

Age-Related Naturally Occurring Depression of Hippocampal Neurogenesis Does Not Affect Trace Fear Conditioning

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ABSTRACT: New neuron production throughout adulthood in granule cell layer (GCL) of rat hippocampus is a well-known phenomenon. A role of new neurons in hippocampal learning has been proposed, but the question is still open. A reduction of neural precursor proliferation in GCL of 2-month-old rats to about 20%, induced by the cytostatic agent methylazoxymethanol, was found to cause impairment in trace conditioning, suggesting a role of immature neurons in this kind of hippocampus-dependent learning (Shors et al., *Hippocampus* 2002;12: 578–584). Neurogenesis decreases with increasing age. In this study, neural precursor proliferation and newborn cell survival were evaluated in GCL of adult rats within a range of ages following development and preceding old age. In 5-month-old rats, neural precursor proliferation was reduced to 57% and newborn cell survival was reduced to 40% in comparison to rats of 2 months of age; in 12-month-old rats, the decrease was to 5 and 4%, respectively. Consistently, the density of immature neurons decreased to 41 and 13% in 5- and 12-month-old rats, respectively. The role of neurogenesis in trace fear conditioning was studied in this natural model of neurogenesis depression. No impairment in trace fear conditioning was found both in 5- and 12-month-old rats in comparison to 2-month-old rats, notwithstanding the decrease of neurogenesis that is marked in 12-month-old rats. This finding shows that a lower rate of neurogenesis is sufficient for learning in 12-month-old rats in comparison to young rats. © 2005 Wiley-Liss, Inc.

KEY WORDS: immature neurons; dentate gyrus; adulthood; rat; learning

INTRODUCTION

New neurons are produced throughout adulthood to the granule cell layer (GCL) of dentate gyrus of rats and of other mammals, including humans (Altman and Das, 1965; Eriksson et al., 1998). New neurons originate from proliferating GFAP-immunoreactive cells (Garcia et al., 2004), presumptive radial glia, located at the border of GCL facing hilus (Seri et al., 2001). A number of newborn cells precociously die (Cecchini et al., 2003), while the surviving ones differentiate into granule cells. Newborn

cells receive synapses and send their axons to CA3 field (Hastings and Gould, 1999; Markakis and Gage, 1999; van Praag et al., 2002; Ambrogini et al., 2004a).

The occurrence of neurogenesis in the hippocampus suggests its possible role in hippocampal function (Gould et al., 1999b). Shors et al. used a model of neural precursor proliferation depression induced by the cytostatic agent methylazoxymethanol (MAM) to reduce newborn neuron number; MAM-treated rats showed an impaired performance in trace conditioning (Shors et al., 2001, 2002), but not in Morris water maze (Shors et al., 2002). Moreover, they found that a MAM-treatment between 8 and 2 days before training did not affect trace conditioning, but did so when the treatment was carried out between 16 and 2 days before training (Shors et al., 2001). The authors interpreted this finding stating that newborn neurons (possibly in the second week after mitosis) are necessary for some kind (trace conditioning) of, but not every (e.g., spatial learning), hippocampus-dependent learning. The findings of Shors et al. are suggestive of a peculiar role of newborn neurons that, due to their “immature” properties (Wang et al., 2000, 2005; Snyder et al., 2001; Ambrogini et al., 2004a), would confer further functional properties to the hippocampus than those due to mature granule cells. However, the effects of MAM on learning might depend on side effects of the drug, other than the depression of neurogenesis (Kempermann, 2002); therefore, a validation of the hypothesis that neurogenesis depression results in trace conditioning impairment when no toxic agent is used should allow the necessary role of neurogenesis in trace conditioning to be demonstrated.

A progressive reduction of neurogenesis rate occurs throughout adult life (Kuhn et al., 1996; Kempermann et al., 1998); therefore, a progressively lower number of immature neurons is added to GCL. This appears to be a good model to study the effects of neurogenesis decrease on hippocampus-dependent learning. Hence, we evaluated the decrease of neurogenesis estimating 5-bromo-2'-deoxyuridine (BrdU)-positive cells 1 day (to study neural precursor proliferation) and 2 weeks (to study newborn cell survival) after BrdU-labeling in 2-, 5-, and 12-month-old rats, a range of age in which hippocampus development

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has ended and old age has not yet begun; in these rats, we studied the performance in trace fear conditioning.

MATERIALS AND METHODS

Animals

Sprague Dawley male rats (Charles River, Italy) of 2, 5, and 12 months of age were used. Animals were housed two per standard size cage, with free access to food and water. Room temperature was $(21 \pm 1)^\circ\text{C}$ and relative humidity was $(50 \pm 10)\%$. Rats underwent light–dark cycle of 12:12 h (lights on at 7 a.m.). Stressful events were carefully avoided. The experimental procedures were carried out following the Italian law and European Community Council guidelines on animal experimentation.

Within each age group, some animals were used for neurogenesis evaluation ($n = 31$), and others for behavioral analysis ($n = 45$).

Neurogenesis Evaluation

Animals were intraperitoneally injected twice a day over three consecutive days with 5-bromo-2'-deoxyuridine (BrdU, Sigma Aldrich, Italy; 50 mg/Kg b.w.) dissolved in 0.01 N NaOH in saline solution. One day or 15 days after the last BrdU injection, rats were anesthetized by intraperitoneal injection of sodium thiopental (45 mg/Kg b.w.) and killed by an intracardiac injection of the same anesthetic, and brains were removed, fixed in Carnoy's solution, and embedded in paraffin (Paraplast Plus, Sigma, St. Louis, MO). BrdU-positive cells were detected by immunohistochemical technique and labeled cells were counted in dorsal hippocampus, where the highest neurogenesis rate was found (Okano et al., 1996), as previously described (Ambrogini et al., 2000); GCL, including the proliferative zone, i.e., the hilar border of GCL, was considered. The cell number was expressed as density (mm^{-2}). To identify newborn cell phenotype, 15 days after the last BrdU injection, a marker of immature neurons, TUC-4, was revealed together with BrdU, as previously described (Ambrogini et al., 2000). Double-labeled cells were counted using a BioRad Radiance 2000 laser-scanning microscope (BioRad Laboratories, Richmond, CA); the percentage of TUC-4-immunoreactive cells in a sample of 25–30 BrdU-labeled cells was calculated.

To quantitatively evaluate immature neurons, the detection of TUC-4 was carried out, as previously described (Cecchini et al., 2003); TUC-4-positive cells were counted and the number was expressed as density (mm^{-2}).

Trace Fear Conditioning

Animals

Rats of each age were randomly divided into two groups, one group (ranging between 8 and 9 rats) underwent trace fear conditioning (shocked); the other (ranging between 6 and 7 rats)

underwent the same paradigm, but without receiving aversive stimuli (nonshocked).

Apparatus

A basic Skinner box module (Modular Operant Cage, Coulbourn Instruments, Lehigh Valley, PA) was used to induce freezing. Box dimensions were $29 \times 31 \times 26$ cm. The top and two opposite sides were made of aluminum panels. The other two sides were made of transparent plastic. The floor was made of stainless steel rods connected to a shock delivery apparatus (Grid Floor Shocker, Coulbourn Instruments, Model E13-08). There was a loudspeaker to emit acoustic stimuli of known intensity, frequency, and duration. The apparatus was connected to a stimulus programming device (Scatola di comando Arco 2340, Ugo Basile, Varese, Italy) to predetermine number, duration, and rate of conditioning stimulus (CS)–unconditioned stimulus (US) couplings. The apparatus was placed in a soundproof room ($3.5 \times 1.8 \times 2.1$ m), kept at a constant temperature of $(20 \pm 1)^\circ\text{C}$. Illumination inside the room was 60 lux.

Context freezing response was measured in the same apparatus that had been used for conditioning. Freezing response to explicit CS (sound) was measured in a totally different apparatus from that used for conditioning. The apparatus was a modified shuttle box apparatus (Ugo Basile) ($20 \times 47 \times 20$ cm). The walls were made of gray opaque plastic, with black vertical stripes (width 1 cm, spaced 3 cm apart). The lid was made of transparent plastic and the floor of black opaque plastic. In the apparatus, there was a loudspeaker to administer acoustic stimuli to the experimental subjects. The apparatus was connected to a stimulus programming unit (Automatic Reflex Conditioner 7501, Ugo Basile) to predetermine CS (number of stimuli, duration of stimuli, and rate of stimulation). The unit could also predetermine intensity and frequency of the acoustic stimuli. The apparatus was placed in a soundproof room ($3.5 \times 3.6 \times 2.1$ m), kept at a constant temperature of $(20 \pm 1)^\circ\text{C}$. Illumination inside the room was 10 lux.

Conditioning

On day 1, the rat was gently taken manually from the home cage, placed in a bucket, and carried from the housing room to the appropriate soundproof room. Once there, it was placed inside the conditioning apparatus. The rat was left undisturbed for 3 min. After this time, CS as an 800 Hz tone from a frequency generator, amplified to 75 dB (Phillips and LeDoux, 1992; Sacchetti et al., 1999) lasting 15 s, was administered 10 times, at 240-s intervals. After the end of each CS (30 s), the US as electric footshock, lasting 1 s, intensity 0.65 mA, was administered. Two minutes after the end of the stimulation pattern, the rats were taken back to the home cage.

Conditioned freezing measurement

The rat's behavior was recorded by means of a closed-circuit TV system. Freezing (immobility) was defined as the complete

absence of somatic motility, except respiratory movements (LeDoux et al., 1983). Measurements were performed by means of a stopwatch by personnel who did not know to which experimental group each animal belonged. Total cumulated freezing time (i.e., total seconds spent freezing during each 3-min period) was measured.

Twenty-four hours after conditioning, to measure acoustic CS freezing, the animals were placed in apparatus different from the one used for conditioning to avoid the facilitation of CS retention caused by contextual cues (Balaz et al., 1982; Corodimas and LeDoux, 1995; Oler and Markus, 1998). Once inside the apparatus, the animal was left undisturbed for 3 min. After this time, during a subsequent 3-min period, a series of four acoustic stimuli (spaced 30 s one from the other) was administered, identical to the series used during the conditioning session (frequency, intensity, and duration). After that time, the animals were taken back to the home cage.

Forty-eight hours after conditioning, the animals were again placed inside the conditioning apparatus and left there for 3 min to measure contextual freezing. While they were there, neither electrical nor acoustic stimuli were administered. After that time, they were taken back to the home cage.

Statistical Analysis

To evaluate neurogenesis results, one-way analysis of variance (ANOVA) test was used; post hoc multiple comparison was carried out by Bonferroni's test. For evaluating trace fear conditioning results, mixed ANOVA test, with different age of rats as a between-subject variable and type of surrounding (training context, new context, and new context + CS freezing) as a within-subject variable (tested by using *F* ratios to determine whether there was a significant quadratic component), and Newman-Keuls multiple comparisons test were used.

RESULTS

Decrease of Neurogenesis With Age

BrdU-positive cells were found in the three groups of rats both 1 ($n = 3, 3,$ and $4,$ respectively, for 2, 5, and 12 months) and 15 days ($n = 4, 3,$ and $5,$ respectively, for 2, 5, and 12 months) after the end of labeling; about 60% of BrdU-positive cells were TUC-4 immunoreactive at 15 days (Fig. 1a). One and 15 days after the last BrdU injection are time points suitable for evaluating proliferation rate of neural precursors and the number of newborn cells surviving after the early phase of cell death (Cecchini et al., 2003), respectively. Moreover, 15 days is a time compatible with age in which newborn cells are necessary for learning, following Shors' results (Shors et al., 2002). In 2-month-old rats, the density of BrdU-positive cells 1 and 15 days after the last BrdU injection was 121.1 ± 6.48 and $80.9 \pm 8.94 \text{ mm}^{-2}$, respectively. In 5- and in 12-month-old rats, the density of proliferated cells was reduced to 57 and 5%, respectively, in comparison to that found in 2-month-old rats; the density of BrdU-positive cells survived 15 days after

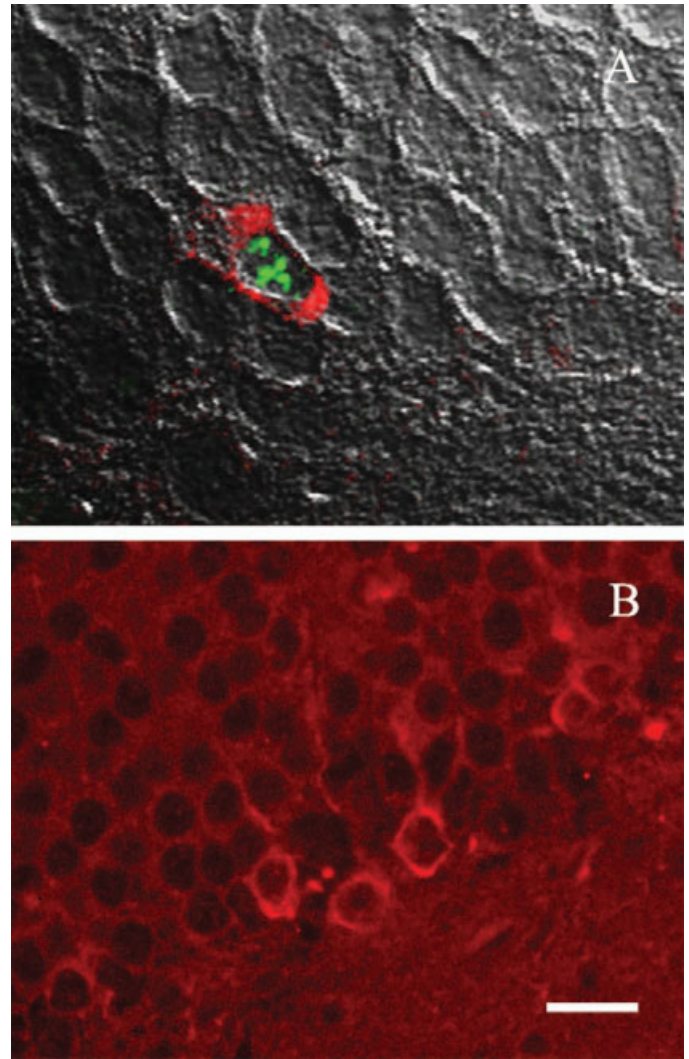


FIGURE 1. A: Confocal microscope image of BrdU (green)-TUC-4 (red)-double-labeled cell in granule cell layer of rat dentate gyrus (bar = 11 μm). B: Fluorescence microscope image of cells showing immunoreactivity for TUC-4 in rat granule cell layer (bar = 20 μm).

BrdU-labeling decreased to 40 and 4%, respectively (Fig. 2). Consistently, the density of immature neurons (TUC-4-positive cells) (Fig. 1b) decreased to 41 and 13% in 5- and 12-month-old rats, respectively, in comparison to 2-month-old rats (Fig. 3). Therefore, at 12 months of age, the decrease of neurogenesis with respect to 2-month-old rats was larger than that obtained by Shors et al. (2002), in rats of an age similar to our youngest rats, with MAM-treatment (about 20%). One-way ANOVA test showed significant age-related differences in neural precursor proliferation ($F(2,7) = 198.87, P < 0.0001$) and in newborn cell survival ($F(2,9) = 46.09, P < 0.0001$). Bonferroni's multiple comparison procedure showed that BrdU-positive cell density 1 day after the last BrdU injection was significantly different in 5-month-old in comparison to 2-month-old rats ($P < 0.0005$) and in 12-month-old in comparison to 2-month-old and in comparison to 5-month-old rats

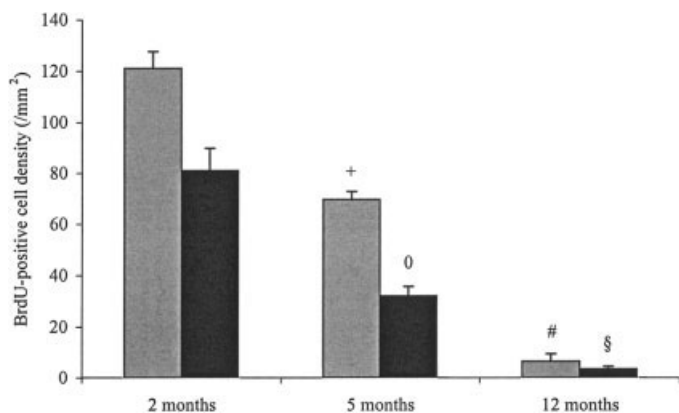


FIGURE 2. Neurogenesis in dentate gyrus of hippocampus of 2-, 5-, and 12-month-old rats, evaluated as BrdU-positive cell density. Rats were killed 1 (white) or 15 (black) days after the last BrdU-injection to evaluate neural precursor proliferation ($n = 3, 3, 3$, and 4 , respectively, for 2, 5, and 12 months) and newborn cell survival ($n = 4, 3$, and 5 , respectively, for 2, 5, and 12 months). One-way ANOVA test showed a significant age-related decrease in neural precursor proliferation ($F(2,7) = 198.87, P < 0.0001$) and in newborn cell survival ($F(2,9) = 46.09, P < 0.0001$). Bonferroni's multiple comparison procedure. Neural precursor proliferation: BrdU-positive cell density 1 day after the last BrdU injection was significantly different in 5-month-old vs. 2-month-old rats ($*P < 0.0005$) and in 12-month-old vs. 2-month-old and vs. 5-month-old rats ($\#P < 0.0005$); proliferated cell survival: BrdU-positive cell density 15 days after the last BrdU injection was significantly different in 5-month-old vs. 2-month-old rats ($^{\circ}P < 0.001$) and in 12-month-old vs. 2-month-old ($^{\S}P < 0.001$) and vs. 5-month-old rats ($^{\S}P < 0.05$).

($P < 0.0005$). Moreover, BrdU-positive cell density 15 days after the last BrdU injection was significantly different in 5-month-old in comparison to 2-month-old rats ($P < 0.001$) and in 12-month-old in comparison to 2-month-old ($P < 0.001$) and in comparison to 5-month-old rats ($P < 0.05$). Besides, one-way ANOVA test showed significant age-related differences in TUC-4-positive cell density ($n = 3$, for each group) ($F(2,6) = 318.92, P < 0.0001$). Bonferroni's multiple comparison procedure showed that TUC-4-positive cell density was significantly different in 5-month-old in comparison to 2-month-old rats ($P < 0.0001$) and in 12-month-old in comparison to 2-month-old ($P < 0.0001$) and in comparison to 5-month-old rats ($P < 0.005$).

Trace Fear Conditioning

Spontaneous and reactive behavior during acquisition training

The 2-, 5-, and 12-month-old rats exhibited a very similar behavior when placed in the apparatus for the first time. As a rule, only very short immobility was recorded during the initial free-exploration 3-min period common to the two paradigms (shocked and nonshocked groups). The mean freezing duration of the six groups (2-, 5-, and 12-month-old rats, shocked and nonshocked) was less than 10% of total time (data not shown).

Rats of all groups uniformly exhibited the same curiosity for the training context. During the 15-s period in which the acoustic CS was presented for the first time, the rats of all groups uniformly exhibited an alerting behavior, as shown by the interruption of the exploratory activity on the Pavlovian "startle reaction." Moreover, the reactions to footshocks (jumping, vocalization) were similar in the shocked groups of the three different ages.

Freezing duration of shocked (conditioned) rats

Figure 4 shows freezing duration during exposition of rats (1) 3 min to the new context without acoustic CS presentation (NCX) (generalization measure), (2) 3 min to the new context with acoustic CS presentation (NCX + CS) (trace fear conditioning measure), and (3) to the training context without acoustic CS presentation (TCX) (contextual fear conditioning measure). The rats of all ages showed similar conditioning levels in all three contingencies. There is a low generalization and a good trace fear conditioning response. Contextual fear conditioning is present in all age groups.

Mixed ANOVA test showed no age-related differences ($F(2,44) = 1.34, N.S.$), but significant differences for the three conditioned responses ($F(2,44) = 25.9, P < 0.001$). There was no significant interaction ($F(4,44) = 0.76, N.S.$). The Newman-Keuls multiple comparisons test revealed significant differences between freezing to new context without and with acoustic CS ($P < 0.05$) and between the new context and the training context ($P < 0.05$).

Freezing duration of nonshocked rats

Figure 5 shows freezing duration of different age rats when exposed to the new context without and with acoustic CS and when again exposed to the training context. Rats of all three

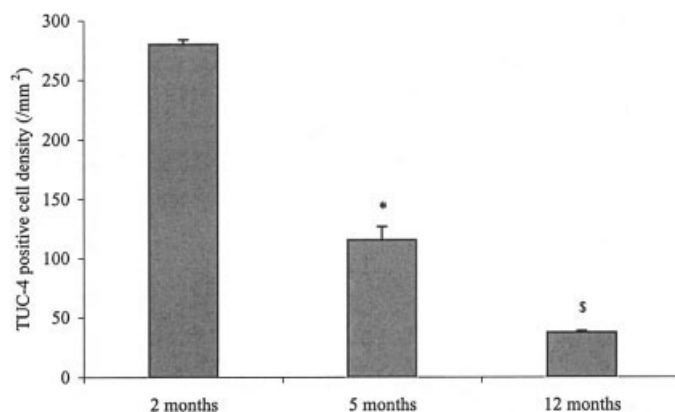


FIGURE 3. TUC-4-positive cell density in GCL of 2-, 5-, and 12-month-old rats ($n = 3$ for each group). One-way ANOVA test showed a significant age-related decrease in TUC-4-positive cell density ($F(2,6) = 318.92, P < 0.0001$). Bonferroni's multiple comparison procedure: TUC-4-positive cell density was significantly different in 5-month-old vs. 2-month-old rats ($*P < 0.0001$) and in 12-month-old vs. 2-month-old ($^{\S}P < 0.0001$) vs. 5-month-old rats ($^{\S}P < 0.005$).

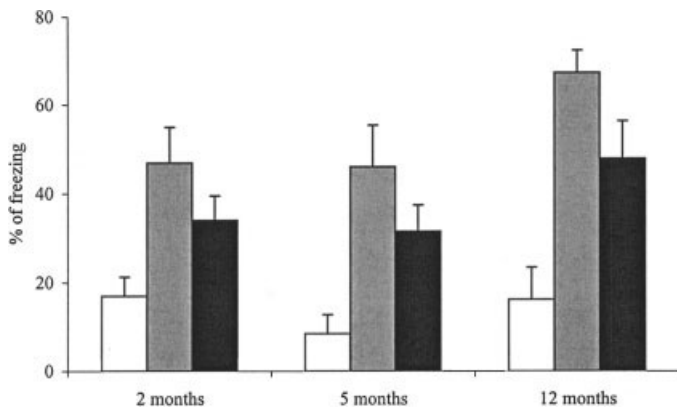


FIGURE 4. Trace conditioning of different age rats expressed as freezing time. Percentage of the total time (mean \pm SEM) during the time spent in the conditioning apparatus without acoustic stimulation (TCX) (black) to measure context responding and in the other apparatus without (NCX) (white) or with acoustic stimulation (NCX + CS) (gray) to measure generalization or conditioning, respectively. $n = 9, 8,$ and $8,$ respectively for 2, 5, and 12 months. Mixed ANOVA test showed no age-related differences ($F(2,44) = 1.34, N.S.$), but significant differences for the three conditioned responses ($F(2,44) = 25.9, P < 0.001$). There was no significant interaction ($F(4,44) = 0.76, N.S.$). The Newman-Keuls multiple comparisons test revealed significant differences between freezing to new context without (NCX) and with (NCX + CS) acoustic CS ($P < 0.05$) and between the new context (NCX) and the training context (TCX) ($P < 0.05$).

age groups showed a very short freezing duration in all three contingencies. Mixed ANOVA test showed no difference among ages ($F(2,34) = 0.41, N.S.$) and among surroundings ($F(2,34) = 0.99, N.S.$). There were no interactions ($F(4,34) = 0.44, N.S.$).

DISCUSSION

The aim of this work was to evaluate whether conditions strongly inhibiting neurogenesis result in an impairment of trace fear conditioning, a kind of hippocampus-dependent learning that has been proposed to depend on neurogenesis (Shors et al., 2002). Neural precursor proliferation decreases with increasing age, giving a natural model for studying the behavioral effects of neurogenesis depression. In this study, we show a decrease in cell proliferation to 5%, and in cell survival to 4% in dentate gyrus of 12-month-old Sprague Dawley rats with respect to 2-month-old rats; this result is similar to that of McDonald and Wojtowicz (2005), showing a decrease in neurogenesis by 94% in 12-month-old Sprague Dawley rats in comparison to 38-day-old rats, mainly due to the decrease of precursor proliferation. The cell proliferation depression is deeper than that induced by MAM (about 20%) in rats of an age similar to that of our youngest rats (Shors et al., 2002). The depression in precursor proliferation and newborn cell survival results in a decrease in immature neuron density, as shown by TUC-4-immunostaining, taking into account that

TUC-4-positive cells provide a more reliable evaluation of the actual number of newborn neurons added to GCL (Seki, 2002).

After training, behavioral results failed to show significant differences either in conditioned freezing responding (Fig. 4) or in footshock responding in the rats of all three age groups; this finding is consistent with the finding that trace eye blink conditioning is similar in 18- to 24-month-old and in 6-month-old rats (Knuttinen et al., 2001). Spontaneous behavior was similar in all three age groups, both as short immobility during the pre-shock period and as short freezing duration in nonshocked animals either in the conditioning apparatus or in the new one, and either without or with acoustic stimulation (Fig. 5). Moreover, the low generalization shows that footshock intensity was low enough; the good trace fear conditioning performance shows a correct employment of conditioning methods (number, duration, intensity of footshock, CS duration, CS-US delay, etc.); the good context fear conditioning performance, even if slightly lower than that to CS, shows a correct choice of conditioning parameters. These behavioral results suggest the independence of hippocampus-dependent trace conditioning from age-related decrease in neurogenesis.

However, theoretical considerations and experimental results suggest a relationship between neurogenesis and hippocampus-dependent learning in adult rats. First, hippocampus is a critical region for occurrence of some types of learning, for transferring information from short-term to long-term memory, and is an informational bottleneck (Kempermann, 2002). Neuron turnover is a form of plasticity that, in principle, may contribute to increasing learning capacity for new information and to clearing old information (Feng et al., 2001; Chambers et al., 2004). Second, hippocampus-dependent learning increases survival of immature neurons in GCL (Gould et al., 1999a;

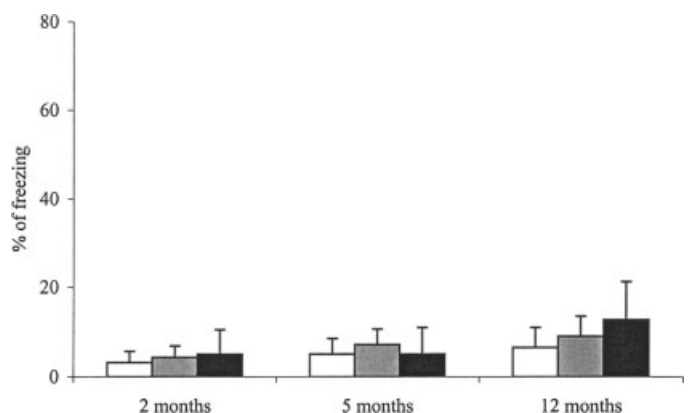


FIGURE 5. Freezing behavior of nonshocked rats of different ages. Percentage of the total time (mean \pm SEM) during the time spent in the conditioning apparatus without acoustic stimulation (TCX) (black) and in the other apparatus without (NCX) (white) or with acoustic stimulation (NCX + CS) (gray). $n = 6, 7,$ and $7,$ respectively, for 2, 5, and 12 months. Mixed ANOVA test showed no difference among ages ($F(2,34) = 0.41, N.S.$) and among surroundings ($F(2,34) = 0.99, N.S.$). There were no interactions ($F(4,34) = 0.44, N.S.$).

Ambrogini et al., 2000), suggesting the possibility that survived neurons may be recruited in coding processes, while hippocampus-dependent learning-induced death of less immature neurons (Ambrogini et al., 2004b) that are potentially already utilized in previous learning may represent the way through which a possible interference is resolved (Anderson, 2000). Finally, several factors modulating neurogenesis also affect hippocampus-dependent learning (corticosterone, estrogens) (Gould et al., 1992; Cameron and Gould, 1994; Bodnoff et al., 1995; Krugers et al., 1997; Luine et al., 1998; Tanapat et al., 1999; Mirescu et al., 2004). Moreover, some studies seem to suggest a quantitative relationship between neurogenesis and learning ability. The enriched environment causes increased survival of new neurons and improves spatial learning in mice (Kempermann et al., 1997), as it does in aged animals too (Kempermann et al., 1998). Running increases neural precursor proliferation and improves performance in Morris water maze (van Praag et al., 1999). In addition, there is a correlation between the rate of spontaneous neural precursor proliferation in different mouse strains and the performance in Morris water maze (Kempermann and Gage, 2002). More recently, it was found that vascular endothelial growth factor, whose hippocampal expression is enhanced by the enriched environment and spatial learning, positively affects neurogenesis and performance in hippocampus-dependent learning (Cao et al., 2004). Altogether these studies suggest a relationship between neurogenesis and hippocampus-dependent learning, but they do not allow the conclusion that there is a causal relationship between neurogenesis and learning ability of the animal to be made, because neurogenesis rate and learning ability might be independent consequences of the same genetic or epigenetic conditions.

Several authors tried to study the role of neurogenesis in spatial learning using the age-related decline of neurogenesis as a natural model of neurogenesis depression. Bizon and Gallagher (2003) did not find any correlation between cell proliferation in GCL of dentate gyrus and individual performance in spatial learning as tested in Morris water maze in Long Evans rats between 7 and 25 months of age and any difference in spatial learning index (averaged proximity) in rats of 7 and 13 months of age. However, cell proliferation does not seem to be a good index of neurogenesis, because a lot of newborn cells die early after mitosis in GCL when they are very immature neurons (Cecchini et al., 2003). Therefore, more recently the same authors correlated the number of GCL newborn cells surviving 1 month after BrdU labeling and performance in spatial learning and, curiously, they found, in 25-month-old rats, a worse performance when the number of survived newborn cells was higher (Bizon et al., 2004). Merrill et al. (2003) did not find any correlation between the decrease of 50% in the number of newborn cells in GCL 1 week after BrdU-labeling in female 21-month-old Fisher 344 rats with respect to 2-month-old rats and their performance in Morris water maze. Altogether these results would lead to the conclusion that hippocampal neurogenesis is not necessary for hippocampus-dependent learning, at least in middle-aged or older rats. On the contrary, Drapeau et al. (2003) showed, in 20-month-old Sprague Dawley rats, a

correlation between neurogenesis rate and performance in Morris water maze. However, an analysis of these data (Drapeau et al., 2003) shows that rats with relatively higher neurogenesis rate give good performances, but the individual performances of rats with low neurogenesis rate are very different. In other words, a depressed neurogenesis seems to be sometimes, but not always, concomitant with bad performance in spatial learning; this situation is not compatible with a causal relationship between depressed neurogenesis and bad performance in spatial learning (and therefore with the hypothesis that, in 20-month-old rats, neurogenesis is necessary for spatial learning).

The studies of Shors et al. (2001, 2002) suggest that more than 20% of neural precursor proliferation of a young rat is necessary for trace conditioning; they, in fact, depressing neural precursor proliferation to about 20% of the controls by MAM, found that trace conditioning was impaired by MAM administered 8–16 days before training, but spatial learning (hippocampus-dependent) and delay conditioning (hippocampus-independent) were not (Shors et al., 2001, 2002). This finding suggests that a number of immature neurons in the second postmitosis week (8–16 days after BrdU-labeling) are necessary for trace conditioning, a hippocampus-dependent learning. On the other hand, our results show that a larger loss of new neurons does not imply an impairment in trace conditioning in 12-month-old rats. Both experimental models (MAM and age), although different, lead to neurogenesis depression through a reduction in precursor proliferation (Cattaneo et al., 1995; McDonald and Wojtowicz, 2005). Therefore, in principle the different results obtained by us and Shors' group are compatible with two possibilities: MAM impairs trace fear conditioning through a mechanism other than neurogenesis depression or, alternatively, the role of neurogenesis changes with increasing age. In the latter case, the crucial question is "why a deeper depression of neurogenesis is compatible with trace conditioning in middle-aged rats, while a minor depression results in an inability to learn in younger rats?" Two hypothesis may be proposed to answer this question: (1) a lower number of new neurons might be sufficient to learn in middle-aged rats; (2) neurogenesis loses its role in learning with increasing age, as previously suggested (Kempermann, 2002; Bizon et al., 2004; Bizon and Gallagher, 2005). In both the cases, the emerging of new morphofunctional features facilitating or replacing the role of neurogenesis in learning should be admitted with increasing age. These morphofunctional features supporting learning correspond to the cognitive concept of meta-learning (Doya, 2002), i.e., of the general procedures the animal must learn to building the individual memory traces (i.e. learning to learn). It is likely that building the cognitive structures for meta-learning (and their neurobiological correspondents) requires many experiences of that kind of learning. When the meta-learning structures are built, then a nonneurogenetic (i.e., without neurogenesis) or a poorly neurogenetic hippocampus might become able to assemble the perceptive inputs in a way suitable for learning; on the contrary, until the meta-learning structures are in construction, an easier pathway within hippocampus may be necessary for building memory traces.

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