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Delayed suppression of hippocampal cell proliferation in rats following inescapable shocks

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Abstract

Adult Sprague-Dawley rats were exposed to a single session of 100 inescapable tail shocks (IS). Bromodeoxyuridine (BrdU) was administered 1 h, 2 d or 7 d later and hippocampal cell proliferation (CP) was assessed after a two-hour survival period. Measures of plasma corticosterone (CORT) levels were also obtained. Despite a large increase in CORT immediately following IS, no associated change in CP was observed. In fact, the only significant change in CP was seen 7 days after IS, at a time when CORT was unchanged from control levels. These data raise questions about the general nature of the relationship between CORT and CP. They also suggest that, under some conditions, changes in hippocampal CP may emerge only after an “incubation period”.

Keywords

Stress; Corticosterone; BrdU; Neurogenesis; Dentate gyrus; Rat

1. Introduction

One of the fundamental tenets regarding theories of clinical depression is that stress is a frequent and important etiological factor in depressive episodes (Kendler et al., 1999; Paykel, 2001). A variety of animal models of depression have incorporated this fact. Thus, after subjecting animals to strong or traumatic conditions, depression-like behaviors and physiological changes are observed, often for lengthy time periods after termination of the stressor. The preponderance of these studies have employed rodents (mice or rats) and have exposed them to either electric shock to the tail or paws, or to some form of social interaction resulting in either defeat or submission (e.g. Desan et al. 1988; Koolhaas et al., 1990; Murua and Molina, 1991; van Dijken et al., 1992 and 1993; Tornatzky and Miczek, 1993; see also recent review by Belda et al., 2004). In addition, since human depression is frequently not an immediate

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Section: Nervous System Development, Regeneration and Aging

response to trauma, but may undergo a period of induction or incubation, this too has been examined in animal studies (Marti et al., 2001; Dal-Zotto et al., 2002; Valles et al., 2003).

We and others have hypothesized that the suppression of hippocampal neurogenesis (NG) is an important factor in the onset and/or maintenance of human depression (Jacobs et al., 2000; Malberg et al., 2000; Santarelli et al., 2003). Reciprocally, it is proposed that the efficacy of several major antidepressant treatments (various categories of antidepressant drugs and electroconvulsive therapy) is based on their capacity to restore hippocampal neurogenesis to a basal or euthymic level (Jacobs et al., 2000; Malberg et al., 2000; Madsen et al., 2000; Santarelli et al., 2003). It should also be noted that this hypothesis has not gone unchallenged (Henn and Vollmayr, 2004; Rosenbrock et al., 2005). However, it seems fair to say that this idea has not been subjected to a critical test, which, of necessity, would directly examine the brains of humans suffering from a major depressive disorder.

One of the widely accepted animal models of depression is the Learned Helplessness paradigm (Weiss et al., 1981; Cryan et al., 2002), where animals are exposed to a series of electric shocks (typically to the paws or tail of rodents) and subsequently manifest a deficit in an adaptive avoidance response (Maier and Seligman, 1976; Maier and Watkins, 2005). Several previous studies have examined the effects of inescapable shock (IS) on hippocampal cell proliferation (CP) in rats (Vollmayr et al. 2003; Malberg and Duman, 2003; Shors et al., 2005; Bland et al., 2006). All have reported reductions in CP at various time points after the shocks, but none of them have examined the simple question of whether shock alone has an effect on CP at various subsequent time points. Here, we exposed rats to a series of inescapable tail shocks in order to study the evolution of the CP response. Following application of IS, we examined CP after 1 hour, or 2 or 7 days later, and examined plasma corticosterone levels immediately after IS and 7 days later (no measure taken at day 2).

2. Results

Groups of animals were exposed to either 100 brief, inescapable shocks to the tail, or were left unhandled in their home cages. At various times after the IS (1 h, 2 d or 7 d) animals were administered BrdU and sacrificed 2 hours later to assess CP using BrdU immunohistochemistry. An example of BrdU labeling in the DG of a representative rat is shown in Figure 1. Data displayed in Figure 2 are the estimated total number of BrdU-labeled cells counted bilaterally in the DG of animals in the different experimental groups. A one-way ANOVA for time after IS was significant ($F_{(3,33)} = 3.84$, $p = 0.018$) and post hoc tests showed that only the 7 day group was significantly different (18% decrease) from the cage control group ($p < 0.05$). A more fine-grained analysis divided the DG into its anterior (dorsal) and posterior (ventral) aspects (Table 1). No significant changes were found in the dorsal DG at any time point ($F_{(3,33)} = 0.61$, $p = 0.611$), however, a significant decrease in CP was seen in the ventral DG at the 7 day time point ($F_{(3,33)} = 5.88$, $p = 0.0025$; post hoc test $p < 0.01$), and the magnitude of this change (24% decrease) was larger than that for the DG as a whole, at this same time point. This suggests that the cellular response to IS may be largely restricted to the ventral DG.

Plasma CORT levels were measured at two different time points in relation to the IS experiment. A two-way ANOVA indicated a significant effect of treatment ($F_{(1,35)} = 106.30$, $p < 0.0001$) and time ($F_{(1,35)} = 93.80$, $p < 0.0001$). As shown in Figure 3, immediately following IS, plasma CORT levels were significantly increased ($p < 0.001$; Bonferroni test) by approximately 10-fold compared to cage control animals. However, when examined seven days later, in a separate group of animals from those used in the CP experiment, there was no significant difference between the IS and cage control animals ($p > 0.05$).

3. Discussion

The etiology of a number of important forms of clinical psychopathology, including post-traumatic stress disorder (PTSD), major depression, and schizophrenia, in many cases, involve initial exposure to traumatic conditions (biological or social/environmental) (for reviews see, Kendler et al., 1999; Paykel, 2001; Vermetten and Bremner, 2002; Davidson et al., 2004; Dinan, 2005; Yeap and Thakore, 2005). The manifestation of this early experience is often not observed until some time later (frequently years or even decades). This implies that the illness growing out of this initial event is dependent either on: 1) a long lasting overt change (for example a hyperreactive hypothalamic-pituitary-adrenal (HPA) axis) that sensitizes the organism to some later occurring stressor; or 2) a covert change (for example, alteration of a gene that is unexpressed at the time) that awaits the occurrence of some important event, perhaps even a naturalistic one, such as puberty. The present data indicate that seven days after IS, the suppression of hippocampal cell proliferation is not attributable to an alteration of the HPA axis, insofar as its expression is reflected in plasma levels of corticosterone at that time. There is at least circumstantial evidence that serotonin may be important in this process. Increases in serotonergic activity are associated with an augmentation of hippocampal CP (Gould, 1999; Santarelli et al., 2003), whereas, reciprocally, decreases are associated with a suppression of CP (Brezun and Daszuta, 1999; Radley and Jacobs, 2002). Unfortunately, only one study has directly examined brain levels of serotonin at a delayed time point after IS and that study reported an increase in frontal cortex serotonin at 24 hours after IS (Petty et al., 1994). If the initial increase in brain serotonin levels seen with IS is later followed by a decrease below baseline, then a disfacilitation of serotonin's promoting action on CP might underlie the delayed suppression of hippocampal CP observed after IS in the present study. Consistent with this idea, treatment with a selective serotonin reuptake inhibitor (fluoxetine) was found to block the decrease in hippocampal CP induced by IS (Malberg and Duman, 2003), possibly by enhancing brain serotonin and restoring its stimulatory effect on CP. The role of other likely candidates, such as changes in mesolimbic dopamine (Puglisi-Allegra and Cabib, 1990; Tidey and Miczek, 1996) or hippocampal norepinephrine (Kulkarni et al., 2002), have not been explored in this context.

A stress-related decrease in CP that is larger in the ventral versus dorsal hippocampus, such as seen here, may be a general phenomenon. We have previously seen this following 56 hours of sleep deprivation (Tung et al., 2005) and following neurotoxic lesions of the median raphe (Sundararaman, 2006). The significance of this lies in the possibility that the dorsal hippocampus is more importantly involved in spatial learning and that the ventral hippocampus is more importantly involved in visceral learning (Moser & Moser, 1998; Faure et al., 2006). Thus, to the degree that stressors or traumatic events affect learning and memory via an effect upon CP, this suggests a greater impact upon emotional processes.

Several studies of IS with some similarity to the present one have been published. Vollmayr et al. (2003) exposed rats to a series of inescapable footshocks (unspecified number) and reported a decrease in hippocampal CP seven days later. However, these animals were also given shock escape testing a day after the initial IS. In a similar study, Malberg and Duman (2003) used 60 inescapable footshocks to rats and reported a decrease in proliferation nine days later. However, similar to Vollmayr's study, these animals were given shock avoidance training a day after the initial IS. Finally, in a recent, brief report, Shors et al. (2006) reported a reduction in hippocampal CP in rats at the end of a seven day series of inescapable footshocks. Thus, it seems clear that exposure to IS can suppress CP for a number of days. Our data show, in addition, that this suppression may only emerge after a delay or "incubation" period of several days.

It is generally believed that there is a direct relationship between acute elevations of plasma corticosterone and a suppression of CP (Cameron and Gould, 1994; Cameron et al., 1998; Tanapat et al., 2001; see Mirescu and Gould (2006) for a review). However, there are now several examples, including the present data, where exposure of animals to acute stressors (with and without concomitant CORT measures) does *not* suppress hippocampal CP (Pham et al., 2003; Van der Borgh et al., 2005; Thomas et al., 2006; Yap et al., 2006). There are no ready explanations for these discrepancies. There may be a temporal disconnect between elevation of plasma corticosterone and suppression of CP and/or, the physiological response to a stressor may also include release of a countervailing or protective factor to the CORT effect. In this respect, Bland et al. (2006), using a yoked tailshock paradigm in rats, have recently demonstrated that behavioral control over the stressor strongly modulates stress-induced changes in CP/NG in the DG, possibly by increasing the expression of the neurotrophin basic fibroblast growth factor (FGF-2) in the hippocampus. Clearly, more important information remains to be uncovered on this topic.

4. Experimental procedures

4.1. Animals

Male Sprague-Dawley rats from Harlan (Indianapolis, IN), weighing approximately 350-400 g at the time of study, were housed in pairs in standard polycarbonate cages with bedding and were maintained in a centralized animal facility under controlled environmental conditions (12-h light/dark cycle, lights on at 07:00 h; 22 ± 1 °C) with free access to food and water. All experiments were conducted in accordance with NIH animal care guidelines and were approved by Rutgers University Institutional Animal Care and Use Committee. Upon arrival, animals were handled for one week prior to experimentation. Pairs of rats housed in the same cage were randomly assigned to the various treatment conditions.

4.2. Inescapable Shock

Pairs of rats were simultaneously submitted to a single session of inescapable shock. Each rat was placed inside a transparent rodent restrainer (Model 554-BSRR; PlasLabs, Inc., Lansing, MI), which had an internal diameter of 6.5 cm and an adjustable interior compartment with openings for adequate ventilation. The animal's tail passed through the bottom rear of the tube and was immobilized with medical adhesive tape to a rigid plastic bar (20 cm long, 2 cm wide, 0.6 cm thick) that protruded from the rear of the restraint tube. Two strips of soft copper (0.005 inch thick, 36 gauge; K & S Engineering, Chicago, IL), measuring 6 cm long and 0.5 cm wide, served as electrodes. The electrodes were fixed around the tail (approximately 5 and 10 cm from the base) and connected to a shock generator (Model E11000A; Grason Stadler Company, West Concord, MA) using a set of alligator clip leads. Prior to attaching the electrodes, a small amount of conductive paste was applied to both the electrode surface and the tail. Each rat received 100 tailshocks of 1.0 mA, 5 seconds of duration, delivered on a variable time schedule with an average inter-trial interval of 60 seconds (range 30-90 sec). All IS treatments were given during the light period, between 10:00 h and 15:00 h. Following the shock session (and collection of any blood samples, see below), rats were returned to their home cages and either moved into a second, unoccupied laboratory room (1 h post IS group) or transported back into the animal facility (2 and 7 day post IS groups). The electrical parameters utilized in this study were identical to those that have previously been shown to induce learned helplessness in rats (Takase et al., 2005). A control group of "unstressed" rats remained at all times in the animal facility until perfusion and were run with the IS animals.

4.3. BrdU administration and animal perfusion

The thymidine analog 5-bromo-2'-deoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO) was dissolved in sterile physiological saline (containing 0.007 N NaOH). Rats received a single

intraperitoneal injection of 200 mg/kg of BrdU at one of three different post-shock intervals (1 h, 2 d, and 7 d) and were killed 2 hours later. Control (unshocked) animals were run with the shocked animals. For perfusions, animals were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and were perfused transcardially with ice-cold physiological saline (containing heparin, 10 IU/ml), followed by paraformaldehyde (4.0% in 0.1 M phosphate-buffered saline, pH 7.4). Brains were removed, postfixed in paraformaldehyde for 24 hours at 4°C, transferred to sucrose (30% in 0.1 M PBS) for 2-3 days until equilibrated and then sectioned.

4.4. BrdU immunohistochemistry

Frozen coronal sections (40 µm) were cut through the entire hippocampus and a 1-in-12 series was then processed for BrdU using a slide-mounted immunoperoxidase technique. Briefly, sections were mounted onto adhesive microscope slides, dried overnight at 37°C, boiled in citric acid (0.01 M, pH 6.0) for 7 min, allowed to cool for 20 min, digested with trypsin (0.1% in 0.1 M Tris buffer, pH 7.5, containing 0.1% CaCl₂) for 8½ min, denatured with 2.4 M HCl (in PBS) for 30 min, and then incubated with a mouse monoclonal antibody raised against BrdU (1:200 in PBS containing 0.5% Tween-20; NCL-BrdU; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) for 24 h at 4°C. The next day, sections were incubated with a biotinylated horse anti-mouse IgG (1:200 in PBS; Vector Laboratories, Burlingame, CA, USA) and with avidin-biotin complex (1:100 in PBS; Vectastain® Elite ABC kit, Vector Laboratories) for 60 min, and then reacted with 3,3'-diaminobenzidine tetrahydrochloride and urea hydrogen peroxide for 10 min, to visualize labeled cells. Sections were then counterstained with cresyl violet, dehydrated and cover-slipped under DPX.

4.5. Tissue analysis

Slides were analyzed blindly, with respect to treatment, using an Olympus BX-60 light microscope. BrdU-positive cells (stained brown) were counted bilaterally in the DG at high magnification (400-1000×). The cell counts for each animal were summed across all sections and then multiplied by 12 (section periodicity) to obtain an estimate of the total number of BrdU-positive cells in the DG. In addition, the DG was divided into anterior (dorsal) and posterior (ventral) portions, as in Guzmán-Marín et al. (2003). Briefly, the boundary separating the anterior and posterior portions of the DG corresponded to the region where the CA2 and CA3 pyramidal cell layers coalesce into a continuous cell layer in the coronal plane [approximately -4.5 mm from Bregma, according to the atlas of Paxinos and Watson (1986)]. Typically, there were 6 anterior and 4 posterior sections for each animal.

4.6. Blood collection and measurement of plasma CORT levels

Blood was obtained from a fresh tail incision (~ 1-2 mm from the tip) and collected into heparin-coated microcapillary tubes. Samples (approximately 70 µl) were centrifuged for 10 min and the resulting plasma was stored at -70°C, until assayed. Blood samples were obtained from both control and IS animals, immediately following the shock session (while rats were still in the restrain tubes) or 7 days later (while rats were gently restrained in a hand towel). The entire sampling procedure usually took about 1-2 min. Blood samples were not collected from the 1 h post IS group, due to the close proximity to the BrdU administration, which could possibly affect the experimental results. For the same reason, blood samples for the 7 day post IS time point were collected from a different group of rats that did not receive BrdU.

Plasma CORT levels were determined using a commercial ¹²⁵I corticosterone radioimmunoassay kit specifically designed for use in laboratory rats and mice (ImmuChem™ Double Antibody; detectability limit, 7.7 ng/ml; cross-reactivity, 0.34% for deoxycorticosterone and <0.10% for all other steroid hormones; MP Biomedicals, Orangeburg, NY).

4.7. Statistical Analysis

Data are expressed as means $\pm$ S.E.M. For statistical analysis of the BrdU data, a one-way analysis of variance (ANOVA) was used, followed by post hoc Newman-Keuls multiple comparison test. For statistical analysis of the CORT data, a two-way ANOVA (treatment $\times$ time) was used, followed by post hoc Bonferroni test. A probability value ≤ 0.05 was taken as statistically significant.

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Abbreviations

ANOVA, analysis of variance; BrdU, bromodeoxyuridine; CC, cage control; CORT, corticosterone; CP, cell proliferation; DG, dentate gyrus; HPA, hypothalamic-pituitary-adrenal; IS, inescapable shock; PTSD, post-traumatic stress disorder.

References

- Belda X, Marquez C, Armario A. Long-term effects of a single exposure to stress in adult rats on behavior and hypothalamic-pituitary-adrenal responsiveness: comparison of two outbred rat strains. *Behav. Brain Res* 2004;154:399–408. [PubMed: 15313027]
- Bland ST, Schmid MJ, Greenwood BN, Watkins LR, Maier SF. Behavioral control of the stressor modulates stress-induced changes in neurogenesis and fibroblast growth factor-2. *Neuroreport* 2006;17:593–597. [PubMed: 16603918]
- Brezun JM, Daszuta A. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* 1999;89:999–1002. [PubMed: 10362289]
- Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* 1994;61:203–209. [PubMed: 7969902]
- Cameron HA, Tanapat P, Gould E. Adrenal steroids and N-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway. *Neuroscience* 1998;82:349–354. [PubMed: 9466447]
- Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol. Sci* 2002;23:238–245. [PubMed: 12008002]
- Dal-Zotto S, Marti O, Armario A. Is repeated exposure to immobilization needed to induce adaptation of the hypothalamic-pituitary-adrenal axis? Influence of adrenal factors. *Behav. Brain Res* 2002;129:187–195. [PubMed: 11809510]
- Davidson JR, Stein DJ, Shalev AY, Yehuda R. Posttraumatic stress disorder: acquisition, recognition, course, and treatment. *J. Neuropsychiatry Clin. Neurosci* 2004;16:135–147. [PubMed: 15260364]
- Desan PH, Silbert LH, Maier SF. Long-term effects of inescapable stress on daily running activity and antagonism by desipramine. *Pharmacol. Biochem. Behav* 1988;30:21–29. [PubMed: 3174746]
- Dinan TG. Stress: The shared common component in major mental illnesses. *Eur. Psychiatry* 2005;20:S326–S328. [PubMed: 16459244]
- Faure A, Conde F, Cheruel F, el Massioui N. Learning-dependent activation of Fra-1: involvement of ventral hippocampus and SNc/VTa complex in learning and habit formation. *Brain Res. Bull* 2006;68:233–248. [PubMed: 16377429]
- Gould E. Serotonin and hippocampal neurogenesis. *Neuropsychopharmacology* 1999;21(Suppl):46S–51S. [PubMed: 10432488]
- Guzman-Marin R, Suntsova N, Stewart DR, Gong H, Szymusiak R, McGinty D. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. *J. Physiol* 2003;549:563–71. [PubMed: 12679377]

- Henn FA, Vollmayr B. Neurogenesis and depression: etiology or epiphenomenon? *Biol. Psychiatry* 2004;56:146–150. [PubMed: 15271582]
- Hoofdakker RH, Beersma DGM. Single social defeat in male rats induces a gradual but long lasting behavioral change: a model of depression? *Neurosci. Res. Comm* 1990;7:35–41.
- Jacobs BL, Praag H, Gage FH. Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol. Psychiatry* 2000;5:262–269. [PubMed: 10889528]
- Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry* 1999;156:837–841. [PubMed: 10360120]
- Koolhaas JM, Hermann PM, Kemperman C, Bohus B, van den Hoofdakker RH, Beersma DGM. Single social defeat in male rats induces a gradual but long lasting behavioral change: a model of depression? *Neurosci. Res. Comm* 1990;7:35–41.
- Kulkarni VA, Jha S, Vaidya VA. Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. *Eur. J. Neurosci* 2002;16:2008–2012. [PubMed: 12453065]
- Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A. Increased neurogenesis in a model of electroconvulsive therapy. *Biol. Psychiatry* 2000;47:1043–1049. [PubMed: 10862803]
- Maier SF, Seligman MEP. Learned helplessness: theory and evidence. *J. Exp. Psych* 1976;105:3–46.
- Maier SF, Watkins LR. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neurosci. Biobehav. Rev* 2005;29:829–841. [PubMed: 15893820]
- Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* 2003;28:1562–1571. [PubMed: 12838272]
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci* 2000;20:9104–9110. [PubMed: 11124987]
- Marti O, Garcia A, Valles A, Harbuz MS, Armario A. Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus-pituitary-adrenal axis that consolidate with time. *Eur. J. Neurosci* 2001;13:129–136. [PubMed: 11135011]
- Mirescu C, Gould E. Stress and adult neurogenesis. *Hippocampus* 2006;16:233–238. [PubMed: 16411244]
- Moser MB, Moser EI. Functional differentiation in the hippocampus. *Hippocampus* 1998;8:608–619. [PubMed: 9882018]
- Murua VS, Molina VA. Antidepressants reduce inactivity during both inescapable shock administration and shuttle-box testing. *Eur. J. Pharmacol* 1991;204:187–192. [PubMed: 1806386]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; 1986.
- Paykel ES. Stress and affective disorders in humans. *Semin. Clin. Neuropsychiatry* 2001;6:4–11. [PubMed: 11172528]
- Petty F, Kramer G, Wilson L, Jordan S. In vivo serotonin release and learned helplessness. *Psychiatry Res* 1994;52:285–293. [PubMed: 7991722]
- Pham K, Nacher J, Hof PR, McEwen BS. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult dentate gyrus. *Eur. J. Neurosci* 2003;17:879–886. [PubMed: 12603278]
- Puglisi-Allegra S, Cabib S. Effects of defeat experiences on dopamine metabolism in different brain areas of the mouse. *Aggressive Behav* 1990;16:271–284.
- Radley JJ, Jacobs BL. 5-HT_{1A} receptor antagonist administration decreases cell proliferation in the dentate gyrus. *Brain Res* 2002;955:264–267. [PubMed: 12419546]
- Rosenbrock H, Bloching A, Weiss C, Borsini F. Partial serotonergic denervation decreases progenitor cell proliferation in the adult rat hippocampus, but has no effect on rat behavior in the forced swimming test. *Pharmacol Biochem Behav* 2005;80:549–556. [PubMed: 15820524]
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003;301:805–809. [PubMed: 12907793]

- Shors TJ, Mathew J, Edgecomb C, Sisti HM. Neurogenesis and depression: uncontrollable, but not controllable stress reduces proliferation in the adult hippocampus. *Soc. Neurosci. Abst.* (no. 648.8). 2005
- Sundararaman N, Ramcharan EJ, Vertes RP, Zhang SX, Fornal CA, Jacobs BL. Effect of neurotoxic lesions of 5HT containing cells in the median raphe on cell proliferation in the dentate gyrus. *Soc. Neurosci. Abstr.* (no. 658.11). 2005
- Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J. Comp. Neurol* 2001;437:496–504. [PubMed: 11503148]
- Takase LF, Nogueira MI, Bland ST, Baratta M, Watkins LR, Maier SF, Fornal CA, Jacobs BL. Effect of number of tailshocks on learned helplessness and activation of serotonergic and noradrenergic neurons in the rat. *Behav. Brain Res* 2005;162:299–306. [PubMed: 15913803]
- Thomas RM, Urban JH, Peterson DA. Acute exposure to predator odor elicits a robust increase in corticosterone and a decrease in activity without altering proliferation in the adult rat hippocampus. *Exp. Neurol.* 2006[Epub ahead of print]
- Tidey JW, Miczek KA. Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Res* 1996;721:140–149. [PubMed: 8793094]
- Tornatzky W, Miczek KA. Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiol. Behav* 1993;53:983–993. [PubMed: 8511216]
- Tung A, Takase L, Fornal C, Jacobs B. Effects of sleep deprivation and recovery sleep upon cell proliferation in adult rat dentate gyrus. *Neuroscience* 2005;134:721–723. [PubMed: 16054767]
- Valles A, Marti O, Armario A. Long-term effects of a single exposure to immobilization stress on the hypothalamic-pituitary-adrenal axis: transcriptional evidence for a progressive desensitization process. *Eur. J. Neurosci* 2003;18:1353–1361. [PubMed: 14511316]
- Van der Borgh K, Meerlo P, Luiten PGM, Eggen BJL, Van der Zee EA. Effects of active avoidance learning on hippocampal neurogenesis and plasma levels of corticosterone. *Behav. Brain Res* 2005;157:23–30. [PubMed: 15617767]
- Van Dijken HH, de Goeij DC, Sutanto W, Mos J, de Kloet ER, Tilders FJ. Short inescapable stress produces long-lasting changes in the brain-pituitary-adrenal axis of adult male rats. *Neuroendocrinology* 1993;58:57–64. [PubMed: 8264856]
- Van Dijken HH, Van der Heyden JA, Mos J, Tilders FJ. Inescapable footshocks induce progressive and long-lasting behavioural changes in male rats. *Physiol. Behav* 1992;51:787–794. [PubMed: 1594677]
- Vermetten E, Bremner JD. Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. *Depress. Anxiety* 2002;16:14–38. [PubMed: 12203669]
- Vollmayr B, Simonis C, Weber S, Gass P, Henn F. Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. *Biol. Psychiatry* 2003;54:1035–1040. [PubMed: 14625145]
- Weiss JM, Goodman PA, Losito BG, Corrigan S, Charry JM, Bailey WH. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. *Brain Res. Rev* 1981;3:167–205.
- Yap JJ, Takase LF, Kochman LJ, Fornal CA, Miczek KA, Jacobs BL. Repeated brief social defeat episodes in mice: Effects on cell proliferation in the dentate gyrus. *Behav. Brain Res.* 2006[Epub ahead of print]
- Yeap S, Thakore JH. Stress axis dysfunction in schizophrenia. *Eur. Psychiatry* 2005;20:S307–S312. [PubMed: 16459241]

Figure 1.

Photomicrograph (1000× magnification) of a representative coronal section through the rat dorsal hippocampus showing BrdU-labeled cells in the DG. Clusters of BrdU+ cells (stained brown) are typically seen at the border between the GCL and the hilus (subgranular zone). GCL, granule cell layer

Figure 2.

Inescapable shock (IS) produces a delayed suppression of cell proliferation in the dentate gyrus of adult rats. Rats received 100 tail shocks and were injected with BrdU at the times designated in the figure and killed two hours later. A group of rats maintained in their home cages served as cage controls (CC). Results are means $\pm$ SEM; numbers in parentheses indicate the number of animals tested at each time point. * $p < 0.05$ vs. CC; † $p < 0.05$ vs. 1 h post IS; by one-way ANOVA and post hoc Newman-Keuls Test.

Figure 3.

Inescapable shock (IS) produces an immediate increase in plasma CORT levels but no change 7 days later. Results are means $\pm$ SEM; numbers in parentheses indicate the number of animals tested at each time period. * $p < 0.001$ vs. CC, immediately post IS; † $p < 0.001$ vs. both CC and IS, 7 days later; by two-way ANOVA and post hoc Bonferroni Test.

Table 1

Time course of effects of inescapable shock (IS) on the number of BrdU-positive cells in the dentate gyrus of adult rats

Region	Cage Control (n = 10)	Time Post IS		
		1 h (n = 12)	2 d (n = 6)	7 d (n = 9)
Dorsal	1153 ± 84	1153 ± 85 (0.0 %)	1224 ± 124 (+ 6.1 %)	1043 ± 65 (- 9.5 %)
Ventral	1687 ± 55	1712 ± 93 (+ 1.5 %)	1496 ± 116 (- 11.3 %)	1279 ± 73 ^{** ††} (- 24.2 %)
Dorsal + Ventral	2840 ± 81	2865 ± 135 (+ 0.9 %)	2720 ± 231 (- 4.2 %)	2321 ± 91 ^{* †} (- 18.3 %)

Values are means ± SEM.

*
p < 0.05

**
p < 0.01 vs Cage Control

†
p < 0.05

††
p < 0.01 vs 1 h; by one-way ANOVA & Newman-Keuls multiple comparison test.