

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

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Early studies investigating the exploratory movements of rodents have remarked on the natural tendency of animals to establish preferred “home” locations from which they make excursions into the remaining environment ([Chance & Mead, 1955](#)). For instance, feral rats maintain home burrows from which they organize their foraging and avoidance of predation ([Barnett, 1963](#); [Whishaw & Whishaw, 1996](#)). [Eilam and Golani \(1989\)](#) provided one of the first experimental characterizations of home base behavior by placing wild rats in a large open environment devoid of a shelter or local cues. Over a 1-h period, rats visited several locations, but restricted their visits to one or two of these locations. Rats tended to spend a disproportionate amount of time stopping at a single location (10 times more than the second location). A stop or pause was defined as the absence of active movement, forward or backwards, and lasting longer than one second. The duration of stops made at this preferred home base increased as a function of test duration. Eilam and Golani additionally observed a particular set of behaviors at the home base. Grooming, for instance, was almost exclusively expressed at the home base. Bouts of grooming were typically followed by excursions into the remaining environment, or prolonged crouching in place. Other behaviors at the home base included long duration rearing movements, and circling or pivoting behavior, that latter of which likely consists of sniffing the maze substrate. In sum, Eilam and Golani’s seminal study described a pattern of regionally restricted behavior characterized by grooming, rearing, and circling behaviors. Home base behavior by rats has been reproduced in subsequent studies using featureless environments or in complete darkness ([Fig. 1A](#)) ([Hines & Whishaw, 2005](#)), but the behavior can also withstand changes in enclosure size and stimulus complexity ([Eilam, 2004](#); [Golani et al., 1993](#); [Whishaw et al., 2006](#)). Although these observations indicate that home base behavior is robust despite changing test situations and contextual features, [Eilam and Golani \(1989\)](#) noted a tendency for behavior to form at the edges of mazes, especially at the corners of square open-fields. Thus, home bases can be modulated to some degree by salient environmental stimuli and environment shape. Whishaw and colleagues investigated this relationship further by placing objects or small shelters in the proximity of the open-field ([Clark et al., 2005](#); [Lehmann et al., 2007](#); [Wallace, Hines, Pellis et al., 2002](#); [Wallace, Hines, Whishaw et al., 2002](#);

[Whishaw et al., 2001](#)). For example, [Hines and Whishaw \(2005\)](#) placed a large dark cue next to an open field, but just out of reach of the rat. It has long been known that rats are attracted to dark locations in an environment ([Whishaw, 1974](#)), and it was therefore hypothesized that animals would establish their home bases near this cued location. As expected, rats rapidly visited the cued location and spent a significant amount of time in that segment of the open-field ([Fig. 1B](#)). Home base behavior at the cued location was similar to home base behavior in featureless environments, including the performance of circling, grooming, rearing, and slow lingering movements. In a follow-up test, the animal was removed from the field and the cue was moved to another location along the edge of the open-field. In response, rats changed their home bases locations so that it was maintained in relation to the moved cue. [Hines and Whishaw \(2005\)](#) reported that in some test sessions, rats would even establish home bases in relation to objects located along the testing room walls, such as a book shelf, suggesting that the behavior can be influenced by distant room cues.

In some studies, a configuration of proximal cues has been provided within the confines of the open-field or just adjacent to the field ([Lehmann et al., 2007](#); [Yaski & Eilam, 2007](#)). Whishaw and colleagues have reported a strong tendency for animals to evenly distribute their home bases across equivalent objects ([Clark, Hamilton, & Whishaw, 2006](#)). However, when competing objects provide different sensory information, there is a clear preference for one cue over the other. For instance, [Lehmann et al. \(2007\)](#) placed a large black box near one side of an open field, but far enough away from the maze such that the rat could not touch the object, but could still be seen. On the opposite side of the maze, a white wall occupied a small segment of the open-field, and was close enough to the field so that it could be used as a tactile cue. The two cues were available in a 30 min exploration session repeated over four days. Although rats formed two home bases, one adjacent to the black box and another next to the white wall, by the fourth day of testing there was a preference for engaging their home base next to the white wall ([Fig. 2](#)). Thus, rats spent a disproportionate amount of time and stopped more frequently at the wall segment. The preference for the wall over the large black box might be related to the perception that the wall offers greater security in the open field, i.e., rats could rest their bodies against the wall but were unable to do so against the black cue ([Whishaw et al., 2006](#)). Alternatively, the preference may reflect a hierarchy of sensory control over home base behavior, with a preference for tactile cues over visual cues. Hierarchical control over behavior by

sensory cues has been observed in other aspects of spatial navigation, with some reports concluding that cues located proximal to the animal, or related to the maze substrate, typically take precedence over other stimulus sources.

Several studies have reported that prior experience with environmental stimuli can modulate regional preferences in home base behavior ([Eilam, 2014](#); [Hines & Whishaw, 2005](#); [Lehmann et al., 2007](#)).

Interestingly, [Hines and Whishaw \(2005\)](#) monitored the exploratory behavior of rats in an open field with a large proximal cue placed next to the arena. Animals were tested in five daily sessions in which the cue occupied the same position, but on the fifth day the proximal cue was removed from the testing room.

Hines and Whishaw observed that despite the removal of the cue, rats continued to dwell in the location of the open-field. This observation indicated that animals can learn the fixed relationship between the cued home base location and the remaining room cues. The persistent behavior in these locations is reminiscent of the place behavior displayed by rats in the Morris water task and in other test procedures ([Morris, Garrud, Rawlins, & O'Keefe, 1982](#); [Poucet, 1989](#)). Similar observations have been reported using a distal cue instead of a proximal cue ([Hines & Whishaw, 2005](#)). Moreover, in studies in which rats established home bases next to two landmarks (e.g., a large black box and a segment of a white wall), animals continue to preferentially dwell in the two locations even in the absence of the cues ([Lehmann et al., 2007](#)).

Nonetheless, it is important to point out that conditioned home base preferences tend to be short lived such that rats make several short trips back to the previously cued location, but do not persevere in visiting these locations during long probe tests. Indeed, [Travis et al. \(2010\)](#) observed that rats tended to orient and make direct trips back to the cued home base location, but the trips were largely concentrated in the first half of the 30 min probe test. The authors concluded that after several visits to the previously cued location, rats likely established home bases in alternative locations.

The fact that rats express a conditioned preference for a previously cued location suggests the possibility that other forms of reinforcement can guide regional preferences for home base establishment. It has previously been speculated that the point of entry into an environment may serve as an organizing spatial feature to solve spatial problems and guide subsequent behavior ([Clark et al., 2015](#); [Eilam & Golani, 1988](#); [Golani, 2012](#); [Martin, Harley, Smith, Hoyles, & Hynes, 1997](#)). In a seminal study by [Golani, Bronchti, Moualem, and Teitelbaum \(1981\)](#), it was shown that when rats are first placed in an unfamiliar location,

they subsequently display a sequence of “warm-up” behaviors composed of successive horizontal and vertical movements that escalate in size from the point of placement in the field. The expression of these warm-ups in relation to the point of entry point suggests that the first point of contact may form an organizational feature for subsequent exploration and home base behavior. [Nemati and Whishaw \(2007\)](#) tested this hypothesis by placing rats on a large circular open-field in the presence or absence of proximal cues, or in complete darkness. Regardless of test condition, rats showed a strong preference in establishing their home bases near the initial place of entry. Specifically, rats organized their excursions from and returns to this location, stopped at that location more frequently, and spent a significantly greater amount of time there. Home base behavior was exhibited at the entry point regardless of where the place was located in the open-field (edge vs. center of the arena). In addition, home base preference at the entry point was modulated by the salience of nearby proximal cues, suggesting that local stimuli can be rapidly associated with home base locations. Again, it is possible that home bases at entry may serve to optimize security, while at the same time organize subsequent exploratory movements. However, the behavior may also be organized in relation to path integration—a navigation strategy utilized in unfamiliar and featureless environments that enables accurate orientation in relation to a home location ([Gallistel, 1990](#); [Etienne & Jeffery, 2004](#); [Whishaw & Tomie, 1997](#)). Because in featureless environments path integration relies on self-movement cues (e.g., vestibular, motor, proprioceptive), this form of navigation can be prone to errors and therefore requires frequent updating in relation to a stable reference. Thus, returns to the point of entry might be linked to a general need to correct inaccuracies in the path integration process ([Hines & Whishaw, 2005](#); [Nemati & Whishaw, 2007](#); [Redish, 1999](#)).

2.2. Mouse home base behavior

It is well documented that wild mice set-up home sites or nests from which they secure resources and avoid predation ([Blanchard, Griebel, & Blanchard, 2001](#)). However, reports on the home base behavior of mice in laboratory settings have largely been inconsistent. Notably, several studies have reported that mice, when placed in a featureless open-field, generally differ from rats in that they fail to restrict their stops, grooming, and rearing to specific locations ([Fig. 3](#)) ([Clark et al., 2006](#); [Gorny, Gorny, Wallace, & Whishaw, 2002](#)). The absence of regionally restricted stopping behavior in featureless environments has been replicated in a number of mouse strains ([Dvorkin, Benjamini, & Golani, 2008](#);

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).

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).

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