a deficiency in forming associations between environmental stimuli that were sampled and attended to over a period of time. However, it must be

emphasized that cues in a complex environment are spatially distributed as well, and there is a large body of evidence showing that learning spatial relations is highly dependent on the hippocampus (O'Keefe and Nadel, 1978). It may be that the impaired context fear conditioning in the irradiated rats was primarily the result of poor spatial processing or, as is quite likely, a more general deficit that involves processing of both spatial and temporal information.

There is a clear need for further research in this area, but a reasonable hypothesis is that hippocampal neurogenesis contributes to a broad range of hippocampal functions. As new cells come online and become integrated into the structure's neuronal circuitry, their role might be to ensure the viability of the existing circuitry and help preserve those functions mediated by the hippocampus, as the mature granule neurons get "saturated" (e.g., relational and configural learning; long-term declarative memory; spatial information processing; context-dependent learning and memory; certain types of remote memory; see reviews by O'Keefe and Nadel, 1978; Sutherland and Rudy, 1989; Eichenbaum, 1994; Rosenbaum et al., 2001; Squire et al., 2004). By this view, preventing neurogenesis, thereby undermining the integrity of hippocampal circuitry, should impact each of these functions, although not necessarily to the same degree.

While this hypothesis must be considered preliminary, at least two lines of evidence provide direction for exploring it further. First, several studies have shown that exposure to an enriched environment (Kempermann et al., 1998; Nilsson et al., 1999; Kempermann et al., 2002) or increased physical activity (van Praag et al., 1999a,b; Cao et al., 2004) leads to increased hippocampal neurogenesis and improved spatial memory. The generality of these findings can be tested by conducting similar experiments with other hippocampal-dependent tasks or by performing these manipulations in animals with suppressed neurogenesis. Second, it has been suggested that the comprehensive loss of hippocampus-dependent memory function in old age is related to reduced neurogenesis and the failure of new cells to become effectively integrated into the unfriendly environment of the aging brain (Nacher et al., 2003; Bizon et al., 2004). In this regard, it has been shown that, in aged rats, spatial memory at least can be predicted by levels of hippocampal neurogenesis (Drapeau et al., 2003). Clearly, this type of analysis could be extended to other tasks.

A nonspecific hypothesis is challenged by variable findings regarding the link between hippocampal neurogenesis and performance on hippocampus-dependent tasks. Thus, neurogenesis suppression was found to affect trace eye-blink conditioning, but not context-fear conditioning (Shors et al., 2002) or spatial learning (Shors et al., 2002; Madsen et al., 2003; Snyder et al., 2005), all of which are impaired by lesions to the hippocampus. As indicated earlier, task-related factors may account for differences between Shors et al.'s results and ours with respect to context-fear conditioning. Another factor may relate to the method of suppressing neurogenesis. In the Shors et al. (2002) study, the antimetabolic agent, methylazoxymethanol acetate (MAM) was used to inhibit neurogenesis, whereas we used low-dose gamma radiation. The importance of this difference,

as yet, is unclear, but stereological analyses of BrdU-labeled cells in the DG of the hippocampus have shown that, while MAM significantly reduces the number of newly generated cells, a substantial number do survive (Shors et al., 2002). By comparison, Snyder et al. (2001, 2005) and others (Monje et al., 2002) have shown that low-dose irradiation, virtually eliminates hippocampal cells with young neuronal phenotype. This was verified in the present study. On this point, it must be noted that Madsen et al., 2003 and Snyder et al., 2005 used irradiation to block hippocampal neurogenesis and, like Shors et al., 2002, found no impairment on a hippocampus-dependent test of spatial learning. Clearly, further research, using consistent neurogenesis depletion techniques and a broader range of behavioral tasks, is needed to resolve these and other outstanding issues, and lead to a better understanding of the role of adult neurogenesis in neurocognitive function.

Acknowledgments

The authors gratefully acknowledge the technical assistance provided by Marie-Ève Couture at the Rotman Research Institute, Dr. H. Dobson and Ms. Kim Stewart at the University of Guelph irradiation clinic and Mr. Ruvim Radik at JMW's laboratory.

REFERENCES

- Aggleton JP, Hunt PR, Rawlins JNP. 1986. The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behav Brain Res* 19:133-146.
- Altman J, Das GD. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319-335.
- Anagnostaras SG, Gale GD, Fanselow MS. 2001. The hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus* 11:8-17.
- Bizon JL, Lee HJ, Gallagher M. 2004. Neurogenesis in a rat model of age-related cognitive decline. *Aging Cell* 3:227-234.
- Cameron HA, McKay RD. 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol* 435:406-417.
- Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, Doring MJ. 2004. VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 36:827-835.
- Drapeau E, Mayo W, Aurousseau C, Le MM, Piazza PV, Abrous DN. 2003. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. *Proc Natl Acad Sci USA* 100:14385-14390.
- Eichenbaum H. 1994. The hippocampal system and declarative memory in humans and animals: experimental analysis and historical origins. In: Schacter DL, Tulving E, editors. *Memory systems*. Cambridge, MA: MIT Press. p 147-202.
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. 1998. Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313-1317.
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. 1999. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* 2:260-265.
- Gross CG. 2002. Genealogy of the "grandmother cell". *Neuroscientist* 8:512-518.

- Hastings NB, Gould E. 1999. Rapid extension of axons into the CA3 region by adult-generated granule cells. *J Comp Neurol* 413:146–154.
- Kempermann G, Kuhn HG, Gage FH. 1998. Experience-induced neurogenesis in the senescent dentate gyrus. *J Neurosci* 18:3206–3212.
- Kempermann G, Gast D, Gage FH. 2002. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* 52:135–143.
- Kim JJ, Fanselow MS. 1992. Modality-specific retrograde amnesia of fear. *Science* 256:675–677.
- Leuner B, Mendolia-Loffredo S, Kozorovitskiy Y, Samburg D, Gould E, Shors TJ. 2004. Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. *J Neurosci* 24:7477–7481.
- Madsen TM, Kristjansen PEG, Bolwig TG, Wörtwein G. 2003. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* 119:635–642.
- McDonald HY, Wojtowicz JM. 2005. Dynamics of neurogenesis in the dentate gyrus of adult rats. *Neurosci Lett* 385:70–75.
- Milner B. 1974. Hemispheric specialization: scope and limits. In: Schmitt FO, Worden FG, editors. *The neurosciences: third study program*. Cambridge, MA: MIT Press. p 75–89.
- Monje ML, Mizumatsu S, Fike JR, Palmer TD. 2002. Irradiation induces neural precursor-cell dysfunction. *Nat Med* 8:955–962.
- Moscovitch M, Winocur G. 1995. Frontal lobes, memory, and aging. *Ann N Y Acad Sci* 769:119–150.
- Nacher J, Alonso-Llosa G, Rosell DR, McEwen BS. 2003. NMDA receptor antagonist treatment increases the production of new neurons in the aged rat hippocampus. *Neurobiol Aging* 24:273–284.
- Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. 1999. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J Neurobiol* 39:569–578.
- O'Keefe J, Nadel L. 1978. *The hippocampus as a cognitive map*. Oxford: Oxford University Press.
- Rosenbaum RS, Winocur G, Moscovitch M. 2001. New views on old memories: re-evaluating the role of the hippocampal complex. *Behav Brain Res* 127:183–197.
- Schmidt-Hieber C, Jonas P, Bischofberger J. 2004. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 429:184–187.
- Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. 2002. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 12:578–584.
- Snyder JS, Kee N, Wojtowicz JM. 2001. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *J Neurophysiol* 85:2423–2431.
- Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM. 2005. A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 130:843–852.
- Squire LR, Stark CEL, Clark RE. 2004. The medial temporal lobe. *Annu Rev Neurosci* 27:279–306.
- Sutherland RJ, Rudy RW. 1989. Configural association theory: the role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology* 17:129–144.
- van Praag H, Christie BR, Sejnowski TJ, Gage FH. 1999a. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA* 96:13427–13431.
- van Praag H, Kempermann G, Gage FH. 1999b. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2:266–270.
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. 2002. Functional neurogenesis in the adult hippocampus. *Nature* 415:1030–1034.
- Wang S, Scott BW, Wojtowicz JM. 2000. Heterogenous properties of dentate granule neurons in the adult rat. *J Neurobiol* 42:248–257.
- Wang S, Kee N, Preston E, Wojtowicz JM. 2005. Electrophysiological correlates of neural plasticity compensating for ischemia-induced damage in the hippocampus. *Exp Brain Res* 165:250–260.
- Winocur G. 1991. Functional dissociation of the hippocampus and prefrontal cortex in learning and memory. *Psychobiology* 19:11–20.
- Winocur G. 1992. A comparison of normal old rats and young adult rats with lesions to the hippocampus or prefrontal cortex on a test of matching-to-sample. *Neuropsychologia* 30:769–781.
- Winocur G. 1997. Hippocampal lesions alter conditioning to conditional and contextual stimuli. *Behav Brain Res* 88:219–229.
- Winocur G, Rawlins JN, Gray JA. 1987. The hippocampus and conditioning to contextual cues. *Behav Neurosci* 101:617–625.
- Zola-Morgan S, Squire LR. 1985. Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav Neurosci* 99:22–34.