Published in final edited form as:

Hippocampus. 2019 September ; 29(9): 848–861. doi:10.1002/hipo.23087.

New Neurons Restore Structural and Behavioral Abnormalities in a Rat Model of PTSD

Timothy J. Schoenfeld¹, Diane Rhee¹, Laura Martin¹, Jesse Smith¹, Anup Sonti¹, Varun S. Padmanaban¹, Heather A. Cameron¹

¹Section on Neuroplasticity, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, 20892

Abstract

Post-traumatic stress disorder (PTSD) has been associated with anxiety, memory impairments, enhanced fear, and hippocampal volume loss, although the relationship between these changes remain unknown. Single-prolonged stress (SPS) is a model for PTSD combining three forms of stress (restraint, swim, and anesthesia) in a single session that results in prolonged behavioral effects. Using pharmacogenetic ablation of adult neurogenesis in rats, we investigated the role of new neurons in the hippocampus in the long-lasting structural and behavioral effects of SPS. Two weeks after SPS, stressed rats displayed increased anxiety-like behavior and decreased preference for objects in novel locations regardless of the presence or absence of new neurons. Chronic stress produced by daily restraint for 2 or 6 hours produced similar behavioral effects that were also independent of ongoing neurogenesis. At a longer recovery time point, one month after SPS, rats with intact neurogenesis had normalized, showing control levels of anxiety-like behavior. However, GFAP-TK rats, which lacked new neurons, continued to show elevated anxiety-like behavior and enhanced serum corticosterone response to anxiogenic experience. Volume loss in ventral CA1 paralleled increases in anxiety-like behavior, occurring in all rats exposed to SPS at the early time point and only rats lacking adult neurogenesis at the later time point. In chronic stress experiments, volume loss occurred broadly throughout the dentate gyrus and CA1 after 6-hour daily stress but was not apparent in any hippocampal subregion after 2-hr daily stress. No effect of SPS was seen on cell proliferation in the dentate gyrus, but the survival of young neurons born a week after stress was decreased. Together, these data suggest that new neurons are functionally important for recovery of normal behavior and hippocampal structure following a strong acute stress and point to the ventral CA1 region as a potential key mediator of stress-induced anxiety-like behavior.

Keywords

single-prolonged stress; adult neurogenesis; GFAP-TK; hippocampal volume; corticosterone; contextual fear conditioning; elevated plus maze; novelty-suppressed feeding; neophagia; object place test; chronic stress

Introduction

Smaller hippocampal volume has been observed in patients suffering from post-traumatic stress disorder (PTSD) (Bremner et al., 1995), and the hippocampus shows extensive plasticity in response to stress (reviewed in Kim et al., 2015), suggesting a critical role for the hippocampus in PTSD. One form of structural plasticity, adult neurogenesis, occurs robustly in the dentate gyrus region of the hippocampus and is regulated by stressful experiences. Stress can affect the proliferation, differentiation, maturation, survival, and activation of newborn neurons (Snyder et al., 2009; Schoenfeld and Gould, 2012), suggesting widespread and potentially powerful alterations in dentate circuitry following stress. In addition, new neurons in the dentate gyrus are functionally important for the regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to stress; animals without adult neurogenesis are more susceptible to anxiodepressive-like behavior following acute stress (Snyder et al., 2011).

A single-prolonged stress (SPS) protocol using three different stressors in the course of one day mimics neurochemical and behavioral effects of PTSD in rodents (Liberzon et al., 1997; Lisieski et al., 2018; Richter-Levin et al., 2018). SPS causes abnormalities in glucocorticoid receptor expression, activation of the HPA axis, and excitatory and inhibitory neurotransmitter levels in the hippocampus after an incubation period of one week following stress (Liberzon et al., 1997; Yamamoto et al., 2009). In addition, SPS leads to widespread changes in hippocampus-dependent learning and emotional behavior, including enhanced contextual fear conditioning, prolonged fear expression during extinction, and increased anxiety-like and depressive behavior (Yamamoto et al., 2009; Ji et al., 2014). One study has suggested that SPS reduces adult neurogenesis in the dentate gyrus (Peng et al., 2013), but the role of new neurons in behavioral effects of SPS and the long-term effects of SPS on adult neurogenesis and hippocampal volume are unknown.

We therefore characterized the effects of SPS on neurogenesis and looked for a role of new neurons in SPS-induced behavioral changes by comparing the behavior of GFAP-TK rats treated with valganciclovir to prevent neurogenesis with that of wild type littermate controls. We also compared the single day SPS to chronic daily unpredictable restraint stress (2 or 6 hr per day for 10 days), another stress model with long-lasting effects.

Materials and Methods

Experimental Animals

Most of the experiments used transgenic male rats expressing Herpes Simplex Virus Thymidine Kinase (HSV-TK) under the control of the human glial fibrillary acidic protein (GFAP) promoter on a Long-Evans background (RRRC #764), which were bred in-house. When phosphorylated by HSV-TK, the anti-viral drug, valganciclovir (VGCV) interferes with DNA replication, preventing production of new neurons from GFAP⁺ radial cells without affecting post-mitotic astrocytes (Heyman et al., 1989; Snyder et al., 2016). VGCV was administered in a peanut butter/powdered chow mixture (4mg VGCV/rat) to all wildtype (WT) and transgenic (TK) rats twice weekly starting at 8 weeks of age. VGCV treatment began 8 weeks prior to stress and continued until the animals were perfused. All

WT and TK rats were meal fed to control weight and given *ad libitum* access to water. For the histological studies in Experiment 5 (described below), male Long-Evans rats (genetically equivalent to wild type rats in the other experiments) were ordered from a vendor (Charles River) and housed with food and water *ad libitum*. All rats were maintained on a 12-hour reverse light-dark cycle (lights off at 9am). All procedures followed the Institute of Laboratory Animal Research guidelines and were approved by the Animal Care and Use Committee of the National Institute of Mental Health.

Experimental Design

Experiment 1 examined the effects of SPS on several behaviors and hippocampal volume in neurogenesis-intact and -deficient rats. WT and TK rats underwent SPS (see below) at 16 weeks of age; controls remained in their cages in the housing room. All rats ($n = 8-9$ per genotype and condition) were tested on the elevated plus-maze (EPM) 9 days after stress followed by novelty-suppressed feeding (NSF) testing the next day. From days 13-16, SPS and control rats underwent object location testing. Starting on day 20 after stress, SPS and control rats underwent shock training and were tested for contextual fear extinction over the next 10 days. All rats were perfused 31 days following stress for hippocampal volume analysis.

Experiment 2 was similar to Experiment 1 but tested the effects of chronic restraint stress instead of one-day SPS, for comparison with Experiment 1. WT and TK rats underwent unpredictable restraint stress (URS; see below) for 6 hours/day $\times$ 10 days. One day after the last restraint session, URS and control rats ($n = 8-10$) began testing on the EPM, and NSF, object location memory, and contextual fear conditioning in the same manner as in Experiment 1. All rats were perfused for hippocampal volume analysis 21 days after the cessation of restraint stress (30 days after the first stress).

Experiment 3 was similar to Experiment 2 but tested the effects of a milder chronic restraint stress paradigm with a shorter restraint period each day. WT and TK rats underwent URS for 2 hours/day for 10 days. Starting one day after the final stress, URS and control rats ($n = 8-10$) were tested on EPM, NSF, object location memory, and contextual fear conditioning as above. 21 days after cessation of restraint (30 days after start), all rats were perfused for hippocampal volume analysis.

Experiment 4a was similar to Experiment 1 but examined a longer recovery time point following SPS. WT and TK rats underwent SPS at 16 weeks of age, and WT rats were injected with the cell division marker bromodeoxyuridine (BrdU; 200 mg/kg) 30 minutes later. SPS and control rats ($n = 10-11$) underwent the same battery of tests as in Experiment 1 beginning 36, rather than 9, days after stress. All rats were perfused 58 days following stress for hippocampal volume and new neuron survival analysis.

In Experiment 4b, an additional cohort of WT and TK rats was tested in the EPM at the long recovery time point after SPS to measure glucocorticoid response to anxiety testing. Previous research suggests that behavioral differences when animals freely explore the EPM limit effects on corticosterone response, whereas forcing rats onto the open arms reveals anxiety-related effects on corticosterone (Landgraf et al., 1999). Thus, some of the rats ($n =$

7–8) were allowed to freely explore throughout the EPM for 5 minutes, while others ($n = 5–6$) were forced onto an open arm of the EPM for 5 minutes. All rats were rapidly decapitated 30 minutes following EPM test and trunk blood was collected for corticosterone analysis (see below).

Experiment 5 examined cell proliferation and survival of new neurons in the adult dentate gyrus of Long Evans rats at various time points after SPS. The WT rats used in Experiment 4 ($n=11$) were injected with the cell division marker bromodeoxyuridine (BrdU; 200 mg/kg) 30 minutes after SPS and perfused 58 days later, after behavioral testing (see above). A second cohort of WT rats was given BrdU 30 minutes after SPS at the same time as the first cohort but was perfused 24 hours after BrdU injection ($n=10$). To examine cells born one week after stress, a separate cohort of Long Evans rats (wild type) was ordered at 12 weeks of age, subjected to SPS at 16 weeks of age, injected with BrdU 1 week after SPS, and perfused 2 hours ($n = 10$) or 3 weeks ($n = 10$) after injection.

Single-Prolonged Stress (SPS)

We used a slightly modified version of SPS (Liberzon et al., 1997). Rats were placed in clear plexiglass restraint tubes (Harvard Apparatus) in a novel procedure room for 2 hours, directly followed by cold water swim (10°C) for 5 minutes. Rats were then placed back in their home cages for 15 minutes, and then exposed to Isoflurane until they reached unconsciousness, all under bright white light conditions. The primary modifications were the use of a short, cold swim in place of a 20-min forced swim in room temperature water and the use of isoflurane in place of ether.

Unpredictable Restraint Stress (URS)

Rats were restrained in clear, plexiglass tubes in brightly lit empty cages for an average of 6 hours a day for 10 days during their active phase (Schoenfeld et al., 2017). The onset (8–11am) and duration (5–7 hours) of restraint were randomized daily. Additionally, a new olfactory cue was presented daily (various oils; LorAnn Oils), and stressed rats were singly housed during the 10 days of stress to add unpredictability and prevent habituation to and social buffering of the stress. For the milder restraint experiment, the protocol was identical except that stress lasted only 2 hours each day. After the 10-day stress period, all rats were placed back with their original cage mates and group housed for the remainder of the experiment. All unstressed rats were group-housed with their original cage mates for the entire experiment.

Behavior Tests

Elevated Plus-Maze (EPM)—Rats were placed on an elevated (20cm high), plus-shaped track for 5 minutes. All maze arms were 20 cm long and 5 cm wide; two opposed closed arms had high walls (20cm high), while the open arms had no wall or lip. The number of arm entries was recorded as a measure of general locomotion, and the amount of time spent in the anxiogenic open arms was used as a measure of anxiety (Walf and Frye, 2007).

Novelty-Suppressed Feeding (NSF)—Rats were food deprived for 24 hours and placed in an open arena (40cm × 40cm) with a pile of regular chow pellets in the center. The

distance traveled was recorded, and the latency to begin eating food pellets was measured. Longer latencies suggest increased anxiodepressive-like behavior (Bodnoff et al., 1988). If they did not eat within 10 minutes of exploration, rats were removed from the arena and assigned a latency of 10 minutes. After the test, the amount of food eaten in the rat's home cage during a 5-minute interval was measured to assess hunger in a safe context.

Object Location Test—The object location test assesses rats' preference for objects that have been moved to a new location. This behavior is hippocampus-dependent (Barker and Warburton, 2011) and is generally interpreted as reflecting memory for the original location, though cases in which rats show marked preference for the familiar stimulus suggest that the effects, particularly of stress, may be on preference rather than memory (Bowman et al., 2009; Wei et al., 2014; Opendak et al., 2016). Rats were habituated in an open field (40cm × 40cm) for 15 minutes per day for 3 days. On the fourth day, two identical objects were placed in adjacent corners of the arena, and rats were allowed to freely explore for 4 minutes. After an inter-trial interval of 3 hours, one of the objects was moved to a different corner, and rats were placed back in the open field and allowed to explore freely for 3 minutes. Object exploration time was measured during both exploration sessions. One rat was excluded for failing to explore the objects; all rats with exploration time > 1s continued on to testing. For the testing session, a discrimination ratio was calculated as (novel object time – stationary object time) / (total object exploration time), with a score of 0 indicating equal preference for both objects, positive values indicating preference for the item in the novel locations, and negative values indicating preference for item in the original location.

Contextual Fear Conditioning and Extinction—On the first day of fear conditioning, rats were placed in conditioning chambers (Coulbourn Instruments) for 9 minutes. After 3 minutes, rats were shocked 5 times (1.5mA, 1.0 second) with a 60 second inter-trial interval. One day later, rats were placed back in the same conditioning box for 8 minutes without being shocked, which served both as a fear conditioning test session and the first day of extinction. Three more identical extinction trials were given on consecutive days. One week after the last extinction session, rats were placed back in the same context for 8 minutes to serve as a test of fear reinstatement. Freezing was measured throughout all sessions. For the training session, freezing during the 30 seconds after each shock was compared across groups.

Histology

Rats were transcardially perfused with 4% paraformaldehyde, and brains were post-fixed in the same fixative for 24 hours. One hemisphere from each brain was then cryoprotected in 20% sucrose/PBS for at least 48 hours and coronally sectioned on a sliding microtome at 40µm throughout the entire extent of the hippocampus. Sections were stored at 4° C in PBS with 0.1% sodium azide, and a 1:12 series of sections was used for each analysis.

For stereological estimates of BrdU-labeled cells, 1:12 series of sections from one hemisphere were mounted on SuperFrost plus slides (ThermoFisher Scientific) and dried, pretreated in heated 0.1M citric acid, pH 6.0, for 10 minutes, digested in trypsin for 10 minutes, denatured in 2N HCl:PBS for 30 minutes, blocked with 0.5% Tween20 and normal

goat serum for 20 minutes, and incubated in mouse anti-BrdU primary antibody (1:250; BD Biosciences) overnight at 4°C. Slides were then incubated in biotinylated goat anti-mouse secondary (1:200; Vector) for 1 hour, 0.03% H₂O₂ for 30 minutes to prevent endogenous peroxidase activity, avidin-biotin complex (ABC kit, Vector Labs) for 1 hour, and cobalt-enhanced DAB for 5 minutes. Slides were coded and analyzed, blinded to treatment group, under an Olympus BX-51 brightfield microscope. The total number of BrdU+ cells counted in all sections from a series was multiplied by 24, twice the section interval, to estimate the total number of BrdU+ cells in the hippocampus of both hemispheres.

For stereological estimates of cells expressing the endogenous proliferation marker PCNA, free floating sections were heated in citric acid (0.1M, 90°C) for 10 minutes, blocked with 0.5% Tween20 and normal goat serum for 20 minutes, and incubated in mouse anti-PCNA (1:20,000; Santa Cruz) for 2 nights. Sections were incubated in biotinylated goat anti-mouse (1:200; Vector) for 30 minutes, then reacted with ABC (Vector) for 1 hour and cobalt-enhanced DAB for 3 minutes. Sections were mounted on SuperFrost plus slides and counterstained with cresyl violet (0.5%; Sigma). Slides were coded and analyzed blind using an Olympus BX-51 brightfield microscope. The total number of PCNA+ cells counted in all sections from a series was multiplied by 24 to estimate the total number of PCNA+ cells in the hippocampus bilaterally.

For BrdU/NeuN double labeling, free-floating sections (1:12 series from each brain) were denatured in 2N HCl:PBS for 1 hour, blocked with 0.5% Tween20 and normal goat serum for 20 minutes, and incubated in rat anti-BrdU (1:200; Accurate) and mouse anti-NeuN (1:250; Chemicon) primaries overnight at 4°C. Sections were then incubated in goat anti-rat Alexa 555 and goat anti-mouse Alexa 488 (both 1:200; Thermo Fisher) for 90 minutes, rinsed, mounted, and coverslipped with ImmunoMount (Thermo Fisher). For each brain, all BrdU+/NeuN+ cells along the entire dorsal-ventral axis were counted using an inverted axiovert Zeiss 780 confocal microscope (Zeiss) and double-labeling was verified in orthogonal planes from 1µm optical stacks. The total number of double-labeled cells was multiplied by 24 to estimate the total number of BrdU+/NeuN+ cells in the hippocampus bilaterally.

Hippocampal Volume Analysis

Unstained sections were mounted, counterstained with 0.5% cresyl Violet (Sigma), dehydrated with 70% (with acetic acid), 95%, and 100% ethanol, cleared, and coverslipped with Permount. Slides were coded and analyzed, blinded to treatment group, using an Olympus BX-51 brightfield microscope. Dorsal and ventral sections of the dentate gyrus, CA3, and CA1 were traced (StereoInvestigator, MBF Bioscience), and subregion and total hippocampal volumes were calculated (NeuroLucida Explorer, MBF Bioscience) as in a previous study (Schoenfeld et al., 2017). Dorsal and ventral regions were separated as in our recent study (Schoenfeld et al., 2017), according to the method of Banasr et al. (2006). When effects were similar in dorsal and ventral portions of the hippocampus (i.e., in the dentate gyrus, CA3, and the hippocampus as a whole), dorsal and ventral data were combined for analysis.

Corticosterone Assessment—Trunk blood was centrifuged 1–2 hours after collection and serum was extracted and frozen. Serum corticosterone was measured using an ELISA kit (Enzo Life Sciences) and analyzed at 405nm on a Victor³ Multilabel Plate Reader (PerkinElmer).

Statistical Analysis

Extinction analysis used 2×2×4 repeated-measures ANOVAs (genotype × stress × extinction trial) and regional analysis of volume used 2×2×2 repeated-measures ANOVAs (genotype × stress × region), both performed in Statistica (Dell Software) and followed by Tukey post hoc tests when necessary. All other behavior and volume analyses used 2×2 ANOVAs (genotype × stress) performed in Prism (Graphpad Software), followed by Newman-Keuls post hoc tests if necessary. In addition, single-sample t-tests were used in object location tests to determine whether or not each group showed significant discrimination. All neurogenesis analyses used unpaired Student's t-tests.

Results

Experiment 1: Single-prolonged stress increases anxiety-like behavior, decreases novel object location preference, and decreases ventral CA1 volume

To measure the effect of SPS on behavior in rats with and without adult neurogenesis, WT and TK rats were stressed and tested on a battery measuring anxiety-like behavior, novel object location preference, and contextual fear learning and extinction (Figure 1A). Stressed rats displayed more anxiety-like behavior than controls, independent of genotype, by spending less time in the open arms of the EPM and having longer latencies to eat in the NSF test (Figure 1B,C). Rats were similar in overall locomotion and hunger levels (Supplemental Figure 1A,B). TK rats took longer to begin eating in the NSF test than WT rats (Figure 1C), but there was no interaction between genotype and stress suggesting that the effects are additive and independent. (EPM – main effect of SPS: $F_{(1,29)} = 4.31$, $p = .047$; main effect of genotype: $F_{(1,29)} = 0.05$, $p = .82$; SPS × genotype interaction: $F_{(1,29)} < 0.01$, $p = .99$. NSF – main effect of SPS: $F_{(1,29)} = 5.78$, $p = .02$; main effect of genotype: $F_{(1,29)} = 10.45$, $p = .003$; SPS × genotype interaction: $F_{(1,29)} = 0.95$, $p = .34$.)

In the object location task, only non-stressed WT and TKs displayed a significant preference for the displaced object (Figure 1D), despite similar exploration behavior by all groups during object acquisition (Supplemental Figure 1C,D). There was a main effect of SPS, and a tendency towards decreased preference in TK rats, with no interaction. (One-sample t-tests – WT control: $t_{(7)} = 5.04$, $p = .001$; TK control: $t_{(7)} = 4.43$, $p = .003$; WT stress: $t_{(7)} = 0.92$, $p = .39$; TK stress: $t_{(8)} = 1.42$, $p = .19$. ANOVA – main effect of SPS: $F_{(1,29)} = 12.90$, $p = .001$; main effect of genotype: $F_{(1,29)} = 3.63$, $p = .07$; SPS × genotype interaction: $F_{(1,29)} = 0.32$, $p = .58$.)

During contextual fear acquisition, prior exposure to SPS decreased pre-shock freezing and increased postshock freezing, while neurogenesis ablation decreased postshock freezing (Supplemental figure 1E,F). However, there were no differences across treatment groups in the contextual fear conditioning test the next day (Trial 1, day 21) or during subsequent

extinction sessions (Figure 1E). One week following the last extinction trial, SPS rats showed increased spontaneous recovery of freezing (fear reinstatement), relative to unstressed controls, with no genotype effect (Figure 1F). (Extinction – main effect of session: $F_{(3,87)} = 62.14$, $p < .0001$; main effect of SPS: $F_{(1,29)} = 1.78$, $p = .19$; SPS $\times$ session interaction: $F_{(3,87)} = 1.45$, $p = .23$. Reinstatement – main effect of SPS: $F_{(1,29)} = 4.31$, $p = .047$; main effect of genotype: $F_{(1,29)} = 0.19$, $p = .66$; SPS $\times$ genotype interaction: $F_{(1,29)} = 0.05$, $p = .82$.)

Because PTSD is associated with smaller hippocampal volume (Bremner et al., 1997), we measured hippocampal volume 31 days following SPS to determine how this model influences hippocampal volume in rats with intact and inhibited neurogenesis (Figure 1G–J). Overall hippocampal volume showed a tendency toward a decrease in TK rats, but no effect of SPS. Investigation of individual hippocampal areas showed that the dentate gyrus was decreased in TK rats, independent of stress, while the volume of CA3 was unaffected by stress or inhibition of adult neurogenesis. SPS reduced CA1 volume, and dorsoventral subregional analysis showed a significant SPS $\times$ region interaction indicating that the volume decrease occurred specifically in the ventral CA1. (Total HIP – main effect of SPS: $F_{(1,28)} = 0.74$, $p = .40$; main effect of genotype: $F_{(1,28)} = 3.66$, $p = .066$; SPS $\times$ genotype interaction: $F_{(1,28)} = 0.69$, $p = .41$. DG – main effect of SPS: $F_{(1,28)} < 0.01$, $p = .95$; main effect of genotype: $F_{(1,28)} = 7.76$, $p = .01$; SPS $\times$ genotype interaction: $F_{(1,28)} = 0.18$, $p = .67$. CA3 – main effect of SPS: $F_{(1,28)} = 0.61$, $p = .44$; main effect of genotype: $F_{(1,28)} = 0.11$, $p = .74$; SPS $\times$ genotype interaction: $F_{(1,28)} = 0.02$, $p = .89$. CA1 3-way ANOVA – main effect of SPS: $F_{(1,28)} = 12.08$, $p = .013$; main effect of genotype: $F_{(1,28)} = 0.94$, $p = .47$; SPS $\times$ region interaction: $F_{(1,28)} = 12.04$, $p = .03$; SPS $\times$ genotype $\times$ region interaction: $F_{(1,28)} = 0.09$, $p = .85$.)

Experiment 2: Unpredictable restraint stress causes behavioral changes similar to single-prolonged stress

To compare the effects of SPS to the classic chronic restraint model of depression, WT and TK rats were stressed 6 hours/day for 10 days and tested on the same behavior battery as in Experiment 1 (Figure 2A). Significant main effects indicated elevated levels of anxiety-like behavior in rats exposed to chronic stress (URS), with decreased time in the open arms of the EPM and slower latencies to feed in NSF (Figure 2B,C). Stressed rats also showed decreased exploration in EPM, but equivalent hunger levels after NSF (Supplemental Figure 2A,B). There were no significant effects of genotype in either of these tests. (EPM – main effect of stress: $F_{(1,32)} = 6.63$, $p = .015$; main effect of genotype: $F_{(1,32)} = 1.01$, $p = .32$; stress $\times$ genotype interaction: $F_{(1,32)} = 0.04$, $p = .85$. NSF – main effect of stress: $F_{(1,32)} = 15.09$, $p = .001$; main effect of genotype: $F_{(1,32)} = 0.41$, $p = .53$; stress $\times$ genotype interaction: $F_{(1,32)} = 0.53$, $p = .47$.)

In the object location task, TK rats explored both objects less than WTs during the acquisition session (Supplemental Figure 2C). However, in the test session (Figure 2D, Supplemental Figure 2D), no group showed a preference for either object (all $p > .05$ in one-sample t-tests comparing to a discrimination ratio of 0), and there were no effects of

genotype or stress. (Test session – main effect of URS: $F_{(1,32)} = 0.10$, $p = .75$; main effect of genotype: $F_{(1,32)} = 0.39$, $p = .54$; URS $\times$ genotype interaction: $F_{(1,32)} = 1.28$, $p = .27$.)

During fear conditioning, all rats froze minimally at baseline, but stressed rats froze less following shocks than controls (Supplemental Figure 2E,F), independent of genotype. However, stressed rats froze more than controls during a context test the next day, as indicated by a significant stress $\times$ extinction interaction (Figure 2E). Fear was rapidly extinguished in all groups, with stressed rats becoming indistinguishable from controls by the second extinction session. When tested one week after the last trial, stressed rats showed greater fear reinstatement than controls (Figure 2F). (Extinction – main effect of session: $F_{(3,96)} = 103.36$, $p < .0001$; main effect of stress: $F_{(1,32)} = 5.03$, $p = .032$; stress $\times$ session interaction: $F_{(3,96)} = 6.20$, $p = .0001$. Reinstatement – main effect of stress: $F_{(1,32)} = 4.26$, $p = .04$; main effect of genotype: $F_{(1,32)} = 0.90$, $p = .35$; stress $\times$ genotype interaction: $F_{(1,32)} = 1.29$, $p = .26$.)

Overall hippocampal volume 30 days following the last day of restraint (Figure 2G–J) did not show significant main effects of stress or genotype, but statistical tendencies and significant post hoc tests suggested that stress and inhibition of neurogenesis, both alone and in combination, decreased hippocampal volume relative to WT controls ($p < .05$). The dentate gyrus was smaller in TK rats, as in previous experiments, and a significant stress $\times$ genotype interaction indicated that stress decreased dentate gyrus volume only in WT rats. CA3 showed no effects of inhibition of neurogenesis or stress on. In CA1, stress decreased volume in both dorsal and ventral subregions, in contrast to the SPS effect, which was limited to ventral CA1. (Total HIP – main effect of stress: $F_{(1,31)} = 3.18$, $p = .08$; main effect of genotype: $F_{(1,31)} = 3.78$, $p = .06$; stress $\times$ genotype interaction: $F_{(1,31)} = 2.83$, $p = .10$. DG – main effect of stress: $F_{(1,31)} = 1.20$, $p = .28$; main effect of genotype: $F_{(1,31)} = 4.71$, $p = .04$; stress $\times$ genotype interaction: $F_{(1,31)} = 4.31$, $p = .046$. CA3 – main effect of stress: $F_{(1,31)} = 0.03$, $p = .87$; main effect of genotype: $F_{(1,31)} = 0.41$, $p = .53$; stress $\times$ genotype interaction: $F_{(1,31)} = 0.88$, $p = .36$. CA1 3-way ANOVA – main effect of stress: $F_{(1,31)} = 6.28$, $p = .018$; main effect of genotype: $F_{(1,31)} = 0.95$, $p = .34$; stress $\times$ subregion interaction: $F_{(1,31)} = 0.002$, $p = .97$; SPS $\times$ genotype $\times$ region interaction: $F_{(1,31)} = 0.09$, $p = .78$.)

Experiment 3: Milder chronic restraint has few effects on behavior

To examine the effects of milder chronic stress on behavior in rats with and without adult neurogenesis, WT and TK rats were stressed 2 hours/day for 10 days (rather than 6 hrs/day as in the previous experiment) and tested on the same battery of behavior tests (Figure 3A). There were no significant effects of stress or genotype on behavior in the EPM and NSF (Figure 3B,C; Supplemental Figure 3A,B). In the EPM, rats in all groups, including controls, spent very little time in the open arms of the maze (Figure 3B), suggesting increased anxiety overall in this cohort relative to previous experiments, which could have obscured effects of restraint stress. (EPM – main effect of stress: $F_{(1,32)} = 0.07$, $p = .79$; main effect of genotype: $F_{(1,32)} = 0.75$, $p = .39$; stress $\times$ genotype interaction: $F_{(1,32)} = 0.04$, $p = .85$. NSF – main effect of stress: $F_{(1,32)} = 0.38$, $p = .54$; main effect of genotype: $F_{(1,32)} = 0.28$, $p = .60$; stress $\times$ genotype interaction: $F_{(1,32)} = 0.02$, $p = .89$.)

In the object location preference task, stressed rats spent more time exploring the objects during the acquisition trial (Supplemental Figure 3C), but only unstressed rats showed a significant preference for the displaced object during the test session, which resulted in a tendency toward a main effect of stress in the preference ratio (Figure 3D; Supplemental Figure 3D). (One-sample t-tests – WT control: $t_{(7)} = 2.42$, $p = .045$; TK control: $t_{(9)} = 2.59$, $p = .03$; WT stress: $t_{(7)} = 0.67$, $p = .52$; TK stress: $t_{(8)} = 0.47$, $p = .65$. ANOVA – main effect of stress: $F_{(1,31)} = 3.83$, $p = .06$; main effect of genotype: $F_{(1,31)} = 0.14$, $p = .71$; stress $\times$ genotype interaction: $F_{(1,31)} = 0.11$, $p = .74$.)

During fear conditioning, all rats froze to the same extent during the pre- and post-shock periods of the training session (Supplemental Figure 3E,F). During testing, however (Figure 3E), a main effect of stress indicated that stressed rats froze less than controls over the course of the extinction trials, and a tendency towards a stress $\times$ session interaction and post hoc testing suggesting that this difference was most prominent in the first extinction session, i.e., the initial context test. In the fear recovery test one week after the last extinction trial (Figure 3F), none of the groups showed spontaneous recovery of freezing. (Extinction – main effect of session: $F_{(3,93)} = 92.04$, $p < .0001$; main effect of stress: $F_{(1,31)} = 9.54$, $p = .004$; stress $\times$ session interaction: $F_{(3,93)} = 2.50$, $p = .065$. Renewal – main effect of stress: $F_{(1,30)} = 1.75$, $p = .20$; main effect of genotype: $F_{(1,30)} = 0.87$, $p = .36$; stress $\times$ genotype interaction: $F_{(1,30)} = 0.34$, $p = .56$.)

Mild chronic stress had no long-lasting effect on hippocampal volume overall or in any area or subregion (Figure 3G–J), including ventral CA1, which was the most vulnerable to stress in Experiment 1. Inhibition of neurogenesis, which continued throughout the experiment, reduced volume of the dentate gyrus, CA3, and hippocampus as a whole, as expected. (Total HIP – main effect of stress: $F_{(1,30)} = 0.24$, $p = .63$; main effect of genotype: $F_{(1,30)} = 8.88$, $p = .006$; stress $\times$ genotype interaction: $F_{(1,30)} = 0.06$, $p = .81$. DG – main effect of stress: $F_{(1,30)} = 0.32$, $p = .58$; main effect of genotype: $F_{(1,30)} = 10.84$, $p = .003$; stress $\times$ genotype interaction: $F_{(1,30)} = 0.21$, $p = .65$. CA3 – main effect of stress: $F_{(1,30)} = 2.55$, $p = .12$; main effect of genotype: $F_{(1,30)} = 4.66$, $p = .039$; stress $\times$ genotype interaction: $F_{(1,30)} = 0.002$, $p = .96$. CA1 3-way ANOVA – main effect of stress: $F_{(1,30)} = 0.02$, $p = .88$; main effect of genotype: $F_{(1,30)} = 0.47$, $p = .50$; stress $\times$ region interaction: $F_{(1,30)} = 0.16$, $p = .70$; SPS $\times$ genotype $\times$ region interaction: $F_{(1,30)} = 0.37$, $p = .55$.)

Experiment 4: New neurons facilitate the recovery of emotional changes and hippocampal volume following single-prolonged stress

To measure behavioral recovery long after SPS, WT and TK rats were stressed and behavior was tested on the same behavior battery as above beginning 5 weeks, instead of one week, after the one-day stress (Figure 4A). In the elevated plus-maze and novelty-suppressed feeding tests (Figure 4B,C), significant stress $\times$ genotype interactions showed that while stressed WT rats no longer showed elevated levels of anxiety-like behavior relative to unstressed controls, stressed TK rats spent less time in the open arms of the EPM and more time before eating in NSF, similar to their behavior shortly after SPS. All rats showed similar exploration of the EPM and hunger levels after NSF (Supplemental Figure 4A,B). (EPM – SPS $\times$ genotype interaction: $F_{(1,39)} = 4.30$, $p = .04$; main effect of SPS: $F_{(1,39)} =$

1.79, $p = .19$; main effect of genotype: $F_{(1,39)} = 5.61$, $p = .023$. NSF – SPS $\times$ genotype interaction: $F_{(1,39)} = 7.09$, $p = .01$; main effect of SPS: $F_{(1,39)} = 6.26$, $p = .017$; main effect of genotype: $F_{(1,39)} = 4.10$, $p = .049$.)

In the object location test, only non-stressed rats (of both genotypes) displayed exploration preferences for the displaced object, resulting in a main effect of stress on preference ratio (Figure 4D, Supplemental Figure 4D). There were no significant genotype main effects or interactions, indicating that effect of stress in this task, unlike those on anxiety-like behavior, were independent of adult neurogenesis. None of the groups differed in their exploration of objects during the acquisition period (Supplemental Figure 4C). (One-sample t-test – WT control: $t_{(9)} = 2.50$, $p = .03$; TK control: $t_{(8)} = 5.09$, $p = .001$; WT stress: $t_{(10)} = 1.36$, $p = .20$; TK stress: $t_{(10)} = 1.13$, $p = .29$. ANOVA – main effect of SPS: $F_{(1,36)} = 8.64$, $p = .006$; main effect of genotype: $F_{(1,36)} = 1.92$, $p = .17$; SPS $\times$ genotype interaction: $F_{(1,36)} = 1.92$, $p = .17$.)

During the contextual fear conditioning session, stressed rats froze significantly more than controls post-shock, independent of genotype (Supplemental Figure 4E,F). However, stress rats showed a tendency to freeze less than controls over the course of extinction (Figure 4E). All groups showed extinction and none showed spontaneous reinstatement of freezing (Figure 4F). (Extinction – main effect of session: $F_{(3,114)} = 52.83$, $p < .0001$; main effect of SPS: $F_{(1,38)} = 3.86$, $p = .056$; SPS $\times$ extinction interaction: $F_{(3,114)} = 0.91$, $p = .44$. Reinstatement – main effect of SPS: $F_{(1,38)} = 0.32$, $p = .58$; main effect of genotype: $F_{(1,38)} = 1.13$, $p = .29$; SPS $\times$ genotype interaction: $F_{(1,38)} = 1.93$, $p = .17$.)

Overall hippocampal volume showed no effect of SPS at this time point, 56 days post-SPS, while inhibition of adult neurogenesis, which continued throughout the post-SPS period, decreased hippocampal volume in TK rats (Figure 4G–J). Ablation of neurogenesis also decreased the volume of the dentate gyrus, independent of stress. CA3 volume showed a small but significant increase in stressed rats, compared to controls. In CA1, a significant 3-way interaction indicated that the volume of ventral CA1 was reduced in stressed TK rats compared to unstressed TK rats, with no parallel change in WT rats or in dorsal CA1 and no change in the CA1 as a whole. (Total HIP – main effect of SPS: $F_{(1,38)} = 1.29$, $p = .26$; main effect of genotype: $F_{(1,38)} = 9.24$, $p = .004$; SPS $\times$ genotype interaction: $F_{(1,38)} = 0.38$, $p = .54$. DG – main effect of SPS: $F_{(1,38)} = 0.72$, $p = .40$; main effect of genotype: $F_{(1,38)} = 11.62$, $p = .002$; SPS $\times$ genotype interaction: $F_{(1,38)} = 0.004$, $p = .95$. CA3 – main effect of SPS: $F_{(1,38)} = 7.54$, $p = .009$; main effect of genotype: $F_{(1,38)} = 2.70$, $p = .11$; SPS $\times$ genotype interaction: $F_{(1,38)} = 0.12$, $p = .74$. CA1 3-way ANOVA – main effect of SPS: $F_{(1,38)} = 0.71$, $p = .41$; main effect of genotype: $F_{(1,38)} = 2.33$, $p = .14$; SPS $\times$ region interaction: $F_{(1,38)} = 1.77$, $p = .19$; SPS $\times$ genotype $\times$ region interaction: $F_{(1,38)} = 6.93$, $p = .012$.)

To investigate whether SPS-exposed rats show differential glucocorticoid responses to anxiogenic experience when neurogenesis is inhibited, we measured cort levels in trunk blood 30 minutes following either free exploration of the EPM or forced exploration of the open arm (Figure 4K,L). After free exploration on the EPM, all rats showed similarly low cort levels. However, after forced open arm exposure, a main effect of SPS and tendency towards an SPS $\times$ genotype interaction indicated that stressed TK rats had higher cort levels

than TK controls, with no effect of stress in WT rats. (Free exploration – main effect of SPS: $F_{(1,26)} = 0.65$, $p = .43$; main effect of genotype: $F_{(1,26)} = 0.34$, $p = .57$; SPS $\times$ genotype interaction: $F_{(1,26)} = 0.05$, $p = .82$. Forced open arm – main effect of SPS: $F_{(1,17)} = 6.35$, $p = .022$; main effect of genotype: $F_{(1,17)} = 2.69$, $p = .12$; SPS $\times$ genotype interaction: $F_{(1,17)} = 4.24$, $p = .055$.)

Experiment 5: Single-prolonged stress decreases survival of new neurons but does not affect proliferation

Cell proliferation and neuronal survival were measured at several time points following SPS (Figure 5A,D). There were no effects of SPS on cell proliferation immediately or 1 week after SPS, as assessed by BrdU+ cell counts, or 4 weeks after SPS, as assessed by PCNA+ cell counts (Figure 5B,E,F). BrdU+/NeuN+ neurons born immediately after SPS showed no change in survival, relative to unstressed controls, after 8 weeks (Figure 5C). However, 30% fewer BrdU+/NeuN+ neurons that were born a week after SPS remained 3 weeks later (Figure 5G). Since proliferation was unchanged a week after SPS, this decrease likely reflects a lower survival rate for neurons born several days following SPS. (Proliferation 30min: $t_{(15)} = 0.35$, $p = .73$; Proliferation 1wk: $t_{(13)} = 0.09$, $p = .93$; Proliferation 4wks: $t_{(17)} = 0.33$, $p = .75$; Survival 30min: $t_{(15)} = 0.65$, $p = .53$; Survival 1wk: $t_{(14)} = 3.26$, $p = .006$).

Discussion

We investigated the long-lasting changes in hippocampal volume and behavior resulting from a single prolonged stress and the role of adult neurogenesis in these changes. Our results indicated that a single prolonged stress increased anxiety-like behavior and enhanced fear recovery, and decreased novel object location preference, when tested one to four weeks after stress, without having any detectable effect on the initial contextual freezing or extinction. These behavioral changes were accompanied by a stress-induced reduction of ventral CA1 volume, with no discernible volume changes elsewhere in the hippocampus, and were unaffected by inhibition of adult neurogenesis. A moderate chronic restraint stress paradigm (6 hours/day for 10 days) produced comparable behavioral changes to SPS, with the addition of increased contextual fear and decreased dorsal, as well as ventral, CA1 volume. A milder restraint paradigm (2 hours/day for 10 days) had no significant effects on anxiety-like behavior or hippocampal volume and actually decreased freezing following contextual fear conditioning. None of these effects of chronic stress were altered in rats lacking adult neurogenesis. However, when we examined WT and TK rats at a longer, 5-week, time point following SPS, anxiety-like behavior, ventral CA1 volume, and corticosterone response to anxiogenic experience were all normal in WT rats, but not in TK rats, suggesting that new neurons are important for recovery. Examination of adult neurogenesis in wild type rats showed no effect of SPS on cell proliferation 30 minutes, 7 days, or 28 days after stress or on the survival of neurons born immediately after SPS. However, neurons born a week after SPS showed impaired survival, relative to those in unstressed controls, suggesting a delayed decrease in neurogenesis after a severe, acute stressor. Although the number of new neurons is decreased after SPS, the young neurons that remain play an important role in recovery of hippocampal structure and behavior.

Long-term effects of acute stress

Several previous studies have observed behavioral effects of SPS that persist for several weeks. Increased anxiodepressive behavior is seen shortly after SPS (Wu et al., 2016a) and persists for at least 2–3 weeks (Khan and Liberzon, 2004; Takahashi et al., 2006; Peng et al., 2010; Ji et al., 2014; Miao et al., 2014; Patki et al., 2014; Lin et al., 2016), consistent with the increase in anxiety-like behavioral changes observed here 1–2 weeks after stress. We found that elevated plus maze behavior and novelty-suppressed feeding returned to normal within 5 weeks of SPS in normal (wild type) rats. However, novel object location preference was altered by SPS even at the longest time point tested, 6 weeks post-stress, indicating that this test may be more sensitive than EPM and NSF for detecting the persistent behavioral effects of stress. Decreased preference for an object in a novel location is often assumed to reflect impaired spatial memory, but previous studies showing SPS-induced decreases in preference for novel objects (Wang et al., 2012; Eagle et al., 2013; Wen, 2017) and novel conspecifics (Eagle et al., 2013) suggest that this behavioral change may reflect decreased preference for novelty rather than impaired ability to remember which stimulus or location is novel. This idea is supported by a preference for the familiar in other stress paradigms, where the differential treatment of two items argues against memory impairment as an explanation (Bowman et al., 2009; Wei et al., 2014; Opendak et al., 2016; Riga et al., 2017) (Bowman et al., 2009; Wei et al., 2014; Opendak et al., 2016; Riga et al., 2017).

Our effects are generally consistent with previous studies, but some differences were observed, potentially related to procedural modifications. We observed decreased preference for objects in novel locations has been observed more than 6 weeks after SPS, consistent with the long-lasting nature of stress effects on preference (Shafia et al., 2017). However, the same study found that anxiety-like changes in elevated plus maze behavior also persisted for 6 weeks after SPS (Shafia et al., 2017), in contrast to what was seen in the current study. Minor differences in behavioral testing paradigms could affect the sensitivity of the tests to detect behavioral changes. Similarly, small changes in the protocols for inducing stress may alter the duration of its behavioral effects, as stronger stressors may have longer-lasting results. One difference between the stress protocol used in the current study and in most previous SPS studies is the replacement of diethyl ether with isoflurane, a less dangerous but less stressful anesthetic (Nagate et al., 2007; Flecknell, 2015). Another study, which used ether, found no effect of SPS on anxiety-like behavior after 24 days, suggesting a relatively rapid recovery, but because this study did not include a shorter time point it is unclear whether behavior resolved or was unaffected by their paradigm (Wu et al., 2016b). The current study also used cold water swim, which has stronger post-stress effects than warmer water (Linthorst et al., 2008), but swim time was decreased, likely offsetting the colder temperature to some degree.

Unlike anxiety and preference behaviors, the effects of SPS on conditioned fear seem to appear only after a delay, with different aspects of the behavior showing different time courses. A previous study found that context-induced freezing was enhanced 14 days, but not 1 day, after SPS (Takahashi et al., 2006). The current study showed no effect of SPS on conditioned freezing 3 or 7 weeks after SPS but found that stressed rats showed more rapid extinction only at the later time point, consistent with delayed appearance of fear-related

behaviors. However, another study observed an effect on extinction 3 weeks after SPS, and the effect was to slow extinction in contrast to the acceleration we observed. Differences in the SPS protocol could alter the overall stressfulness of the experience and thus the timing of recovery, as described above. In addition, differences in fear conditioning paradigms could alter behavioral outcomes. Here we used a different conditioning paradigm than most previous studies (Takahashi et al., 2006; Miao et al., 2014; Wu et al., 2016b), with more numerous and higher amplitude, but shorter duration, shocks, likely enhancing memory strength and decreasing ambiguity, which may enhance freezing in the non-SPS controls and eliminate differences. Another acute stress paradigm, “stress-enhanced fear learning” (Perusini et al., 2016), uses multiple shock exposures in a single session to produce a PTSD-like enhancement of fear response to a future shock, which lasts up to 90 days after the initial stress session, suggesting that enhancement of responses to a new stressor may persist longer than changes in anxiety-like behavior. Taken together, these results indicate that each behavior is altered by SPS with a different time course, making it difficult to define specific time points at which animals show maximal behavioral changes or have fully recovered. Understanding the factors controlling the duration of behavioral abnormalities following SPS is vital for relating these effects to PTSD.

Role of neurogenesis in stress effects

Mice without neurogenesis show increased anxiety-like behavior for several days after acute or chronic stress (Snyder et al., 2011; Seo et al., 2015; Glover et al., 2017; Anacker et al., 2018). The current study found increased anxiety-like behavior for at least 10 days after SPS regardless of the presence or absence of new neurons. Adult neurogenesis only affected behavior at a later time point, more than a month after SPS, when only rats with ongoing neurogenesis demonstrated behavioral recovery. Impaired recovery of sucrose preference and anxiety-like behavior following chronic mild stress has previously been observed in rats lacking adult neurogenesis during the recovery period (Mateus-Pinheiro et al., 2013), suggesting a similar role for new neurons in recovery from these two forms of stress.

Mirroring the behavioral effects, we also found an increase in HPA response that was specific to TK rats at this later time point after SPS. When rats were allowed to freely explore the elevated plus maze, stressed TK rats showed greater avoidance of the open arms relative to other groups but no difference on corticosterone. However, when rats were forced to stay on an open arm (Liebsch et al., 1998; Landgraf et al., 1999), corticosterone levels were higher in SPS-exposed TK rats compared to the other groups, suggesting that neurogenesis affects the level of perceived threat in this apparatus and modulates their exploratory behavior to limit exposure to this threat. This is consistent with previous work showing that new neurons are activated by stress and hasten recovery of the stress response to acute stressors (Snyder et al., 2011). Taken together, these findings support the idea that new neurons enhance the ability to update behavior and adapt to new safer, contextual situations following a traumatic stressor, preventing generalization of anxiety from persisting beyond when it is adaptive (Cameron and Schoenfeld, 2018).

Enhanced generalization that increases fear and anxiety behavior toward normally non-threatening cues and environments is a key component of PTSD (Morey et al., 2015). Fear

generalization has been observed in other rodent models of PTSD-like behavioral changes induced by stress, such as those using strong shock (Kamprath and Wotjak, 2004; Ghosh and Chattarji, 2015; Seo et al., 2015). Increased anxiety-like behavior in most standard rodent tests is likely to reflect generalization, as long as the testing apparatus is placed in a novel environment that does not share cues with the environment in which the animals were stressed. Enhanced anxiety-like behavior in these models is dependent on both new neurons in the dentate gyrus and synaptic plasticity in the amygdala (Ghosh and Chattarji, 2015; Seo et al., 2015). Unlike SPS, however, the behavioral changes produced by these strong shock paradigms are typically observed 1–3 days after stress but are gone within 7 days (Kamprath and Wotjak, 2004), presumably because the stressor is less severe than in the SPS model.

Another hallmark of PTSD is an inability to suppress traumatic memories such that they are easily triggered and become intrusive, producing anxiety (Catarino et al., 2015). Increasing neurogenesis in adult mice is reported to promote the forgetting of learned fear responses, while ablating neurogenesis appears to preserve recent fear memories (Akers et al., 2014; Gao et al., 2018). In our study, rats were tested in novel contexts with cues that were novel (elevated plus maze and novel object location) or familiar but not associated with stress (novelty-suppressed feeding), so memories for cues or locations associated with SPS should not affect behavior in these tests. Instead, the observed behavior changes likely reflect nonassociative effects of the aversive stress experience that sensitize the animals to novel cues and/or cause them to generalize anxiety to novel situations (Glover et al., 2017). In line with this idea, new neurons may allow rats to suppress anxiety-like responses, possibly by compartmentalizing fear and limiting generalization responses in novel situations, without forgetting their negative experience.

Effects of SPS on neurogenesis

The effects of stressors on adult neurogenesis are highly variable, with effects that are unpredictable but likely dependent on the type of stress as well as the time point relative to stress that is examined (Schoenfeld and Gould, 2012; 2013). The only previous study to investigate adult neurogenesis following SPS found reduced numbers of new cells born several days after SPS (Peng et al., 2013), which the current work suggests is the result of decreased cell survival. Chronic social defeat, shock stress, and acute social defeat produce no detectable effects on cell proliferation one day later (Westenbroek et al., 2004; Dagyte et al., 2009), consistent with the lack of effects observed at multiple time points in the current study. However, decreased cell proliferation has been observed following acute and chronic stressors in other studies (Tanapat et al., 2001; Malberg and Duman, 2003), indicating that additional work is needed to clarify specific factors controlling the effect of stress on the birth of new neurons in adulthood.

Current evidence suggests that stress has divergent, and even opposite, effects on the survival of young neurons that may depend on their age at the time of stress. The survival of cells born before stress appears unaffected by daily stress and can actually be increased by brief daily restraint (Dagyte et al., 2009; Snyder et al., 2009). However, similar stress protocols decrease the survival of neurons born during the period of chronic stress (Westenbroek et al., 2004; Czéh et al., 2007; Dagyte et al., 2009). The current findings

indicate that cells born up to a week after a one-day stress continue to show decreased survival. A recent study found that cells born at the end of chronic stress period showed increased survival relative to cells born in unstressed rats (De Miguel et al., 2018), suggesting that the removal of stress may have a positive effect on survival of a cohort of neurons maturing during the post-stress recovery period. L-type calcium channels containing the $\text{Ca}_v1.2$ subunit, which are upregulated in the hippocampus following SPS and chronic stress and have been implicated in PTSD (Maigaard et al., 2012; Ji et al., 2014; Krzyzewska et al., 2018), may be an important part of the mechanism regulating the survival of new neurons following SPS. A post-stress period of enhanced new neuron survival may have started more than a week after SPS in the current study, a possibility that should be investigated in the future. A more complete time course of the effects of stress on differentiation and survival of new neurons will hopefully provide a clearer picture of how stress-induced changes in new neurons relate to their function in behavioral recovery.

Relationship of volume shrinkage to behavior

Prolonged URS was the only treatment in the current study to enhance contextual fear conditioning and the only experimental manipulation that reduced volume in the dorsal CA1. Although this parallel cannot be taken as evidence for a causative connection between these two effects, previous studies have found that manipulations of the dorsal hippocampus, and the dorsal CA1 region in particular, affect contextual fear memories more reliably than ventral manipulations (Lee and Kesner, 2004; Fanselow and Dong, 2010). Taken together, results from this study together with previous work from our group (Schoenfeld et al., 2017) suggest a progression of susceptibility to stress-induced volumetric decrease from ventral CA1 (decreased by a 1-day stress) to dorsal CA1 and the dentate gyrus (decreased after 10 days of stress) to CA3 (decreased only after 4 weeks of stress).

Lesion studies (reviewed in Fanselow and Dong, 2010) and circuit-based approaches suggest that ventral regions of hippocampus are uniquely involved in anxiety-like behavior in rodents (Adhikari et al., 2010; Felix-Ortiz and Tye, 2013). The ventral CA1 region in particular has been implicated in this behavior by the recent finding that this region contains pyramidal neurons that generate anxiety-like behavior, labeled “anxiety cells”, that are absent in the dorsal portion of CA1 (Jimenez et al., 2018). Shrinkage of the ventral CA1 has also been implicated in human mood disorders by a recent MRI study showing that women at high risk for depression had decreased hippocampal volume specific to the ventral CA1 region (Durmusoglu et al., 2018). The current study demonstrates that stress on a single day reduces volume in this same subregion while increasing anxiety-like behavior. In fact, the ventral CA1 was reduced in volume in all groups that showed elevated anxiety-like behavior – WT and TK rats in short-term SPS experiment, WT and TK rats in the 6 hours/day URS experiment, and TK rats in the long-term SPS experiment. Importantly, ventral CA1 returned to normal size in WT rats 8 weeks after stress, indicating that changes in volume, like the behavioral effects, are reversible.

Normally, shrinkage of ventral CA1 following SPS may act in an adaptive manner to prime an organism for more cautious behavior in a dangerous environment. However, in the absence of adult neurogenesis, cautious, or anxious, behavior persists longer than it does in

normal controls. This prolonged caution, in the absence of further stressors, is likely to be maladaptive as it decreases opportunities for reward-seeking (Glover et al., 2017). In addition to behavioral changes, rats lacking adult neurogenesis in the current study showed persisting enhancement of glucocorticoid responses to anxiogenic experience 5 weeks after stress. The ventral subiculum, which receives direct innervation from ventral CA1, is particularly important in providing negative feedback on the HPA axis following stress (Herman et al., 1995), suggesting that vCA1 shrinkage may be directly involved in this neuroendocrine change as well in the behavioral effects. Altogether, our data support the idea that the ventral CA1 region of the hippocampus is particularly important for modulating HPA axis and behavioral responses to the environment and that ongoing neurogenesis in the dentate gyrus is critical for recovery of ventral CA1 volume and associated anxiety-like behaviors following an acute stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant sponsor: Intramural Program of the NIH, National Institute of Mental Health

Grant number: ZIAMH002784 (H.A.C.)

References

- Adhikari A, Topiwala MA, Gordon JA. 2010 Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. *Neuron* 65:257–269. [PubMed: 20152131]
- Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang H-LL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards BA, Miyakawa T, Josselyn SA, Frankland PW. 2014 Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* 344:598–602. [PubMed: 24812394]
- Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC, Chen B, Hen R. 2018 Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature* 476:458.
- Banasr M, Soumier A, Hery M, Mocaër E, Daszuta A. 2006 Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. *Biol Psychiatry* 59:1087–1096. [PubMed: 16499883]
- Barker GRI, Warburton EC. 2011 When Is the Hippocampus Involved in Recognition Memory? *J Neurosci* 31:10721–10731. [PubMed: 21775615]
- Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. 1988 The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology (Berl)* 95:298–302. [PubMed: 3137614]
- Bowman RE, Micik R, Gautreaux C, Fernandez L, Luine VN. 2009 Sex-dependent changes in anxiety, memory, and monoamines following one week of stress. *Physiology & behavior* 97:21–29. [PubMed: 19419681]
- Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB. 1995 MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Am J Psychiatry* 152:973–981. [PubMed: 7793467]
- Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS. 1997 Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse--a preliminary report. *Biol Psychiatry* 41:23–32. [PubMed: 8988792]

- Cameron HA, Schoenfeld TJ. 2018 Behavioral and structural adaptations to stress. *Front Neuroendocrinol* 49:106–113. [PubMed: 29421158]
- Catarino A, Küpper CS, Werner-Seidler A, Dalgleish T, Anderson MC. 2015 Failing to forget: inhibitory-control deficits compromise memory suppression in posttraumatic stress disorder. *Psychological science : a journal of the American Psychological Society / APS* 26:604–616.
- Czéh B, Müller-Keuker JIH, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E. 2007 Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. *Neuropsychopharmacology* 32:1490–1503. [PubMed: 17164819]
- Dayte G, Van der Zee EA, Postema F, Luiten PGM, Boer Den JA, Trentani A, Meerlo P. 2009 Chronic but not acute foot-shock stress leads to temporary suppression of cell proliferation in rat hippocampus. *Neuroscience* 162:904–913. [PubMed: 19482059]
- De Miguel Z, Haditsch U, Palmer TD, Azpiroz A, Sapolsky RM. 2018 Adult-generated neurons born during chronic social stress are uniquely adapted to respond to subsequent chronic social stress. *Mol Psychiatry* 287:1433.
- Durmusoglu E, Ugurlu O, Akan S, Simsek F, Kizilates G, Kitis O, Ozkul BA, Eker C, Coburn KL, Gonul AS. 2018 Hippocampal shape alterations in healthy young women with familial risk for unipolar depression. *Compr Psychiatry* 82:7–13. [PubMed: 29367060]
- Eagle AL, Fitzpatrick CJ, Perrine SA. 2013 Single prolonged stress impairs social and object novelty recognition in rats. *Behav Brain Res* 256:591–597. [PubMed: 24036168]
- Fanselow MS, Dong H-W. 2010 Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65:7–19. [PubMed: 20152109]
- Felix-Ortiz AC, Tye KM. 2013 BLA to vHPC Inputs Modulate Anxiety-Related Behaviors. *Neuron* 79:658–664. [PubMed: 23972595]
- Flecknell P 2015 Basic principles of anaesthesia In: *Laboratory Animal Anesthesia*. Fourth Edition Oxford p 1–73.
- Gao A, Xia F, Guskjolen AJ, Ramsaran AI, Santoro A, Josselyn SA, Frankland PW. 2018 Elevation of Hippocampal Neurogenesis Induces a Temporally Graded Pattern of Forgetting of Contextual Fear Memories. *J Neurosci* 38:3190–3198. [PubMed: 29453206]
- Ghosh S, Chattarji S. 2015 Neuronal encoding of the switch from specific to generalized fear. *Nat Neurosci* 18:112–120. [PubMed: 25436666]
- Glover LR, Schoenfeld TJ, Karlsson R-M, Bannerman DM, Cameron HA. 2017 Ongoing neurogenesis in the adult dentate gyrus mediates behavioral responses to ambiguous threat cues. *PLoS Biol* 15:e2001154. [PubMed: 28388632]
- Herman JP, Cullinan WE, Morano MI, Akil H, Watson SJ. 1995 Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *J Neuroendocrinol* 7:475–482. [PubMed: 7550295]
- Heyman RA, Borrelli E, Lesley J, Anderson D, Richman DD, Baird SM, Hyman R, Evans RM. 1989 Thymidine kinase obliteration: creation of transgenic mice with controlled immune deficiency. *Proc Natl Acad Sci USA* 86:2698–2702. [PubMed: 2539597]
- Ji L-L, Tong L, Xu B-K, Fu C-H, Shu W, Peng J-B, Wang Z-Y. 2014 Intra-hippocampal administration of ZIP alleviates depressive and anxiety-like responses in an animal model of posttraumatic stress disorder. *Behav Brain Funct* 10:28. [PubMed: 25178800]
- Jimenez JC, Su K, Goldberg AR, Luna VM, Biane JS, Ordek G, Zhou P, Ong SK, Wright MA, Zweifel L, Paninski L, Hen R, Kheirbek MA. 2018 Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron* 97:670–683.e6. [PubMed: 29397273]
- Kamprath K, Wotjak CT. 2004 Nonassociative learning processes determine expression and extinction of conditioned fear in mice. *Learn Mem* 11:770–786. [PubMed: 15537742]
- Khan S, Liberzon I. 2004 Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology (Berl)* 172:225–229. [PubMed: 14586539]
- Kim EJ, Pellman B, Kim JJ. 2015 Stress effects on the hippocampus: a critical review. *Learn Mem* 22:411–416. [PubMed: 26286651]
- Krzyzewska IM, Ensink JBM, Nawijn L, Mul AN, Koch SB, Venema A, Shankar V, Frijling JL, Veltman DJ, Lindauer RJL, Olff M, Mannens MMAM, van Zuiden M, Henneman P. 2018 Genetic

variant in CACNA1C is associated with PTSD in traumatized police officers. *Eur J Hum Genet* 26:247–257. [PubMed: 29362489]

Landgraf R, Wigger A, Holsboer F, Neumann ID. 1999 Hyper-reactive hypothalamo-pituitary-adrenocortical axis in rats bred for high anxiety-related behaviour. *J Neuroendocrinol* 11:405–407. [PubMed: 10336720]

Lee I, Kesner RP. 2004 Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. *Hippocampus* 14:301–310. [PubMed: 15132429]

Liberzon I, Krstov M, Young EA. 1997 Stress-restress: effects on ACTH and fast feedback. *Psychoneuroendocrinology* 22:443–453. [PubMed: 9364622]

Liebsch G, Linthorst AC, Neumann ID, Reul JM, Holsboer F, Landgraf R. 1998 Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior. *Neuropsychopharmacology* 19:381–396. [PubMed: 9778660]

Lin C-C, Tung C-S, Liu Y-P. 2016 Escitalopram reversed the traumatic stress-induced depressed and anxiety-like symptoms but not the deficits of fear memory. *Psychopharmacology (Berl)* 233:1135–1146. [PubMed: 26740318]

Linthorst ACE, Flachskamm C, Reul JMH. 2008 Water temperature determines neurochemical and behavioural responses to forced swim stress: an in vivo microdialysis and biotelemetry study in rats. *Stress (Amsterdam, Netherlands)* 11:88–100.

Lisieski MJ, Eagle AL, Conti AC, Liberzon I, Perrine SA. 2018 Single-Prolonged Stress: A Review of Two Decades of Progress in a Rodent Model of Post-traumatic Stress Disorder. *Front Psychiatry* 9:196. [PubMed: 29867615]

Maigaard K, Hageman I, Jørgensen A, Jørgensen MB, Wörtwein G. 2012 Electroconvulsive stimulations prevent chronic stress-induced increases in L-type calcium channel mRNAs in the hippocampus and basolateral amygdala. *Neurosci Lett* 516:24–28. [PubMed: 22465249]

Malberg JE, Duman RS. 2003 Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* 28:1562–1571. [PubMed: 12838272]

Mateus-Pinheiro A, Pinto L, Bessa JM, Morais M, Alves ND, Monteiro S, Patrício P, Almeida OFX, Sousa N. 2013 Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl Psychiatry* 3:e210. [PubMed: 23321807]

Miao Y-L, Guo W-Z, Shi W-Z, Fang W-W, Liu Y, Liu J, Li B-W, Wu W, Li Y-F. 2014 Midazolam ameliorates the behavior deficits of a rat posttraumatic stress disorder model through dual 18 kDa translocator protein and central benzodiazepine receptor and neurosteroidogenesis. *PLoS ONE* 9:e101450. [PubMed: 24988461]

Morey RA, Dunsmoor JE, Haswell CC, Brown VM, Vora A, Weiner J, Stjepanovic D, Wagner HR, VA Mid-Atlantic MIRECC Workgroup, LaBar KS. 2015 Fear learning circuitry is biased toward generalization of fear associations in posttraumatic stress disorder. *Transl Psychiatry* 5:e700. [PubMed: 26670285]

Nagate T, Chino T, Nishiyama C, Okuhara D, Tahara T, Maruyama Y, Kasahara H, Takashima K, Kobayashi S, Motokawa Y, Muto S-I, Kuroda J. 2007 Diluted isoflurane as a suitable alternative for diethyl ether for rat anaesthesia in regular toxicology studies. *J Vet Med Sci* 69:1137–1143. [PubMed: 18057828]

Opendak M, Offit L, Monari P, Schoenfeld TJ, Sonti AN, Cameron HA, Gould E. 2016 Lasting Adaptations in Social Behavior Produced by Social Disruption and Inhibition of Adult Neurogenesis. *J Neurosci* 36:7027–7038. [PubMed: 27358459]

Patki G, Li L, Allam F, Solanki N, Dao AT, Alkadhi K, Salim S. 2014 Moderate treadmill exercise rescues anxiety and depression-like behavior as well as memory impairment in a rat model of posttraumatic stress disorder. *Physiology & behavior* 130:47–53. [PubMed: 24657739]

Peng Y, Feng S-F, Wang Q, Wang H-N, Hou W-G, Xiong L, Luo Z-J, Tan Q-R. 2010 Hyperbaric oxygen preconditioning ameliorates anxiety-like behavior and cognitive impairments via upregulation of thioredoxin reductases in stressed rats. *Prog Neuropsychopharmacol Biol Psychiatry* 34:1018–1025. [PubMed: 20493230]

- Peng Z, Zhang R, Wang H, Chen Y, Xue F, Wang L, Yang F, Chen Y, Liu L, Kuang F, Tan Q. 2013 Ziprasidone ameliorates anxiety-like behaviors in a rat model of PTSD and up-regulates neurogenesis in the hippocampus and hippocampus-derived neural stem cells. *Behav Brain Res* 244:1–8. [PubMed: 23384713]
- Perusini JN, Meyer EM, Long VA, Rau V, Nocera N, Avershal J, Maksymetz J, Spigelman I, Fanselow MS. 2016 Induction and Expression of Fear Sensitization Caused by Acute Traumatic Stress. *Neuropsychopharmacology* 41:45–57. [PubMed: 26329286]
- Richter-Levin G, Stork O, Schmidt MV. 2018 Animal models of PTSD: a challenge to be met. *Mol Psychiatry* 2:47.
- Riga D, Schmitz LJM, Hoogendijk WJG, Smit AB, Spijker S. 2017 Temporal profiling of depression vulnerability in a preclinical model of sustained depression. *Sci Rep* 7:8570. [PubMed: 28819243]
- Schoenfeld TJ, Gould E. 2012 Stress, stress hormones, and adult neurogenesis. *Experimental Neurology* 233:12–21. [PubMed: 21281629]
- Schoenfeld TJ, Gould E. 2013 Differential effects of stress and glucocorticoids on adult neurogenesis. *Curr Top Behav Neurosci* 15:139–164. [PubMed: 23670817]
- Schoenfeld TJ, McCausland HC, Morris HD, Padmanaban V, Cameron HA. 2017 Stress and Loss of Adult Neurogenesis Differentially Reduce Hippocampal Volume. *Biol Psychiatry*.
- Seo D-O, Carillo MA, Chih-Hsiung Lim S, Tanaka KF, Drew MR. 2015 Adult Hippocampal Neurogenesis Modulates Fear Learning through Associative and Nonassociative Mechanisms. *J Neurosci* 35:11330–11345. [PubMed: 26269640]
- Shafia S, Vafaei AA, Samaei SA, Bandegi AR, Rafiei A, Valadan R, Hosseini-Khah Z, Mohammadkhani R, Rashidy-Pour A. 2017 Effects of moderate treadmill exercise and fluoxetine on behavioural and cognitive deficits, hypothalamic-pituitary-adrenal axis dysfunction and alternations in hippocampal BDNF and mRNA expression of apoptosis - related proteins in a rat model of post-traumatic stress disorder. *Neurobiol Learn Mem* 139:165–178. [PubMed: 28137660]
- Snyder JS, Glover LR, Sanzone KM, Kamhi JF, Cameron HA. 2009 The effects of exercise and stress on the survival and maturation of adult-generated granule cells. *Hippocampus* 19:898–906. [PubMed: 19156854]
- Snyder JS, Grigereit L, Russo A, Seib D, Brewer M, Pickel J, Cameron HA. 2016 A transgenic rat for specifically inhibiting adult neurogenesis. *Eneuro*:1–13.
- Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. 2011 Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 476:458–461. [PubMed: 21814201]
- Takahashi T, Morinobu S, Iwamoto Y, Yamawaki S. 2006 Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. *Psychopharmacology (Berl)* 189:165–173. [PubMed: 17031709]
- Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E. 2001 Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J Comp Neurol* 437:496–504. [PubMed: 11503148]
- Walf AA, Frye CA. 2007 The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2:322–328. [PubMed: 17406592]
- Wang H, Zuo D, He B, Qiao F, Zhao M, Wu Y. 2012 Conditioned fear stress combined with single-prolonged stress: A new PTSD mouse model. *Neurosci Res* 73:142–152. [PubMed: 22426496]
- Wei J, Yuen EY, Liu W, Li X, Zhong P, Karatsoreos IN, McEwen BS, Yan Z. 2014 Estrogen protects against the detrimental effects of repeated stress on glutamatergic transmission and cognition. *Mol Psychiatry* 19:588–598. [PubMed: 23835908]
- Wen L 2017 PERK signalling pathway mediates single prolonged stress- induced dysfunction of medial prefrontal cortex neurons. *Apoptosis* 22:753–768. [PubMed: 28391375]
- Westenbroek C, Boer Den JA, Veenhuis M, Horst Ter GJ. 2004 Chronic stress and social housing differentially affect neurogenesis in male and female rats. *Brain Res Bull* 64:303–308. [PubMed: 15561464]
- Wu Z, Tian Q, Li F, Gao J, Liu Y, Mao M, Liu J, Wang S, Li G, Ge D, Mao Y, Zhang W, Liu Z, Song Y. 2016a Behavioral changes over time in post-traumatic stress disorder: Insights from a rat model of single prolonged stress. *Behav Processes* 124:123–129. [PubMed: 26772783]

- Wu Z-M, Zheng C-H, Zhu Z-H, Wu F-T, Ni G-L, Liang Y. 2016b SiRNA-mediated serotonin transporter knockdown in the dorsal raphe nucleus rescues single prolonged stress-induced hippocampal autophagy in rats. *J Neurol Sci* 360:133–140. [PubMed: 26723990]
- Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, Liberzon I. 2009 Single prolonged stress: toward an animal model of posttraumatic stress disorder. *Depress Anxiety* 26:1110–1117. [PubMed: 19918929]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

SPS increases anxiety-like behavior, decreases object location memory, and reduces volume of ventral CA1. A) Timeline showing experimental paradigm. B-D) Stressed rats displayed increased anxiety-like behavior in the elevated plus-maze (B) and novelty-suppressed feeding (C) tests and decreased preference for an object in a new location (D). E,F) SPS had no impact in a contextual fear conditioning test (day 1) or subsequent extinction (E); however stressed rats did show higher spontaneous fear recovery one week after fear training (F). G-J) A month after SPS, there was no detectable effect of stress on the volume of the hippocampus overall (G), or in the dentate gyrus (H) or area CA3 (I). Although there were no volume effects in dorsal sections of area CA1, stress reduced volume in the ventral portion of area CA1 (J). * $p < .05$ compared to control or WT, # $p < .05$ in one-sample t -test compared to 0.

Figure 2.

Six hours a day of unpredictable restraint stress (URS) for 10 days increases anxiety-like behavior, enhances contextual fear conditioning, and reduces volume of the entire hippocampus, including dentate gyrus and dorsal and ventral regions of area CA1. A) Timeline showing experimental paradigm. B,C) Stressed rats displayed increased anxiety-like behavior in the elevated plus-maze (B) and novelty-suppressed feeding (C) tests. D) None of the groups showed a preference for the displaced object. E,F) Stress enhanced freezing in a contextual fear conditioning test without affecting extinction (E), but stressed rats later showed greater spontaneous reinstatement of freezing (F). G-J) A month after SPS, hippocampal volume was reduced in the hippocampus as a whole (G) and in the dentate gyrus (H) and throughout the CA1 region (J). Loss of neurogenesis decreased volume in the dentate gyrus and hippocampus as a whole, but neither stress nor inhibition of neurogenesis affected CA3 volume (I). * $p < .05$ compared to control.

Figure 3.

A mild chronic stress comprising two hours a day of unpredictable restraint stress (URS) for 10 days has no effect on anxiety-like behavior nor hippocampal volume, but reduces object location memory and contextual fear conditioning. A) Timeline showing experimental paradigm. B,C) All rats showed relatively high anxiety-like behavior but no group differences in time spent in open arms or latency to eat in the novelty-suppressed feeding test. D) Restrained rats showed no increase in exploration of the displaced object during testing. E) Mild chronic stress reduced contextual freezing during initial testing without affecting extinction. F) Stress had no effect on spontaneous reinstatement of fear when placed back in the shock context. G-J) Brief daily restraint had no lasting effects on the volume of the hippocampus as a whole (G), the dentate gyrus (H), area CA3 (I), or area CA1 (J), though inhibition of neurogenesis decreased dentate gyrus and CA3 volume. * $p < .05$ compared to control. @ $p < .10$ compared to control, # $p < .05$ in one-sample t -test compared to 0.

Figure 4.

WT rats recover from SPS within 8 weeks, while effects on anxiety-like behavior and CA1 volume persist in TK rats. A) Timeline showing experimental paradigm. B,C) Stressed TK rats show increased anxiety-like behavior, but WT rats no longer show effects of stress in the elevated plus-maze (B) and novelty-suppressed feeding (C) tests. D) Stress decreased preference for a displaced object in rats with and without adult neurogenesis. E,F) SPS had little impact on contextual freezing or extinction (E) or on spontaneous reinstatement of freezing (F). G-J) Stress had no effect on overall hippocampal volume (G) or dentate gyrus volume (H), but increased volume in CA3 (I). Stressed TK rats showed reduced volume in ventral CA1, while stressed WT rats had normalized to control levels (J). K,L) All groups had similarly low levels of serum corticosterone after freely exploring the elevated plus-maze (K), but stressed TK rats had elevated serum corticosterone when confined on an open arm (L). * $p < .05$ compared to control or WT, @ $p < .10$ compared to control, # $p < .05$ in one-sample t -test compared to 0.

Figure 5.

SPS reduces survival of neurons born one week after stress. A) Timeline showing experimental paradigm for labeling cells born immediately after SPS. Stress had no impact on the number of BrdU+ cells in the dentate gyrus born immediately after SPS at either a short time point reflecting proliferation (B) or at a longer time point reflecting both proliferation and survival (C). D) Timeline showing experimental paradigm for labeling cells born a week after SPS. Cell proliferation was unchanged 7 days after SPS, as seen using BrdU labeling with a short post-injection survival (E) or 28 days after SPS, as seen with the endogenous cell proliferation marker PCNA (F). However, stress decreased the survival of cells born one week after stress (G). * $p < .05$ compared to control.

Table 1.

Summary of stress effects

	Summary of Stress Effects^I			
	SPS	SPS break	6hr URS	2hr URS
EPM (anxiety)	↑	–/↑	↑	–
NSF (anxiety)	↑	–/↑	↑	–
Object location	↓	↓	–	↓
Extinction (fear)	–	–	↑	↓
Fear recovery	↑	–	↑	–
dCA1 Volume	–	–	↓	–
vCA1 Volume	↓	–/↓	↓	–

^I Symbols show changes relative to unstressed controls. A single symbol indicates effects are the same in wild type (WT) and TK rats; different effects are shown as WT/TK. ↑, increase relative to unstressed; ↓, decrease; – no change. SPS, single prolonged stress; URS, unpredictable restraint stress; EPM, elevated plus maze; NSF, novelty-suppressed feeding.