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Social instability, hippocampal plasticity and resilience

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SOCIAL INSTABILITY, HIPPOCAMPAL PLASTICITY AND RESILIENCE

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A DISSERTATION PRESENTED TO THE FACULTY OF PRINCETON UNIVERSITY IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

RECOMMENDED FOR ACCEPTANCE BY THE PRINCETON NEUROSCIENCE INSTITUTE

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Abstract

Experience-dependent changes in the production and function of new neurons may serve as a means to fine-tune the hippocampus to the predicted environment. Here we describe a series of studies done to examine the effects of naturalistic social disruption on structural plasticity and behavior in adult rats. Our results provide novel mechanistic evidence that social disruption shapes behavior in an adaptive way by reducing adult neurogenesis in the hippocampus.

First, we developed an ethologically relevant model of social instability by forming stable dominance hierarchies in a visible burrow system and then switching dominant rats between hierarchies, a manipulation that increased aggression. Next, we examined the brains of rats subjected to social disruption and compared them to those living in a stable hierarchy and to those living in standard laboratory cages. We found that social disruption dramatically reduced the number of neural stem cells, as well as the number of new neurons, in the hippocampus regardless of social position. Instead of producing the predicted deleterious consequences of social stress, like impaired cognition and increased anxiety, we found no change in cognition and reduced anxiety-like behavior in rats from a disrupted hierarchy. We also found that socially disrupted rats did not show impairments in the ability to detect novelty in a social setting, but they did show a preference for familiar, as opposed to novel, conspecifics. Taken together, this behavioral profile suggests a potential "stress inoculation" or resilience, as opposed to the emergence of pathology. Next, we used two approaches to investigate whether these behavioral effects were causally linked to the reduction in adult neurogenesis in the hippocampus. We used oxytocin to stimulate adult neurogenesis just after social disruption occurred and found that this manipulation prevented the preference for familiar conspecifics. Then we used transgenic conditional neurogenesis knockout (GFAP-TK) rats to reduce adult neurogenesis in rats without

social disruption experience and found that these rats preferred familiar rats, similar to what we observed in social disrupted wild-types. The mechanisms by which new neurons influence social preference remain to be determined.

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As I prepare to leave Princeton, I reflect on being a member of only the second entering class of graduate students ever to matriculate in the Princeton Neuroscience Institute. It has been

an honor to participate in the beginnings of this incredible program and I look forward to the next steps of our journey towards understanding.

All the choir of heaven and furniture of earth - in a word, all those bodies which compose the frame of the world - have not any subsistence without a mind.

- *George Berkeley, 1710*

Chapter 1: Introduction

Shaping the adult brain through experience

More than one hundred years ago, Santiago Ramon y Cajal wrote, "Any man could, if he were so inclined, be the sculptor of his own brain," (Ramon y Cajal, 1999). This statement was amazingly prescient given how little was known at the time about the plasticity inherent to the adult brain. After a virtual explosion in research focused on neural plasticity in recent years, it has become increasingly clear that environmental influences, including specific experiences, have a profound effect on adult brain structure and function (Leuner & Gould, 2010). Much attention has focused on the hippocampus in this regard, both because of the role it plays in important functions, like certain types of learning and memory, and its impressive degree of structural plasticity. The hippocampus displays a high rate of adult neurogenesis, specifically within the dentate gyrus where new granule cells are produced throughout life (Altman & Das, 1965; Gould et al., 1992; Snyder & Cameron, 2012) (Fig. 1). Adult neurogenesis in the dentate gyrus has been demonstrated in a wide range of mammalian species, including humans, (Gould et al., 1999; Snyder & Cameron, 2012; Spalding et al., 2013) and the process of adult neurogenesis has been shown to be strongly influenced by the environment (Leuner & Gould, 2010; Schoenfeld & Gould, 2012). Evidence suggests that experience-dependent changes in adult neurogenesis are associated with major changes in hippocampal function, both for better and for worse. The relationship between experience and functional neurogenesis has been studied extensively, but questions remain with respect to how refinements in neuron numbers integrate with other types of structural change to produce large scale behavioral modifications.

Identification of experiences that alter adult neurogenesis in the hippocampus may provide clues into the functional significance of new neurons. Findings from the overall literature on experiential modulation of adult neurogenesis seem to converge on a basic model: Aversive

experiences, such as social defeat or predator odor exposure, tend to decrease the production of new neurons, whereas more rewarding experiences, such as physical activity or mating, tend to increase the production of new neurons (Leuner & Gould, 2010; Schoenfeld & Gould, 2012). Taken together with results demonstrating a role for adult neurogenesis in hippocampal function, this model suggests that new neurons may serve as a substrate by which experience can shape the hippocampus so that it is fine-tuned to the environment. To reach this conclusion, however, a clear understanding of how experience regulates adult neurogenesis, as well as the known behaviors linked to new neurons, is necessary. A vast literature has explored the influence of various experiences on adult neurogenesis (Fig. 2); of these, the majority has focused on two types of experience, namely, stress and physical activity, each producing generally opposite effects on the number of new neurons.

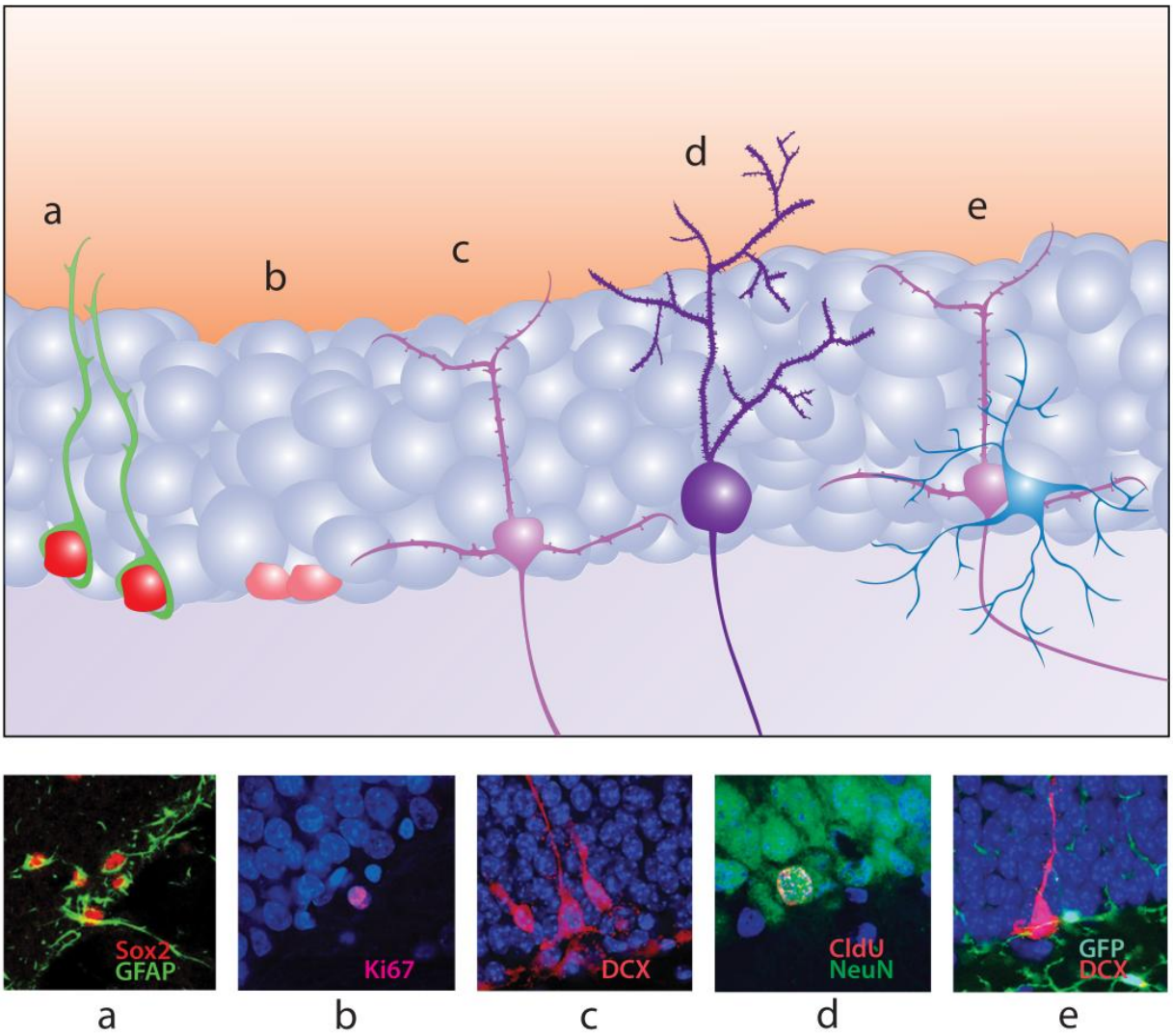


Figure 1. Stages of adult neurogenesis in the dentate gyrus. Much research has explored the stages involved in the production of new neurons in the adult dentate gyrus. Top panel: Schematic diagram of adult neurogenesis in the granule cell layer of the dentate gyrus showing relevant cell types at different stages in the process. First, new neurons originate from precursor cells that have glial-like characteristics (A). These cells proliferate (B) and produce daughter cells that differentiate into immature neurons (C). Some of these immature neurons will survive and differentiate into mature neurons (D) while others will degenerate and their debris will be cleared by microglial phagocytosis (E). Bottom panel: confocal microscopic images of different cell types in the process of adult neurogenesis: A) Glia-like precursor cells; Sox2 (red), GFAP (green). B) Proliferating precursor cells; Ki67 (red), Hoechst 33342 (blue). C) Immature granule cells labeled with doublecortin (DCX) (red), Hoechst 33342 (blue). D) Mature granule cells labeled with NeuN (green), CldU, a thymidine analog incorporated by proliferating cells (red), Hoechst 33342 (blue). E) Immature neuron near microglial cell, labeled with green fluorescent protein (GFP). DCX (red), GFP (green), Hoechst 33342 (blue).

Stress effects on adult neurogenesis and hippocampal function

Stressful experiences, such as restraint, social defeat, predator odor exposure, inescapable foot shock, sleep deprivation, as well as a regimen of different stressors combined, have been shown to decrease the number of new neurons in the dentate gyrus (Gould et al., 1992; Tanapat et al., 1999; Snyder et al., 2009; Lucassen et al., 2010; Leuner & Gould, 2010; Schoenfeld & Gould, 2012; Kreisel et al., 2014; Fuchs & Flugge, 2014). Although a few studies have reported enhanced adult neurogenesis with certain stress paradigms (Parihar et al,. 2011; McEwen, 2012), the stressors in these cases were predictable, mild and may have added enriching complexity to an environment, an experience known to enhance adult neurogenesis. When the stressor is unpredictable and relatively intense, however, the effect on adult neurogenesis is typically negative. The literature on this topic is large, with studies indicating multiple stages in the adult neurogenesis process during which stress has an inhibitory effect (Fig. 2).

First, stress has been shown to suppress the proliferation of progenitor cells that produce new neurons. This effect has been demonstrated in a wide range of mammalian species, including rats, mice, marmosets and macaques (Gould et al., 1997; Schoenfeld & Gould, 2012; McEwen, 2012; Fuchs & Flugge, 2014). Not only does stress result in a net decrease in the number of proliferating cells, but some evidence suggests that stress shifts neural stem cells in the hippocampus away from the production of neurons and toward the generation of oligodendrocytes (Chetty et al., 2014). It is not clear how a combined decrease in new neurons and increase in new oligodendrocytes affects hippocampal function, but the latter effect may alter myelination of axons coursing through the dentate gyrus. It remains unknown, however, whether new oligodendrocytes would contribute to myelination of axons of new neurons, which are generally known to be unmyelinated (Zhao et al., 2012). It is additionally unknown whether

the effects of stress on new neurons and oligodendrocytes would follow similar or different time courses. Second, stress has been shown delay neuronal differentiation, or the maturation of a cell into a specific type of neuron (McEwen 2012; Schoenfeld & Gould, 2012; Fuchs & Flugge, 2014). Third, stress reduces the survival of neurons produced prior to the stressful experience. While the mechanism that underlies this effect remains unknown, stress is known to reduce expression of brain derived neurotrophic factor (BdNF), a molecule known to enhance cell survival (McEwen, 2012). A reduction in the survival of new neurons is also likely to engage another population of non-neuronal cells, the microglia, which are known to engulf new neurons in the dentate gyrus. Stress has been shown to have a profound effect on the number of microglia, as well as their state of damage-induced reaction (Kreisel et al., 2014). Although stress-induced reaction of microglia may be important for cleaning up the debris left behind by dead new neurons, it remains possible that microglia play an active role in reducing new neuron survival, either by releasing cytokines with neurotoxic effects, or by actively engulfing new neurons prior to their definitive demise.

Evidence suggests that elevated levels of glucocorticoids are, at least in part, responsible for the effects of stress on new neuron production (Gould et al., 1992; Schoenfeld & Gould, 2012). Exogenous glucocorticoid administration has similar effects as stress on cell proliferation, neuronal differentiation and cell survival, as well as on oligodendrocyte production and microglial reaction (Kreisel et al., 2014; Chetty et al., 2014). Furthermore, some evidence suggests that stress effects on new neuron production can be prevented by interfering with stress-induced increases in glucocorticoid levels (Cameron & McKay, 1999; Schoenfeld & Gould, 2012).

Stress-induced suppression of adult neurogenesis has been associated with impaired performance on cognitive tasks that require the hippocampus, such as spatial navigation learning and object memory (Conrad et al., 2009; Schoenfeld & Gould, 2012; Trofimiuk & Braszko, 2013; Said Mohammadi et al., 2014). It should be noted that stress has been shown to facilitate certain types of learning but these effects are typically observed within a shorter time frame than what would be expected for the involvement of new neurons (Bangasser & Shors, 2010). Stressful experiences have also been shown to increase anxiety-like behaviors that are associated with the hippocampus, including those measured with the elevated plus maze, open field and novelty suppressed feeding tasks (McEwen, 2012; Schoenfeld & Gould, 2012) (Fig. 2).

The extent to which these behavioral changes are directly linked to reduced numbers of new neurons remains unknown (Schoenfeld & Gould 2012), but data from studies examining other types of experiences that have the opposite effect on adult neurogenesis and behaviors related to the hippocampus provide further indirect evidence in support of a causal relationship (Fig. 2).

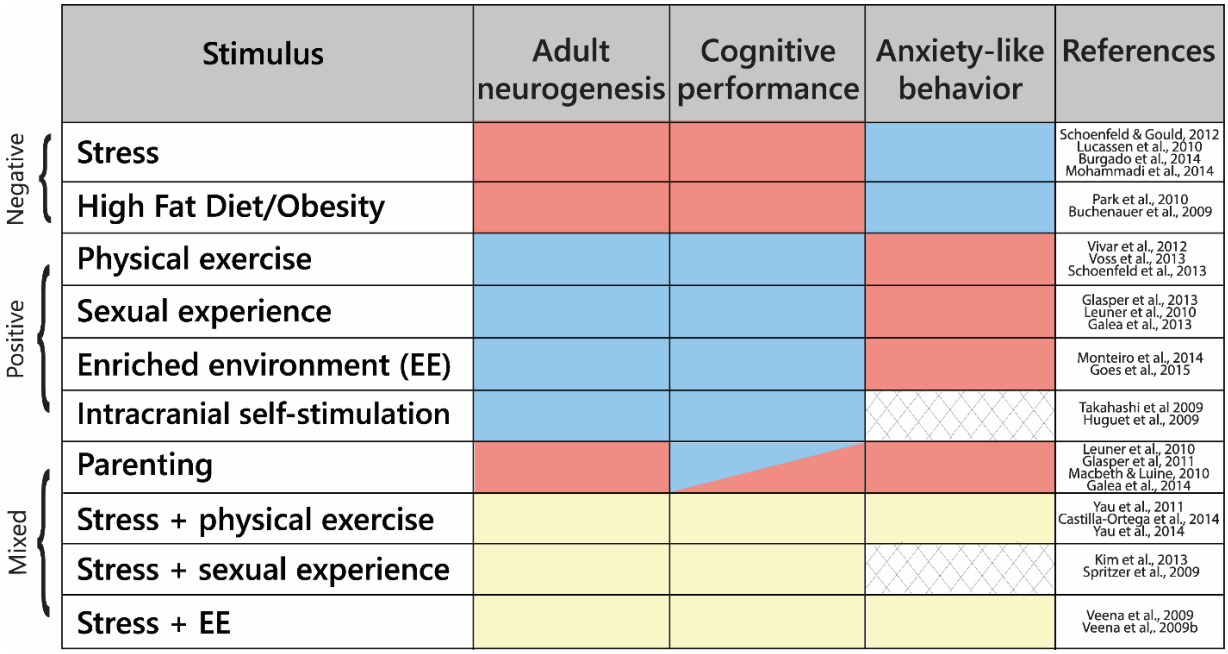


Figure 2. Experience- dependent effects on adult neurogenesis in the hippocampus, as well as on cognition and anxiety-like behavior. This figure summarizes findings from studies examining the effects of experiences with different emotional valences on adult neurogenesis and behaviors associated with the hippocampus. Red boxes indicate negative experiences or effects; blue boxes indicate positive experiences or effects; yellow boxes indicate no change; white boxes indicate no data. Negatively-valenced experiences (red) are associated with reduced adult neurogenesis (red) and impaired cognition (red) and but increased anxiety-like behavior (blue). Positively-valenced experiences (blue) are associated with increased adult neurogenesis (blue), improved cognition (blue) but decreased anxiety-like behavior (red). Mixed valence experiences are shown as red/blue and may lead to mixed outcomes such as inhibited adult neurogenesis (red) and decreased anxiety-like behavior (red) or no effect (yellow), presumably due to the additive nature of experiences of opposite valence. These null outcomes seem unlikely to reflect that complex experiences have no effects on the brain and behavior. Sample citations are provided for each effect.

Physical exercise effects on adult neurogenesis and hippocampal function

A large body of literature has explored the effects of physical exercise on adult neurogenesis in the hippocampus and found effects for both voluntary running on a wheel and forced running on a treadmill (van Praag et al., 1999; Schoenfeld & Gould, 2012; Vivar et al., 2013). In general, the effects of physical exercise are opposite to those of stress. Whereas stress suppresses adult neurogenesis, physical exercise generally promotes it. There are, however,

exceptions to this observation. These include running in isolation and intense bouts of physically taxing exercise, both of which have a suppressive effect on adult neurogenesis, most likely due to stress (Schoenfeld & Gould, 2012; Vivar et al., 2013). Similar to the stress literature, physical exercise seems to have a global effect in the promotion of adult neurogenesis, targeting different stages, including cell proliferation, neuronal differentiation and cell survival (Schoenfeld & Gould, 2012; Vivar et al., 2013; Voss et al., 2013; Farioli-Vecchioli et al., 2014). Running has been shown to enhance cell proliferation by shortening the cell cycle of progenitor cells (Farioli-Vecchioli et al., 2014; Brandt et al., 2010). It also enhances neuronal differentiation by hastening the expression of mature neuronal characteristics, and it prevents the death of new neurons (Snyder et al., 2009; Yau et al., 2011; Glasper et al., 2010; Kohman et al., 2012). In contrast to the effects of stress on adult neurogenesis, running produces fewer microglia in the hippocampus (Kohman et al., 2012; Gebara et al., 2013), an effect that may contribute to enhanced neuronal survival.

Physical exercise has profound effects on the biochemical milieu of the hippocampus, as it is associated with increases in levels of neurotransmitters and growth factors (Vivar et al., 2013). This is perhaps not surprising, given that physical exercise greatly improves blood flow, promotes angiogenesis, as well as the delivery of oxygen and nutrients to the brain (Vivar et al., 2013; Voss et al., 2013). Many of the molecules that are increased by running, including serotonin (Klempin et al., 2013), insulin-like growth factor (Glasper et al., 2010) and BdNF (Marlatt et al., 2012), have been causally linked to running-enhanced neurogenesis. Here again, these effects appear to be the converse of stress effects, which generally result in decreased levels of these molecules in the hippocampus (McEwen, 2012; Wilson et al., 2014; Basta-Kaim et al., 2014). One family of signaling molecules that does not follow this consistent pattern is

glucocorticoids. Physical exercise is known to activate the hypothalamic pituitary adrenal axis, leading to increased levels of circulating glucocorticoids (Schoenfeld & Gould, 2012) and, paradoxically, increased neuronal growth in the hippocampus. Taken together, these findings suggest that other factors associated with running override the actions of elevated glucocorticoid levels.

One aspect of the running experience that may engage growth-promoting signaling molecules is that of reward. For laboratory rodents, voluntary running appears to be a universally rewarding experience, as evidenced by its ability to produce a place preference as well as physiological signs of withdrawal (Kanarek et al., 2009; Greenwood et al., 2011). The rodent experience of running stands in contrast to the human experience of running, where the degree to which physical exercise is perceived as rewarding varies greatly across individuals. This raises the possibility that the universal appeal of running in rodents may be an artifact of living in captivity. However, a recent study showing that wild rodents voluntarily run in a wheel suggests that such behavior may be naturally rewarding, as opposed to a side effect of living in captivity (Meijer et al., 2014). While the distinction between the universality of the rewarding aspects of running between rodents and humans raises questions about the translational validity of these findings, studies of voluntary running in rodents may be generalizable to other experiences which individual humans are strongly motivated to pursue.

Consistent with the possibility that the rewarding aspect of running is responsible for neuronal growth, dopamine, a neurotransmitter central to reward learning circuitry, has a positive influence on adult neurogenesis (Takamura et al., 2014). Additional experiences that are known to be rewarding, such as enriched environment living, sexual experience, intracranial self-stimulation and cocaine administration, are known to promote adult neurogenesis (Kempermann

et al., 1998; Glasper et al., 2010; Schoenfeld & Gould, 2012) (Fig. 2). An exception to this relationship can be observed, however, with parenting behavior, which has a strong hedonic component but is associated with reduced, as opposed to enhanced, adult neurogenesis (Leuner et al., 2010; Glasper et al., 2011). Since parenting is a complicated experience comprising stress, enrichment and profound hormonal changes (Leuner et al., 2010), it is perhaps not surprising that its neurogenesis profile differs from those of more controlled rewarding experiences.

The behavioral changes observed in animals exposed to rewarding experiences that enhance neurogenesis also appears to be the opposite of what has been observed with stress (Fig. 2). That is, improved performance on cognitive tasks involving the hippocampus has been reported following running (Schoenfeld & Gould, 2012; Vivar et al., 2013), sexual experience (Glasper et al., 2013; Glasper et al., 2012) and intracranial self-administration (Schoenfeld & Gould, 2012; Aldavert-Vera et al., 2013). Some of these experiences are also associated with reduced anxiety-like behavior (Glasper et al., 2011; Schoenfeld & Gould 2012; Schoenfeld et al., 2013). The behavioral profile for parenting is, like the adult neurogenesis findings, mixed. Some studies report improved cognitive performance while others do not (Leuner et al., 2010; Tronel et al., 2010; Franssen et al., 2011; Kim et al., 2012).

A rich literature indicates that learning itself alters the number and development of new neurons in the dentate gyrus (Gould et al., 1999; Tronel et al., 2010; Koehl & Abrous, 2011; Curlik & Shors, 2013). These studies are more difficult to assign to aversive or rewarding categories because learning paradigms vary and most involve a combination of both. It is also very difficult to assess whether training on a specific learning task alters learning on an altogether different task. Although proponents of mental training would suggest that it does, the literature on this subject is controversial and highly mixed (Curlik & Shors, 2013).

Functional significance of new neurons

Adult-generated cells can be activated by a wide range of experiences, including exposure to a stressor, running, exposure to a novel environment and recall of previously learned information (Clark et al., 2012; Schoenfeld et al., 2013; Chow et al., 2013). Most of the experiences that activate new neurons are also known to alter the number of new neurons, as well as to change behaviors linked to the hippocampus. Taken together, these findings strongly suggest that new neurons participate in experience-dependent changes in hippocampal function.

These findings raise the question of whether new neurons generated under specific experiential conditions are somehow geared toward the recognition of that particular experience. In other words, do these new cells represent a neuroanatomical mechanism for experience-specific learning or do they provide a more naïve pool of new neurons that are positioned to enable environmental adaptation in the future? In this regard, the literature presents a mixed set of findings. On the one hand, evidence suggests that new neurons generated during a period of spatial navigation learning are more likely to be activated by repeat exposure to relevant spatial cues (Kee et al., 2007; Chow et al., 2013). On the other hand, evidence suggests that new neurons generated during running respond to a broad set of experiences relevant to the hippocampus, including stress, novelty, spatial cues, and running itself (Clark et al., 2012; Schoenfeld et al., 2013). The proportion of new cells that respond to these cues does not differ based on whether the cells were generated during running or sedentary living, indicating that the cells are not specifically tuned to the running experience.

Whether new neurons are tagged by the experiences surrounding their generation remains an open question, but recent evidence suggests that environmental conditions present during the time when new neurons are formed can influence later activation of those neurons. Specifically,

new neurons generated during running are less likely to be activated by stress than new neurons generated during sedentary living (Schoenfeld et al., 2013) (Fig. 3). This lack of stress-induced activation seems attributable to increases in activity of GABA, a major inhibitory neurotransmitter, in the dentate gyrus itself (Schoenfeld et al., 2013), suggesting that plasticity in other neuronal populations that communicate with new neurons is important for understanding how hippocampal circuitry is changed by experience. It should be noted, however, that studies of experiential modulation of activity in new neurons have mostly used immediate early gene expression as a proxy for neuronal activity. Immediate early genes are rapidly increased in neurons when they fire action potentials but this method is an indirect and very general way of assessing neuronal activation that has poor temporal resolution and provides no information about how often a neuron is activated or its pattern of activation. Thus, it remains entirely plausible that new neurons respond to general experiences with one activity pattern and to specific experiences related to the conditions under which they were produced with another, distinctly different, activity pattern.

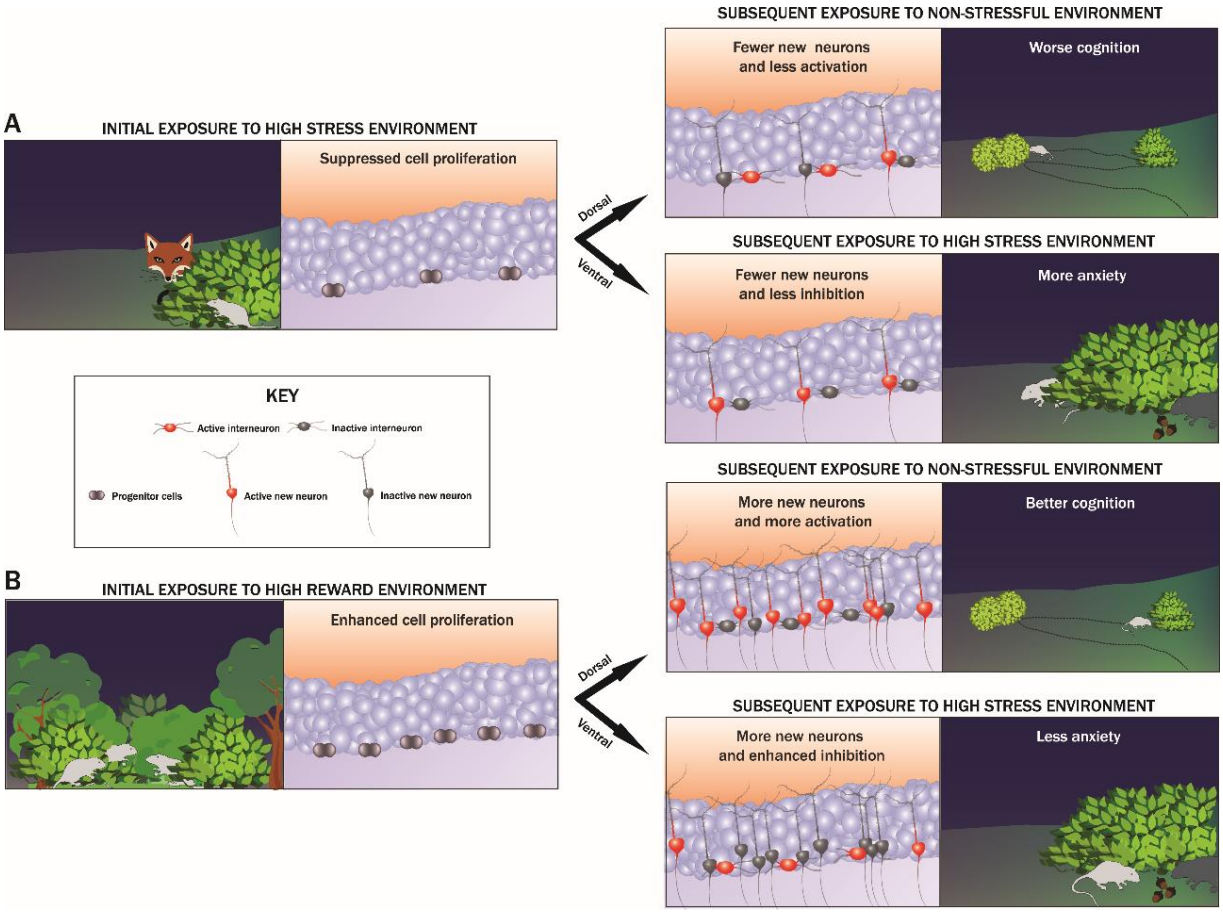


Figure 3. Experience modifies the dentate gyrus to prepare it for adaptive responses This schematic depicts dentate gyrus-shaping scenarios rodents might encounter in the wild and their likely outcomes in terms of neuronal activation and behavior. ***A,*** Initial exposure to a high stress environment, such as one with predatory risk (in this scenario, a fox), suppresses adult neurogenesis throughout the dentate gyrus. At a subsequent time, under no stress conditions, the dorsal dentate gyrus would have fewer new neurons available to participate in spatial learning tasks, thus producing worse cognition (in this scenario, inability to remember food foraging locations). Despite having fewer new neurons as well, the ventral dentate gyrus responds to stress with activation of such neurons, thus producing more anxiety (in this scenario, failure to forage for food in the presence of a novel conspecific). ***B,*** Initial exposure to a high reward environment, such as one providing ample opportunities for enrichment (in this scenario, prosocial interactions with conspecifics and a complex landscape to explore), enhances adult neurogenesis throughout the dentate gyrus. At a subsequent time, under no stress conditions, the dorsal dentate gyrus would have more new neurons available to participate in spatial learning tasks, thus producing better cognition (in this scenario, ability to remember food foraging locations). Despite having more new neurons as well, the ventral dentate gyrus responds to stress with inactivation of such neurons, through the engagement of inhibitory neurons, thus producing less anxiety (in this scenario, willingness to forage for food in the presence of a novel conspecific).

A large body of literature has focused on understanding the function of new neurons, mostly without reference to experience-dependent change. Efforts to understand the behavioral impact of experience-dependent changes in adult neurogenesis need to consider both the function of the hippocampus itself and the electrophysiological properties of granule cells. Using a wide variety of technical approaches, the majority of which involve deleting new neurons, studies have addressed the behavioral consequences of new neurons directly. These studies have implicated new neurons in virtually all of the known functions of the hippocampus, including its learning and memory capabilities (Feng et al., 2001; Snyder et al., 2005; Snyder et al., 2009; Wu & Hen 2014), as well as in anxiety (Wu & Hen, 2014) and stress regulation (Snyder et al., 2011).

Within the hippocampus, new neurons appear to be exclusively added to the dentate gyrus. Therefore, any discussion of the function of new neurons in the hippocampus must consider the function of this specific sub-region. The dentate gyrus has been implicated in pattern separation, a computational process by which similar inputs are converted to more distinct outputs (Yassa & Stark, 2011). Behavioral pattern separation is used to describe discrimination between highly similar stimuli, usually in different contexts. New neurons have been implicated in pattern separation; cognitive behaviors reflecting this process can be altered by environmental stimuli that increase or decrease the number of new neurons in the dentate gyrus (Aimone et al., 2010; Creer et al., 2010; Sahay et al., 2011; Kheirbek et al., 2012; Niibori et al., 2013; Bekinschtein et al., 2014). It is important to note that a recent detailed study using several methods to reduce the number of new neurons in the dentate gyrus failed to demonstrate any substantial deficit in learning or behavioral pattern separation (Groves et al., 2013). Additional studies will be needed to determine whether subtle differences in testing paradigms or baseline stress exposure underlie these discrepancies.

The involvement of new neurons in pattern separation may rely on their unusual electrophysiological properties, including enhanced synaptic plasticity and a lower threshold for long term potentiation than mature neurons (Wang et al., 2000; Snyder et al., 2001; Aimone et al., 2010; Chancey et al., 2013). The addition of new functional units, with their temporally dynamic profiles of functionality and excitability, and their differential responses to theta rhythms, may additionally separate information according to temporal features (Aimone et al., 2010). Such modulation of hippocampal circuitry may rely on the addition of highly excitable functional units to an otherwise silent network. In other words, new neurons may be given starring roles in the function of the dentate gyrus, at least until they transition into a state of maturity associated with their strong inhibition, when they become more like supporting characters.

Although only one type of neuron, i.e., the granule cell, is created through the process of adult neurogenesis in the dentate gyrus, regional differences in hippocampal function are likely to determine their role in behavior. The hippocampus can be divided into dorsal and ventral regions in rodents, or anterior and posterior regions in humans (Fanselow & Dong, 2010). These regions serve different functions; in rodents, the dorsal aspect has been associated with encoding spatial information, whereas the ventral aspect has been associated with anxiety and stress regulation (Fanselow & Dong, 2010; Yassa & Stark, 2011; Hawley et al., 2012; Wu & Hen, 2014). Likewise, new neurons in the dorsal and ventral dentate gyrus may have distinct functions in terms of their behavioral output (Lacar et al., 2014). For example, selective deletion of new neurons in the dorsal, but not ventral, hippocampus impairs context fear conditioning. By contrast, selective deletion of new neurons in the ventral, but not dorsal, hippocampus prevents some of the anxiolytic actions of antidepressants (Wu & Hen, 2014). This dissociation, however,

seems to disappear under more challenging situations, such as in the presence of altered temporal cues or elevated levels of glucocorticoids, both circumstances in which new neurons throughout the dentate gyrus become capable of supporting tasks normally relegated just to a subdivision (Wu & Hen, 2014). That is, under more taxing circumstances, regional specificity gives way to overall new neuron recruitment.

With regard to the regional specificity of new neuron function, it is relevant to note that experiential regulation of adult neurogenesis sometimes targets one subdivision more than others. For example, both stress and sexual experience seem to alter adult neurogenesis in the ventral dentate gyrus more profoundly than in the dorsal dentate gyrus (Hawley et al., 2012; Schoenfeld & Gould, 2012; Tanti et al., 2012). Evidence indicates that new neurons in the ventral dentate gyrus are not only less numerous, but they also undergo differentiation at a slower rate than those in the dorsal dentate gyrus (Snyder et al., 2012). This suggests that different time windows of sensitivity may exist for experience-dependent effects on new neurons of one subdivision of the dentate gyrus versus another.

Despite being linked to different behavioral outputs, presumably because of different target sites of the dorsal and ventral hippocampus, new neurons throughout the dentate gyrus may use similar computational mechanisms. In this regard, it is relevant to note that pattern separation has been linked not only to the cognitive functions of the hippocampus, but also to its anxiety and stress regulation functions, in that these likely require the ability to distinguish familiar from novel, as well as safe from potentially threatening, circumstances (Kheirbek et al., 2012). In cases where the differences between known versus unknown or between safe versus unsafe situations are small, pattern separation may be critical for making accurate distinctions and setting a level of anxious behavior that is appropriate for the environment. It will be

illuminating to ascertain whether or not a common computational mechanism exists for new neurons throughout the entire hippocampus. Regardless, the observation that certain experiences, such as stress, that regulate adult neurogenesis in turn activate new neurons and are directly related to new neuron functions, raises the possibility of an adaptive feedback loop that may shape the hippocampus to facilitate both cognitive and anxiety-like behavioral responses most appropriate to the environment.

Adaptive significance of new neurons

The relationship between experiential regulation of new neuron number and the involvement of new neurons in functions related to that experience has been considered in previously described theories (Gould et al., 1999; Glasper et al., 2012; Kempermann et al., 2012; Kheirbek et al., 2012; Konefal et al., 2013). In particular, we have proposed that stress-induced decreases in new neuron formation may improve chances of survival by increasing anxiety and inhibiting exploration – impaired cognition may be a necessary trade off for this benefit (Glasper et al., 2012). It is also worth considering the possibility that although stress may impair learning, it may also have the advantage of diminishing forgetting, a phenomenon positively linked to the number of new neurons (Akers et al., 2014). This may produce a maladaptive outcome under certain circumstances, as in the case of PTSD, where memories that should diminish over time do not (Deppermann et al., 2014). Conversely, reward-induced increases in new neuron number associated with diminished anxiety may facilitate exploration and learning, leading to greater reproductive success (Glasper et al., 2012). The ability of the hippocampus to continually produce a new pool of neurons, the numbers and organization of which are responsive to environmental conditions, strongly suggests that new neurons are a substrate for sculpting the brain to produce behaviors that are adaptive for the organism. Because the past is often the best

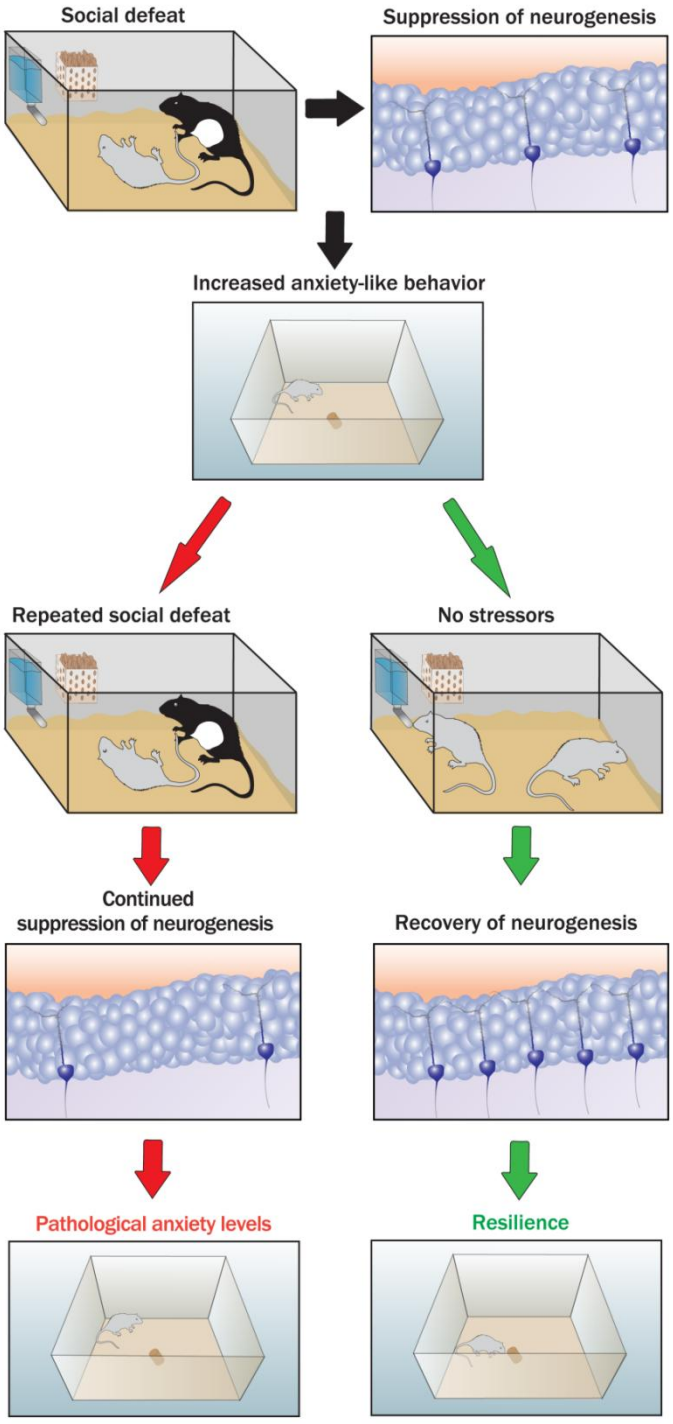
predictor of the future, a stress-modeled brain may facilitate adaptive responses to life in a stressful environment, whereas a reward-modeled brain may do the same but for life in a low stress, high reward environment. Thus, experience may optimize hippocampal circuitry through effects on adult neurogenesis by either priming a mode of avoiding punishment or seeking rewards. The influence of experience on hippocampal circuitry in adulthood may be a diminished version of what occurs during development when the base structure of the hippocampus is being formed. Studies have shown that early life experience can shape the developing hippocampus and also produce lasting changes in the rate of adult neurogenesis (Mirescu et al., 2004; Oomen et al., 2010; Korosi et al., 2012; Naninck et al., 2014).

Although stress-induced decreases in adult neurogenesis may have adaptive benefits, they remain potentially problematic for adaptive flexibility; if an organism encounters stressful circumstances, is it doomed to avoid situations that could be rewarding even when circumstances change? Such a behavioral bias could hardly be considered optimal, except in the short-term. Recent studies suggest that a more flexible adaptation scheme may be engaged in certain challenging situations. As discussed above, the region-specific involvement of new neurons in cognitive versus anxiety-related functions appears to dissipate in favor of overall hippocampal involvement when conditions are especially challenging (Wu & Hen, 2014). In the laboratory, stress-induced inhibition of adult neurogenesis has been shown to be reparable in that new neuron production typically returns to normal once the stressful period ends (Fig. 4). By contrast, repeated stress produces continued reduction in adult neurogenesis and ultimately, the emergence of depressive-like symptoms, including anhedonia, along with heightened anxiety (Joëls et al., 2007) (Fig. 4). Such a scenario could represent processes that are engaged under pathological conditions and may be somewhat akin to what humans experience when exposed to

repeated traumatic stress. Thus, when aversive experiences far outnumber rewarding ones in both quantity and intensity, the system may reach a breaking point and produce a maladaptive outcome. It seems clear, however, that the typical response to stress is one of resilience in which adjustments in hippocampal structure and function to better suit the environment do not interfere with the ability to take advantage of rewarding opportunities that may arise in the future.

Consideration of the adaptive significance of adult neurogenesis raises questions about conditions that animals typically experience in the wild. Meaningful tests of new neuron function may require different experimental approaches including a focus on more naturalistic environments that provide cues necessary for naturally occurring levels of brain plasticity.

Figure 4. Stress-induced modifications in hippocampal circuitry lead to resilience or pathology depending on the duration of stress exposure.



This schematic shows the brain and behavioral response to short-term versus persistent stressor exposure. Short-term exposure to laboratory stressors, such as social defeat, suppresses adult neurogenesis. These changes are associated with increased anxiety-like behavior, such as avoidance of novel food in a novelty suppressed feeding task. If conditions change and no further stressors are introduced, adult neurogenesis levels should recover and increased anxiety subsides. If stressful conditions continue, adult neurogenesis will be further suppressed and anxiety-like behavior will persist. This outcome is particularly disadvantageous if conditions become safe, as persistent anxiety could prevent acquisition of rewards that would help restore adult neurogenesis levels.

New neurons in the real world

There is little doubt that adult neurogenesis and new neurons themselves are sensitive to experience. However, laboratory studies from which this conclusion has been drawn typically investigate adult neurogenesis in animals that are essentially devoid of most experiences that were present during mammalian brain evolution. This potential confound raises the question of whether studying an experience-dependent process under controlled laboratory conditions provides information with any relevance to understanding the brains of animals living in their natural habitats. Animals in the wild have complex, challenging, and stimulating lives, whereas those living in standard laboratory settings have limited, static lives lacking stress or stimulation. Furthermore, animals in the wild have varied experiences that can serve to accentuate individual differences in brain and behavior, whereas those living in standard laboratory settings have similar experiences that undoubtedly narrow the range of brain and behavioral measures (Fig. 5). Research exploring the effects of stress on adult neurogenesis would suggest that laboratory control animals, with their relatively stress-free existences, have more new neurons than their wild living counterparts. Conversely, research exploring the effects of physical exercise on adult neurogenesis would suggest that laboratory control animals, with their sedentary lifestyles, have many fewer new neurons than their wild living counterparts.

Clearly, living in the wild involves exposure to multiple experiences known to alter adult neurogenesis. Some studies have sought to address this issue by combining exposure to positive and negative regulators of adult neurogenesis, e.g., stress and running or stress and sexual experience, and have found the effects seem to cancel one another out (Fig. 2). That is, running or sexual experience reverses stress-induced suppression of neurogenesis such that there is no net change compared with neurogenesis in control animals (Yau et al., 2011; Kim et al., 2013;

Castilla-Ortega et al., 2014). Perhaps in a similar vein, wild caught rodents exposed to a running wheel do not exhibit increased adult neurogenesis (Hauser et al., 2009), possibly due to the combined actions of stress and physical exercise on this population. Does the lack of change in overall new neuron number in laboratory animals exposed to two experiences with seemingly opposite emotional valences suggest that adult neurogenesis, and indeed, the hippocampus itself, remains unchanged by these experiences? Although it is possible that experiential regulation of adult neurogenesis only matters for hippocampal function when the emotional valence of an organism's overall experience is highly biased in one direction or the other, as opposed to mixed, this seems unlikely especially given the fact that the number of new neurons is only one aspect of adult neurogenesis that can be modulated by experience. To better understand how new neurons refine brain function in animals living in the wild, studies examining adult neurogenesis under conditions that are as similar to the wild as possible would be useful.

Enriched environment studies in the laboratory are important steps in the right direction toward understanding adult neurogenesis in the brains of animals living natural lives in that they increase complexity and provide opportunities for cognitive, social and physical challenges relative to laboratory cage controls. These studies, however, typically work to minimize competition among conspecifics and almost always involve animals of one sex, a highly unnatural social arrangement for rodents. The use of a visible burrow system can provide a hybrid between the wild and the enriched environment living in the laboratory (Blanchard et al., 1995; Leedy et al., 2013). The visible burrow system, a structure consisting of tubes, chambers, and an open field, has allowed researchers to re-create the conditions that allow for the production of dominance hierarchies that rats naturally form in the wild, replicating also the stressors and rewards that accompany this social lifestyle. Social experiences, including

dominance hierarchy formation, engage a variety of cognitive systems, including those that underlie learning and memory and decision-making. As such, these scenarios provide a more realistic model for studying functional modifications in neuron production and recruitment.

Previous studies have shown that when mixed sex rats live in a dominance hierarchy in a visible burrow system, individual differences emerge in the number of new neurons in the dentate gyrus. Dominant male rats produce more new neurons than subordinate male rats (Kozorovitskiy & Gould, 2004). A similar relationship between dominance status and adult neurogenesis has been demonstrated for baboons (Wu et al., 2014). For Sprague Dawley rats at least, this outcome is not directly related to stress, as glucocorticoid levels do not differ between dominants and subordinates (Kozorovitskiy & Gould, 2004). These findings are illuminating because they involve adult neurogenesis in a more realistic setting that combines previously isolated stimuli such as social interaction, physical exercise, environmental enrichment, sexual experience and social stress. These findings also suggest that animals living in similar circumstances may have different levels of adult neurogenesis depending on individual differences in the degree to which they engage in certain behaviors.

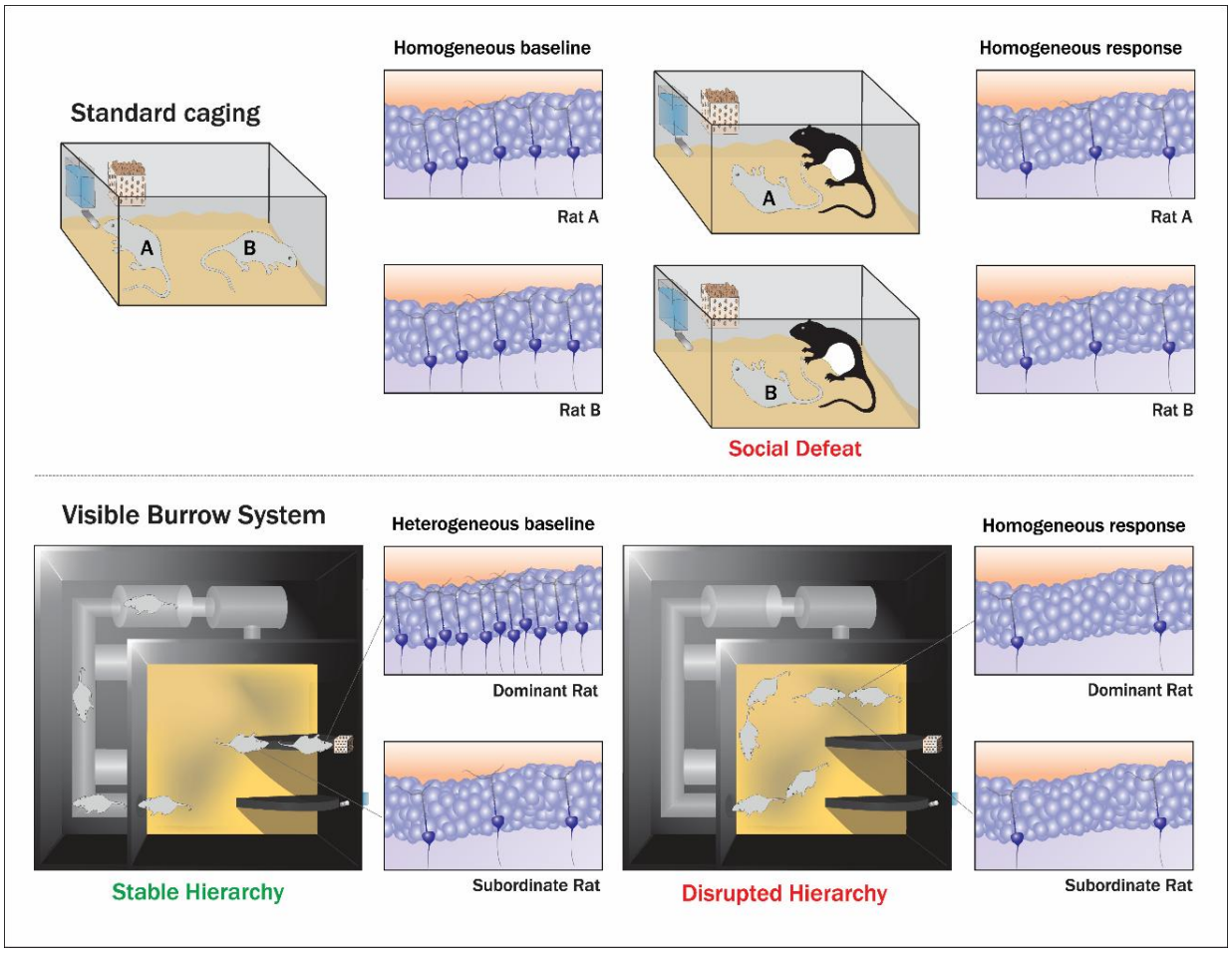


Figure 5: The benefits of a more naturalistic setting for studying adult neurogenesis. Schematic diagrams showing the effect of different types of laboratory housing (standard caging or visible burrow system) on new neurons in the dentate gyrus at baseline and after social defeat stress. Standard housing produces a homogeneous adult neurogenesis baseline and a homogeneous stress-induced adult neurogenesis suppression (both rat A and B have the same response). This uniformity may be an artifact of living in artificial laboratory housing. Visible burrow system living in a stable social hierarchy produces a heterogeneous baseline with more neurogenesis in the dominant compared to subordinate rats. This community profile of adult neurogenesis and its related behaviors is more likely to reflect that of animals living in the wild. This wide range of individual differences can be eliminated by an extreme intervention imposed on the entire group. Social hierarchy disruption, which produces increased aggression, results in a substantial decline in new neuron number in all animals, regardless of their social position.

While use of the visible burrow system allows researchers to observe adult neurogenesis in a naturalistic setting, it also allows for ethologically relevant perturbations of this setting. In the wild, dominance hierarchies can be destabilized if a dominant animal dies or leaves the community. This scenario can be replicated in the lab by interchanging dominants from stable hierarchies, a manipulation that disrupts the social order and produces renewed fighting. Regardless of social position before the disruption, animals living in a disrupted social hierarchy show decreased survival of adult-born neurons (Opendak et al., 2013). Thus, social disruption trumps the individual differences in new neuron number that emerge as a result of stable hierarchy living. As mentioned above, conditions under which neurons are generated may "program" these neurons to be activated by and functionally involved in tasks of the same type. The extent to which neurons generated during a disrupted social experience are activated by and involved in stress regulation and social behavior remains unknown. Nonetheless, the visible burrow system presents an unusual opportunity to explore the effects of complex experiences on adult neurogenesis and hippocampal function in a meaningful social setting that more closely mimics wild habitats than most laboratory conditions. The utility of this approach, however, requires the use of appropriate species and strains of animals to minimize physical aggression and easily distinguish among animals so that their behavior can be analyzed accurately. For example, male mice may be too aggressive for such experiments and pigmented rats may be too difficult to distinguish from one another (albino rats can be tagged with dye in easily distinguishable patterns). Furthermore, experiments designed to increase individual differences require larger *n* sizes to obtain enough power for meaningful statistical analyses. In effect, such environmental living conditions make studies on experimental animals prone to some of the

same practical limitations as those of studies on humans, which may not be optimal for many experimental questions.

Studies have shown that the human hippocampus exhibits a substantial amount of adult neurogenesis (Spalding et al., 2013) and that neuronal growth in the human hippocampus may be stunted by stress (Wang et al., 2010) and stimulated by physical activity (Voss et al., 2013). While such results in humans are severely limited by technical and ethical constraints, they are nonetheless encouraging indicators that laboratory research on adult neurogenesis in experimental animals may be on the right track toward understanding how experience alters plasticity in the human brain. Taking findings from laboratory animals to the next level by exploring complex social interactions in settings that maximize individual variability, a hallmark of the human experience, is likely to be especially illuminating.

Towards this end, my experimental work was designed to explore the regulation of adult neurogenesis by naturalistic experiences and to determine what mechanisms underlie experience-dependent changes in behavior. Using a visible burrow system, I modeled the ethologically relevant scenario of de-stabilization of an established dominance hierarchy of rats. Changes in adult brain plasticity and behavior were then assessed with the aim of providing a mechanistic description of the role of adult born neurons in behavioral adaptations following disruptive social experience.

Chapter 2:

Social disruption produces lasting behavioral adaptations by reducing adult neurogenesis.

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, as 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 employ various coping strategies even in response to chronic instability (Khoshaba et al., 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 & 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 resilience and its cellular mechanisms (McCormick et al., 2012; 2015; Branchi et al., 2013).

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 as non-dominant 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 rat, a rogue rat joining the group, or a change in habitat conditions (Barnett et al., 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). The VBS has been used to examine the effects of living in a stable hierarchy on the brain and the results have demonstrated that dominant rats exhibit a higher rate of adult neurogenesis in the

hippocampus compared to subordinate rats (Kozorovitskiy & Gould, 2004) but no previous studies have explored the effects of hierarchy destabilization on the brain and behavior. Since 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 & Gould 2015), it is likely that such changes could have behavioral consequences. To investigate this further, we explored the effects of living in stable and unstable hierarchies on new neuron production in the hippocampus, as well as on behaviors that have been associated with this brain region. We found expected differences between dominants and subordinates in adult neurogenesis that 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 a disrupted hierarchy displayed reduced anxiety-like behavior and no cognitive deficits. These rats also show a marked change in social behavior. While control rats and rats from stable hierarchies prefer novel over familiar rats, rats from disrupted hierarchies exhibit the converse preference of familiar over novel rats. Next, we used two approaches to investigate whether these behavioral effects were causally linked to the reduction in neurogenesis. We used the neuropeptide oxytocin to stimulate adult neurogenesis (Leuner et al., 2012) immediately after social disruption and 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 knockout (GFAP-TK) rats to reduce adult neurogenesis in rats (Snyder et al., 2011) without social disruption experience and found that these rats had control levels of anxiety-like behavior but social preference for familiar conspecifics. Our results show that social disruption produces a resilient phenotype, with reduced anxiety,

preserved cognition and a change in social preference, and that suppressed neurogenesis in the hippocampus is likely to play a role in this latter outcome.

Methods

Animals

All animal procedures were approved by the Princeton University IACUC and conformed to the guidelines of the National Research Council. Adult male Sprague-Dawley rats (Taconic Farms, NY) were housed on a 12h light/dark reverse light cycle with lights on 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 visible burrow system without risks of injurious fighting. For neurogenesis knockout experiments in rats not subjected to VBS living, adult male Long Evans GFAP-TK transgenic rats generated at the NIMH transgenic core were used (Snyder et al., 2011). Controls for studies using neurogenesis knockout rats were GFAP-TK rats not treated with drug that activates the transgene (valganciclovir).

Animal housing

Sprague-Dawley rats were housed in groups of 4 males and 2 females in a modified version of the VBS (Kozorovitskiy & Gould, 2004) (Fig.1*B*). 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”x 36”), 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 hr 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 days, each colony of 4 males was videotaped for 10 hr 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 & Gould, 2004). In each cohort, a dominant rat was selected based on the quantity of offensive and defensive behavior. 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 & Gould 2004).

For each male, the number of defensive acts was subtracted from the number of offensive acts over the entire 300 minute sampling period, yielding one score. The rat with the highest score was designated as the dominant, and the other 3 males in the colony were considered subordinate. On day 4, the dominant rats were switched between 2 VBS communities. Following 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 minutes as described above, and each rat's aggressive and defensive actions were tallied to determine post-switch changes in aggression and hierarchy dynamics. On day 7, rats were returned to individual home cages until perfusion. Individual housing was used post-

VBS to constrain social experience to the VBS. For comparison purposes with this study, additional cohorts of rats lived in either standard laboratory cages (control) or in non-disrupted hierarchies (stable). The rats living in standard cages were housed in pairs for 3 days 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 days and then were moved to individual housing, as described above. Rats living in stable hierarchies remained in their initial cohorts for 6 days before being returned to individual housing.

Rats from all groups (disrupted VBS, stable VBS, cage control) were perfused at either a 2 week or a 6 week time point. The first time point was used to examine effects on immature neuron production while 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 (Song et al., 2005).

Drug treatments and perfusions

Rats received equimolar injections of thymidine analogs iododeoxyuridine (IdU, 57.5 mg/kg i.p.; MP Biomedicals, cat. No. 100357) on day 3 and chlorodeoxyuridine (CldU, 42.5 mg/kg i.p.; Sigma Aldrich C6891) on day 6. Selective BrdU antibody sensitivities for IdU versus CldU allow for distinct assays of cell proliferation before and after our manipulation (Vega & Peterson 2005; Leuner et al., 2009).

Separate groups of rats living in either stable or disrupted hierarchies were treated with the neuropeptide oxytocin (1 mg/kg; Bachem Americas Inc.) for 7 days after return to individual caging at a dose shown to increase adult neurogenesis in the hippocampus (Leuner et al., 2012).

Rats were either perfused at 2 weeks to assess the effects of oxytocin treatment on immature neurons or remained in individual caging until behavioral testing at a 6 week time point to allow for adult-born neurons to affect performance on hippocampus-dependent tasks.

For GFAP-thymidine kinase experiments, beginning at 8 weeks of age, TK rats were treated with 4.0 mg valganciclovir (Valcyte, Roche) per half-gram peanut butter ball treat. Previous studies have shown that this is an effective and stress-free way to deliver this drug (Snyder et al., 2011). Each rat was fed the peanut butter treat twice weekly for 2 weeks, and on the day following the second drug treatment, rats received injections of BrdU (200mg/kg i.p.; Roche) and were perfused after a 6 week time point was over. Control TK rats received the peanut butter treats without the valganciclovir.

Hormone assays

Additional cohorts of rats were used to examine the effects of social disruption on basal levels of corticosterone and testosterone. These rats lived in either cage control, stable VBS, or disrupted VBS conditions and received sham injections on day 3 and trunk blood was collected on day 6 at lights on. After the blood was centrifuged, plasma was collected and circulating corticosterone and testosterone (in nanograms per decaliter) were measured using a radioimmunoassay (Coat-A-Count; Siemens Healthcare Diagnostics, Malvern, PA). Assay sensitivity was 98.5%.

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 employed because they are known to involve the hippocampus and appear to be affected by changes in adult neurogenesis (Dupret et al., 2008; Mak & Weiss, 2010; Schoenfeld et al., 2013). Rats were tested

on these 3 measures over 2 days, with the order of testing counterbalanced. Rats were perfused the day following the last test. Each testing day, rats were habituated to the testing room for at least 30 minutes prior to 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”x17.5”x17.5”) with two identical objects (plastic, each approx. 36 $in.^3$) for 3 minutes (Barker & Warburton, 2011). Rats were removed for 5 minutes 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 minutes. 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 non-moved object is considered to be evidence for object location memory.

Anxiety testing.

To assess anxiety-like behavior, the elevated plus maze was used (Lister, 1987; Walf & Frye, 2007; Schoenfeld et al., 2013; 2014). Rats were placed on an elevated plus-shaped track (44”x44”x20”). Two of the arms had high walls (13”) while the other two arms and central intersection were open. All arms were 20” in length, dimly lit by reflected light. Exploratory behavior was measured for 5 minutes 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 & 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”x10”x12”. Removable Plexiglas dividers perforated with 1 cm. diameter holes were placed on each side of the apparatus to create two chambers that were 10”x10”x10”. These walls allowed the test rat to see, smell, and hear stimulus rats while preventing the possibility of physical interaction. Prior to testing, test rats were placed in a standard cage with a novel rat for 5 minutes, as this time is sufficient to produce social recognition 30 minutes later (Thor & Holloway 1982; Popik et al., 1992). 25 minutes after the end of the 5 minute exposure, test rats were placed in the social recognition apparatus for a 5 minute 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 & Sandi, 2012). After a 10 minute 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.

Histology

At 2 or 6 weeks, rats were anesthetized with Euthasol and perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer. After 48 hr post-fixation, coronal sections (40 μm) were cut throughout the entire rostrocaudal extent of the dentate gyrus on a vibratome into a bath of 0.1 M phosphate buffered saline (PBS), pH 7.5. Brains were analyzed for the following: IdU, to assess the survival of new neurons generated before social disruption; CldU, to assess the proliferation and survival of new neurons generated after social disruption; BrdU, to assess the number of new neurons generated in GFAP-TK rats fed valcanciclovir or vehicle; DCX, to label immature neurons; GFAP, to determine changes in glial cells; Sox2, to determine changes in pluripotent progenitors and Sox2/GFAP co-labeling to assess changes in radial glia-like precursors. Because receptors for oxytocin and vasopressin are expressed in the hippocampus and both neuropeptides are involved in social behavior, stress responses and neurogenesis (Albeck et al., 1997; Alonso et al., 2004), we examined OTR and V1aR expression in the hippocampus and OTR/Ki67+ and V1aR/Ki67+ co-labeling, to assess the expression patterns of oxytocin receptors on proliferating neuronal precursors. All immunolabeling was performed on sections from groups to be compared simultaneously to minimize variability.

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 twelfth half-section through the dentate gyrus with 100x oil objective on an Olympus (Tokyo, Japan) BX-60 microscope. The numbers were tallied for the dentate gyrus, GCL–SGZ, and multiplied by 24 to obtain a stereological estimate for the brain region.

Double labeling with immunofluorescence for IdU/CldU and the neuronal marker doublecortin (DCX) or for BrdU and the neuronal marker neuronal nuclei (NeuN) was carried out 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 and HeNe 543). For examining endogenous markers of immature neurons and progenitor cells, the tissue was incubated in primaries against DCX, Sox2, Ki67 and GFAP according to previously published protocols (Suh et al., 2007; Lugert et al., 2010).

For labeling OTR and V1aR receptors, free floating tissue was incubated with rabbit anti-OTR (1:150, Alpha Diagnostics, Israel, cat. no. OTR-11A), rabbit anti-V1aR (1:150, Alpha Diagnostics, Israel; cat. no. AVP1A11-A). For cell density measurements, tissue was counterstained using Hoechst 33342 (1:1000, Molecular Probes, Eugene OR; cat no. H-3570,) and volume measurements of the dentate gyrus were taken using StereoInvestigator software (MBF Bioscience). Total DCX+, Sox2+ and GFAP+ (radial and horizontal morphologies) were counted on every twelfth half-section through the dentate gyrus with 100 oil objective on an Olympus (Tokyo, Japan) BX-60 microscope. The raw counts for each section were divided by the volume of the DG to obtain a density measurement. 50 Sox2+ cells in the DG (25 dorsal, 25 ventral) per brain were scanned and analyzed for GFAP+ co-labeling using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; Argon 458/488 and HeNe 543). 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-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).

Oxytocin receptor and vasopressin receptor expression was measured for mean optical intensity using 1 µm thick optical sections of the dentate gyrus and CA3 regions. For these analyses, the dentate gyrus and CA3 within a single hemisection was imaged in its entirety, with 10 images per brain region obtained for each brain. Five representative 1µm optical slices from the dorsal and ventral dentate gyrus and CA3 regions were scanned and measured for optical intensity from each brain using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; Argon 458/488 and HeNe 543). Intensity was calculated using LSM 510 software. Regions of interest (ROI) were manually defined to include the dorsal granule cell layer, ventral granule cell layer, dorsal pyramidal cell layer of the CA3 and ventral pyramidal cell layer of the CA3. The fornix was used for a measure of background staining as this region had no OTR 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 (Singer et al., 2011). 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 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 dentate gyrus within a hemisection (25 dorsal, 25 ventral) . Using LSM 510 software, each Ki67+ cell body was traced and the brightness of OTR or V1aR of pixels was determined within the tracing. This was assessed with respect 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, one or two-way ANOVA followed by Bonferroni post-hoc comparisons were used.

Results

Social disruption results in increased aggression and dominance hierarchy destabilization

Throughout the 6 day period of living in the VBS, the overall level of aggression changed significantly ($F_{2,14=}$12.97, p=0.001) (Fig 1*C*). Between days 1-3, the level of aggression decreased for both groups (stable and disrupted) (p=0.005). In stable cohorts, aggression did not change from days 3-6 (p=.821) but in disrupted cohorts, after the dominant rats were switched between two hierarchies, the level of aggression increased significantly from 3-6 days (p=.001). Post-disruption aggression levels were also significantly higher than day 1 aggression levels (p=.0001) (Fig 2*A*). 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 factors phase & dominance score, $F_{1,38}$=10.294, p=0.003) (Fig. 2*B*). 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 naïve rats placed in the VBS for the first time, as well as to a reorganization of the social hierarchy and loss of position by previously dominant rats.

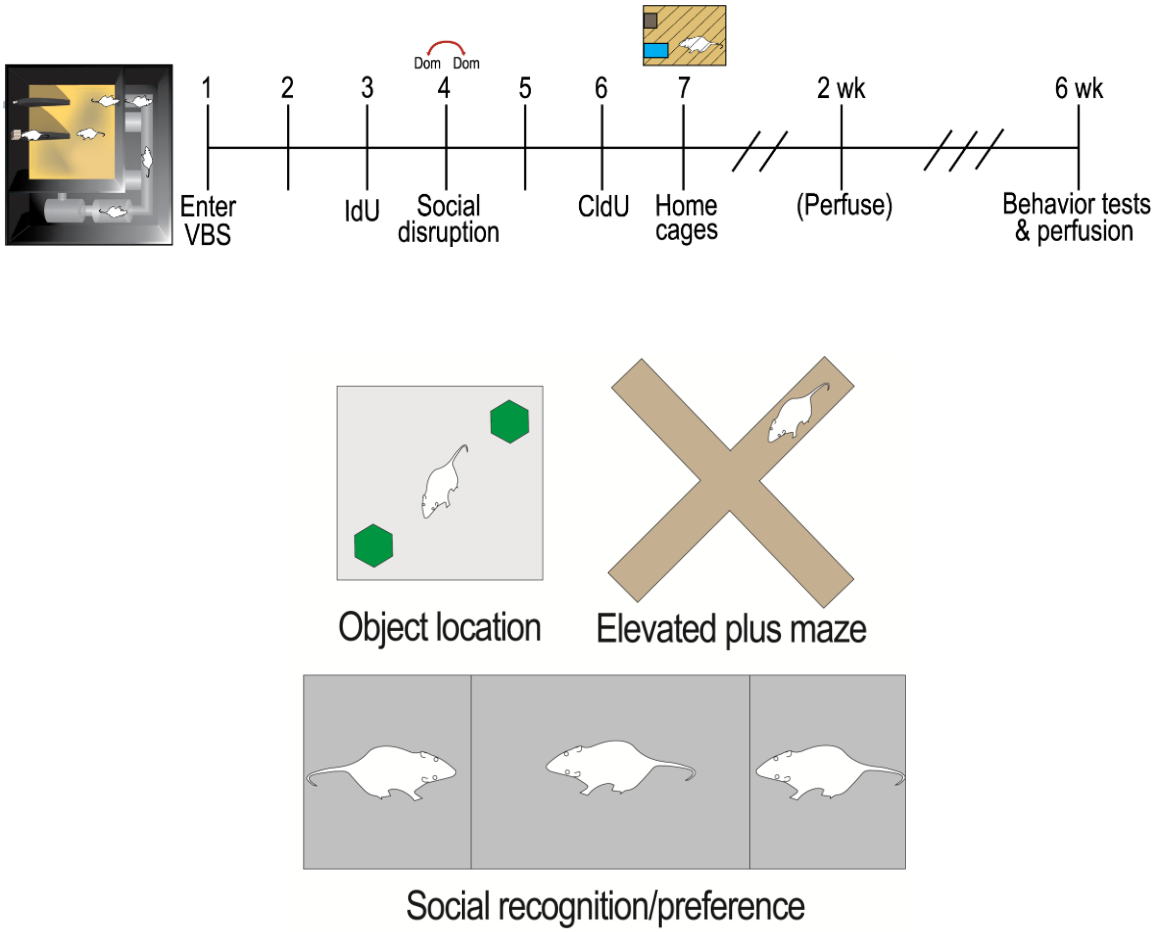


Figure 1. ***Top,*** Timeline for hierarchy, neuronal birth-dating, drug treatment and behavioral testing in rats living in a dominance hierarchy. ***Bottom,*** Schematics of behavioral assays used at the 6 week time point.

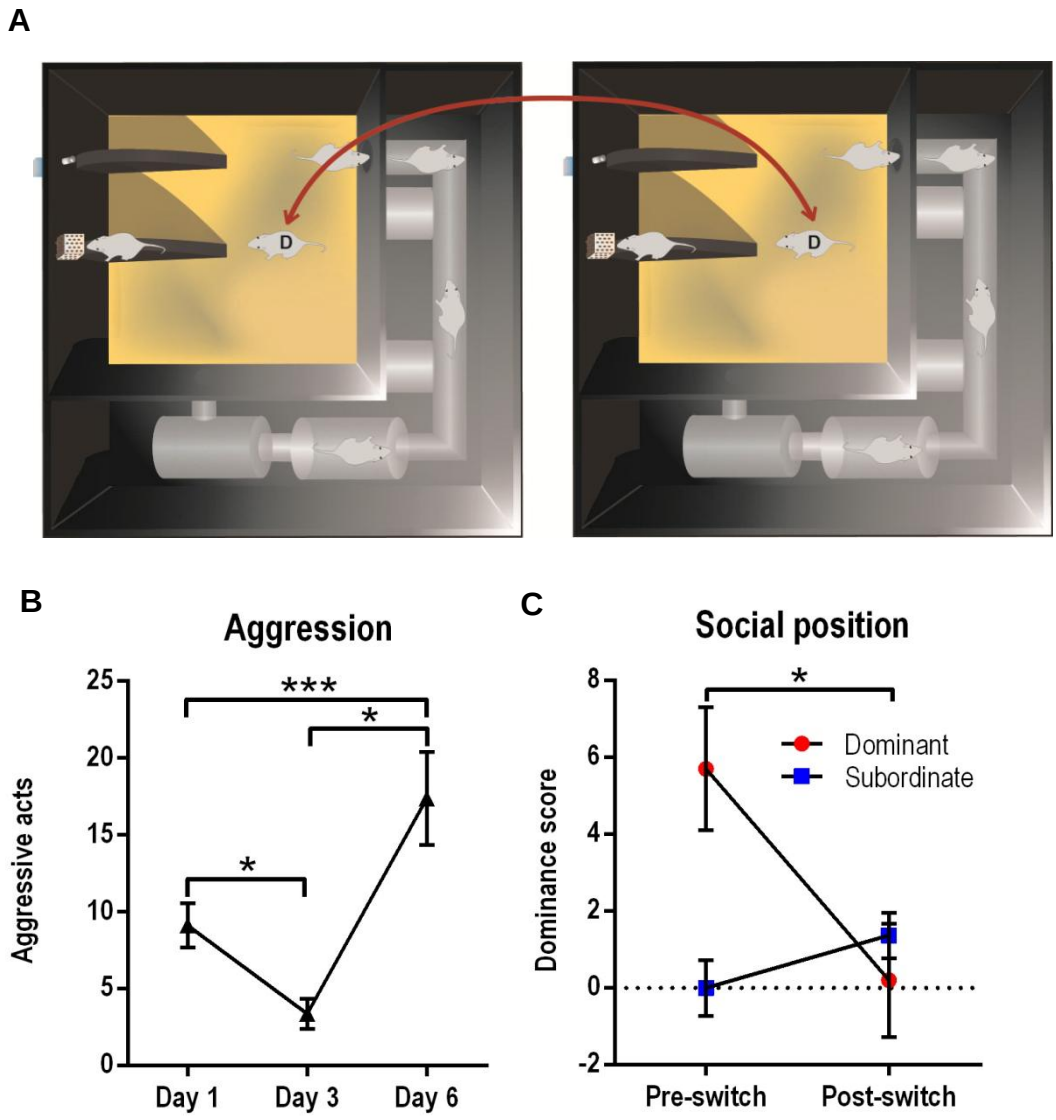


Figure 2. Social disruption increases aggression and dissolves a dominance hierarchy of rats. ***A,*** Schematic of dominant ("D") switch on day 4 in a disrupted hierarchy.***B,*** Individual aggressive acts decrease by the third day in a VBS, but switching dominants on day 4 produces an increase in aggression. ***C***, Following the switch, former dominants lose their status as measured by dominance score (aggressive actions-defensive actions). Error bars indicate SEM. *p<0.05. **p<0.01. ***p<0.001.

Social disruption suppresses adult neurogenesis in the hippocampus

Consistent with previous results (Kozorovitskiy & Gould, 2004), we found that dominants in a stable hierarchy had more new neurons in the ventral dentate gyrus than subordinates (IdU: 6552± 840 in dominants versus 4448 ± 480 in subordinates, t_{13}=2.41; p=0.032). Social disruption, however, eliminated the advantage of being dominant in dominants (IdU: 3300 ± 607 in dominants versus 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. No differences were observed between rats that were group-housed in standard cages and those that were switched between cages, suggesting that switching location or being housed with new conspecifics did not alter baseline levels of neurogenesis (e.g., for ventral dentate gyrus IdU: 4824$\pm$363 in switched versus 5580$\pm$1222 in non-switched; t_6=0.593; p=0.57). Furthermore, no differences were observed between control and stable VBS cohorts 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. Thus, subsequent comparisons were made between non-switched control and disrupted groups.

Social disruption produced a decrease in the numbers of neuronal precursor cells and immature neurons compared to 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 dentate gyrus following social disruption compared to control (Figs 3,4*A*). 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 co-labeling of Sox2 and GFAP (p=0.26) with social disruption, indicating that the observed decreases were in Sox2+/GFAP+ radial glia-like precursor cells (Fig. 4*C,D)*. In addition, no changes were observed on any of these measures in the dorsal dentate gyrus (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 dentate gyrus compared to controls at the 2 week time point (t_6= 3.073, p=0.022) (Figs 3,4*A*). Similar to what was observed for neuronal precursors, the suppressive effects of social disruption on DCX+ cells were not seen in the dorsal dentate gyrus (p=0.21). Social disruption also decreased the number of new cells in the ventral dentate gyrus labeled prior to the disruption (IdU: t_{21}= 4.31, p=0.002), as well as the number of new cells in the

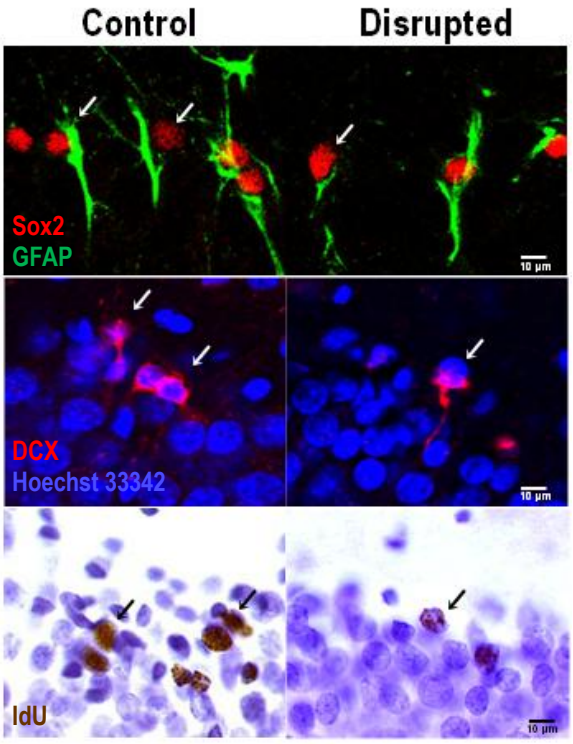


Figure 3. Social disruption produces a decrease in the number of neuronal progenitors and adult-born neurons in the ventral dentate gyrus.
Top, Representative images of Sox2/GFAP labeled radial glia-like progenitor cells in DG. ***Middle,*** Representative images of DCX-labeled immature neurons in DG, indicated by arrows. ***Bottom,*** Representative images of IdU-labeled cells in DG with Cresyl violet counterstain, indicated by arrows.

ventral dentate gyrus labeled after the disruption (CldU: t_{22}= 4.32, p=0.001) compared to controls at the 2 week time point (Figs. 3,4*B*).

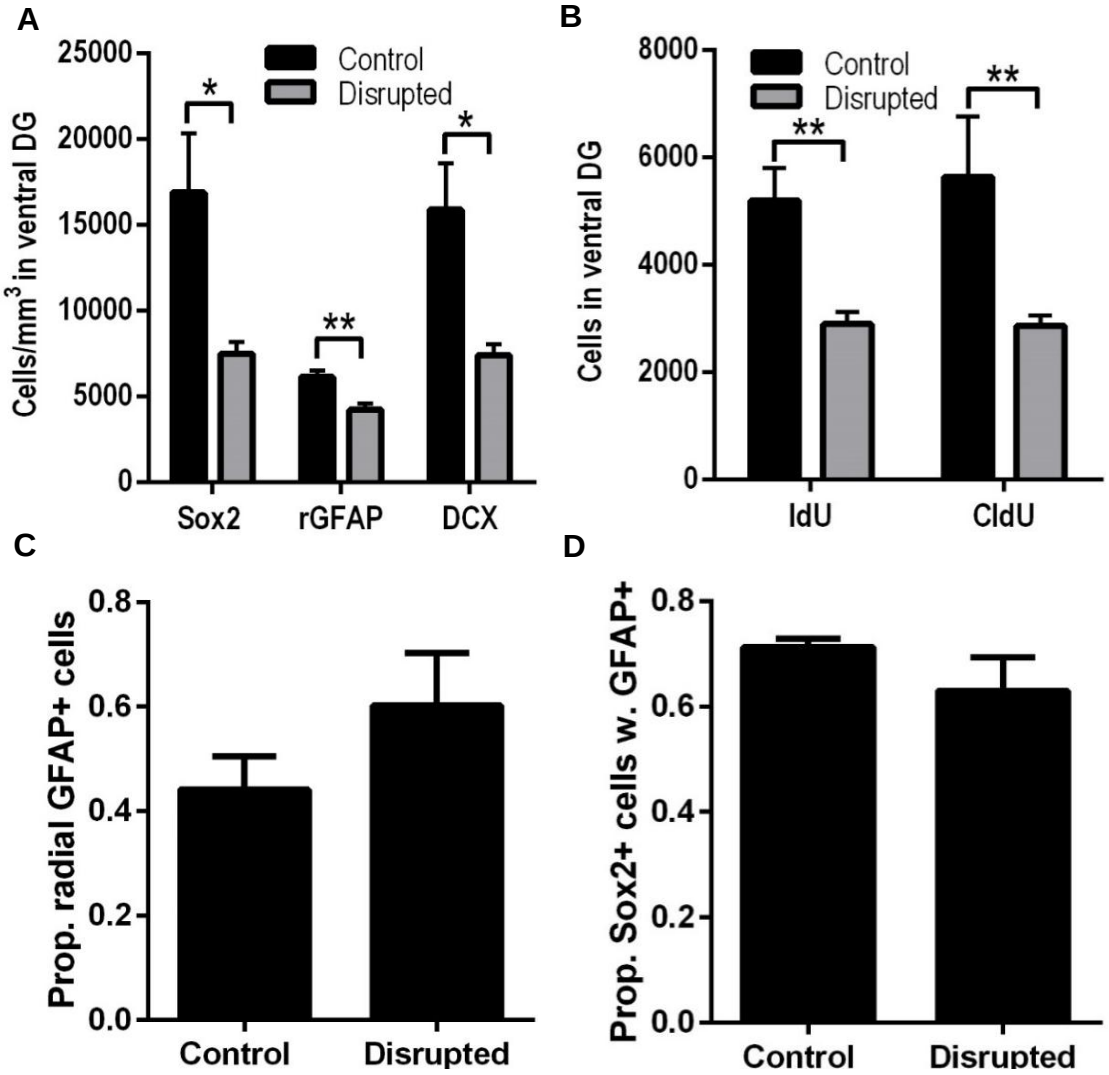


Figure 4. Social disruption suppresses adult neurogenesis in the ventral dentate gyrus. *A,* Radial GFAP+ cells, Sox2+ cells, and DCX+ cells are suppressed in rats from a disrupted hierarchy. ***B,*** IdU+ and CldU+ cells in the dentate gyrus are suppressed in rats from a disrupted hierarchy. ***C,*** The proportion of radial to horizontal GFAP+ cells is does not differ between rats in control vs disrupted conditions. ***D,*** The proportion of Sox2+ cells that co-label with GFAP does not differ between rats in control and disrupted conditions. Error bars indicate mean ± SEM. *p<0.05. **p<0.01.

More than 80% of IdU cells and CldU cells co-labeled 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 dentate gyrus no longer existed for cells generated before the disruption (IdU: 3141±295 in control versus 2621±323 in disrupted $t_{14}= 1.15$, $p=0.268$), but a persistent decrease in the number of cells generated after the disruption (CldU: 3174±416 in control versus 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 dentate gyrus (4293±374 in control versus 5327±1129 in disrupted; $t_9= -0.94$, $p=0.372$) suggesting that the rate of immature neuron production had returned to baseline. At the 6 week time point, there were no significant differences between control and disrupted groups in the dorsal dentate gyrus for any measure ($p>0.05$).

Social disruption does not alter OTR+ or V1aR+ expression by proliferating cells in the dentate gyrus

OTR and V1aR expression was observed on Ki67+ proliferating cells in the dentate gyrus but the proportion of Ki67+ cells that expressed either of these receptors did not differ between groups in dorsal or ventral dentate gyrus ($p>0.05$ for all comparisons). Optical intensity for OTR was increased in disrupted hierarchy rats compared to controls in the ventral dentate gyrus ($t_{10}=2.716$, $p=0.022$), but not dorsal dentate gyrus, dorsal CA3 or ventral CA3 ($p>0.05$) (Fig. 5). There were no differences in V1aR expression in dorsal or ventral dentate gyrus or the CA3 region between disrupted and control groups ($p>0.05$ for all comparisons).

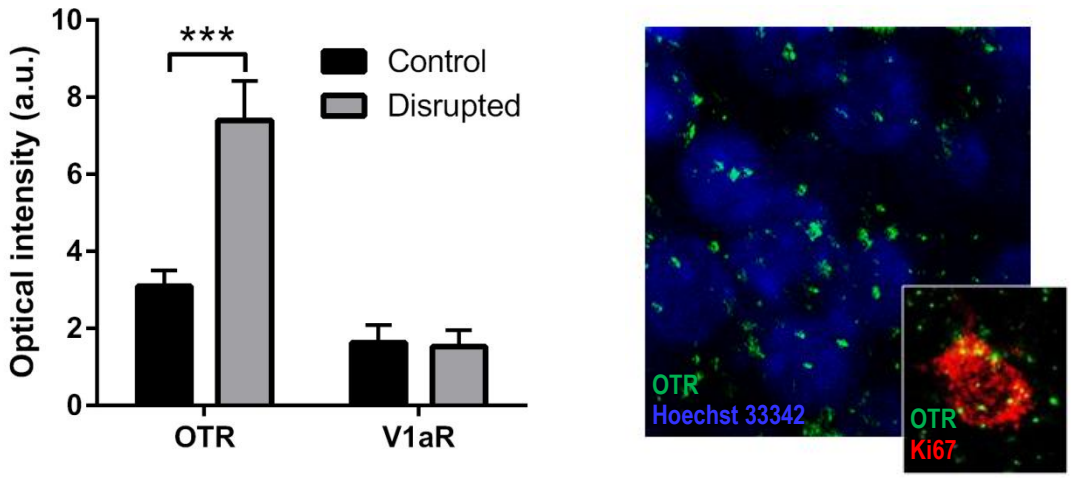


Figure 5. Social disruption increases oxytocin receptor expression in the ventral dentate gyrus. ***Left***, Oxytocin receptor staining is increased in the ventral DG in rats from a disrupted hierarchy. ***Right,*** Representative image of staining of oxytocin receptors in ventral DG. Green: OTR, Blue: Hoechst 33342. Inset: OTR expression on Ki67-labeled neuronal precursor cells. Green: OTR, Red: Ki67. Error bars indicate mean ± SEM. ***p<0.001

Social disruption does not result in persistently elevated levels of basal stress hormones

Baseline CORT levels differed among rats living in different social settings on day 6 but not in a predicted manner. While we anticipated that social disruption would result in elevated CORT levels, we found that it did not, at least at the 6 day time point. Instead, social disruption resulted in decreased baseline CORT levels (99.47 ± 36.1 ng/dl in control, 214.4 ± 47.5 ng/dl in stable, 31.96 ± 22.5 ng/dl in disrupted; $F_{2,21}$ =6.636, p=0.007). Plasma testosterone levels did not differ significantly among groups (106.1 ± 27 ng/dl in control, 210.53 ± 67.7 ng/dl in stable, 243.4 ± 88.8 ng/dl in disrupted; $F_{2,23}$ =1.169, p=0.330) (Fig. 6).

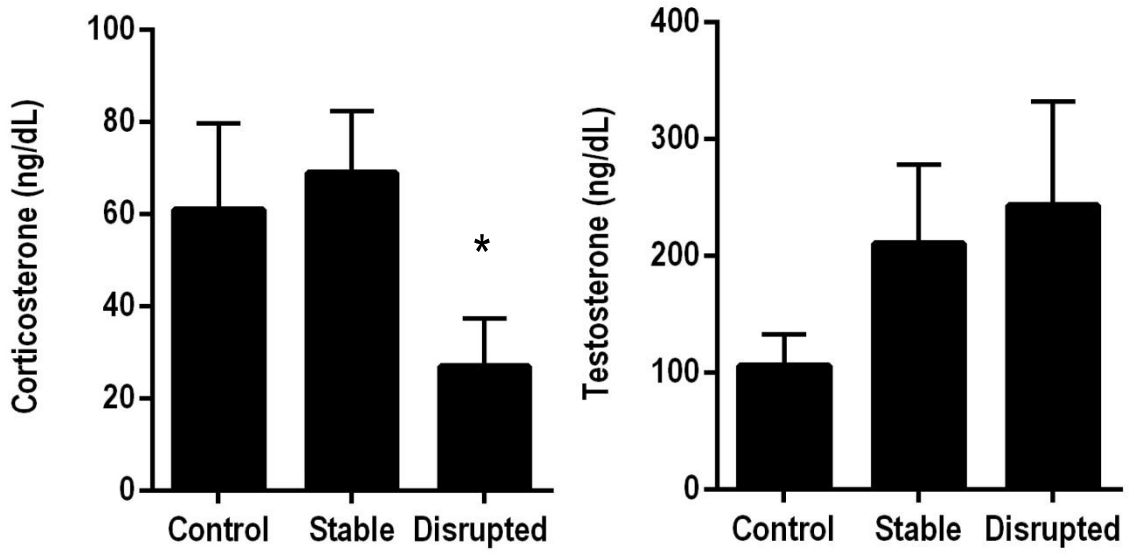


Figure 6. Social disruption did not increase levels of plasma corticosterone ***(Left)*** or testosterone ***(Right)*.**

Social disruption produces lasting changes in social behavior without impairing performance on hippocampus-dependent tasks.

Performance on the object location task did not differ between rats from disrupted hierarchies and rats from standard group housing, while rats from a stable hierarchy showed improved performance (DR=0.252 ± .046 for control, DR = 0.61 ± 0.15 for stable, DR=0.271 ± 0.076 for disrupted; $F_{2,18}$=3.917, p=0.039). On the elevated plus maze, rats from both stable and disrupted hierarchies were more active overall than rats from standard group housing, and both stable and disrupted groups spent a greater proportion of their time in the open, rather than closed arms (0.105±0.052 for control, 0.310±.05 for stable, 0.237±.04 for disrupted; $F_{2,30}$=3.710, p=0.037) (Fig. 7).

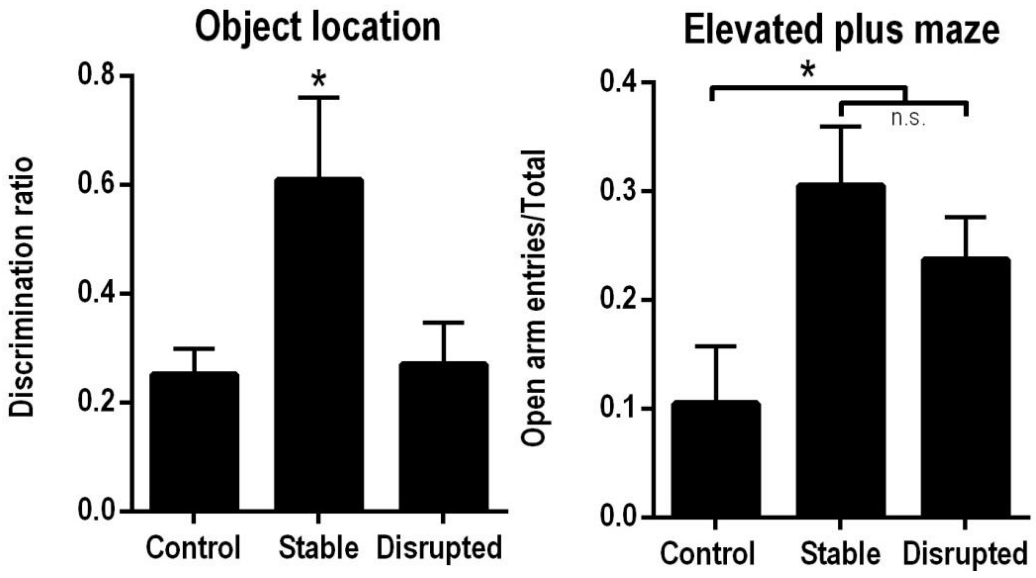


Figure 7. Social disruption does not produce impairments in cognitive or anxiety tasks linked to the hippocampus 6 weeks after disruption. ***Left,*** Rats from a disrupted hierarchy do not show impairments in their ability to discriminate between an object in a previously encountered location versus an object in a new location. Bars indicate mean discrimination ratio (DR = time spent investigating relocated object – time spent investigating non-moved object/ total investigation time). ***Right,*** Social hierarchy produces changes in anxiety-like behavior. Rats from both stable and disrupted hierarchies enter the open arms of an elevated plus maze more than rats that were group-housed in standard cages. Error bars indicate mean ± SEM. *p<0.05.

Social recognition did not differ between the control and disrupted groups with all groups (control, stable, disrupted) showing more time spent investigating a novel conspecific compared to a familiar conspecific ($F_{1,55}$=9.285, p=0.004) (Fig. 8*B*). However, substantial differences were noted among the groups when social resting preference was analyzed. Whereas control rats preferred to rest in the proximity of the novel rat, rats from a disrupted hierarchy preferred to rest in the proximity of a familiar rat (Fig. 8*C*). These findings suggest that social disruption alters social preference without adversely affecting social recognition, object location memory or anxiety levels.

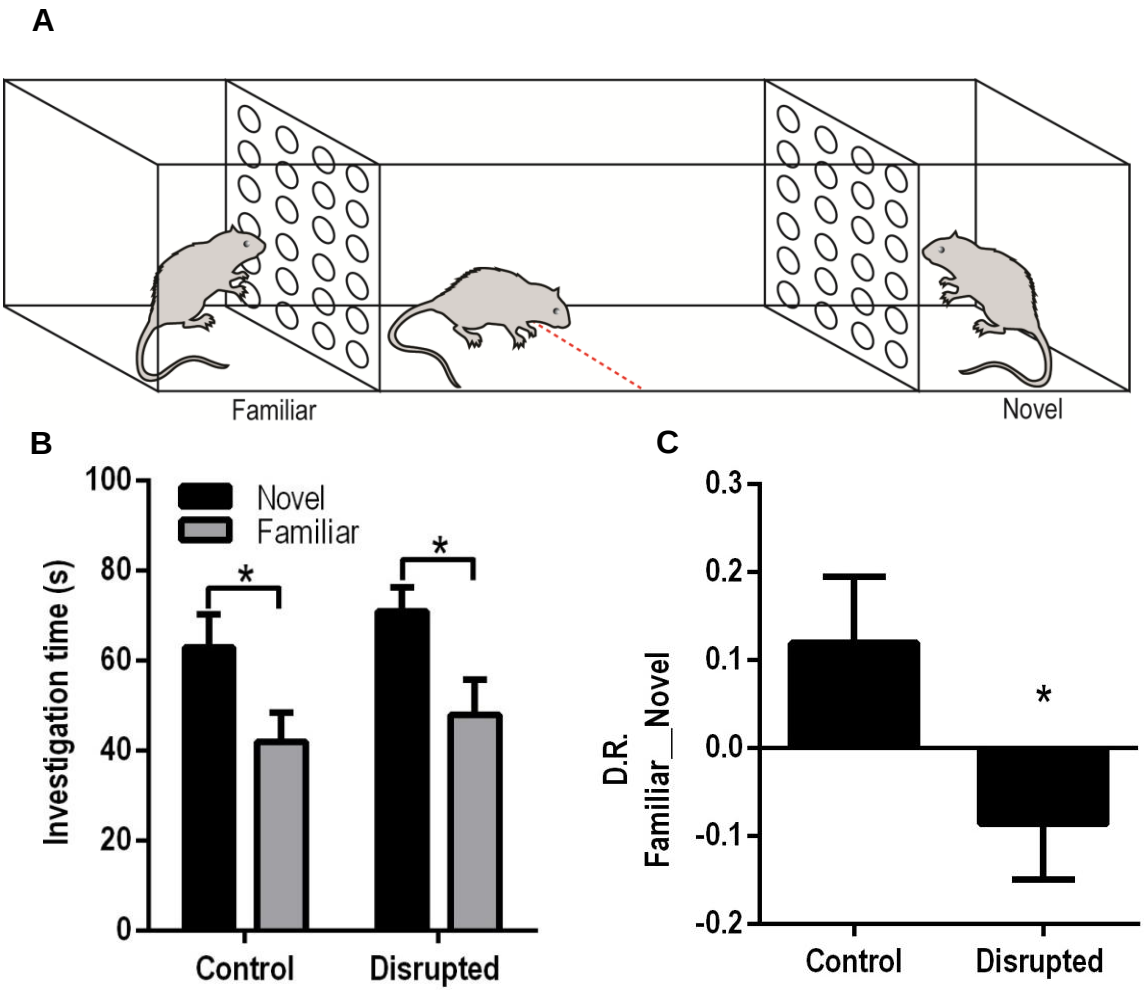


Figure 8. Social disruption produces changes in social preference without impairing social recognition. ***A,*** Schematic of apparatus used to test social recognition and social preference. ***B***, Social disruption produces no deficits in the ability to recognize a familiar conspecific, as rats from both groups investigate a novel rat more than a familiar rat. ***C,*** Whereas rats in control conditions prefer to rest in the proximity of a novel rat, rats from a disrupted hierarchy prefer to rest in the proximity of a familiar rat. Bars indicate mean relative social preference expressed as a discrimination ratio (D.R.=time spent near novel rat- time spent near familiar rat/total time). Values > 0 indicate a preference for the novel rat; values < 0 indicate a preference for the familiar rat. Error bars indicate mean ± SEM. *p<0.05.

Oxytocin restores adult neurogenesis and social preference in socially disrupted rats

As expected from previous findings (Leuner et al., 2012), daily oxytocin treatment for one week following social disruption increased DCX+ cells in the ventral DG (t_{16}=2.365, p=0.031) but not the dorsal dentate gyrus (t_{18}=0.56, p=0.58) (Fig. 9*B*).

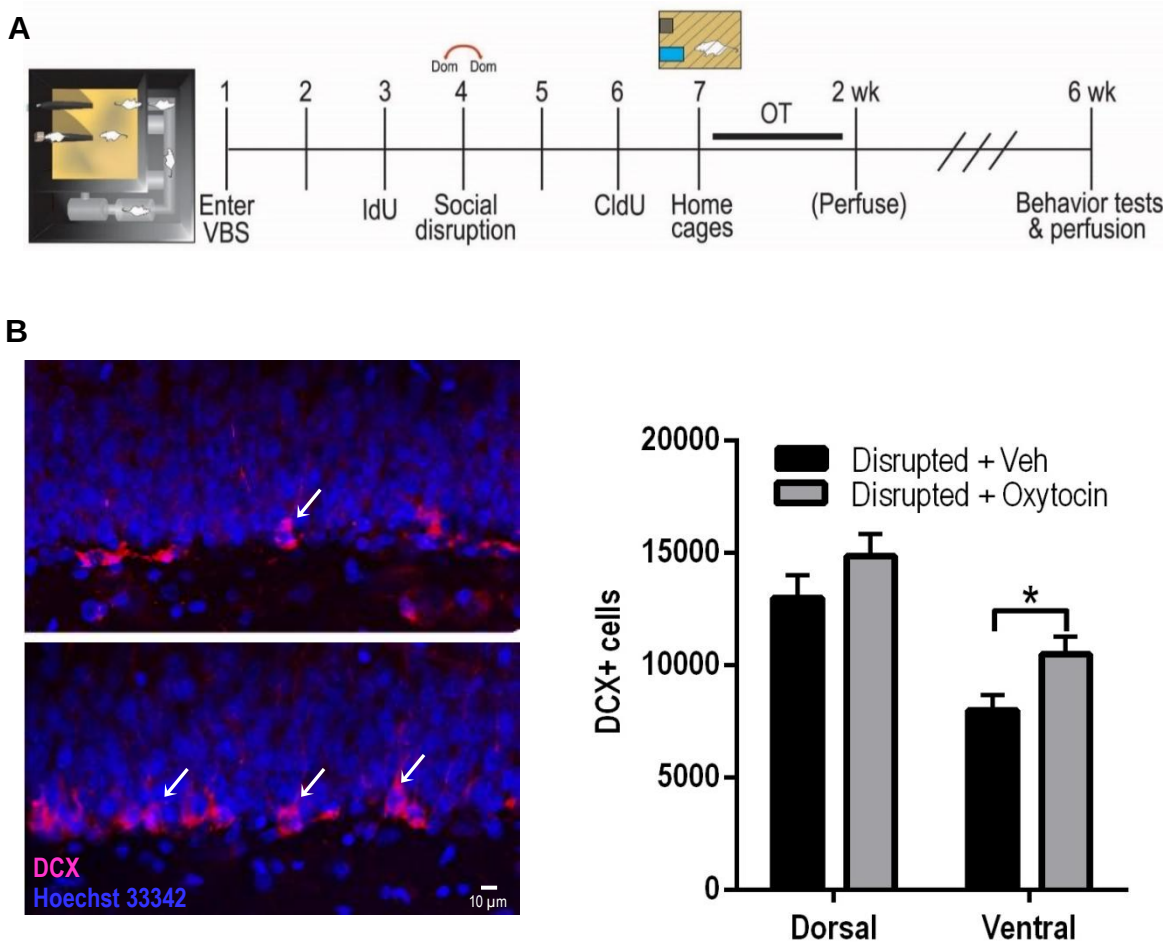


Figure 9. Chronic oxytocin (OT) treatment following social disruption increases the number of immature neurons in the ventral dentate gyrus. Timeline for hierarchy, neuronal birth-dating, drug treatment, and behavioral testing. ***B,*** Representative image of ventral dentate gyrus in rat from a disrupted hierarchy treated with vehicle (top) or 1 mg/kg oxytocin (bottom) for a week following exiting the VBS, with DCX+ immature neurons indicated with arrows. Red: DCX; Blue: Hoechst 33342. ***C,*** Rats treated with oxytocin following social disruption showed an increase in immature neurons in the ventral dentate gyrus. Error bars indicate mean ± SEM. *p<0.05.

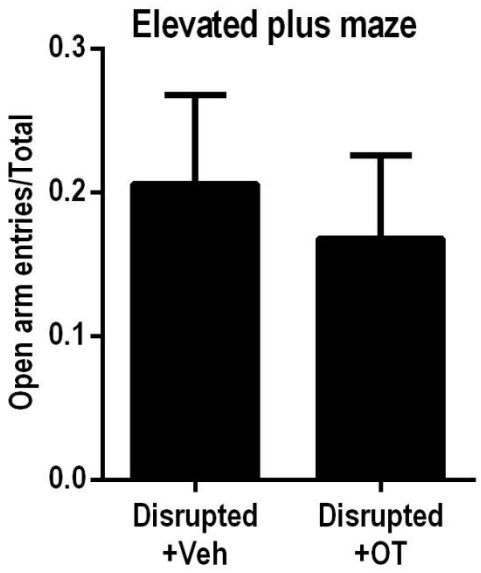


Figure 10. OT administration to disrupted rats did not produce changes in anxiety-like behavior on the elevated plus maze at 6 weeks. Error bars indicate mean ± SEM.

This treatment had no effect on anxiety-like behavior in the elevated plus maze (0.21±0.06 in disrupted+vehicle versus 0.17±0.06 in disrupted+oxytocin; t_{14} =0.44, p=0.66) (Fig. 10). 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, $F_{(1,13)}$ =6.588, p=0.028) (Fig. 11). As previously observed for socially disrupted rats (Fig. 8), rats from a disrupted hierarchy that received vehicle injections displayed a preference for resting near a familiar, as opposed to novel, rat (Fig. 11). This preference was reversed in socially disrupted rats with oxytocin treatments (Fig. 11). Rats that received oxytocin showed a preference for resting near a novel, as opposed to a familiar, rat (t_{30}=3.13, p=0.003), similar to what was observed in naïve rats without social disruption (compare with Fig. 8*C*). These findings suggest that oxytocin treatment after social disruption restores levels of adult neurogenesis as well as social resting preference behavior without changing social recognition or anxiety-like behavior.

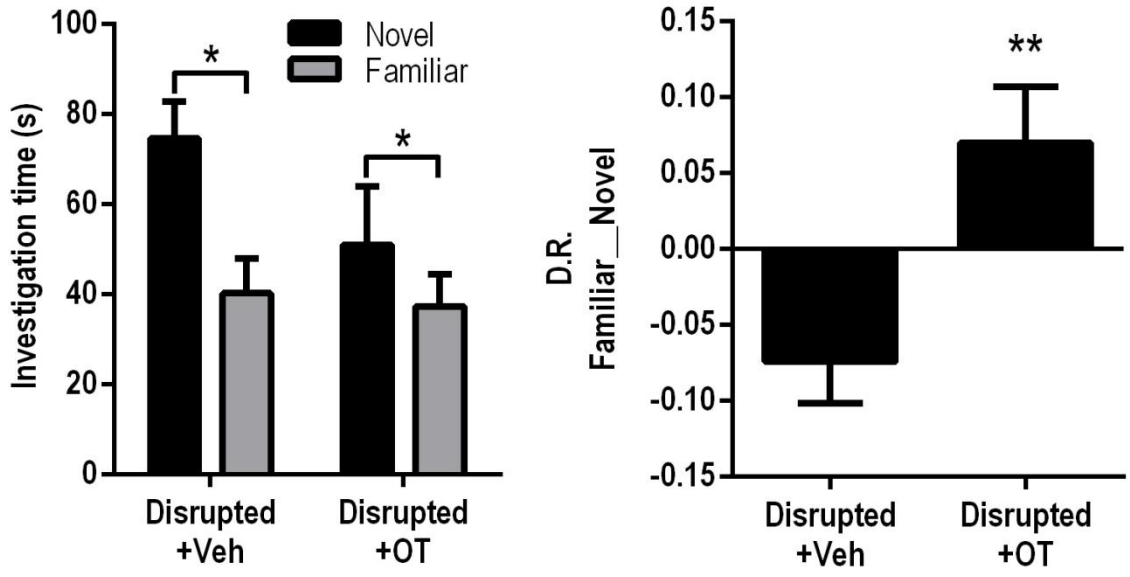


Figure 11. Restoring adult neurogenesis after social disruption reverses social resting preference. Social recognition is not affected by OT administration after social disruption (*Left*), whereas rats treated with OT prefer to rest near the novel rat (*Right*). Error bars indicate mean ± SEM. *p<0.05 **p<0.01.

Suppressed adult neurogenesis in socially naïve GFAP-TK rats reverses social preference

Valganciclovir (GCV) treatment was effective in reducing adult neurogenesis in GFAP-TK rats, as BrdU+ cells were lower in both the dorsal and ventral dentate gyrus compared to in GFAP-TK rats treated with vehicle (t_{15}=2.56, p=0.022 for dorsal dentate gyrus; t_{15}=3.18, p=0.006 for ventral dentate gyrus) (Fig. 12*B*). Importantly, the decrease in new neurons was not complete with this treatment of GCV and instead, quantitatively resembled the decrease we observed following social disruption in wildtypes (compare Fig. 12*B* with Fig 4*B*).

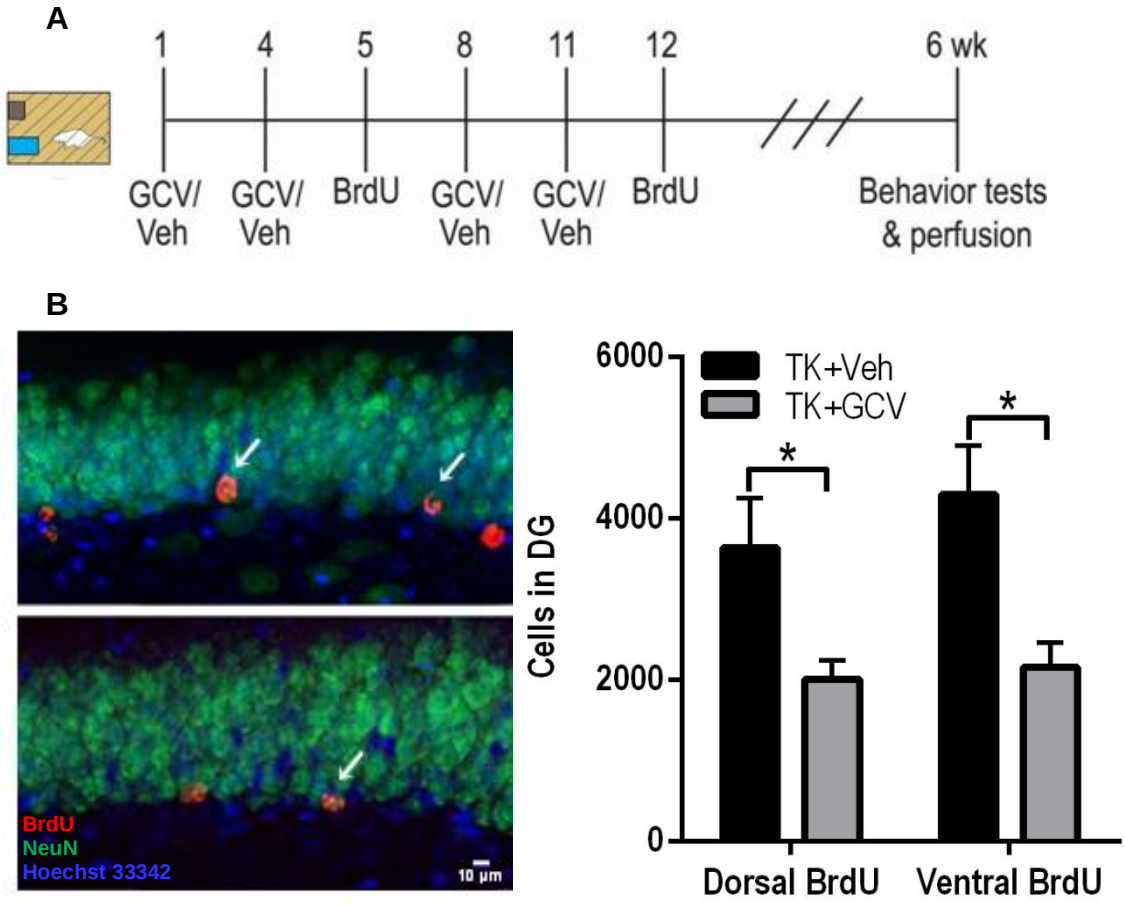


Figure 12. GFAP-TK rats treated with GCV show a suppression in adult neurogenesis in the dentate gyrus. ***A,*** Timeline for neuronal birth-dating, drug treatment and behavioral testing for GFAP-TK rats. ***B,*** *Left,* Representative image of ventral DG of GFAP-TK treated with vehicle (top) or valganciclovir (bottom) for a 2 week period ; Red: BrdU; Green: NeuN; Blue: Hoechst 33342. *Right,* GFAP-TK rats treated with valganciclovir (GCV) showed a suppression in BrdU+ neurons in the dentate gyrus. Error bars indicate mean ± SEM. *p<0.05

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 (0.223 ± 0.189 for TK+Veh versus 0.363 ± 0.305 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 (.43±.03 for TK+Veh versus .49±.01 for TK+GCV; t_{20}=1.46, p=0.15) (Fig.13).

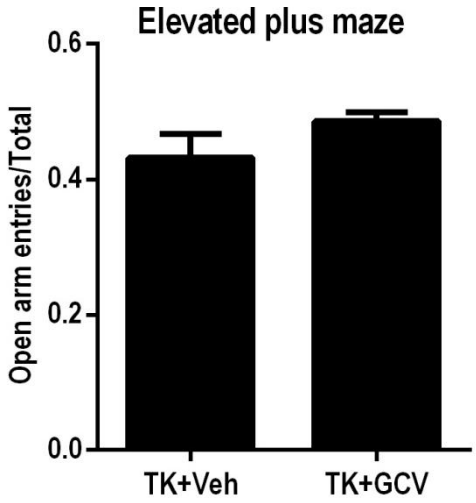


Figure 13. GCV administration does not produce changes in anxiety-like behavior in GFAP-TK rats at 6 weeks. Error bars indicate mean ± SEM.

In addition, social recognition did not differ between vehicle- and GCV-treated rats (two way ANOVA of condition vs novelty, ME of novelty, $F_{(1,15)}$=6.509, p=0.025) (Fig 12). Like socially disrupted wildtype rats, socially naïve GFAP-TK rats treated with GCV showed a preference for resting near a familiar rat, whereas socially naïve GFAP-TK rats treated with vehicle showed a preference for resting near the novel rat (t_{18}=2.6, p=0.019) (Fig. 14).

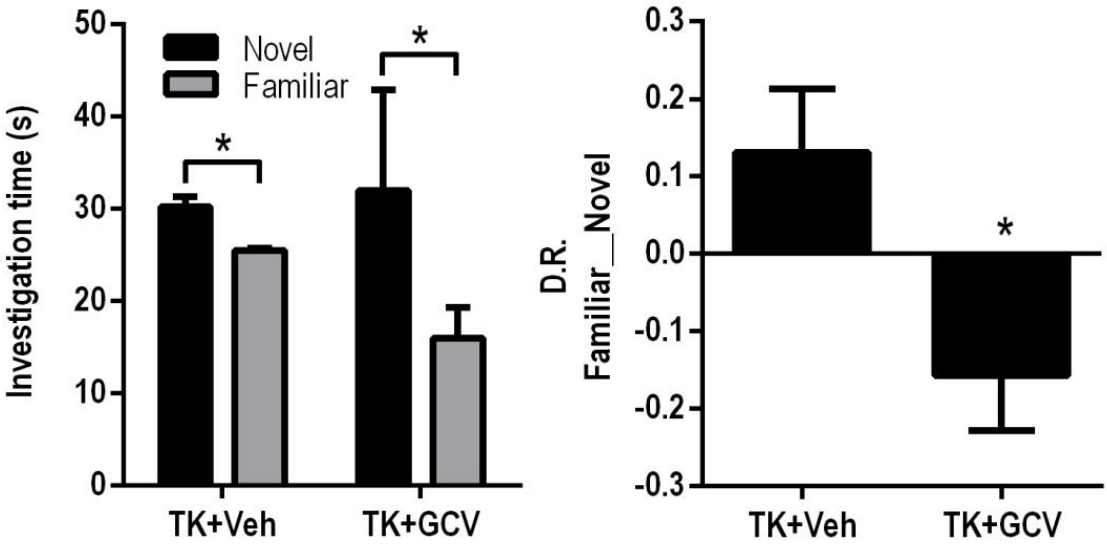


Figure 14. Suppressing adult neurogenesis in socially naïve rats changes social resting preference. GFAP-TK rats with a suppressed pool of 6 week old neurons show no impairments recognizing a familiar rat *(Left)* and exhibit a resting preference for a familiar rat (*Right)*. Error bars indicate mean ± SEM. *p<0.05.

Chapter 3: Discussion

Our results show that disrupting an established dominance hierarchy of rats changes the rate of adult neurogenesis in the hippocampus and that these changes are sufficient to produce lasting adaptations in social behavior. Social disruption suppresses the numbers of neuronal progenitor cells and new neurons for all members of a dominance group, and increases oxytocin receptor expression in the hippocampus. Six weeks later, at a time when new neurons generated during social disruption have had time to integrate into the circuitry, rats from a disrupted hierarchy exhibit no change in social recognition but a preference for resting near a familiar, rather than novel, rat, without impairments in cognition or increases in anxiety-like behavior. Increasing adult neurogenesis with oxytocin administration after social disruption reverses social preference behavior, and suppressing adult neurogenesis with transgenic manipulation produces a familiar preference in socially naïve rats. These data suggest that social disruption may fine-tune subsequent social behavior by reducing the number of new neurons in the dentate gyrus.

Other studies have modeled social instability by switching animals between cages (Herzog et al., 2009; McCormick et al., 2012), but our manipulation takes advantage of the natural propensity of rats to form stable dominance hierarchies in the wild (Blanchard et al., 1991) and allows us to observe the response to perturbations of this setting. In standard group-housing, rats do not create hierarchies and therefore introducing instability by switching cage-mates is limited in its implications for complex social behavior. Our observation that group-housed rats that undergo a switch of cage-mates were virtually identical to group-housed rats without such a switch in terms of adult neurogenesis suggests that this model is unlikely to have a major effect on brain plasticity. By contrast, we found a robust decrease in neuronal progenitors and immature neurons in rats living in a disrupted VBS hierarchy. This effect was observed regardless of dominance position and may have occurred as a result of the stressful

effects of persistent aggression, which has been shown to decrease adult neurogenesis (Czeh et al., 2002). However, somewhat paradoxically, rats from a disrupted VBS exhibit lower, as opposed to higher, levels of plasma corticosterone than rats from stable hierarchies and control conditions, which may reflect hypocortisolism, a phenomenon wherein chronic stress produces a down-regulation of the hypothalamic-pituitary-adrenal (HPA) axis response (Yehuda et al., 1990; Rohleder et al., 2004). Although this can lead to psychopathology in some cases, researchers have argued that hypocortisolism can reflect an adaptive response to chronic stress (McEwen, 1998; Fries et al., 2005). The hippocampus is dense with oxytocin receptors which may play a role in shutting down the HPA axis after stress (Gimpl & Fahrenholz, 2001). The increase in OTR we observed with social disruption may optimize the hippocampus for diminishing corticosterone levels. Along these lines, proactive stress-coping styles can also lead to a blunted HPA response (Koolhaas 2009); the increased aggression following social disruption and suppressed anxiety-like behavior supports a proactive phenotype (Veneema & Neumann, 2007) in our disrupted rats.

Previous studies have shown that experimental manipulations that produce suppressed adult neurogenesis are often associated with increased anxiety-like behavior (Bessa et al., 2009; Revest et al., 2009, Snyder et al 2011; Opendak & Gould, 2015) and impaired performance on some memory tasks including parental recognition of offspring (Mak & Weiss, 2010) and spatial memory (Dupret et al., 2008; Jessberger et al., 2009). As rats from a disrupted hierarchy had fewer new neurons in our study, we predicted that they would show behavioral deficits several weeks later as a result of having a smaller pool of functional units in the hippocampus. We were surprised to find no such impairments, as rats from disrupted hierarchies showed no impairment on the object location task, social recognition, or increased anxiety-like behavior despite reduced

numbers of new neurons. In fact, both rats from a disrupted and stable hierarchy showed reduced anxiety-like behavior compared to rats from standard group-housing.

Our observation that rats from a disrupted hierarchy show long-term changes in social preference behavior can be interpreted several ways. A lack of novelty preference is typically used as a diagnostic marker in disease models of depression and autism, in which the hippocampus has been implicated (Moy et al., 2004; Nadler et al., 2004; Irle et al., 2010, but see Naert et al 2011). Social defeat paradigms in rodents can lead to decreased motivation to interact with a novel conspecific due to long-term changes in the appeal of novelty as an expression of anhedonia (Von Frijtag et al., 2000). As socially disrupted rats in our studies showed no impairment recognizing a familiar rat and control-level investigation times, a familiar preference due to impaired novelty detection and/or suppressed sociability seems highly unlikely.

Another possible interpretation of these results is that preference for a familiar, rather than novel, rat is as an analog of classical stress-induced social avoidance, a marker of social anxiety (Berton et al., 2006; Krishnan et al., 2007). Social disruption may produce a socially avoidant phenotype through an impairment in pattern separation by the hippocampus, in which adult born neurons have been implicated (Yassa & Stark, 2011). Behaviorally, this corresponds to an ability to distinguish between highly similar contexts (Creer et al 2010; Tronel et al., 2012). In the absence of sufficient adult-born neurons, over-generalization may occur (Kheirbek et al., 2012; Dranovsky & Leonardo, 2012) whereby a rat is impaired in distinguishing between a threatening and non-threatening stimulus; the novel rat, in this case, would be grouped as a threat with the previously encountered intruder dominant in the VBS. If this were the case, however, we would also expect to see less investigation of the novel rat, which was not observed. Furthermore, the lack of a general increase in anxiety also argues against this possibility. The

possibility that suppressed adult neurogenesis produces a socially avoidant phenotype through over-generalization is further challenged by the fact that social avoidance correlates with *enhanced* adult neurogenesis in chronically defeated rats (Lagace et al., 2010). In the chronic social defeat paradigm, more neurons generated following chronic social defeat survived in rodents that exhibited persistent social avoidance of a novel non-threatening conspecific, leading Lagace et al. (2010) to suggest that new neurons are important for long-term maintenance of social avoidance. In our study, the total number of six week- old neurons was lower, not higher, in the ventral dentate gyrus of disrupted versus control rats further arguing against this interpretation. It should also be noted that between the histories of those neurons are quite different, indicating that the identity and involvement of adult-born neurons may be more important than their quantity. This point is reminiscent of several studies showing a dissociation between neuron numbers and behavioral changes (Branchi et al., 2006; Jayatissa et al., 2010; Lyons et al., 2010; Parihar et al., 2011). Others have shown that social instability stress in adolescence increases the survival rate of immature neurons in the hippocampus (McCormick et al., 2012). In our study, virtually every new neuron generated within a disrupted VBS survived up to the expression of social behavior six weeks later, whereas up to half of the neurons born in control conditions were eliminated. If experience in a disrupted dominance hierarchy led to learning new social strategies, the neurons generated during that learning in adulthood may act as a substrate for the expression of that behavior.

It has been suggested that animal researchers reconsider the interpretation of all behaviors that differ from controls as "aberrant", as stress-induced changes in behavior are likely to be adaptive in some circumstances (Lagace et al., 2010; Petrik et al., 2012; Blanchard et al., 2013). Social disruption-induced changes in novelty preference may belong in this category. Socially

isolated mice have exhibited enhanced novelty preference, has been interpreted as diagnostic of an impairment in an ability to assess danger (Naert et al., 2011). 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). For rats from a highly aggressive and dynamic disrupted dominance hierarchy, preference for a familiar rat may reflect enhanced social risk assessment without concomitant impairments in general anxiety or the ability to detect novelty.

In most models of stress-induced changes in behavior, a loss of social novelty preference typically occurs with increases in generalized anxiety and depressive behavior (Saavedra-Rodriguez & Feig 2013; Zheng et al 2014). We found a dissociation of these, as rats from a disrupted hierarchy and rats with a suppressed pool of six week-old neurons showed no increases in anxiety-like behavior. This resilience may be due in part to the VBS as a form of environmental enrichment, which has been shown to interact with adult neurogenesis to remediate the effects of chronic stress on behavior (Schloesser et al., 2010); environmental enrichment helped form resilience in chronically socially defeated rat with intact, but not ablated, adult neurogenesis. Other studies using chronic social instability have produced anxiogenic effects in mice and rats (Herzog et al., 2009; McCormick et al., 2010; Saavedra-Rodriguez & Feig 2013), but these models typically involve unpredictable periods of social isolation and switching cage-mates without the added complexity of re-establishing a dominance hierarchy within a visible burrow system. In our model, the enriching aspects of hierarchy life may buffer the effects of social disruption on generalized anxiety but not be sufficient to protect against changes in social behavior, supporting a dissociation between the mechanisms of social and general anxiety (Vialou et al., 2014).

We observed a marked reduction in anxiety-like behavior in rats from both stable and disrupted hierarchies. These changes were not due to changes in new neurons or in oxytocin receptor expression in the hippocampus, as rats from a stable hierarchy exhibited decreased anxiety without changes in these measures. Although the blunted HPA response in rats from a disrupted hierarchy may suggest that suppressed anxiety is linked to changes related to hypocortisolism, rats from a stable hierarchy did not exhibit persistent changes in glucocorticoid levels. The cellular mechanisms underlying these effects remain unclear, although known mechanisms of anxiety regulation in the ventral hippocampus are likely to play a role. Certain aspects of life in a dominance hierarchy may engage these systems; access to an enriched environment can decrease anxiety and physical exercise has been shown to decrease anxiety through increases in local GABA-ergic inhibition in this region (Schoenfeld et al., 2013; Goes et al., 2015). Our results are atypical as most research in the visible burrow system involves the more aggressive Long Evans rat species that produces an anxious phenotype in subordinate rats (Blanchard et al., 1991; 1995). As such, more studies will be necessary in order to understand how experience in a dominance hierarchy and/or visible burrow system produces decreases in anxiety-like behavior in more docile rodents.

Suppressed neurogenesis in our model exhibited a marked regional specificity, occurring predominantly in the ventral, as opposed to dorsal, dentate gyrus. This outcome lends support to the notion of functional dissociation between the dorsal and ventral regions (Fanselow & Dong, 2010). Given the emotionally disruptive nature of losing a stable environment, it is likely that brain regions involved in emotional processing, including the ventral dentate gyrus, would be primarily affected by its consequences. However, it is somewhat surprising that rats from a disrupted hierarchy with a robust suppression of new neurons in this region failed to show an

anxious phenotype at the six week time point. These data suggest a dissociation between the regional specificity of experience-induced effects on adult neurogenesis and the substrate for long-term deficits.

To determine whether suppressed neurogenesis was necessary and sufficient for producing long-term changes in social preference behavior, we increased neurogenesis in rats following social disruption using oxytocin and suppressed the pool of six-week old neurons in naïve GFAP-TK rats. Oxytocin was used because this neuropeptide has been shown to increase neurogenesis when administered chronically (Leuner et al., 2012). Rats treated with oxytocin showed a reversal of social preference towards the novel rat, whereas rats treated with vehicle maintained a preference for the familiar rat. It is possible that these effects may have been due to oxytocin administration, rather than the restoration of adult neurogenesis, because oxytocin can restore social novelty preference in chronically stressed and autism-model rats (Witt et al., 1992; Lee et al., 2007; Lukas et al., 2011) although oxytocin was administered weeks before behavioral testing and was not likely elevated at this time. It is also unlikely that oxytocin itself, as opposed to changes in new neurons, is responsible for this effect because socially naïve GFAP-TK rats with suppressed neurogenesis also showed impaired novelty preference without changes in social recognition, object location, or anxiety-like behavior. Thus, the most likely explanation is that the effects of social disruption on social preference, but not anxiety-like, behavior are the result of suppressed adult neurogenesis.

A large literature has explored the link between experience-induced changes in adult neurogenesis and long-term behavioral changes (Opendak & Gould, 2015). A proportionately large amount of these studies have focused on behavioral enhancements following increases in adult neurogenesis following access to voluntary running or enriched environment (Nilsson et

al., 1999; Lucassen et al., 2010; Schoenfeld & Gould, 2012). Several correlational studies have explored the involvement of suppressed neurogenesis in models of stress-induced changes in behavior (Goshen et al., 2008; Hulshof et al., 2011; Lajud et al., 2012) but the findings have been mixed and have failed to show a causal link between adult neurogenesis and a stressed behavioral phenotype (Vollmayr, 2007; Ho & Wang, 2010).

Taken together, these data suggest that a suppression in neurogenesis during adulthood may produce lasting changes in social behavior without causing obvious impairments in hippocampal functions, such as cognition and anxiety regulation. Our results challenge the simplistic established model wherein negative experiences tend to suppress adult neurogenesis and impair cognitive performance. Social disruption indeed produces a suppression in adult neurogenesis, but this does not impair performance on tasks known to engage the hippocampus and instead produces 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 functional and adaptive significance of social preference for familiarity.

Concluding remarks

For centuries, philosophers have argued over the extent to which biology is destiny, or, to what extent experience can change the brain and behavior. Discoveries of experience-dependent change in adult neurogenesis and other forms of plasticity suggest that the brain can refine its function based on experience. Encountering environments in which exploration is rewarding seems to lead to a brain primed for exploration and improved cognition through enhanced neurogenesis and decreased anxiety. On the other hand, punishment and stress seem to lead to a brain primed to prioritize safety and avoidant behavior over cognitive optimization and increased production of new neurons.

Our data indicate that a brief experience during adulthood can have lasting consequences for social behavior without concomitant impairments in cognition or emotional regulation. This result is informative in several ways. First, it suggests that social behavior can be modified after a critical period in development, in contrast to studies showing the importance of early life socialization in programming behavior during adulthood (McCormick et al., 2012; 2015; Kirshenbaum et al., 2014). Second, this modification in social behavior is accomplished at least in part through refinements in a small number of new neurons, indicating a role for adult neurogenesis in social behavior that was previously limited to social recognition or stress-induced social avoidance (Lagace et al., 2010; Mak & Weiss, 2010). Third, despite the highly disruptive nature of the hierarchy destabilization stimulus, adult rats exhibited resilience, failing to express an impaired phenotype. Although social preference was altered in these rats, there was no evidence that this behavior was maladaptive, and, instead, may reflect an optimization of hippocampal function to favor safety over novelty.

Ours is the first study to causally demonstrate an experience-induced change in behavior following experience-induced suppression in adult neurogenesis. Surprisingly, we did not

observe a specialized impairment reflecting the nature of the stressor i.e. impaired social recognition or enhanced social anxiety following destabilization of a dominance hierarchy. On the contrary, suppressed anxiety and a change in social preference may reflect a specialized refinement of hippocampal function to suit the predicted environment, following a regionally specific suppression in adult neurogenesis. This provides direct experimental evidence of resilience following acute stress instead of a negative feedback loop, wherein an injurious experience suppresses new neuron production and leads to behaviors that preclude opportunities to restore normal levels of adult brain plasticity and behavior (Chapter 1, Fig. 4).

It is important to note that rats in our study only lived in a complex environment for six days over the course of their lives. Nonetheless, both the opportunity to live in this environment and the nature of the experience within the environment had long-term effects on behavior. In particular, living in a VBS for six days was sufficient to suppress anxiety and possibly buffer the effects of social disruption on anxiety in destabilized hierarchies. The rats from disrupted hierarchies showed an adaptation in social behavior without concomitant anxiogenesis. These data suggest that the combination of a naturalistic scenario and an ethologically relevant stressor can optimize, rather than impair, behavior through small changes in adult neurogenesis.

Given contemporary views about the need to reduce stress and increase physical activity in order to optimize human brain health, it may be that Ramon y Cajal's claim about the ability to sculpt one's own brain, by controlling one's experiences, has become a scientific and cultural reality. Seeking out rewarding experiences and avoiding stressful experiences may help each individual optimize his own brain. But this is not always easy or even possible; the real-world scenarios people face most often involve a complement of stressful and rewarding conditions. Tightly controlled laboratory studies of adult neurogenesis have been informative, but new data

suggest qualitative differences in the type and complexity of experiences that affect adult brain plasticity in the hippocampus. Naturalistic experimental scenarios may prove invaluable in this regard, in order to understand the scope of this neurobiological process and its functional consequences.

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