

Lasting Adaptations in Social Behavior Produced by Social Disruption and Inhibition of Adult Neurogenesis

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Research on social instability has focused on its detrimental consequences, but most people are resilient and respond by invoking various coping strategies. To investigate cellular processes underlying such strategies, a dominance hierarchy of rats was formed and then destabilized. Regardless of social position, rats from disrupted hierarchies had fewer new neurons in the hippocampus compared with rats from control cages and those from stable hierarchies. Social disruption produced a preference for familiar over novel conspecifics, a change that did not involve global memory impairments or increased anxiety. Using the neuropeptide oxytocin as a tool to increase neurogenesis in the hippocampus of disrupted rats restored preference for novel conspecifics to predisruption levels. Conversely, reducing the number of new neurons by limited inhibition of adult neurogenesis in naive transgenic GFAP–thymidine kinase rats resulted in social behavior similar to disrupted rats. Together, these results provide novel mechanistic evidence that social disruption shapes behavior in a potentially adaptive way, possibly by reducing adult neurogenesis in the hippocampus.

Key words: dominance hierarchy; GFAP-TK transgenic rats; hippocampus; neurogenesis; oxytocin; social behavior

Significance Statement

To investigate cellular processes underlying adaptation to social instability, a dominance hierarchy of rats was formed and then destabilized. Regardless of social position, rats from disrupted hierarchies had fewer new neurons in the hippocampus compared with rats from control cages and those from stable hierarchies. Unexpectedly, these changes were accompanied by changes in social strategies without evidence of impairments in cognition or anxiety regulation. Restoring adult neurogenesis in disrupted rats using oxytocin and conditionally suppressing the production of new neurons in socially naive GFAP–thymidine kinase rats showed that loss of 6-week-old neurons may be responsible for adaptive changes in social behavior.

Introduction

Social instability is associated with an increased risk of mental illness, including depression and anxiety disorders in humans (Vives et al., 2011; Bossarte et al., 2013). Although instability can be a health risk, social disruption is a normal part of life, because peer groups and interpersonal relationships change with age and circumstances. Despite acute risks to mental health, studies show

that humans display long-term resilience and use various coping strategies even in response to chronic instability (Khoshaba and Maddi, 1999; Gunnar et al., 2009; Cutuli et al., 2013). Similar to humans, rodents display negative consequences to short-term social disruption but often recover completely unless social interactions are predominantly aversive, as in the case of chronic social defeat (McCormick and Green, 2013; Bourke et al., 2014). Nonetheless, the vast majority of studies on social instability in rodents have been designed to ensure negative outcomes, with relatively little research focused on adaptation and its cellular mechanisms (McCormick et al., 2012, 2015; Branchi et al., 2013a,b).

In the wild, rats form social hierarchies in which a period of intense fighting is followed by stabilization when the most aggressive rat emerges as the dominant (Krames et al., 1969). This arrangement coincides with a reduction in fighting, because nondominant members of the group adjust their behavior in response to the dominant. Although hierarchy stability can be lasting, destabilization is common, occurring as a result of death, aging, or illness of a dominant

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rat, a rogue rat joining the group, or a change in habitat conditions (Barnett, 1958). Because such behavior is difficult to study in the wild, the visible burrow system (VBS) was designed to mimic semi-naturalistic social living for rats in a laboratory setting (Blanchard et al., 1991, 1995, 2013). Dominant rats in a stable hierarchy in the VBS exhibit a higher rate of adult neurogenesis in the hippocampus compared with subordinate rats (Kozorovitskiy and Gould, 2004), but no previous studies have explored the effects of hierarchy destabilization on the brain and behavior. Because adult neurogenesis in the hippocampus has been linked to a variety of functions, including anxiety/stress regulation and cognition (Dupret et al., 2008; Snyder et al., 2011; Opendak and Gould, 2015), it is likely that changes related to dominance hierarchy destabilization could have important behavioral consequences. To investigate this further, we explored the effects of living in stable and unstable hierarchies on new neuron production in the hippocampus and on behaviors that have been associated with this brain region. We found that differences between dominants and subordinates in adult neurogenesis were eliminated when the hierarchy was disrupted. We further found that new neuron production in the hippocampus was consistently lower after social disruption. Despite suppressed neurogenesis, rats from disrupted hierarchies displayed reduced anxiety-like behavior without global cognitive deficits. These rats also show a marked change in social behavior. Although control rats and rats from stable hierarchies prefer novel over familiar rats while resting, rats from disrupted hierarchies exhibit the converse preference of familiar over novel rats.

Next, we used two approaches to investigate whether these behavioral effects are linked to a reduction in the number of new neurons. We used the neuropeptide oxytocin to stimulate adult neurogenesis (Leuner et al., 2012) immediately after social disruption and tested behavior several weeks later, at a time when new neurons would have incorporated into the circuitry. We found that this manipulation had no effect on anxiety-like behavior but prevented the social preference for familiar conspecifics. Then, we used transgenic conditional neurogenesis knock-out [GFAP–thymidine kinase (TK)] rats to reduce new neuron number in rats (Snyder et al., 2016) without social disruption experience and found that these rats had control levels of anxiety-like behavior but social resting preference for familiar conspecifics. Our results show that social disruption produces a resilient phenotype, with reduced anxiety, generally preserved cognition, and a change in social preference and that suppressed neurogenesis in the hippocampus may play a role in this latter outcome.

Materials and Methods

Animals

All animal procedures were approved by the Princeton University Institutional Animal Care and Use Committee and conformed to guidelines of the National Research Council. Adult male Sprague Dawley rats (6–8 weeks of age, Taconic Farms) were housed on a 12 h light/dark reverse light cycle with lights off at 7:00 A.M. and *ad libitum* access to food and water. Adult female Sprague Dawley rats (Taconic) were used as stimulus animals in the VBS studies (see below). Sprague Dawley rats were used because this relatively docile strain can be group-housed in a VBS without risks of injurious fighting. For neurogenesis knock-out experiments in rats not subjected to VBS living, adult male Long–Evans GFAP-TK transgenic rats were used (Snyder et al., 2016). Controls for behavioral studies using neurogenesis knock-out rats were GFAP-TK rats not treated with the drug that activates the transgene [valganciclovir (GCV)]. Controls for the corticosterone (CORT) study using neurogenesis knock-out rats were Long–Evans wild-type rats.

Animal housing

Sprague Dawley rats were housed in groups of four males and two females in a modified version of the VBS (Kozorovitskiy and Gould, 2004; Fig. 1B). The VBS was made from wood and Plexiglas, with opaque wood side panels and transparent Plexiglas tunnels and chambers. The VBS consisted of an open field area (36 × 36 inches), covered by bedding material lit by an overhead fluorescent light during the light phase and a red light bulb during the dark phase of the reverse 12 h light/dark cycle. Red light illumination allowed for videotaping in the dark to determine dominance status of the rats. Standard laboratory rat cages were used for controls and the post-VBS housing portions of the experiments. GFAP-TK rats were also housed in standard cages.

Social disruption manipulation

Rats were marked with black dye in different patterns for identification on videotapes before placement in the VBS. To determine which rat emerged as the dominant by the end of 3 d, each colony of four males was videotaped for 10 h during the dark phase for each day in the VBS. Three days is sufficient time for establishing dominance hierarchies among male Sprague Dawley rats (Kozorovitskiy and Gould, 2004). In each cohort, a dominant rat was selected based on the quantity of offensive relative to defensive behaviors. Behavioral measurements were sampled hourly from videotapes, in 10-min-long intervals, adding up to the total of 300 min per colony. Male-to-male dyadic interactions involving chasing, lateral attacks, boxing, and fighting on ramps were scored as instances of offensive behavior; fleeing the open field and underside exposure were scored as defensive actions (Blanchard et al., 1995; Kozorovitskiy and Gould, 2004). For each male, the number of defensive acts was subtracted from the number of offensive acts over the entire 300 min sampling period, yielding one score. The rat with the highest score was designated as the dominant, and the other three males in the colony were considered subordinate. On day 4, the dominant rats were switched between two VBS communities. After the disruption, rats were videotaped during the dark phase for 3 more days to determine whether stable hierarchies re-emerged among the new configurations. Behavior during the active period was sampled for 300 min as described above, and each rat's aggressive and defensive actions were tallied to determine postswitch changes in aggression and hierarchy dynamics. On day 7, rats were returned to individual home cages until perfusion. Individual housing was used after the social disruption paradigm to prevent effects of additional social experience after group living in the VBS.

For comparison purposes with this study, additional cohorts of rats lived in standard laboratory cages (control) or in nondisrupted hierarchies (stable). Rats living in stable hierarchies remained in their initial cohorts for 6 d before being returned to individual housing. The rats living in standard cages were housed in pairs for 3 d with *ad libitum* access to food and water. On day 4, one rat from each pair was switched with another from a separate cage to determine whether a change in cage mate was sufficient to induce social disruption effects. Rats continued to live in these configurations for 3 d and then were moved to individual housing, as described above.

Rats from all groups (disrupted VBS, stable VBS, cage control) were perfused at either a 2 or 6 week time point relative to the start of the experiment. The first time point was used to examine effects on immature neuron production, whereas the second time point was selected because it is sufficient for neurons generated during social experience to become functionally integrated into the existing hippocampal circuitry and affect performance on hippocampus-dependent tasks (Snyder et al., 2009).

Drug treatments and perfusions

Rats received equimolar injections of thymidine analogs iododeoxyuridine (IdU; 57.5 mg/kg, i.p.; MP Biomedicals) on day 3 and chlorodeoxyuridine (CldU; 42.5 mg/kg, i.p.; Sigma-Aldrich) on day 6. Selective BrdU antibody sensitivities for IdU versus CldU allow for distinct assays of cell proliferation before and after our manipulation (Vega and Peterson, 2005; Leuner et al., 2009).

Separate groups of rats living in disrupted hierarchies were treated with the neuropeptide oxytocin (1 mg/kg, i.p.; Bachem) for 7 d after

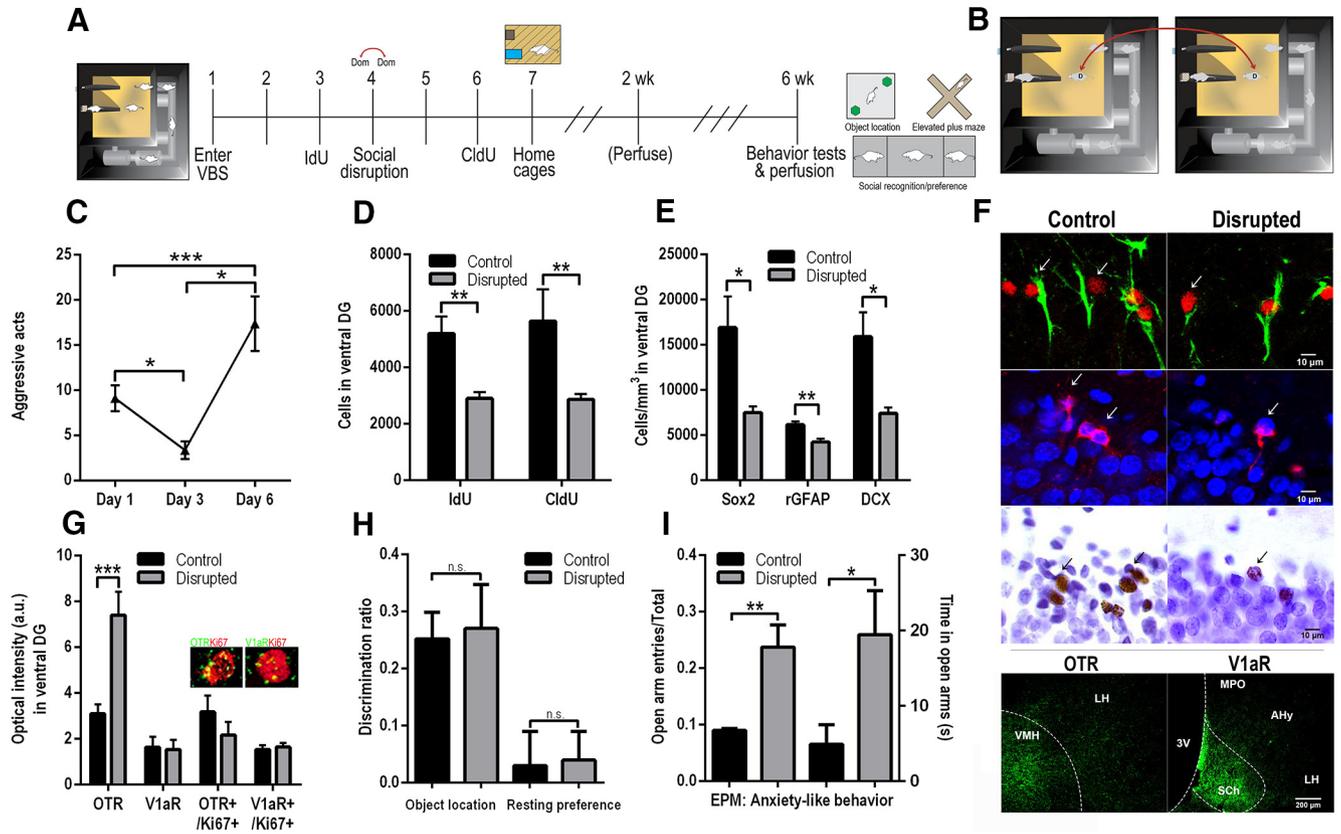


Figure 1. Social disruption suppresses adult neurogenesis without impairing performance on hippocampus-dependent tasks. **A**, Timeline for hierarchy, neuronal birthdating and behavioral testing in rats living in a dominance hierarchy. **B**, Schematic of dominant (D) switch on day 4 in a disrupted hierarchy. **C**, Individual aggressive acts decrease by day 3 in a VBS, but disrupting a hierarchy by switching dominants on day 4 produces an increase in aggression. **D**, IdU⁺ and CldU⁺ cells in the ventral DG are decreased in number in rats from a disrupted hierarchy. **E**, Sox2⁺, radial GFAP⁺ (rGFAP⁺), and DCX⁺ cells in the ventral DG are decreased in number in rats from a disrupted hierarchy. **F**, First row, Representative images of Sox2/GFAP-labeled radial glia-like progenitor cells in DG. Red, Sox2; green, GFAP. Second row, Representative images of DCX-labeled immature neurons in DG, indicated by arrows. Red, DCX; blue, Hoechst 33342. Third row, Representative images of IdU-labeled cells in DG with cresyl violet counterstain, indicated by arrows. Fourth row, Representative images of OTR and V1aR staining patterns in the hypothalamus. **G**, OTR staining, but not V1aR staining, is increased in the ventral DG in rats from a disrupted hierarchy, but no changes were observed in the number of cells colabeled for Ki67 and OTR or Ki67 and V1aR. Inset, Representative images of Ki67-stained progenitor cells colabeled with OTR or V1aR. **H**, There were no differences in rats' object location memory performance, because both groups investigate an object in a novel location more than an object in a familiar location. Neither group showed a preference for resting near objects located in novel or familiar locations. Bars indicate mean relative recognition and resting preference expressed as a discrimination ratio [discrimination ratio = (time spent near novel object – time spent near familiar object)/total time]. Values >0 indicate a preference for the novel object. **I**, Rats from disrupted hierarchy spent more time in the open arms of an elevated plus maze than controls, in terms of both absolute time and proportion of entries in the open arms. Bars indicate mean + SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. n.s., No significant difference; VMH, ventromedial hypothalamus; LH, lateral hypothalamus; Sch, suprachiasmatic nucleus; 3V, third ventricle; MPO, medial preoptic area; AHy, anterior hypothalamic area.

return to individual housing at a dose shown to increase adult neurogenesis in the hippocampus (Leuner et al., 2012). Rats were either perfused at 1 week after the last oxytocin injection (2 weeks after social disruption) to assess the effects of oxytocin treatment on immature neurons or remained in individual housing until behavioral testing at a 6 week time point to allow for adult-born neurons to affect performance on hippocampus-dependent tasks.

For GFAP-TK experiments, beginning at 8 weeks of age, pair-housed TK rats were treated with 4.0 mg of GCV (Valcyte; Roche) per half-gram of peanut butter. Previous studies have shown that oral administration is an effective way to deliver this drug (Snyder et al., 2011; 2016). Rats were fed the peanut butter treat twice weekly for 2 weeks and received injections of BrdU (200 mg/kg, i.p.; Roche) after the second drug treatment each week. The rats remained pair-housed and were perfused 6 weeks after the first GCV treatment. Control TK rats received peanut butter without GCV.

Hormone assays

Additional cohorts of rats were used to examine the effects of social disruption on basal levels of CORT and testosterone. These rats lived in either cage control or disrupted VBS conditions and received sham injections on day 3, and trunk blood was rapidly collected within 3 min of capture on day 6 at lights on. After the blood was centrifuged, plasma was

collected, and circulating CORT and testosterone (in nanograms per milliliter) were measured using a radioimmunoassay (Coat-A-Count; Siemens). Assay sensitivity was 98.5%. To determine whether GFAP-TK rats with inhibited neurogenesis have altered baseline stress hormone levels, additional pair-housed GFAP-TK rats and wild-type controls were treated with GCV as described above. Blood was collected from the tail vein within 3 min of cage opening, and the serum was analyzed for CORT as described above.

Behavioral measures

For behavioral analysis at the 6 week time point, rats were tested on object location, elevated plus maze, and social recognition/preference. These tests were used because they are known to involve the hippocampus and/or appear to be affected by changes in adult neurogenesis (McHugh et al., 2004; Mak and Weiss, 2010; Morris et al., 2013). Rats were tested on these three measures over 2 d, with the order of testing counterbalanced. Rats were perfused the day after the last test. On each testing day, rats were habituated to the testing room for at least 30 min before the first test of the day.

Object location. To assess recognition of a novel location for a previously encountered object, rats were placed in an open field with opaque walls (17.5 × 17.5 × 17.5 inches) with two identical objects (plastic, each ~36 cubic inches) for 3 min (Barker and Warburton, 2011). Rats were

removed for 5 min while one of the objects was moved to the opposite side of the chamber. The left/right position of the moved object was counterbalanced between each rat. Rats were then returned to the chamber for 3 min. A discrimination ratio was calculated for time spent investigating the moved object versus the object that was not moved. Investigating the moved object more than the nonmoved object is considered to be evidence for object location memory.

Anxiety testing. To assess anxiety-like behavior, the elevated plus maze was used (Pellow and File, 1986; Lister, 1987; Walf and Frye, 2007; Staples and Cornish, 2014). Rats were placed on an elevated plus-shaped track (44 × 44 × 20 inches). Two of the arms had high walls (13 inches), whereas the other two arms and central intersection were open. All arms were 20 inches in length, dimly lit by reflected light. Exploratory behavior was measured for 5 min and analyzed. Number of entries into the open and closed arms and time spent in the open arms were measured for each rat. Because the open arms are more exposed to the light and open air, more time spent in the open arms is considered a measure of reduced anxiety (Sun et al., 2013). Total entries into both closed and open arms are considered a measure of locomotion (File and Aranko, 1988).

Social recognition and novelty preference testing. Social recognition and novelty preference were tested using an apparatus based on previous social behavior studies done in mice, with dimensions scaled up to accommodate rats (Engelmann et al., 1995; Moy et al., 2004; Crawley, 2007; Eagle et al., 2013). This apparatus was made of Plexiglas and was 40 × 10 × 12 inches. Removable Plexiglas dividers perforated with 1-cm-diameter holes were placed on each side of the apparatus to create two side chambers that were each 10 × 10 × 10 inches. These walls allowed the test rat to see, smell, and hear stimulus rats while preventing the possibility of physical interaction. Before testing, test rats were placed in a standard cage with a novel rat for 5 min, because this time is sufficient to produce social recognition 30 min later (Thor and Holloway, 1982; Popik et al., 1992). Twenty-five minutes after the end of the 5 min exposure, test rats were placed in the social recognition apparatus for a 5 min habituation; during this period, the side chambers were empty. After this habituation, the familiar rat was placed in one chamber and a novel rat was placed in the other chamber. The side of the chamber with the familiar rat was counterbalanced for each rat. Behavior of the test rat was recorded and analyzed for grooming, rearing, investigation, and resting on each half of the apparatus. Investigation was defined as active sniffing of each Plexiglas wall and was analyzed to assess recognition of the familiar rat (van der Kooij and Sandi, 2012). After a 10 min testing phase, all rats were returned to home cages. The discrimination ratio of time spent investigating each rat over the total time investigating both rats was calculated. The discrimination ratio was also calculated for total time spent resting near each rat, excluding investigation time, to assess rats' resting social novelty preference behavior. To assess social novelty preference behavior in additional detail, resting behavior after investigation (crossing to the other side of the chamber vs staying on the same side) was measured for each rat. In addition, we assessed incomplete and reversed crossings from one side of the testing apparatus to the other as a potential measure of vicarious trial and error (VTE; Redish 2016).

Ultrasonic vocalizations (USVs) were measured for the duration of the social preference study using the UltraSoundGate 116Hb recorder (116Hb/41; Avisoft Bioacoustics) and analyzed using Avisoft-RECORDER and SASLab Lite software. Recordings were analyzed for 22 and 50 kHz vocalizations, typically associated with threatening and rewarding stimuli, respectively (Brudzynski, 2013).

Histology

At 2 or 6 weeks, rats were anesthetized with Euthasol and perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer. At 48 h after fixation, coronal sections (40 μm) were cut throughout the entire rostrocaudal extent of the dentate gyrus (DG) on a vibratome into a bath of 0.1 M PBS, pH 7.5. Brains were analyzed for the following: (1) IdU, to assess the survival of new neurons generated before social disruption; (2) CldU, to assess the proliferation and survival of new neurons generated after social disruption; (3) BrdU, to assess the number of new neurons generated in GFAP-TK rats fed GCV or vehicle; (4) doublecortin (DCX), to label immature neurons; (5) GFAP, to determine changes in glial cells; (6)

Sox2, to determine changes in pluripotent progenitors; and (7) Sox2/GFAP colabeling to assess changes in radial glia-like precursors. Because receptors for oxytocin and vasopressin are expressed in the hippocampus (both neuropeptides are involved in social behavior, stress responses, and neurogenesis; Albeck et al., 1997; Alonso et al., 2004; Leuner et al., 2012) and oxytocin receptor (OTR) binding has been shown to increase in the ventral hippocampus with stress (Liberzon and Young, 1997), we examined OTRs and vasopressin 1a receptors (V1aRs), the main vasopressin receptor type expressed in the hippocampus. We also analyzed OTR/Ki67⁺ and V1aR/Ki67⁺ colabeling to assess the expression patterns of OTRs and V1aRs on proliferating neuronal precursors. All immunolabeling was performed on sections from groups to be compared simultaneously to minimize variability. We also examined the immunolabeling pattern for OTR and V1aR in the hypothalamus to verify that both antibodies produce staining results that are consistent with binding data (Tribollet et al., 1988; Kremarik et al., 1995). Dorsal and ventral distinctions were made within hippocampal hemisections according to published guidelines (Banar et al., 2006).

For IdU/CldU/BrdU peroxidase staining, a 1:12 series of sections was mounted onto glass slides and stained according to established protocols (Leuner et al., 2009). For microscopic data analysis, the slides were coded, and IdU/CldU/BrdU-labeled cells in the granule cell layer (GCL), subgranular zone (SGZ), and the hilus were counted on every 12th half-section through the DG with 100× oil objective on an Olympus BX-60 microscope. The numbers were tallied for the DG and GCL-SGZ and multiplied by 24 to obtain a stereological estimate for the brain region.

Double labeling with immunofluorescence for IdU/CldU and the immature neuronal marker DCX or BrdU and the neuronal marker neuronal nuclei (NeuN) was performed on free-floating sections to determine whether the newly labeled cells were immature neurons according to established protocols (Kempermann et al., 2003). For double labeling, the percentage of IdU/CldU-labeled GCL cells expressing DCX was determined by analyzing 25 IdU/CldU-labeled cells per brain using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; argon 458/488 nm and helium–neon 543 nm). For examining endogenous markers of immature neurons and progenitor cells, the tissue was incubated in primary antibodies against DCX, Sox2, Ki67, and GFAP according to previously published protocols (Suh et al., 2007; Lugert et al., 2010).

For labeling OTRs and V1aRs, free-floating tissue was incubated with rabbit anti-OTR (1:150; Alpha Diagnostics) and rabbit anti-V1aR (1:150; Alpha Diagnostics). For cell density measurements, tissue was counterstained using Hoechst 33342 (1:1000; Invitrogen), and volume measurements of the DG were taken using StereoInvestigator software (MBF Bioscience). Total DCX⁺, Sox2⁺, and GFAP⁺ (radial and horizontal morphologies) were counted on every 12th half-section through the DG with 100× oil objective on an Olympus BX-60 microscope. The raw counts for each section were divided by the volume of the DG to obtain a density measurement. Fifty Sox2⁺ cells in the DG (25 dorsal, 25 ventral) per brain were scanned and analyzed for GFAP⁺ colabeling using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; argon 458/488 nm and helium–neon 543 nm). A distinction was made between GFAP cells with radial and horizontal morphology because previous studies have shown that GFAP⁺/Sox2⁺ cells with radial morphology represent radial glia-like precursor cells with stem cell-like properties, whereas GFAP⁺/Sox2⁺ cells with horizontal morphology represent type 2a intermediate/transit amplifying progenitor cells that are not yet neuronally committed (Lugert et al., 2010).

OTR and V1aR expression was measured for mean optical intensity using 1-μm-thick optical sections of the DG. For these analyses, the DG within a single hemisection was imaged in its entirety, with 10 images obtained for each brain. Five representative 1 μm optical slices from the dorsal and ventral DG were scanned and measured for optical intensity from each brain using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; argon 458/488 nm and helium–neon 543 nm). Intensity was calculated using LSM 510 software. Regions of interest (ROIs) were manually defined to include the dorsal GCL and ventral GCL. The fornix was used for a measure of background staining because this region had no OTR or V1aR staining in any of the brains analyzed. Raw ROI intensity values were divided by fornix intensity within the same pixel-size

ROI on the same scan of each slice to control for variability in staining intensity between sections. Normalized brightness per ROI was determined for each pixel of a given ROI as the fractional increase in brightness at that pixel over the mean brightness of the fornix, according to the following formula: (mean intensity of the region – mean intensity background)/mean intensity background. To analyze OTR and V1aR staining on individual Ki67⁺ proliferating cells, 50 Ki67⁺ cells were scanned from the DG within a hemisection (25 dorsal, 25 ventral). Using LSM 510 software, each Ki67⁺ cell body was traced, and the brightness of OTR or V1aR pixels was determined within the tracing. This was assessed with respect to the background brightness within an area of the same size of the tracing adjacent to each labeled cell (Siegel et al., 1993; Schoenfeld et al., 2014).

Statistical analysis

Depending on the number of groups in an experiment, unpaired Student's *t* tests or one or two-way ANOVA, followed by Bonferroni's *post hoc* comparisons were used.

Results

Social disruption results in increased aggression and dominance hierarchy destabilization

Throughout the 6 d period of living in the VBS, the overall level of aggression changed significantly ($F_{(2,14)} = 12.97, p = 0.001$; Fig. 1C). Between days 1 and 3, the level of aggression decreased for both groups (stable and disrupted; $p = 0.005$). In stable cohorts, aggression did not change from day 3 to 6 (3.87 ± 0.56 vs 4.09 ± 0.60 acts/rat, $t_{(8)} = -0.27, p = 0.821$), but in disrupted cohorts, after the dominant rats were switched between two hierarchies, the level of aggression increased significantly from day 3 to 6 ($p = 0.001$; Fig. 1C).

Postdisruption aggression levels were also significantly higher than day 1 aggression levels ($p = 0.0001$; Fig. 1C). Switched dominants showed a significant decrease in dominance score (aggressive acts – defensive acts) from 5.7 ± 1.6 to 0.2 ± 1.5 [two-way repeated-measures ANOVA, interaction of phase (before switch vs after switch), and dominance score, $F_{(1,38)} = 10.294, p = 0.003$]. In every instance, switching dominants led to a loss of dominant status for these animals. In other words, hierarchy disruption led to an increase in aggression that exceeded that of naive rats placed in the VBS for the first time, as well as to a reorganization of the social hierarchy with no evidence of a clear dominant, and loss of position by previously dominant rats.

Social disruption suppresses adult neurogenesis in the hippocampus

Consistent with previous results (Kozorovitskiy and Gould, 2004), we found that dominants in a stable hierarchy had more new neurons in the ventral DG than subordinates (IdU, 6552 ± 840 in dominants vs 4448 ± 480 in subordinates, $t_{(13)} = 2.41, p = 0.032$). However, social disruption eliminated the advantage of being dominant in dominants (IdU, 3300 ± 607 in dominants vs 2762 ± 218 in subordinates, $t_{(13)} = -1.07, p = 0.3$). Because the dominant – subordinate difference in new cell number in stable groups was no longer present in socially disrupted groups and was irrelevant for control groups, data were collapsed across dominance status for subsequent analyses, and experiments focused on control compared with disrupted hierarchy groups. No differences were observed between rats that were pair-housed in standard cages and those that were switched between cages, suggesting that switching location or being housed with a new conspecific did not alter baseline levels of neurogenesis (e.g., for ventral DG IdU, 4824 ± 363 in switched vs 5580 ± 1222 in nonswitched; $t_{(6)} = 0.59, p = 0.57$). Furthermore, no differences were observed between control and stable VBS cohorts (exclud-

ing dominant rats) on any measure of cell proliferation or adult neurogenesis ($p > 0.05$ for all comparisons), suggesting that living in a VBS, even under stable conditions, did not produce any measurable change in neurogenesis. As shown previously (Kozorovitskiy and Gould, 2004), cage controls and subordinates in a stable hierarchy did not differ on measures of adult neurogenesis. Thus, subsequent comparisons were made between nonswitched control and disrupted groups.

Social disruption produced a decrease in the numbers of neuronal precursor cells and immature neurons compared with control groups at the 2 week time point. The number of Sox2⁺ cells ($t_{(6)} = -2.68, p = 0.037$) and GFAP⁺ cells with radial morphology ($t_{(6)} = -3.73, p = 0.01$) were decreased in the ventral DG after social disruption compared with control (Fig. 1E,F). No changes were observed in horizontal GFAP⁺ cells ($p = 0.53$), the proportion of radial to horizontal cells ($p = 0.23$), or the degree of colabeling of Sox2 and GFAP ($p = 0.26$) with social disruption, indicating that the observed decreases were in Sox2⁺/GFAP⁺ radial glia-like precursor cells. In addition, no changes were observed on any of these measures in the dorsal DG ($p > 0.05$ for all such comparisons). Social disruption also produced a decrease in the number of immature neurons (labeled with DCX) in the ventral DG compared with controls at the 2 week time point ($t_{(6)} = 3.07, p = 0.022$; Fig. 1E,F). Similar to what was observed for neuronal precursors, the suppressive effects of social disruption on DCX⁺ cells were not seen in the dorsal DG ($p = 0.21$). Social disruption also decreased the number of new cells in the ventral DG labeled before the disruption (IdU, $t_{(21)} = 4.31, p = 0.002$), as well as the number of new cells in the ventral DG labeled after the disruption (CldU, $t_{(22)} = 4.32, p = 0.001$) compared with controls at the 2 week time point (Fig. 1D,F). No differences were observed in the numbers of IdU⁺ or CldU⁺ cells in the SVZ, suggesting that these effects may have been specific to the hippocampus ($p > 0.05$ for all comparisons).

More than 80% of IdU⁺ and CldU⁺ cells colabeled with the immature neuronal marker DCX. There were no differences in the percentage of IdU- or CldU-labeled cells that expressed DCX among any of the groups examined ($p > 0.05$), suggesting that the changes in IdU- or CldU-labeled cell number associated with disruption represent suppressed adult neurogenesis.

At the 6 week time point, the difference between control and disrupted groups in the ventral DG no longer existed for cells generated before the disruption (IdU, 3141 ± 295 in control vs 2621 ± 323 in disrupted, $p > 0.05$), but a persistent decrease in the number of cells generated after the disruption (CldU, 3174 ± 416 in control vs 1854 ± 169 in disrupted; $t_{(6)} = -2.94, p = 0.026$) remained in this region. At the 6 week time point as well, there were no persistent differences in the number of DCX⁺ cells in the ventral DG (4293 ± 374 in control vs 5327 ± 1129 in disrupted; $t_{(9)} = -0.94, p = 0.372$), suggesting that the rate of immature neuron production had returned to baseline. The lower numbers of DCX cells in this experiment may be attributable to the switch in housing from group living to individual living. At the 6 week time point, there were no significant differences between control and disrupted groups in the dorsal DG for any measure ($p > 0.05$).

Social disruption did not alter OTR⁺ or V1aR⁺ labeling in proliferating cells in the DG

The staining pattern of both OTR and V1aR in the hypothalamus (Fig. 1F) was similar to what has been reported in binding studies (Tribollet et al., 1988; Kremarik et al., 1995), including high OTR labeling in the ventromedial hypothalamus compared with the

lateral hypothalamus, as well as high V1aR in the suprachiasmatic nucleus compared with the surrounding regions, including the medial preoptic area, suggesting that this labeling reflects receptors for oxytocin and vasopressin. Also consistent with previous reports showing that OTR binding was increased in the ventral hippocampus of stressed rats (Liberzon and Young, 1997), we observed increased optical intensity for OTR in disrupted hierarchy rats compared with controls in the ventral DG ($t_{(10)} = 2.72$, $p = 0.022$) but not dorsal DG ($p > 0.05$; Fig. 1G). There were no differences in V1aR expression in dorsal or ventral DG between disrupted and control groups ($p > 0.05$ for both comparisons). OTR and V1aR expression was observed on Ki67⁺ proliferating cells in the DG, but the proportion of Ki67⁺ cells that expressed either of these receptors did not differ between groups in dorsal or ventral DG ($p > 0.05$ for all comparisons; Fig. 1G).

Social disruption results in lower baseline levels of CORT

Plasma CORT levels differed among rats living in different social settings on day 6 but not in a predicted manner. Although we anticipated that social disruption would result in elevated CORT levels, we found that it did not, at least persistently. Instead, social disruption resulted in decreased baseline CORT levels (102 ± 36.1 ng/ml in control, 31.96 ± 22.5 ng/ml in disrupted; $t_{(12)} = 1.96$, $p = 0.036$). Plasma testosterone levels did not differ significantly between groups (106.1 ± 27 ng/ml in control, 243.4 ± 88.8 ng/ml in disrupted; $p > 0.05$).

Social disruption produced lasting changes in social behavior without impairing performance on some hippocampus-dependent tasks

Performance on the object location task did not differ between rats from disrupted hierarchies and rats from standard group housing (discrimination ratio, $t_{(12)} = 0.21$, $p > 0.05$; Fig. 1H). Preference for resting near objects located in novel versus familiar locations was not detected in either group. On the elevated plus maze, rats from disrupted hierarchies entered the open arms more than controls and spent more time in the open arms than controls (proportional entries into open arms, $t_{(29)} = 3.04$, $p = 0.004$; time in open arms, $t_{(20)} = -2.26$, $p = 0.03$; Fig. 1I).

Social recognition did not differ between the control and disrupted groups, with both groups showing more time spent investigating a novel conspecific compared with a familiar conspecific (two-way ANOVA of condition vs novelty, main effect of novelty, $F_{(1,55)} = 9.29$, $p = 0.004$), and total number of investigations of novel or familiar rats did not differ between groups. However, substantial differences were noted among the groups when social resting preference was analyzed. Whereas control rats tended to rest in the proximity of the novel rat, rats from a disrupted hierarchy preferred to rest in the proximity of a familiar rat (control preference compared with disrupted preference, $t_{(14)} = 2.83$, $p = 0.013$; Fig. 2B). That is, disrupted rats had a greater propensity to cross the chamber to rest near a familiar rat after investigation of the novel rat (Fig. 2C). In contrast, control rats preferred to rest near the novel rat after investigation of the novel rat (proportional crosses to familiar after investigating novel, $t_{(9)} = 4.24$, $p = 0.002$; Fig. 2C). We further analyzed the behavior of rats after investigation of the novel rat and found that control rats often initiated a movement path toward the opposite side of the social chamber, in the direction of the familiar rat, but hesitated and turned back to rest by the novel rat, suggesting a behavior similar to what has been described as reflecting VTE in maze studies (Redish, 2016). In contrast, disrupted rats displayed significantly less VTE-like behavior and most often turned from the novel rat

after investigation and took a direct path to the familiar rat to rest. In controls, VTE-like behavior occurred to a much lesser extent after investigation of the familiar rat compared with the novel rat, and this was unchanged in disrupted rats (see Fig. 5). Thus, compared with controls, disrupted rats exhibited fewer instances of VTE-like behavior after investigating the novel stimulus rat but not the familiar stimulus rat (see Fig. 5; social disruption group \times stimulus rat interaction, $F_{(1,16)} = 8.67$, $p = 0.001$). These results appear independent of general anxiety measures within the social preference apparatus, because we did not observe differences in behavior during habituation to the social chamber (in the absence of conspecifics), including center crossing and active time (Fig. 2D). After addition of conspecifics to the chamber, rearing and grooming behavior, sometimes used as measures of anxiety (Daniels et al., 2008), did not vary between control and disrupted groups in either duration of these behaviors or side preference (Fig. 2E,F; $p > 0.05$ for all comparisons). Furthermore, in the presence of conspecifics, no test rats from either control or disrupted groups emitted USVs in the 22 kHz range, which are typically associated with threatening stimuli (Fig. 2G,H). Instead, rats in both groups emitted 50 kHz vocalizations, which are typically associated with neutral or rewarding stimuli (Brudzynski, 2013). Together, these findings suggest that social disruption alters social resting preference without adversely affecting social recognition, object location memory, or social anxiety levels.

Oxytocin restored adult neurogenesis and social preference in socially disrupted rats

As expected from previous findings (Leuner et al., 2012), daily oxytocin treatment for 1 week after social disruption increased DCX⁺ cells in the ventral DG ($t_{(16)} = 2.37$, $p = 0.031$) but not the dorsal DG ($t_{(18)} = 0.56$, $p = 0.58$; Fig. 3B,C). Four weeks after the end of oxytocin treatment, oxytocin-treated rats did not show any difference in anxiety-like behavior in the elevated plus maze compared with vehicle-treated rats (proportional time in open arms, 0.21 ± 0.06 in disrupted + vehicle vs 0.17 ± 0.06 in disrupted + oxytocin; $t_{(14)} = 0.44$, $p = 0.66$). Likewise, rats preferred to investigate a novel rat over a familiar rat, regardless of whether they had been treated with oxytocin (two-way ANOVA of condition vs novelty; main effect of novelty, $F_{(1,13)} = 6.59$, $p = 0.028$). As previously observed for socially disrupted rats (Fig. 2B), rats from a disrupted hierarchy that received vehicle injections displayed a preference for resting near a familiar, as opposed to novel, rat (Fig. 3C). This preference was reversed in socially disrupted rats with oxytocin treatment (Fig. 3C). Rats that received oxytocin lost their preference for familiarity, changing strategy to rest near the novel, as opposed to the familiar, rat ($t_{(30)} = 3.13$, $p = 0.003$), similar to what was observed in naive rats without social disruption (compare with Fig. 2B). These differences were driven by both increased propensity to cross to the familiar side to rest after novel investigation and remaining on the familiar side to rest after investigating the familiar rat (Fig. 3D). In addition, rats that had restored neurogenesis after oxytocin treatment showed an increase in VTE-like behavior after investigating the novel stimulus rat that was comparable with controls (see Fig. 5), whereas no change was observed in VTE-like behavior after investigating the familiar stimulus rat (see Fig. 5; oxytocin treatment \times stimulus rat interaction, $F_{(1,12)} = 18.62$, $p = 0.001$). These findings suggest that oxytocin treatment after social disruption restores levels of adult neurogenesis, and, 1 month after oxytocin treatment, social resting preference behavior is restored to control values without changing social recognition or general anxiety-like behavior.

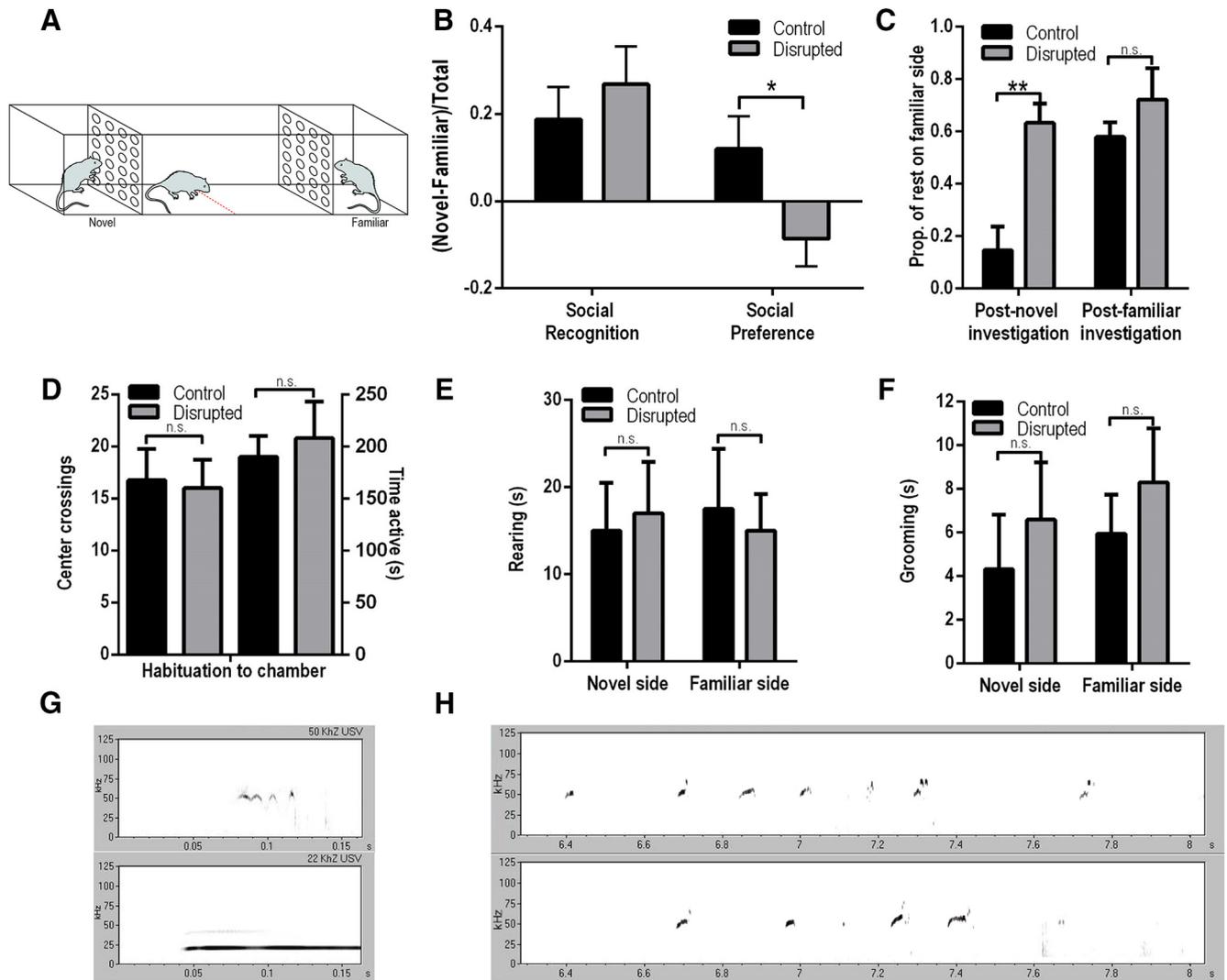


Figure 2. Social disruption has a lasting effect on social behavior. **A**, Schematic of social testing apparatus. **B**, Social disruption produces no deficits in the ability to recognize a familiar conspecific, because both control and disrupted groups investigate a novel rat more than a familiar rat. Whereas rats from standard group housing showed a preference for resting near a novel rat, rats from a disrupted hierarchy prefer to rest near a familiar rat. Values >0 indicate a preference for the novel rat; values <0 indicate a preference for the familiar rat. **C**, Differences in social preference are driven by behaviors after investigation of the novel rat: rats from a disrupted hierarchy prefer to cross the chamber to rest near the familiar rat, whereas both groups show similar resting behaviors after investigation of the familiar rat. **D**, Before the addition of stimulus rats, groups exhibited the same amount of activity, in terms of both center crossings and time spent mobile in the chamber. **E**, There were no group differences in side localization or duration of rearing behavior. **F**, There were no group differences in side localization or duration of grooming behavior. **G**, Examples of the two USV types that rats emit; 50 kHz USVs are primarily associated with neutral or positive valence, whereas 22 kHz USVs are associated with danger or negative valence. **H**, Sample USVs emitted by rats from the control group (top) and the disrupted group (bottom) after introduction of the stimulus animals. No rats emitted 22 kHz USVs during the social test. Bars indicate mean + SEM. * $p < 0.05$, ** $p < 0.01$. n.s., No significant difference.

Suppressed adult neurogenesis in socially naive GFAP-TK rats reversed social preference

GCV treatment was effective in reducing adult neurogenesis in GFAP-TK rats, because BrdU⁺ cells were lower in both the dorsal and ventral DG compared with in GFAP-TK rats treated with vehicle ($t_{(15)} = 2.56, p = 0.022$ for dorsal DG; $t_{(15)} = 3.18, p = 0.006$ for ventral DG; Fig. 4B). There was no effect of GCV treatment on the proportion of BrdU⁺ cells colabeled with NeuN in the DG (0.91 ± 0.04 for TK + vehicle vs 0.88 ± 0.01 for TK + GCV; $t_{(8)} = -0.77, p = 0.46$), indicating that the number of new neurons was decreased. Importantly, the decrease in new neurons was not complete with this treatment of GCV and instead quantitatively resembled the decrease we observed after social disruption in wild types (compare Figs. 4B, 1D). GCV treatment also decreased the number of BrdU-labeled cells in the SVZ from 3704 ± 240 to 2436 ± 180 ($t_{(6)} = -4.28, p = 0.008$). When tested

behaviorally 6 weeks after the last GCV treatment, no significant differences were observed between GCV- and vehicle-treated TK rats on object location (0.22 ± 0.19 for TK + vehicle vs 0.36 ± 0.31 for TK + GCV; $t_{(17)} = 1.21, p = 0.2$). There were also no effects on the proportion of time spent in the open arms of the elevated plus maze, indicating no difference in anxiety-like behavior (0.43 ± 0.03 for TK + vehicle vs 0.49 ± 0.01 for TK + GCV; $t_{(20)} = 1.46, p = 0.15$). In addition, social recognition did not differ between vehicle- and GCV-treated rats (two-way ANOVA of condition vs novelty; main effect of novelty, $F_{(1,15)} = 6.51, p = 0.03$). Like socially disrupted wild-type rats, socially naive GFAP-TK rats treated with GCV showed a preference for resting near a familiar rat, whereas socially naive GFAP-TK rats treated with vehicle showed a preference for resting near a novel rat ($t_{(18)} = 2.6, p = 0.02$; Fig. 4C). These differences were driven by both increased propensity of GCV-treated rats to cross to the familiar side to rest

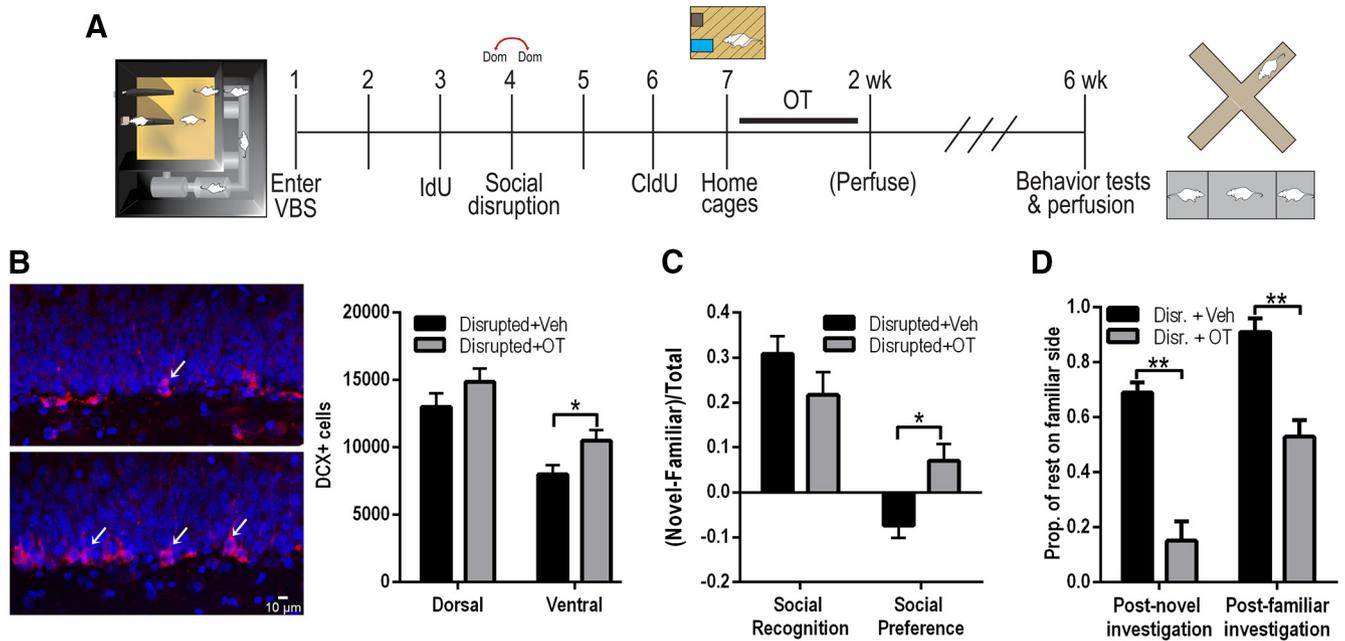


Figure 3. Oxytocin treatment after social disruption increases the number of immature neurons in the ventral DG and restores a resting preference for novelty. **A**, Timeline for hierarchy, neuronal birthing, oxytocin (OT) treatment, and behavioral testing. **B**, Left, Representative image of ventral DG in a rat from a disrupted hierarchy treated with vehicle (Veh; top) or 1 mg/kg OT (bottom) daily for 1 week after leaving the VBS, with DCX⁺ immature neurons indicated with arrows. Red, DCX; blue, Hoechst 33342. Right, Rats treated with OT after social disruption showed an increase in immature neurons in the ventral DG. **C**, Social recognition is not affected by OT administration after social disruption, whereas rats treated with OT prefer to rest near the novel rat. Values >0 indicate a preference for the novel rat; values <0 indicate a preference for the familiar rat. **D**, OT-treated rats are more likely to rest near the novel rat after investigation of either side. Bars indicate mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

after novel investigation and to remain on the familiar side to rest after investigating the familiar rat (Fig. 4D). In addition, GFAP-TK rats with lowered neurogenesis (after GCV treatment) showed fewer incidents of VTE-like behavior after they investigated the novel stimulus rat compared with rats with control values of neurogenesis (after no GCV treatment) and no such change in VTE-like behavior after they investigated the familiar stimulus rat (Fig. 5; GCV group \times stimulus rat interaction, $F_{(1,16)} = 13.79$, $p = 0.002$). No significant differences in baseline CORT levels were observed between GFAP-TK rats treated with GCV and wild-type controls (158.8 ± 39.8 ng/ml in WT, 206.0 ± 34.1 ng/ml in GFAP-TK; $t_{(9)} = 0.87$, $p = 0.41$).

Discussion

Our results show that disrupting an established dominance hierarchy changes the rate of adult neurogenesis in the hippocampus and that these changes may be related to lasting adaptations in social behavior. Social disruption suppressed the numbers of progenitor cells and new neurons for all members of a dominance group. At a later time when new neurons generated during social disruption had time to integrate into the circuitry, rats from a disrupted hierarchy exhibited a preference for resting near a familiar, rather than novel, rat, without notable impairments in social recognition, cognition, or anxiety regulation. Increasing adult neurogenesis with oxytocin administration after social disruption restored control-typical social preference behavior, and suppressing adult neurogenesis with transgenic manipulation produced a familiar preference in socially naive rats. These data suggest that social disruption may fine tune subsequent social behavior, at least in part, by reducing the number of new neurons in the DG.

The reduction in the number of new neurons with social disruption was observed regardless of dominance position and may

have occurred as a result of the stressful effects of persistent aggression (Czéh et al., 2002). However, somewhat paradoxically, rats from a disrupted VBS exhibit lower levels of plasma CORT than rats from stable hierarchies and control conditions. This may reflect hypocortisolism, a phenomenon wherein chronic stress downregulates the hypothalamic–pituitary–adrenal axis response (Yehuda et al., 1990; Rohleder et al., 2004). Although this can lead to psychopathology, hypocortisolism may also reflect an adaptive response to chronic stress (McEwen, 1998; Fries et al., 2005). The hippocampus is dense with OTRs, which may play a role in lowering CORT levels after stress (Gimpl and Fahrenholz, 2001). The increase in hippocampal OTR immunolabeling we observed with social disruption may be linked to lower CORT levels. Along these lines, proactive stress-coping styles can suppress basal CORT levels (Korte et al., 1996; Koolhaas et al., 2010); increased aggression after social disruption and suppressed anxiety-like behavior also suggest that our disrupted rats have developed a proactive phenotype (Veenema and Neumann, 2007). Our observations are consistent with data showing proactive coping (Garrett et al., 2015) and suppressed anxiety (Tsai et al., 2015) in adult mice with conditional suppression of immature neurons (but see Snyder et al., 2011; Deng and Gage, 2015).

We used oxytocin administration to stimulate neurogenesis after social disruption and found a reversal of the social behavior change. Together with our findings that reduced adult neurogenesis in transgenic socially naive rats mimics the social behavior change we observed with social disruption, these findings suggest that a similar adult neurogenesis mechanism influences social behavior in each case. However, we cannot rule out the possibility that similar changes in social behavior are occurring through different mechanisms in these conditions. Although oxytocin manipulation ended 4 weeks before behavioral testing, postdis-

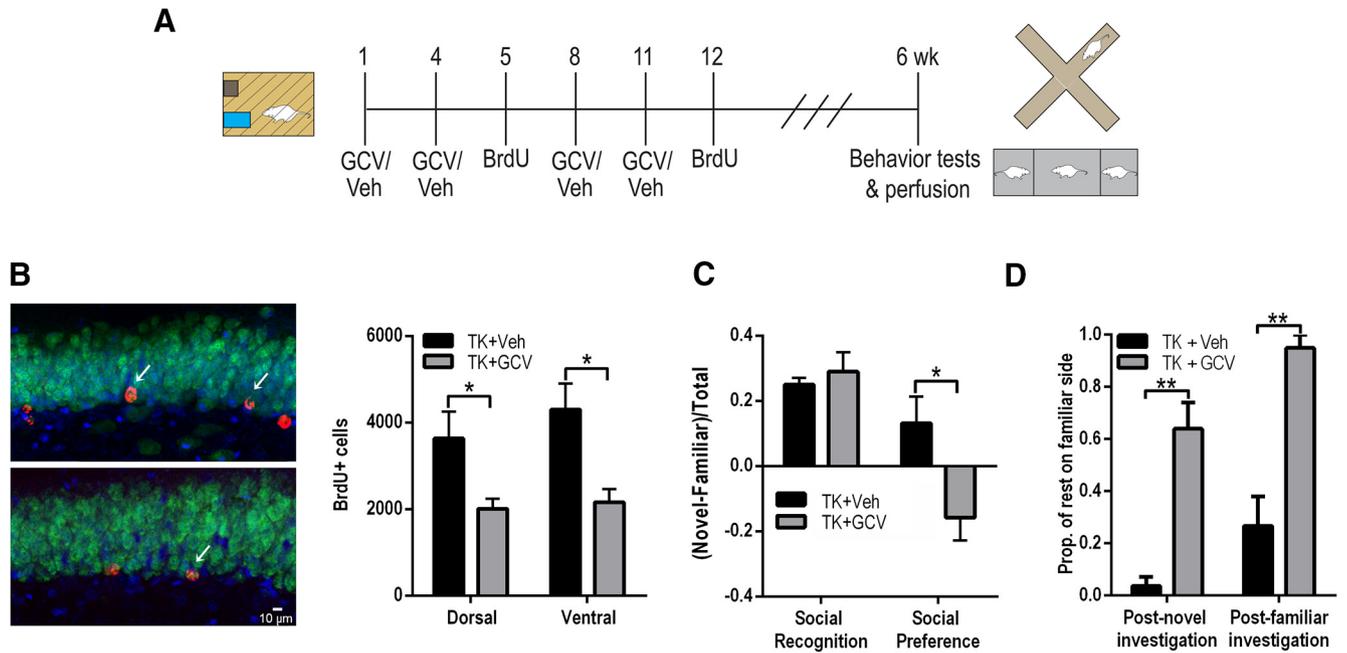


Figure 4. GFAP–TK rats treated with GCV show a suppression in adult neurogenesis in the DG and exhibit a resting preference for a familiar rat. *A*, Timeline for neuronal birthdating, drug treatment, and behavioral testing for GFAP–TK rats. *B*, Left, Representative image of ventral DG of GFAP–TK rat treated with vehicle (Veh; top) or GCV (bottom) for a 2 week period. Red, BrdU; green, NeuN; blue, Hoechst 33342. Right, GFAP–TK rats treated with GCV showed a suppression in BrdU⁺ neurons in the DG. *C*, GFAP–TK rats with a suppressed pool of 6-week-old neurons show no impairments to recognizing a novel rat but exhibit a resting preference for a familiar rat. Values >0 indicate a preference for the novel rat; values <0 indicate a preference for the familiar rat. *D*, GFAP–TK rats with a suppressed pool of 6-week-old neurons prefer to rest near the familiar rat after investigation of either side. Bars indicate mean + SEM. **p* < 0.05, ***p* < 0.01.

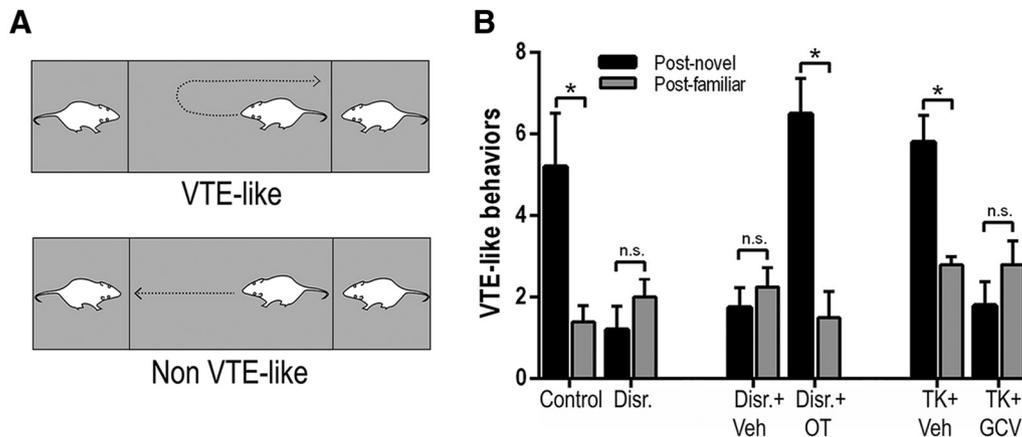


Figure 5. VTE behavior in the social preference test changes in groups with suppressed adult neurogenesis. *A*, Diagram showing VTE-like behavior defined by hesitating and turning back after initiating a movement across the social chamber, as well as a non-VTE-like (straight) trajectory. *B*, After investigation of the novel rat, disrupted VBS rats and GFAP–TK rats exhibited fewer instances of VTE-like behavior, resulting in fewer turns back to the novel side, than controls. Oxytocin treatment reversed the effects of social disruption on VTE-like behavior so that the values were similar to socially naive controls. No significant changes were observed in post-familiar VTE-like behavior in any group. Bars indicate mean post-investigation VTE-like behavior. Error bars indicate SEM. **p* < 0.05. Disr., Disruption; n.s., no significant difference; Veh, vehicle.

ruption oxytocin administration may still produce changes in social behavior by acting through other mechanisms (Witt et al., 1992; Lee et al., 2007; Lukas et al., 2011). Additional experiments will be necessary to determine whether oxytocin treatment would produce long-term changes in social behavior if adult neurogenesis levels were kept constant.

Changes in social preference behavior after social disruption could reflect one of the following possibilities: (1) an inability to distinguish novel from familiar conspecifics; (2) a decrease in the natural preference for novelty; or (3) anxiety that causes stressed rats to avoid novel rats. These possibilities seem unlikely, given that socially disrupted rats in our studies showed no impairment

in recognizing novel rats, they investigated novel more than familiar rats (and for durations of time that were indistinguishable from controls), and they demonstrated no obvious increase in general anxiety-like behavior. In fact, socially disrupted rats appeared to be less anxious than control rats on the elevated plus maze. The possibility that preference for a familiar, rather than a novel, rat reflects stress-induced social anxiety also seems unlikely because, if this were the case, we would also expect to see less investigation of the novel rat during initial exposure, which was not observed.

A more detailed analysis of behavior in the social apparatus suggested that disrupted rats may have diminished ability to op-

timize decision making. This possibility is consistent with literature suggesting that the hippocampus participates in resolving conflicts between different goals, particularly when they have similar value, for use in decision-making (Johnson et al., 2007; Bannerman et al., 2012; Doll et al., 2015; Palombo et al., 2015). In our study, after bouts of social investigation, rats either sat without moving to a different location or turned away from the investigated rat and began to traverse the testing apparatus. When control rats turned away after investigating the novel rat, rather than continuing to the other side of the testing apparatus, they often hesitated and turned back toward the novel rat and returned to rest there. In maze training, movements back and forth at difficult choice points have been described as VTE behavior and are considered as a reflection of working memory-dependent deliberative decision-making (Tolman, 1938; Hu et al., 1997; Redish, 2016). This behavior is diminished by lesions to the hippocampus (Hu and Amsel, 1995; Bett et al., 2012). The turning back behavior in our study may also reflect the outcome of a VTE process, in which the rat predicts likely outcomes of different actions. In controls, this VTE-like behavior was displayed to a much lesser extent after investigating the familiar rat, suggesting that its occurrence may reflect a greater difficulty in choosing to leave the more rewarding novel rat. When rats with reduced levels of adult neurogenesis (as a result of social disruption or transgenic inhibition) turned away from the investigated novel rat, they engaged in significantly less turning back to rest by the novel rat; instead, they more frequently continued to traverse the testing apparatus and rested by the familiar rat. This pattern of behavior raises the possibility that new neurons may be involved in choosing between moving back toward the rat that was investigated less recently or returning to the rat that is generally more novel. Because the investigation rate was higher on the novel side for both groups, uninterrupted traversals led the experimental group to rest more frequently near the familiar rat. Some evidence suggests that new neurons can be activated in response to novelty (Sandoval et al., 2011; Clark et al., 2012; Marrone et al., 2012; Meconi et al., 2015) and are important in tasks requiring flexible decision-making (Burghardt et al., 2012; Swan et al., 2014), which has been associated with VTE (Redish, 2016). It may be relevant to note that aging, which is associated with reduced adult neurogenesis (Kuhn et al., 1996; Cameron and McKay, 1999; Heine et al., 2004; McDonald and Wojtowicz, 2005), also results in diminished VTE maze behavior (Breton et al., 2015), suggesting that this may be a mechanism that extends beyond social behavior.

Studies have shown that stress-induced changes in behavior may be adaptive in many circumstances (Lagace et al., 2010; Petrik et al., 2012; Blanchard et al., 2013). In this light, a decrease in novelty preference may signal a “survival-mode” optimization of hippocampal function through stress-induced changes in adult neurogenesis (Glasper et al., 2012). It is also possible that control rats may have an exaggerated focus on novelty given that their housing conditions lack much of the stimulation that is typically present when living in the wild. The possibility that individual housing interacts with the relevance of novelty is also an additional important consideration. Although we housed rats individually after the social disruption to avoid interference of additional social experience, we cannot rule out the possibility that some of our effects resulted from an interaction between social disruption and social isolation. However, because our GFAP-TK rats did not experience social disruption or social isolation at any point in the study, this interpretation seems unlikely.

These findings demonstrate experience-induced change in

behavior after experience-induced suppression of adult neurogenesis. Together, these data suggest that suppression in neurogenesis in the DG during adulthood may produce lasting changes in social behavior without causing obvious impairments in hippocampal functions, such as object location memory and anxiety regulation. The lack of effect on object location memory and anxiety-like behavior with reduced adult neurogenesis is consistent with data from naive mice lacking immature neurons (Garrett et al., 2015; Seo et al., 2015). Our results challenge the more established pattern of results wherein negative experiences tend to suppress adult neurogenesis and impair cognitive performance, as well as increase anxiety-like behavior (Leuner and Gould, 2010). Social disruption indeed produces a suppression in adult neurogenesis, but this does not appear to globally impair hippocampal function and instead is associated with a social preference phenotype that may be adaptive rather than pathological. Future work will be necessary to determine the mechanisms underlying social disruption-induced suppression of adult neurogenesis, as well as the long-term consequences of altered social preference behavior.

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