SEX DIFFERENCES IN PHYSICAL SKILL LEARNING WITH CONSEQUENCES FOR NEUROGENESIS AND CELL SURVIVAL IN THE HIPPOCAMPUS

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ABSTRACT OF THE DISSERTATION

Sex differences in physical skill learning with consequences for neurogenesis and cell survival in the hippocampus

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In animal models of learning it has been proposed that males and females use different pathways to perform particular behaviors and thus perform differently on a variety of training tasks. These sex differences in performance and learning have consequences for numerous measures of plasticity and brain function. The dentate gyrus (DG) of the adult hippocampus gives rise to thousands of new neurons every day, yet a majority of these cells die off within one to two weeks of their birth. Our laboratory has previously reported that learning increases the number of new neurons in the brain by rescuing them from death. These new neurons are capable of being rescued from apoptosis using the accelerating rotarod, a physical skill learning task, in adult male rats. In the first experiment here, we tested the hypothesis that a modified version of this task, which increases motivation, will rescue a greater number of new cells than
training with the traditional motor skill task. We trained adult male and female rats on the standard rotarod and the modified “motirod” task. The trained males successfully learned their respective tasks and retained significantly more new cells in the DG than untrained males. On the other hand, females performed better on the motirod than they did on the rotarod, and thus, female trained on the motirod retained a significantly greater number of cells than females trained on the rotarod and untrained females. To our knowledge this was the first demonstration of sex differences in performance on the rotarod and motirod, both gross motor skill tasks. In the second experiment we performed the same procedures as in the first but examined pubescent males and females. The trained pubescent males and females successfully learned both tasks and as a result rescued significantly more newly born DG cells than untrained animals. The data did not indicate any sex differences in learning the tasks, which is in agreement with the literature stating that many sex differences in learning tend to emerge after puberty.

Adolescence, and, more importantly, the impact of stress during adolescence, is the least studied of the developmental stages of life. Sexual aggression during the adolescent years is one of the most traumatic and stressful of life experiences and approximately 30% of young women worldwide are the victims of such abuse. In the third set of experiments we employed a novel animal model developed in our laboratory that mimics early life trauma in pubescent female rodents. We developed this animal model in order to examine the effects of this type of aggressive stress experienced by adolescent women.
hereafter known as Sexual Conspecific Aggressive Response (SCAR). During the SCAR experience females were repeatedly exposed to an aggressive adult male for 30 minutes every third day throughout pubescence in order to mimic chronic stress and we examined how this impacted future learning on the motirod task. We also examined the effects of motirod training on cell survival in the DG of the hippocampus in these same female animals. There were no differences in learning the motirod task between pubescent females exposed to an adult male during pubescence and those that were not. However, the SCAR animals rescued significantly less newly born DG cells as a result of training compared to NO SCAR animals. There were also no differences discovered between the SCAR and No SCAR females in either cell proliferation or cell survival in the DG.

Based on our behavioral and hormone analyses, the SCAR experience was indeed stressful to the females that were exposed to the adult males during adolescence. Our findings that the SCAR animals were not susceptible to the positive effects of learning on cell survival are particularly interesting because the majority of research has demonstrated that successful learning increases the retention of newly born cells in the DG and rescues them from cell death. It is likely that the stress of the SCAR procedures interacted in a complex manner with the effects of learning and cell survival. Future research on the interplay among stress, neurogenesis and learning will be necessary to elucidate our findings as well as designing experiments that include both males and females in order to better understand the impact early life stress and vulnerability to certain psychological disorders. Our SCAR model will serve as a useful tool in order to
better understand how the female brain responds to sexual trauma and aggression that occurs during pubescence. Our model could ultimately lead to clinical interventions for young women who have experienced the severely detrimental trauma caused by sexual abuse and aggression.
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GENERAL INTRODUCTION

*History of adult neurogenesis*

Neurogenesis is the birth and growth of functional neurons in the brain. In the past this process was thought to only occur during development and there was controversy over whether or not this process occurred in the adult brain (Altman & Das, 1965). However, based on the past few decades of research on neurogenesis it has become widely accepted in the scientific community that the adult brain does in fact produce new functional neurons. Adult neurogenesis is reported to occur in a variety of species, such as birds (Goldman & Nottebohm, 1983), insects (Cayre, Malaterre, Scotto-Lomassese, Strambi, & Strambi, 2002), rodents (Altman, 1962; Gage, 2002; Kaplan and Hinds, 1977; Kempermann et al., 1998), primates (Gould et al., 1999), and humans (Eriksson et al., 1998). The two brain regions in mammals, where adult neurogenesis has been discovered to occur, are the olfactory bulb and the hippocampus. The hippocampus is a brain structure with a high degree of functional and structural plasticity, and is known to be crucial in the processes of learning and in the formation of certain forms of memories, namely episodic in humans and spatial memories (Squire, 1992). Therefore, the hippocampus is an important brain region to study in order to gain a better understanding of adult neurogenesis and how learning may impact this process.

With the advent of 5-bromo-2-deoxyuridine (BrdU), investigations on adult neurogenesis have become more refined. BrdU is a thymidine analog that
incorporates itself into the DNA of cells during the S-phase of mitosis and, once labeled with BrdU, newly born cells can then be visualized through immunohistochemistry techniques and quantified by stereology (Miller & Nowakowski, 1988). Neurogenesis is a complex process and in the adult rat hippocampus it begins with the proliferation of neural progenitor cells (NPC’s) in the subgranular zone (SGZ) of the dentate gyrus (DG) (Cameron, Woolley, McEwen, & Gould, 1993). Through a combination of BrdU and cell-type specific markers, such as neuron-specific nuclear protein (NeuN), the phenotype of these NPC’s can be determined through this double labeling using confocal microscopy (Kuhn, Dickinson-Anson, & Gage, 1996). Most of these newly born cells will differentiate into dentate granule cells (DGCs), but a small portion of them become glial cells. Once they have differentiated, these newborn neurons undergo a long developmental period of maturation, involving distinct morphological and physiological changes, before becoming synaptically integrated into the hippocampal circuitry (van Praag et al., 2002).

It is estimated that the DG of the adult hippocampal formation generates approximately ten thousand new neurons every day (Cameron & McKay, 2001; Gould et al., 1999). Under “normal” conditions, most of the new cells die, even before they have fully matured into neurons; therefore it is important to distinguish between cell proliferation and cell survival when examining the factors that are involved in the regulation of adult neurogenesis. Cell proliferation can be defined simply as the division or mitotic activity of NPCs in the SGZ of the DG. Because more than half of these newly born cells die within weeks of their birth,
the rate of cell proliferation is not indicative of the rate of cell survival, which is
the incorporation of these newly born cells into the existing circuitry of the adult
hippocampus.

**Factors modulating cell proliferation**

Neurogenesis in the hippocampus changes with age; the number of cells
in the DG in the rodent decreases by at least half between the time of
pubescence and adulthood (Klempin & Kempermann, 2007; Kuhn et al., 1996).
In one of the first studies to investigate an age related decline in adult
hippocampal neurogenesis, it was reported that there is a significant decrease in
cell proliferation from when comparing six month old and 21 month old female
rats (Kuhn et al., 1996). In this same study, they also compared the number of
proliferating cells in 6, 12, and 27-month-old rats and observed a significant
decrease from 6-12 months but there were no significant decreases from 12-27
months. Rats are considered to be in adulthood at 6 months of age, middle-aged
at 12 months, and senescent at 27 months. Through BrdU labeling of the dividing
newly born cells, the researchers determined that it was likely the reduced
proliferative activity in the DG that contributes to the age related decline in
neurogenesis. In a related study, Kempermann and colleagues (1998) also
observed a decline in cell proliferation in female mice from 6-18 months. The
mechanisms underlying the age-related decline in hippocampal neurogenesis still
remain poorly understood, although a number of factors have been implicated.
These include a general loss of the number of neural precursor (stem) cells in the
DG, deficits and impairments in cell survival, as well as impaired commitment to
a neuronal fate (Encinas et al., 2011; Kuhn et al., 1996; Rao, Hattiangady, & Shetty, 2006).

The decrease in neurogenesis throughout the lifespan is likely a consequence of normal aging processes, there are a number of extrinsic factors that are thought to play a role in the proliferation of adult hippocampal neurons. Some of the earliest studies that examined how experience modulates aspects of neurogenesis involved the use of an environmental enrichment (EE) paradigm (Kempermann, Kuhn, & Gage, 1997; Kempermann et al., 1998). The EE paradigm was developed in the 1960’s in an attempt to study how environmental complexities might influence brain plasticity and behavioral measures in the rodent (Rosenzweig, 1966; Rosenzweig et al., 1962). An EE involves raising multiple animals in a housing environment that contains such items as toys, tunnels, and running wheels in order to stimulate social interaction and exploratory behavior. This housing environment is often varied to allow for novel experiences and learning in the animals. Studies using an EE to investigate neurogenesis have revealed that this type of housing environment reduces the amount of cell death in the DG but will also increase neurogenesis when compared to control animals raised in standard housing conditions (Brown et al., 2003; Bruel-Jungerman, Laroche, & Rampon, 2005; Kempermann et al., 1997; van Praag, Kempermann, & Gage, 1999; Young, Lawlor, Leone, Dragunow, & During, 1999). In terms of neuronal structure, the main findings of these studies demonstrated that animals living in an EE displayed an increase in the number, density, branching and arborization of the newly born granule cell dendrites in the
DG. These early studies demonstrate that an EE has a profound impact on the synaptic plasticity of the hippocampus and the newly born cells in the DG are associated with these positive effects. The following studies that will be discussed demonstrated that it is likely that exercise on a running wheel is the positive contributing factor involved in proliferation in an EE paradigm.

An early study by Kempermann and colleagues (1997) investigated the effects of raising animals in an EE compared to standard housing and what effect this has on different components of neurogenesis. In order to study cell proliferation, mice were housed in either an EE or standard cages for 40 days and were then injected for 12 consecutive days with BrdU and sacrificed 24 hours after the last injection. This study revealed that raising animals in an EE did not have an impact on cell proliferation. Two separate groups of animals, one group of EE and one group of standard housed animals, were also housed for 40 days prior to the 12 days of BrdU injections and sacrificed 4 weeks later to investigate cell survival. It was determined that the EE mice had a greater number of surviving new neurons compared to the standard housed mice. These mice were also tested on a spatial memory task during the 4-week period following the BrdU injections. The EE animals learned to perform faster than controls on the Morris Water Maze (MWM), a spatial learning task (Morris, 1984). These results suggested that the EE-induced increase in cell survival may be a contributing factor to the enhanced cognition observed in the EE animals. An important component in the EE paradigm that must be considered when analyzing any aspect of neurogenesis due to this manipulation is the inclusion of
a running wheel in the enriched housing environment. In a follow up to this initial study, the researchers then attempted to elucidate which specific components of an EE were responsible for the resulting neurogenic impact (van Praag, Kempermann, et al., 1999). This study examined cell proliferation and cell survival in 5 different groups of animals: MWM learners, yoked control swimmers, voluntary wheel runners, EE housed, and standard housed animals. The important findings from this study revealed that cell proliferation was significantly increased in the wheel running group only and that the surviving number of newborn cells in the DG was higher in both animals that ran in a running wheel and EE housed rats when compared to the MWM learners, swimmers and standard housed rats. These findings highlight the fact that it is likely that physical activity on a running wheel in an EE is the contributing neurogenic component of this housing paradigm. Indeed, in a more recent study that examined groups of animals housed in either an EE with or without a running wheel, concluded only those given access to voluntary running displayed enhanced cell proliferation and neurotrophin levels (Kobilo et al., 2011). This was determined for both animals, either living in the EE that were isolated or housed with other animals, further evidence of it being the physical activity and not the social aspect of the EE as the reason for the positive neurogenic effects.

Numerous studies since have supported that exercise and physical activity increased cell proliferation in the DG of rodents (Fabel et al., 2003; T Kitamura, Mishina, & Sugiyama, 2003; Kronenberg et al., 2006; H. van Praag et al., 2007). Whereas many of these studies examined voluntary exercise by providing...
animals with free access to a running wheel, studies have also demonstrated that forced exercise, such as treadmill running, also increased cell proliferation in the DG (Kim et al., 2002; Lou, Liu, Chang, & Chen, 2008; Trejo, Carro, & Torres-Aleman, 2001). When evaluating the effects of exercise on cell proliferation it is important to determine what is the necessary amount of physical activity required to obtain these positive effects. Research has shown that one day of physical activity was sufficient to increase cell proliferation (Steiner, Zurborg, Hörster, Fabel, & Kempermann, 2008), and it appeared that this increase in proliferation peaked after 3 days and continued to be positively effective for up to 10 days of running consecutively (Kronenberg et al., 2006). These findings highlighted the plasticity of the hippocampus and how important the role of experience is in neurogenic processes.

The mechanisms through which exercise exerts proliferative effects have been studies extensively, and a number of factors have been implicated in this phenomenon. Most importantly, the positive effects of exercise on cell proliferation have been strongly associated with an enhancement in the synaptic plasticity of the hippocampus (Bliss & Collingridge, 1993). A number of these studies concerned the effect of exercise on long-term potentiation (LTP), which is a physiological and putative model of learning and memory (Bliss & Lomo, 1973). LTP in the hippocampus is the facilitation of synaptic pathways by high frequency stimulation that resulted in a prolonged increase in the amplitude of excitatory postsynaptic potentials in the target neurons (Bliss & Collingridge, 1993). Recordings from hippocampal DG slices have revealed an increase in LTP
amplitude in animals given access to running wheels when compared to controls (van Praag, Kempermann, et al., 1999). In studies that examined recordings in vivo, LTP significantly increased in the DG of rodents that were both given voluntary access to a running wheel and forced to run on a treadmill, over sedentary animals (Farmer et al., 2004; O'Callaghan, Ohle, & Kelly, 2007).

Studies have also implicated neurotrophic growth factors as being the key mediators of this proliferative effect of exercise. These studies have revealed that neurotrophins, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1), are all upregulated by exercise or physical activity (Kohara, 2001; Trejo et al., 2001; Wu et al., 2008).

**Factors regulating cell survival**

As previously mentioned, the rates of cell proliferation in the adult DG did not necessarily or always predict the number of cells that ultimately survive. Much of the research implicated certain forms of learning as a critical factor that increased the survival of these newly born adult DG cells. Before discussing the role of learning in cell survival, it is important to understand some of the early physiological and morphological changes that newly born cells undergo. During the first week after their birth, these cells, in the SGZ, differentiate and begin a short migration to the inner granule cell layer (GCL) of the DG. Once in the GCL, and at approximately 10 days old, these newly born cells extend short radial processes, or spineless dendrites, that reach into the inner molecular layer of the DG. Then, between 10-14 days old, the immature neurons project axons through
the mossy fiber pathway to form efferent synapses with hilar cells and pyramidal cells in the CA3 area of the hippocampus.

Figure 1. Diagram of the rat hippocampus.

At this point, immature cells are receiving synaptic gamma-Aminobutyric acid (GABA)-ergic input from interneurons of the hilus, which depolarizes the cell and is dependent on a Na+-K+-2Cl- co-transporter called NKCC1. The role of NKCC1 is to regulate the resting membrane potential of the immature neurons (Ge et al., 2006). Thus, GABA-dependent depolarization is likely one of the first processes that regulates survival of adult born neurons. For example, knockdown of NKCC1 in vivo delays dendritic development and reduces the number of newly born cells that survive in the DG (Ge et al., 2006; S. Z. Young et al., 2012). The dendritic growth observed in these new cells were facilitated by a GABA receptor agonist
Thus, in the early stages of development in the newly born adult DG cells, GABA is the primary neurotransmitter involved in these processes.

By the end of the second week, newly generated cells are considered to be in a very critical period, because about half of the newly born cells die at this time (Cameron et al., 1993). At approximately 14 days old, the newly born adult cells begin to develop elaborate dendritic processes along with beginning to receive functional glutamatergic synaptic inputs via ipsi- and contra-lateral mossy fiber cells and from mature granule cells. Because the number of efferent and afferent target neurons does not change in the brain, these adult born cells must compete for these sites of synaptic contact with the pre-existing population of DG cells. Thus it appears that the survival of adult born cells in the DG is highly dependent upon and regulated by this activity-driven competition (Ge, Sailor, Ming, & Song, 2008; Jessberger & Kempermann, 2003). At this stage, the electrophysiological properties of the immature adult born neurons are different from the mature neurons; they are highly excitable and can respond to weaker stimulation (Ge, Yang, Hsu, Ming, & Song, 2007). The stimulation of perforant path fibers to the DG, using a high-frequency stimulation, can be induced in the DG, along with enhancing the survival of the newly born neurons in this same region (Bruel-Jungerman, Davis, Rampon, & Laroche, 2006). The enhanced plasticity of the newly born neurons is mediated by N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs), which are located on the membranes of these adult born cells (Ge et al., 2007; Snyder, Kee, & Wojtowicz,
One study, which utilized an NMDAR antagonist, to inhibit LTP induction, examined the survival rate of these adult born neurons (Kitamura, Saitoh, Murayama, Sugiyama, & Inokuchi, 2010). This same study demonstrated the survival effect, induced by high-frequency stimulation of perforant path fibers to the DG, was restricted to the 7-10 day old period of these cells. Moreover, when the same researchers pharmacologically blocked LTP with an NMDAR antagonist, this resulted in a suppression of enhanced cell survival by the high-frequency stimulation. These findings suggested that the survival effect is modulated by the glutamatergic input and NMDARs. However, they do not rule out the activity of surrounding neurons and how they might indirectly impact the survival of newly born cells. Therefore, a study was conducted using a retrovirus-mediated, single-cell gene knockout technique to investigate whether the survival effect was input-dependent and regulated by a neurons own NMDA-type glutamate receptor (Tashiro, Sandler, Toni, Zhao, & Gage, 2006). This study demonstrated that there is a critical time period (1-2 weeks old) in which the survival of a newly born neuron is regulated through their own NMDARs in an input-dependent, cell-specific manner. Thus, it has been established that the survival of newly born hippocampal DG neurons is influenced by factors, such as GABA and glutamate signaling, NMDA receptor subtypes, and LTP-induced structural plasticity. In addition, there appears to be a critical time period in which experiential factors play a role in the survival of adult born DG neurons.

*Learning and neurogenesis*
Research has revealed that there is an increase in apoptosis levels at specific sites, such as the DG, where the birth and proliferation of these adult born cells occurs (Biebl, Cooper, Winkler, & Kuhn, 2000). This further substantiated the fact that the adult born cells in the DG might be replacing or competing for survival with the existing mature neurons in these areas. It has also been determined that the survival of the newborn cells is highly sensitive to experience. This has been backed up by research demonstrating that the structural plasticity of the hippocampus is highly modulated by experience, where certain types of learning have been implicated in the process of rescuing the adult born cells from death. One of the first studies to implicate environmental factors influencing cell survival in the DG were performed in the avian songbird species (Paton & Nottebohm, 1984). These studies examined adult born DG neurons in vivo and discovered that they integrated into the existing hippocampal circuitry and were responsive to learned auditory stimuli. However, this was before the advent of BrdU labeling. Therefore these cells could not be visualized for their specific properties. As mentioned earlier, through the use of BrdU labeling techniques, early researchers, such as van Praag and colleagues, were able to perform their pioneering work on neurogenesis and the observations of animals raised in an enriched environment (Kempermann, Gast, & Gage, 2002; Praag, Christie, Sejnowski, & Gage, 1999; Praag et al., 2002). Activity on the running wheel in the EE paradigm increased cell proliferation in the hippocampus, but did not impact cell survival. Therefore, other components of the EE must be increasing the survival of these cells, and it is likely that the
numerous opportunities for learning provided by the EE that are responsible for this phenomenon (Kempermann et al., 1997, 1998).

Gould and colleagues (1999) were the first to demonstrate an association between learning and the survival of adult born neurons in the DG. This study examined whether associative learning was capable of rescuing adult born neurons and if this differed between hippocampal- and non-hippocampal-dependent tasks. The two hippocampal-dependent tasks used were trace eyeblink conditioning and spatial navigation in the Morris water maze (MWM), whereas the non-hippocampal-dependent tasks employed were delay eyeblink conditioning and a cued version of the MWM task. They demonstrated that hippocampal-dependent tasks were capable of enhancing the survival of the newly born cells in the DG. Moreover, hippocampal-dependent learning rescued cells that were born 1-2 weeks prior to the time of training, suggesting that this is the critical time period during which learning must occur and coinciding with the time when newly born adult cells undergo apoptosis (Cameron et al., 1993). This critical time period for experience-dependent modification involving newly born adult neurons and how this affected their survival has been supported by a study that examined an EE and later activation of the adult born neurons (Tashiro, Makino, & Gage, 2007). This study revealed that a one-week exposure to an EE only resulted in cell survival when it occurred during the first 3 weeks of the birth of the cells. They also examined the expression of immediate early gene (IEG) products, which is indicative of neuronal activation. They discovered that surviving neurons are responsive when the animals are re-exposed to the EE,
which suggests that the selective survival of the newly born neurons may be mediated through experience-dependent modification of the existing hippocampal circuitry.

A more recent study supported the finding that there is a critical time period for learning to enhance the survival of adult born cells. This study examined through an associative learning task, trace eyeblink conditioning, the impact on cell survival at three different training time points after being labeled with BrdU. These training time points included 30 minutes, 1 week, or 3 weeks (Anderson, Sisti, Curlik, & Shors, 2011). Training on this associative learning task only rescued adult born cells when they are approximately 1-2 weeks of age. However, when training occurred either before or after this time period, learning did not impact the survival. A similar finding was also observed in the case of spatial learning in the Morris water maze. In this study, rats were trained on the task 1 day, 6 days, or 11 days after receiving an injection of BrdU and only training that occurred from days 6-10 rescued a significant number of cells (Epp, Spritzer, & Galea, 2007).

The survival of newly born adult neurons increased when animals were trained on certain tasks, however, this did not directly indicate that it was due to learning. Not all animals learn task that they are trained on and even if they do, there are still individual differences among the animals to consider. However, our laboratory and others (Curlik & Shors, 2013; Gould et al., 1999; Waddell & Shors, 2008) have consistently observed positive correlations between learning performance and cell survival. We have also determined that not all types of
learning are capable of rescuing these newly born DG cells from death. The different types of learning not capable of rescuing these newly born cells from death include delay eyeblink conditioning, and a cued version of the MWM, both of which are hippocampal dependent tasks (Beylin et al., 2001; Gould et al., 1999). In addition, not all hippocampal dependent tasks are capable of rescuing cells either. Using a shorter trace interval in eyeblink conditioning still requires the hippocampus, but did not enhance cell survival (Waddell, Anderson, & Shors, 2011). Moreover, even non-hippocampal dependent tasks, such as contiguous trace eyeblink conditioning, rescued newly born adult DG cells from apoptosis (Dalla, Bangasser, Edgecomb, & Shors, 2007). These findings suggested that the hippocampus is neither necessary nor sufficient to enhance cell survival. As a result, for learning to positively impact the survival of these cells, the learning must be successful and difficult to achieve (Curlik and Shors, 2013; Dalla et al., 2007; Leuner et al., 2006; Leuner et al., 2004; Waddell and Shors, 2008).

Sex Differences in Learning

Sex differences in learning have been well documented in the literature. It is widely reported that males outperform females in spatial learning tasks (Galea, Kavaliers, & Ossenkopp, 1996; Galea & Kimura, 1993) and certain fear conditioning paradigms (Maren, De Oca, & Fanselow, 1994; Pryce, Lehmman, & Feldon, 1999). In humans, males made fewer errors and required fewer trials to learn while learning a new route on a map (Galea & Kimura, 1993). The authors suggested that this was likely due to sex differences in learning strategy, rather than learning ability. Similarly, males often outperform females during training on
the spatial navigation task using the Morris Water Maze (Beiko, Lander, Hampson, Boon, & Cain, 2004; Galea et al., 1996; Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996). During fear conditioning, an animal learns to associate a contextual cue with an aversive stimulus, typically a footshock, it is reported that females generally express less conditioned freezing behavior than males during both the acquisition and testing phases of the procedure (Maren et al., 1994; Pryce et al., 1999). Meanwhile, adult females tend to outperform males in associative learning tasks such as trace and delay eyeblink conditioning (Dalla et al., 2009; Leuner et al., 2004; Waddell et al., 2008). Most relevant to the present research study are studies that reported females to outperform males during training on operant conditioning tasks requiring active avoidance (Dalla, Edgecomb, Whetstone, & Shors, 2008; Shors et al., 2007).

**Importance of studying sex differences**

Much of the research discussed thus far has relied on results of studies only performed on male rodents. The reports in the pre-clinical research and the reliance on findings from only male animals obscures the fact that there are significant sex differences in brain morphology and various aspects of emotion, behavior, and cognition. It is important to not only increase the number of female only studies, but also conduct studies that compare both male and female animals in order to target sex-specific clinical interventions, whether psychological or pharmacological. The inclusion of sex differences in pre-clinical research could have important implications in sex-skewed neurological disorders such as stroke, Parkinson’s disease, depression, and anxiety. The primary
concern that is most often cited as a reason for studying only males in animal research is that the female estrous cycle might be a significant confounding variable when interpreting findings. However, a recent meta-analysis of rodent studies has provided evidence that there does not appear to be any more variability in studies of females in different phases of their estrous cycles than in studies using only male animals (Prendergast, Onishi, & Zucker, 2014). These findings are important to consider because based on the complexity of the brain, in general, it would be difficult to suggest that any one factor, such as the female estrous cycle, could play a crucial and solitary role in any type of neuroscientific finding. We know that multiple brain regions, systems and pathways contribute to almost every aspect of behavior and cognition; therefore it is unlikely that controlling for stage of estrous cycle would have any significant impact on data collected in animal research.

**Stages of development in the adolescent rodent brain**

Adolescence in rodents has been classified as having 3 stages: prepubescence from post natal day (PND)21-34, mid-adolescence from PND34-46, and late adolescence from PND46-59 (Tirelli et al., 2003). There are significant morphological changes that occur during this time of maturation in the adolescent rodent brain. Many studies have revealed that this developmental time period in the brain is marked by a high incidence of plasticity, both functionally and structurally (Romeo & McEwen, 2006). The three brain regions most important to learning and memory, the medial prefrontal cortex (mPFC), hippocampus, and amygdala go through extensive remodeling during
adolescence. It is important to note that these brain regions that undergo significant changes during adolescence are high in the expression of corticosteroid receptors, particularly the glucocorticoid receptor (GR). These receptors are involved in the regulation of the negative feedback system of the hypothalamic-pituitary-adrenal (HPA) axis and conversely this system then influences the structure of these brain systems. During adolescence, the synapses in these regions, especially the hippocampus, become more complex and the brain structures increase in volume. For our purposes here, the changes in the hippocampus during this developmental period include high rates of neurogenesis that decreases up until adulthood, as well as decreases in the density of dendritic spines (He & Crews, 2007). The decreases in neurogenesis and morphology of dendritic spines during adolescence suggest that the hippocampus must be going through a period of significant pruning in its circuitry and since the adolescent brain is highly plastic, any experiential factors could have profound effects. Numerous studies have concluded that adolescence is a time in which the brain is extremely sensitive to activation of the HPA axis, typically through the stress response system of the brain (Green & McCormick, 2013; McCormick & Mathews, 2010; Romeo & McEwen, 2006). Therefore, during this time, if the brain is exposed to stressors it will undoubtedly affect these developing stress systems and likely lead to long-lasting changes or disturbances in such systems and alter their function in later stages of life. It is surprising that there are so few studies investigating the long-term effects of stress during adolescence, and furthermore, these studies rarely investigate...
female rodents, therefore it is important to perform experiments in which we investigate both of these factors.

**Animal models of early life stress**

The majority of studies on “early life stress” thus far have focused on the long-lasting effects of prenatal and neonatal stressors on neurobiology and behavior later in life, with little attention to the adolescent time period. In order to interpret the present findings and relate them to the previous literature, it is important to take into consideration that there is a large degree of variability in studies on the effects of stress in early life. In animal models of chronic stress, the most commonly used manipulations are physical and psychological. Some typical physical stressors that have been used are damp bedding, restraint, tilting of the cage, and footshock. The most commonly used psychological manipulations used for stress paradigms are loud noise, predator odor, overnight illumination and isolation housing. In addition to these types of stressors, animal models of social stress have also been employed. One study in particular used a variation of the social defeat model, which was specifically the maternal defeat stress (Bourke & Neigh, 2011). This model exposed an intruding female rat to a postpartum, lactating female, a phenotype known for aggressive behavior, because the new mother attempts to defend her newborn pups. This study found that the female rat exposed to the aggressive female exhibited behaviors indicative of depression and an enhanced startle response. While this study examined adult female response, the important factor was about the use of an ethologically relevant stressor that resulted in behavioral deficits. In addition to
the physical and psychological aspects of this type of stress, there is a social aspect that makes the experience more ethologically relevant.

One other example of an ethologically relevant stressor used in animal studies is the social instability stress (SS) model, which can involve the changing of cage mates, crowding, and isolation (Herzog et al., 2009; Oines, Murison, Mrdalj, Grønli, & Milde, 2012). These procedures can be used alone or in combination, and can last between a few days and a few months. Another variation of social stress is the resident-intruder paradigm (Buwalda, Scholte, de Boer, Coppens, & Koolhaas, 2012). This paradigm involves pairing a young rat with a larger adult male for a particular amount of time over the course of the study. Studies that have used different types of psychosocial manipulations have revealed that this type of stressor is capable of activating a more complex response involving multiple neurotransmitter and hormonal systems that is not possible using the more traditional methods.

Another important factor to consider, when investigating early life adversity, is the age at which the animals experience the stressor. A majority of the research thus far has focused on the effects of stress in early postnatal life, usually within 1 to 2 weeks of birth. The most commonly used stressor during this time period is maternal deprivation, where the dam is removed from the cage containing her pups for a set period of time. This type of stressor is significant, because it has been well established that maternal behavior is an important factor in shaping the behavior and responsiveness of the offspring as adults (Meaney, 2001). The results from studies on early life stress and its impact on
measures of learning and neurogenesis are mixed and highly dependent upon
the duration of the stressor and more importantly, the exact time in which the
stressful experience occurs. However, the majority of the studies do indicate that
early life stress does cause lasting reductions in neurogenesis in the DG as well
as impairments in cognitive and emotional measures.

It has been established that environmental factors will exert their greatest
impact on brain structures during the cascade of developmental processes (Rice
& Barone, 2000). The hippocampus, and, especially, the DG, is in the process of
developing and differentiating during these first 2 weeks after the animal is born.
Therefore, adverse manipulations are likely to have their most prominent and
lasting effects during this time period. However, adolescence is also a sensitive
period for stress to exert an impact because the HPA axis and related brain
regions, like the hippocampus, are continuing to mature over this developmental
period. Adolescence, and more importantly the impact of stress during
adolescence, is the least studied of the developmental stages of life. Therefore, it
is very important to examine the effects of stress during this critical time period.

**Adolescent stress and impact on learning in adulthood**

There are large variations in the design of experimental procedures that
make it difficult to compare between studies of early life stress. In addition to this
major factor, which includes type of stressor, duration of stressor, number of
exposures, and the exact age at which the stress occurs, studies differ in time of
testing the effect of stress on learning. Not only are the effects on cognition
stressor-specific, these effects may also differ if tested immediately after the
stress occurs or at a later time in adulthood. Some forms of chronic stress during adolescence do appear to affect some forms of learning in adulthood. A previous study (Isgor, Kabbaj, Akil, & Watson, 2004) examined the effects of either a daily variable physical or social stress regimen in adolescent male rats from PND 28-PND 56 on spatial navigation of the MWM. The physical stressors in this study included forced swim, restraint, cold, noise, and ether as well as social stressors, which included isolation, crowding, novel environment, and litter shifting. This study placed the animals into two stress groups, physical stress and social stress, as well as a control group. These groups were tested on the MWM 24 hours after the last stress exposure and a different set of animals were tested three weeks later following the last exposure. Both the physical and social stress groups performed better than the controls when tested 24 hours later. However at the third week follow up testing, the physical stress group expressed impaired performance compared to the other two groups. This group also expressed a significant decrease in hippocampal volume. These results suggested that the physically stressful experience during adolescence alters the ongoing maturation of the hippocampal circuitry.

Another study (Avital & Richter-Levin, 2005) revealed a delayed deficit in performance on the MWM that was demonstrated using only 30 minutes on an elevated platform, which was considered a brief stressor, from PND 28-PND 30. The animals that were stressed during adolescence were impaired on the MWM in adulthood compared to control animals. Interestingly, if the stressed animals were given an acute stressor just prior to the testing in adulthood, their
performance was enhanced when compared to controls as well as animals that were not exposed to any acute stressor. This research study’s findings were interpreted as the animals having a better coping ability to stress later in life, adulthood, only because they had experienced stress during adolescence. Experience with a stressor in the past and learning from it will result in better coping and understanding of stress later.

Contradictory to the previous findings in delayed deficits, other studies, (Sterlemann et al., 2008; Toth et al., 2008; Tsoory, Cohen, & Richter-Levin, 2007) did not observe this. In a recent study (Sterlemann et al., 2008), Male mice exposed to a chronic social stressor for seven weeks over adolescence and into adulthood displayed deficits in MWM performance compared to non-stressed controls. However, these same mice did not differ from controls on object recognition or social recognition memory testing. Another study (Toth et al., 2008) exposed animals to variable mixed stressors, such as 23 hr food deprivation, acute swim stress and an elevated platform stressor, which began in adolescence and were administered for 60 days into adulthood, did not differ from non-stressed controls on shuttle avoidance learning in adulthood. On the other hand, if exposed to only 3 days of these variable mixed stressors in adolescence, the stressed animals demonstrated impaired shuttle avoidance learning when compared to controls in adulthood (Tsoory, Cohen, & Richter-Levin, 2007). These mixed findings highlight the fact that when studying adolescent stress and the effect on learning, many variables must obviously be taken into consideration, including length of stressor, age of stressor, and type of
learning and memory tests. Furthermore, the studies discussed thus far have only described the effects of stress in adolescent males while other studies (Toledo-Rodriguez & Sandi, 2007) have revealed that these effects can be varied between the two sexes. When male and female rats underwent a varied stress procedure from PND 28 – PND 30 and were trained in an auditory fear conditioning paradigm, which was then followed by context and cued recall testing soon after in adolescence and then again during adulthood expressed no gender differences (Toledo-Rodriguez & Sandi, 2007). There were no differences between the stressed males and non-stressed males during training, however the stressed females froze to the conditioned stimulus (CS) more than non-stressed females. When tested in adolescence and adulthood, there were no differences between stressed and control animals of either sex in context and cued recall, but the stressed females displayed less freezing behavior than controls during adulthood.

In other studies (McCormick et al., 2012; McCormick, Nixon, Thomas, Lowie, & Dyck, 2010) that involved a social instability stressor daily from PND 30 – PND 45, both male and female stressed rats displayed deficits in a spatial object location task during adulthood compared to their unstressed controls. These findings accentuate the importance of age, sex, and stressor type when investigating the consequences of adolescent stress on learning. Specifically, stressors that involve a social aspect in which aggressive behavior occurs is a more ecologically valid animal model of human interaction. One of the most traumatic experiences for an adolescent female is the exposure to sexual abuse
or aggression by an adult male. Based on the research thus far, we know very little about how these forms of experiences alter the female brain. Therefore, we need to create animal models that can mimic these types of circumstances in order to better understand how the experience will affect the young females brain and behavior later in life.

**Summary and Aims**

The current literature has revealed that sex differences exist in different types of learning and this is typically dependent on the type of learning task. Furthermore, it has been established that learning that is successful and difficult to achieve is capable of rescuing newly born adult DG hippocampal cells from death. The first experiment presented will discuss findings on how males and females perform on a physical skill learning task and what impact this will have on cell survival in the DG. The rotarod is a rotating cylindrical rod that is used to measure physical skill learning performance in rodents, since performance on this skill has been proven to increase over consecutive days of training (Buitrago, Schulz, Dichgans, & Luft, 2004). Using an accelerating version of rotarod procedure, we reported that acquisition of new physical skills required to perform the task increases the number of these cells that survive (Curlik, Maeng, Agarwal, & Shors, 2013). This procedure requires many trials to acquire the physical skill to remain on the rod. It is, however, distinctly different than aerobic exercise because it does not increase cell number but rather rescues cells that are already present from death (Curlik et al., 2013). In addition to the standard accelerating rotarod, we modified the task by placing a shallow pool of cold water
beneath the rod as a motivational feature. The cold water appeared to motivate
the animals to learn to remain on the rod for longer periods of time rather than
simply stepping off the rod. In the present experiment, we will examine whether
sex differences in learning these physical motor skill tasks exist and what
consequences this might have on cell survival in the DG. It was hypothesized
that training with the addition of this motivating feature, which we refer to as
“motirod” training, would enhance performance and thereby increase the number
of surviving cells in the hippocampus. To our knowledge this will be the first
demonstration of sex differences in learning on the rotarod, a gross motor skill
task (Buitrago et al., 2004).

The Office of Research on Women’s Health (ORWH) and the NIH has
recently developed a strategic plan that includes studies on sex and gender
differences in many areas of biomedical and behavioral basic research (Clayton
& Collins, 2014). One of the goals was to determine how sex contributes to the
vulnerability to disease and then translating this information into new drugs and
therapies for the sexes, including personalized approaches. It is clear that animal
models of sex differences are necessary to reach these goals. Thus, studies,
such as the present studies, that examine sex differences in learning, and how
those sex differences influence brain structure, are important.

In addition, we will also be examining sex differences throughout
development by examining these differences in learning and neurogenesis in a
male and female population of pubescent rodents. The third hypothesis, of the
present study, looked to examine if there would be no differences in learning the
rotarod and motirod tasks between male and female pubescent rats, and that learning these tasks in adolescence would rescue a significantly greater number of newly born hippocampal cells compared to untrained animals. Previous research (Hodes & Shors, 2005; Kanit et al., 2000) has reported that sex differences in learning tend to emerge after the pubertal period, where male and female pubescent rodents perform similarly on tasks, such as trace eyeblink conditioning and Morris water maze learning. In addition, our laboratory (Curlik, DiFeo, & Shors, 2014) has recently demonstrated that pubescent male rodents produce a significantly greater number of newly born cells in the hippocampus than adults and, as a result, rescue a significantly greater number of these cells when they learned a trace eyeblink task successfully. The second experiment in the present study investigated the performance of adolescent male and female rats on the rotarod and motirod tasks, which are the same physical skill learning tasks that we used to examine in the adult animals. As in the adult study, we also investigated what impact these physical skill learning tasks had on cell survival in the DG of newly born pubescent hippocampal cells.

Adolescence, and, more importantly, the impact of stress during adolescence, is the least studied of the developmental stages of life. Therefore animal models that examine the effects of stress during this time period are critical in preclinical research. Sexual aggression, during the adolescent years, is one of the most traumatic and stressful of life experiences and approximately 30% of young women worldwide are the victims of such abuse. The third set of experiments will reveal our findings using a novel animal model developed in our
laboratory that mimics early life trauma in pubescent female rodents. We have
developed an animal model in order to examine the effects of this type of
aggressive stress experienced by adolescent women hereafter known as Sexual
Conspecific Aggressive Response (SCAR). Our SCAR model consists of
exposing a pubescent female rat to a sexually experienced adult male
conspecific for 30 minutes in a novel context from both animals’ home cages. It is
hypothesized that this exposure will be a stressful experience for the adolescent
female and that there will be negative consequences on behavior, learning, and
neurogenesis as a result of chronic SCAR exposures.

Past research (McCormick et al., 2012, 2010; Toledo-Rodriguez & Sandi,
2007) that has investigated the impact of chronic stress during adolescence thus
far has been mixed but many studies have demonstrated that animals that
undergo stress during this developmental period do display deficits in learning
tasks during adulthood. These studies revealed that rats that were exposed to
chronic stressors, such as auditory fear conditioning and spatial object location
tasks, during adolescence were impaired on learning tasks performed during
adulthood when compared to unstressed animals. Therefore, the researchers in
the present study hypothesized that female rats exposed to our SCAR procedure
during adolescence would show impairments in performance on the motirod
physical skill learning task when compared to females that did not undergo the
SCAR exposures. We also hypothesized that the SCAR females trained on the
motirod would rescue a significantly less number of newly born cells in the DG
compared to the unstressed females as a result of this impaired learning. The
data presented here will provide evidence that SCAR is a useful animal model for understanding the negative consequences in young women resulting from sexual trauma and aggression experienced during adolescence.
GENERAL METHODS FOR ALL EXPERIMENTS

Subjects

Male and female Sprague-Dawley rats were bred at Rutgers University in the Department of Psychology. At 28 days after birth, animals were weaned and housed in groups of 2-3 males and 2-4 females in standard plastic shoebox style cages (44.5 cm long by 21.59 cm wide by 23.32 cm high). Animals were given access to food and water *ad libitum* and were maintained on a 12:12 hr light-dark cycle; the light cycle began at 7 a.m. and ended at 7 p.m. All handling and experiments were carried out in the light portion of the cycle. All experiments were conducted with full compliance with the rules and regulation specified by the PHC Policy on Human Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals. The Rutgers University Animal Care and Facilities Committee approved all procedures.

BrdU immunohistochemistry and quantification

The majority of adult born hippocampal cells die within 1 and 3 weeks of birth unless they are rescued from death during that time through new and effortful learning (M. L. Anderson et al., 2011; Daniel M Cursik et al., 2013; Daniel M Cursik & Shors, 2011). Therefore, twenty-one days after the BrdU injection all animals were deeply anaesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were extracted and post-fixed in 4% paraformaldehyde at 4 degrees Celsius for 24-48 hrs to preserve the tissue structure before being transferred to phosphate buffered saline (PBS). A vibratome was used to obtain 40 µm coronal
sections through the entire rostral-caudal extent of the DG in one hemisphere. This is the standard practice in our laboratory, as no differences in neurogenesis have been observed in the DG of the right or left hemisphere (Anderson et al., 2011; Dalla et al., 2007). Every twelfth slice was mounted onto a superfrost glass slide (Fisher, Suwane, GA, USA) and allowed to air dry. Once dry, the tissue was then stained using standard peroxidase methods to visualize the cells that incorporated BrdU as described previously (Curlik & Shors, 2011). Briefly summarized, the tissue was pretreated with heated 0.1 M citric acid (pH 6.0). The tissue was then rinsed with 0.1 M PBS, incubated in trypsin for 10 minutes, and denatured in 2N HCl for 30 minutes with PBS rinses in between. The tissue was then incubated overnight in primary mouse anti-BrdU (1: 200, Becton-Dickinson, Franklin Lakes, NJ, USA) and 0.5% Tween-20 (Vector Laboratories, Burlingame, CA, USA). The next day, tissue was rinsed and incubated in in biotinylated anti-mouse antibody (1: 200, Vector Laboratories) for 60 minutes and then placed in avidin-biotin-horseradish peroxidase (1: 100, Vectastain ABC Kit, Vector Laboratories) for 60 minutes. The tissue was then placed in diaminobenzidine (DAB SigmaFrost tablets, Sigma, Atlanta, GA, USA) for four minutes, rinsed, counterstained with 0.1% cresyl violet, dehydrated, cleared, and coverslipped with Permount glue (Fisher Scientific, Fair Lawn, NJ, USA).

Quantitative analysis was performed blind to the experimental condition by coding each slide. Estimates of the total number of BrdU-positive cells were determined using a modified unbiased stereology protocol (Gould et al., 1999; West, Slomianka, & Gundersen, 1991). The number of BrdU-positive cells in the
DG of each slice (granule cell layer and hilus) were counted by hand at 1000X on a Nikon Eclipse 80i light microscope. The number of cells was multiplied by 24 to obtain an estimate of the total number of BrdU-positive cells in the entire DG of both hemispheres.

**Physical Skill Training**

The rotarod (Four-station Rotarod for rat, Model #ENV575, MED Associates Inc., Georgia, VT, USA) is a cylindrical rod that is elevated 26.75 cm above a platform. The rod can either accelerate or maintain a constant velocity over a five-minute period. Previous studies have shown that performance on the accelerating rotarod improves over several days (Buitrago et al., 2004; Curlik et al., 2013), and we have previously demonstrated that adult male rats trained on the accelerating rotarod retain significantly more new hippocampal cells (Curlik et al., 2013). It is presumed that rodents acquire this task because of a natural inclination to run. In a recent study researchers set up exercise wheels in the wild and monitored the wheels with automated cameras and motion detectors in order to determine whether mice would voluntarily run on the wheels (Meijer & Robbers, 2014). This study reported that wild mice displayed similar running wheel behavior to mice captive in a laboratory setting. These results suggest that rodents have a natural inclination for running activity and that wheel running can be ruled out as pathological phenomenon developed only in animals housed in a captive laboratory setting. To further enhance their motivation to perform the task, we modified the standard version of the rotarod by placing ice cold (3-6°C) water directly under the rod (approximately 55 mm deep). Animals were
removed from the water as soon as they fell off the rod and immediately dried off with a towel in order to prevent hypothermia. We termed this modified version of the task the “motirod”.

Groups of animals were trained on either the standard accelerating rotarod (“rotarod”) or the modified motirod task. Rotarod and motirod training consisted of four trials per day over four consecutive days. Animals in both training conditions were placed on the rotarod while stationary, facing in the opposite direction that the rod began rotating. Thus, animals had to move forward in order to remain on the rotating rod. Each trial began once all animals were placed on the rod in the correct orientation. In all trials the rotarod linearly accelerated from 1.47 cm/sec to 14.74 cm/sec over a five-minute period. After the first five minutes of a trial, the rod no longer accelerated and remained at a constant maximum velocity of 14.74 cm/sec. The latency to fall from the rod (in seconds) was the recorded behavioral measure of the task. Animals were allowed to remain on the rod until they fell off or until 10 minutes had passed. The time from the start of one trial to the start of the next trial was twenty minutes.
CHAPTER 1

Introduction

Over the past 15 years, our laboratory has reported a number of rather dramatic sex differences in rodent models of learning. In these studies (Shors, 2001; Wood, Beylin, & Shors, 2001; Wood & Shors, 1998), female rats learned a classically-conditioned eyeblink response faster than males, but this exposure to a stressful event can suppress the same type of learning in females while actually enhancing performance in males. However, during operant conditioning tasks, females often outperform males and do not express the performance deficits typically observed in males, a phenomenon referred to as “learned helplessness”, a putative model of depression (Dalla et al., 2008; Shors et al., 2007). These sex differences in performance and learning have consequences for numerous measures of plasticity and brain function. In the case of classical eyeblink conditioning, females retained more new neurons in their hippocampus than did males, simply as a result of sex differences in learning (Dalla et al., 2008). In the present experiment, we report yet another example of a sex difference in learning and performance. In this particular instance, the sex difference only emerges as the task demands change, thereby illustrating the importance of attending to sex differences while remaining aware of their dynamic response to changing experimental conditions.

As discussed, learning increased the number of new neurons in the adult brain by rescuing them from death (Shors, 2014). These cells are generated in the hippocampus of the adult brain. Under “normal” conditions, most of the new
cells die, even before they have fully matured into neurons. Recently, we revealed that acquisition of new physical skills required to perform the accelerating rotarod procedure increases the number of these cells that survive (Curlik et al., 2013). Despite the positive effects of rotarod training on cell survival, there were performance outcomes that diminished our enthusiasm for using this particular task. Specifically, many rats would simply step off the rod once it began to accelerate. Since there was no consequence for this behavior, we hypothesized that the rats were not motivated to perform the task. To address this problem we placed a shallow pool of cold water under the rod. It was hypothesized that training with the addition of this motivating feature, which we refer to as Motirod Training, would enhance performance and thereby increase the number of surviving cells in the hippocampus. In line with the recent provision by the NIH in partnership with the Office of Research on Women’s Health (ORWH) for increased research on sex differences (Clayton & Collins, 2014), we examined performance in both males and females and observed robust sex differences, as described below. Moreover, it will be discussed that the sex differences in learning produced significant consequences for the survival of new cells in the DG of the hippocampus.

**Methods**

**Subjects**

Four groups of animals were used to investigate whether sex differences were observed during training with a gross motor skill task (the "rotarod"; Fig.2a)
and whether there were differences among and between adult male and female rats when this task was modified by including ice water as a motivating factor (the “motirod”; Fig. 2b). These four groups consisted of males trained on the rotarod (n=10), males trained on the motirod (n=8), females trained on the rotarod (n=11), and females trained on the motirod (n=10). Rotarod and motirod training was as described in the general methods and consisted of 4 trials per day over 4 consecutive days.

Figure 2: Physical skill training for Experiment 1. (A) The standard accelerating rotarod apparatus. (B) The “motirod: apparatus, which was similar to the standard rotarod, however, cold water was placed below the rod to motivate animals to remain on the rod. Groups of animals were trained on either the standard accelerating rotarod or motirod task. Training consisted of 4 trials per day over 4 consecutive days. Animals were allowed to remain on the rod for each trial until they fell off the rod or until 10 minutes had passed. The time from the start of one trial to the start of the next trial was 20 minutes, meaning there was a 10-minute intertrial interval (ITI) between each of the trials.

Experimental timeline

All groups received one single intraperitoneal injection of 5-bromo-2-deoxyuridine (BrdU; 200 mg/kg) at the start of the experiment when they were approximately 60 days of age (PND 60). An additional male and female group also received the BrdU injection at this time point and served as untrained
controls, they did not receive any training; male “no training” (n=9) and female “no training” (n=10). See experimental timeline in Fig. 3. BrdU immunohistochemistry and quantification was performed as described in general methods section.

![Experimental timeline](image)

**Figure 3: Experimental timeline for Experiment 1.** All rats received one single intraperitoneal injection of BrdU at approximately PND 60. Training began exactly one week after the BrdU injection. All groups (untrained and trained) were sacrificed three weeks after the BrdU injection.

**Data Analyses**

We first determined how well both the males and females learned the rotarod and motirod tasks, and whether performance would differ between the sexes and two training procedures. A repeated-measures analysis of variance (ANOVA) was used to analyze the behavioral data, with training condition (rotarod, motirod) and sex (male, female) as between-subject factors, and training trial (1-16) as the within-subject factor. We then examined whether the rotarod and motirod training increased the survival of newly born cells in the DG, and whether more cells were rescued in the trained animals compared to those that were not trained. The number of surviving BrdU-positive cells was assessed using a univariate analysis of variance with the rotarod condition (no training,
rotarod, motirod) as the independent measure, and the number of the surviving BrdU-positive cells in the DG as the dependent measure. Because the volume and density of the DG in males are significantly larger than females (Chow, Epp, Lieblich, Barha, & Galea, 2013; Dalla & Shors, 2009), all analyses of cell survival were performed separately for each sex. Pearson correlations were calculated to examine the relationships between motor skill performance and the number of surviving BrdU-positive cells in the DG. Post-hoc tests were performed using Tukey’s procedure.

Results

Physical skill learning

Repeated-measures analysis of variance of performance during physical skill training revealed a significant 3-way interaction between trial, sex, and rotarod condition \( F_{(15,35)} = 2.21 \ p < 0.001 \) with a main effect of trial \( F_{(15,35)} = 25.91, \ p < 0.001 \) and training condition \( F_{(1,35)} = 12.76, \ p < 0.01 \). Separate repeated-measures ANOVA’s conducted for each sex revealed main effects of trial for both males \( F_{(15,240)} = 10.39, \ p < 0.001 \) and females \( F_{(15,285)} = 17.5, \ p < 0.001 \). These results suggest that both sexes increased their latency to fall from the rod as training progressed. Separate repeated measures analysis of variance revealed that there was an increased latency to fall from the rod as training progressed over the 16 trials in all four trained groups: male rotarod \( F_{(15,135)} = 5.14, \ p < 0.001 \), male motirod \( F_{(15,105)} = 5.56, \ p < 0.001 \), female rotarod \( F_{(15,150)} = 1.81, \ p < 0.05 \) and female motirod \( F_{(15,135)} = 21.22, \ p < 0.001 \). These results
indicate that each of the four groups successfully learned the particular motor skill task.

To detect sex differences in learning, we compared the performance of males versus females during training on the two tasks. Separate repeated-measures ANOVA’s revealed a significant interaction between trial and rotarod condition for females ($F_{(15,285)} = 5.68$, $p < 0.001$; Fig. 4a) but not for males ($F_{(15,240)} = 0.72$, $p > 0.05$; Fig. 4b). These results indicate that females trained on the motirod outperformed females trained on the rotarod. However, there was no difference in performance in males trained on the rotarod compared to the motirod. These results present a clear and significant sex difference in acquisition of the two tasks, wherein the addition of the motivating stimulus had a more pronounced enhancing effect on learning in females but not in males.

Figure 4: Physical skill learning on the rotarod and motirod tasks in adult males and females. (A) Females trained on the motirod outperformed females trained on the rotarod ($p < 0.0001$). (B) Male rats trained on the motirod performed comparably to male rats trained on the rotarod ($p > 0.05$).

Body weights differ significantly between male and female rodents. To determine whether these differences account for the performance differences, we
conducted a Pearson correlation analysis between individual body weights and latency to fall from the rod. There was no correlation between average latency to fall from the rod in females ($r = -0.22$, $p > 0.05$), males ($r = -0.47$, $p > 0.05$), or in both sexes combined ($r = -0.29$, $p > 0.05$). These results suggest that body weight did not account for the sex difference in performance during training on the motor skill tasks.

**Neurogenesis: Effect of training on cell survival in females**

Animals were sacrificed three weeks following the BrdU injection, a time point when most cells that would have died would have already done so and the cells remaining tend to survive. Analysis of cell survival was performed separately for males and females due to differences in volume and density of their hippocampi. In the first analysis, we examined the effects of training versus no training on the number of surviving cells in either sex. We used a one-way analysis of variance, with training condition (no training, rotarod, motirod) as the independent measure, and the number of surviving BrdU+ cells in the total DG as the dependent measure. Results indicated a significant interaction between the number of BrdU+ cells and training condition ($F_{2,28} = 15.32$, $p < 0.001$; Fig. 5a). Post-hoc Tukey comparisons revealed that females trained on the motirod retained significantly more cells than females that were not trained ($p < 0.001$) and those trained on the rotarod ($p < 0.05$). Females trained on the rotarod retained more BrdU+ cells than the untrained females ($p < 0.05$). These differences between the three female training conditions were also observed in both the GCL ($F_{2,28} = 10.27$, $p < 0.001$) and the hilus ($F_{2,28} = 5.47$, $p < 0.05$).
Taken together, these results suggest that females that were trained with an accelerating rotarod task rescued more cells than untrained animals, and that when this task was modified to include a motivating factor the animals retained even more cells in the DG. These results are consistent with a previous study which reported that acquisition of a motor skill not dependent on the hippocampus will nonetheless increase the number of cells that survive in the adult DG (Curlik et al., 2013), and go beyond these data to indicate a more stressful and motivating task can further enhance the number of surviving cells. These likewise agree with reports that more effortful training conditions tend to rescue the most number of new neurons (Curlik et al., 2014, 2013; Curlik &
Although both trained groups of females successfully learned the motor skill, the females trained on the motirod outperformed those on the rotarod, and thus retained a greater number of cells. The overall impact that training had on cell survival is displayed in Fig. 5b, where an independent samples t-test suggests that females trained in either task rescued a significantly greater number of BrdU-labeled cells than in those that were not trained ($t_{(29)} = 28.25$, $p < 0.001$).

**Neurogenesis: Effect of training on cell survival in males**

In males a one-way ANOVA, with training condition as the independent measure (no training, rotarod, motirod), and the number of surviving BrdU+ cells in the total DG as the dependent measure, revealed no significant interaction between the number of BrdU+ cells and training condition ($F_{(2,24)} = 2.58$, $p = 0.10$; Fig. 6a). There was no interaction between the number of surviving cells and training condition in either the GCL ($F_{(2,24)} = 2.05$, $p = 0.15$) or in the hilus ($F_{(2,24)} = 3.11$, $p = 0.06$). However, an independent samples t-test between the number of cells in animals untrained versus trained on the motirod was significant ($t_{(15)} = 11.84$, $p < 0.05$). We did not test separate untrained control groups for each training condition and therefore cannot conduct an ANOVA with independent variables according to conditions on the rotarod test. However, we were able to use a t-test to further assess the overall impact of training (irrespective of the type of training) on cell survival. The t-test was used to compare the number of BrdU-labeled cells in trained male rats (motirod and rotarod) to those that were present in males that were not trained. A t-test revealed that the males that were
trained on either the rotarod or motirod retained significantly more cells in the DG than males that were not trained ($t_{(25)} = 23.11$, $p < 0.05$; Fig. 6b). The trained males also retained more cells than untrained males in the GCL ($t_{(25)} = 23.40$, $p < 0.05$) and the hilus ($t_{(25)} = 23.62$, $p < 0.05$). These results are consistent with the previous findings reporting that successful acquisition of a motor skill will increase the number of surviving newly born cells in the DG (Curlik et al., 2013). Nonetheless, training with the motivating feature did not further increase the number of surviving cells in the male hippocampus, even though it did in the female hippocampus.

![Figure 6: Neurogenesis: Effect of training condition on cell survival in adult males.](image)

(A) In males no significant differences were observed between training condition (no training, rotarod, motirod) and number of surviving cells in the dentate gyrus (p’s > 0.05). (B) In males, those that were trained (on either the rotarod or motirod) rescued significantly more cells than males that were not trained ($p < 0.05$).
Correlations between behavioral performance and cell survival

In females that were trained on either of the tasks, average performance across the 4 days of training correlated with the number of cells retained in the DG ($r=0.56$, $p<0.01$; Fig. 7). In trained females significant correlations were also observed between the number of surviving cells in the DG and the average performance on the first day of training ($r=0.61$, $p<0.01$), the third day of training ($r=0.58$, $p<0.01$), and the last day of training ($r=0.50$, $p<0.05$). The correlation between the number of cells rescued and the average performance across all training sessions was likely stronger in females due to the fact that the female motirod group outperformed all other groups, and therefore retained proportionately more cells.

![Figure 7: Correlation between average performance on physical skill task and the number of BrdU+ cells retained in adult females.](image)

In females trained on either the rotarod or motirod, the average latency to fall from the rod across the 4 days of training positively correlated with the number of cells retained in the dentate gyrus ($r=0.56$, $p<0.01$).
In the males trained with the motirod and rotarod, no significant correlations were observed between the average latency to fall from the rod across all four days of training and the number of surviving cells in the DG during rotarod \((r= 0.54, \ p=0.11)\) and motirod training \((r= 0.40, \ p= 0.32)\). This was also observed in females when examining the correlations for both training conditions separately; female rotatod \((r= 0.32, \ p= 0.36)\) and female motirod \((r= 0.51, \ p= 0.14)\). However, it was observed in both sexes that the correlations were all in a positive direction. Therefore, all trained males were combined and all trained females were combined for correlation analyses to give a larger sample size and to get a more distinct effect of training in general. In all trained males there was a significant positive correlation between the average performance across the four days of training and the number of cells surviving in the DG \((r= 0.49, \ p< 0.05; \ \text{Fig. 8})\). In trained males significant correlations were also observed between number of cells retained in the DG and average performance on last day of training \((r= 0.57, \ p< 0.05)\) and the last day average minus the first day average \((r= 0.56, \ p< 0.05)\).
Discussion

The present data indicated that sex differences in acquisition of a physical motor skill have consequences for neurogenesis in the adult brain, specifically by increasing cell survival in the adult DG. Groups of male and female rodents were trained on two different types of a physical skill learning task in order to determine how well they learned these tasks and whether these forms of learning were capable of preventing the death of new cells generated in the adult DG. Males performed similarly on both the standard rotarod task as well as on the motirod, which is a modified version in which cold water was included at the bottom of the platform to make the task more motivating. These trained males successfully learned their respective tasks and retained significantly more new cells in the DG than males that did not undergo any training. On the other hand,
females performed better on the motirod than they did on the rotarod, and thus, female trained on the motirod task retained a significantly greater number of cells than both females trained on the rotarod and untrained females. These results are in agreement with existing literature reporting that cells are capable of being rescued from death by successful learning (Curlik et al., 2013; Curlik & Shors, 2011; Dalla et al., 2007; Shors, 2014). To our knowledge this was the first demonstration of sex differences in performance on the rotarod and motirod, both gross motor skill tasks (Buitrago et al., 2004). However, as noted, this particular task was developed in our laboratory, and therefore no other studies have been conducted using it to study learning.

**Sex Differences in Learning**

Sex differences in learning have been well documented in the literature. It is widely reported that males outperform females in spatial learning tasks (Galea & Kimura, 1993; Galea et al., 1996) and certain fear conditioning paradigms (Maren et al., 1994; Pryce et al., 1999). In humans, males made fewer errors and required fewer trials to learn than did females while learning a new route on a map (Galea & Kimura, 1993). The authors suggested that this was likely due to sex differences in learning strategy, rather than learning ability. Similarly, males often outperform females during training on the spatial navigation task using the Morris Water Maze (Beiko et al., 2004; Galea et al., 1996; Perrot-Sinal et al., 1996). During fear conditioning, an animal learns to associate a contextual cue with an aversive stimulus, typically a footshock. It is reported that females generally express less conditioned freezing behavior than males during both the
acquisition and testing phases of the procedure (Maren et al., 1994; Pryce et al., 1999). Meanwhile, adult females tend to outperform males in associative learning tasks such as trace and delay eyeblink conditioning (Dalla & Shors, 2009; Leuner, Mendolia-Loffredo, & Shors, 2004; Waddell et al., 2008). Most relevant to the present findings are studies, which reported that females outperform males during training on operant conditioning tasks requiring active avoidance (Dalla et al., 2008; Shors et al., 2007). The sex differences reported here are largely consistent with these findings because animals trained on the motirod learned to avoid the aversive water condition through an operant response.

**Sex Differences in Learning versus Performance**

Sex differences in learning are oftentimes influenced and sometimes misinterpreted because of sex differences in performance. As past research revealed (Beatty & Fessler, 1977; Hyde & Jerussi, 1983), sex differences in fear itself can modify performance during fear conditioning. Also, female rats are more active than males, as indicated in running wheels and the open-field test. These differences can contribute to sex differences in fear conditioning, especially when the absence of movement rather than freezing is used to assess performance. Performance effects play an even more significant role in operant conditioning, because the animal must initiate an overt motor response. A classic example is learned helplessness, during which animals are first subjected to a one-way avoidance task [fixed ratio 1 (FR1)]. In this task, the animal must pass through the door of a shuttle box in order to avoid a footshock. Female rats tend
to escape sooner than males during training with a FR1 task (Dalla et al., 2008; Shors et al., 2007). Their performance is facilitated by their increase in activity when compared to males. During the test of helplessness, animals must then transverse the shuttle-box two times in order to avoid the footshock [fixed ratio 2 (FR2)]. As noted, females are more active and are less likely to freeze and consequently learn the correct response. In contrast, males are more likely to freeze and much less likely to enter the side of the shuttlebox in which the shock originally occurred, which one could argue shows that males are “smarter” (Beatty & Beatty, 1970; Shors, 2008; van Haaren, van Hest, & Heinsbroek, 1990). However, to avoid a female deficit interpretation, one might also conclude that females do not demonstrate learned helplessness (Dalla et al., 2008; Dalla & Shors, 2009).

Sex differences in performance likely contribute to the sex differences in motirod behavior as well. As described above, the motirod differs from the standard accelerating rotarod in that animals are presumably “motivated” to remain on the rod and thus avoid dropping into cold water just under the rod. The addition of this motivating feature increased the performance in females, but not in males. There are several explanations for these differences. It could be due to females being more active, although there were no sex differences in the rotarod, which is a task that similarly depends on activity. It could also be that the fear of the cold water is more potent in females.

Looking in a different direction, there is more likely to be sex differences in weight. Females weigh substantially less than do males and this weight
difference may give them an advantage for remaining on the rod. However, we conducted a correlation between body weight and performance and the analyses revealed no statistically significant correlations.

**Hormonal Regulation of Sex Differences in Learning**

Sex differences in learning are often influenced and sometimes mediated by sex hormones. During the classical eyeblink conditioning, a task in which females outperform males, the sex difference is most robust when females are trained in proestrus, a stage of the estrous cycle when estrogen levels are particularly high (Dalla & Shors, 2009; Shors, Chua, & Falduto, 2001). Females that are ovariectomized (without estrogen) perform similarly to males, suggesting the activational effects of estrogen and perhaps progesterone is important for enhancing performance in females (Wood & Shors, 1998). Although estradiol concentrations are clearly important, they do not appear to be acting alone to enhance performance (Leuner et al., 2004). Ovariectomized females that were provided physiological replacement of estradiol performed similarly to males (Leuner et al., 2004). That said, sex differences in eyeblink conditioning only emerge after puberty and are not observed during or before puberty (Hodes & Shors, 2005), which suggested that they are dependent on the emergence of a mature estrous cycle. Testosterone, which is present at higher concentrations in males, is not required to express sex differences in eyeblink conditioning but it does organize them in females. Females that are given testosterone at birth perform like males when they become adults (Shors, Miesegaes, et al., 2001). It would be of interest to determine whether the sex differences in motirod training
reported here are evident in pre-pubescent males and females, before they have sexually matured, and whether they are organized by the presence of testosterone during very early development. That experiment is the focus of the following chapter here.

**Cellular Proliferation versus Survival**

The present experiment focused on the effects of physical skill learning on cell survival and not on cell proliferation. To be specific, we examined the effects of physical skill training on new cells that were already present at the time training began. It is important to distinguish between these two aspects of neurogenesis, because they are differentially regulated and influenced by different conditions, such as growth factors (Aberg, Aberg, Hedbäcker, Oscarsson, & Eriksson, 2000), learning (Gould et al., 1999), exercise (van Praag et al., 1999), and environmental enrichment (Kempermann et al., 1997). Sex differences in neurogenesis have been reported, and these studies indicated that gonadal hormones may influence cell proliferation (Galea, 2008). One study (Galea & McEwen, 1999) examined the rate of cell proliferation in the DG of adult meadow moles as a function of sex and seasonal differences. Both proliferation and survival was enhanced in males and correlated with the concentrations of testosterone. On the other hand, large fluctuations in proliferation were observed in female voles across seasons, which also correlated with endogenous hormone concentrations. In laboratory rodents, however, females produced more new cells than did males with no apparent difference in cell survival (Tanapat, Hastings, Reeves, & Gould, 1999). In addition, high levels of estrogen were
positively correlated with cell proliferation in the female rats. These and other studies (Chan, Chow, Hamson, Lieblich, & Galea, 2014; Duarte-Guterman, Yagi, Chow, & Galea, 2015; Galea, 2008; Spritzer & Galea, 2007; Tzeng, Chen, Cherng, Tsai, & Yu, 2014) indicated that gonadal hormones modulate aspects of neurogenesis including cell survival. However, these studies are assessing the effects of hormones on how many cells survive as a consequence of enhanced proliferation, not how many survive that were already present when the hormonal manipulation occurred. As is evident, these are highly complex and dynamic systems and making generalizations about them is not realistic.

Two studies examined whether sex differences influenced learning then discussed this influence on cell survival. In the first study, adult animals were injected with BrdU once and then trained one week later with trace eyeblink conditioning. Females outperformed males during conditioning, which further increased the number of cells that survived (Dalla et al., 2009). In the second study (Chow et al., 2013), adult animals received a single BrdU injection and were then trained 6-10 days later on a spatial learning task. This study utilized the Morris Water Maze, which produced a different pattern of findings but a similar effect of learning; that is, males outperformed females and subsequently rescued a greater number of cells in the DG. The present study is in agreement with these findings, such that females outperformed males on the motirod task and thus rescued more cells in the DG, as a result. Although these studies used different types of training tasks, the findings are consistent because the
enhanced performance rescued more new cells from death, leading to an increase in cell survival.

It is well established that exercise increased neurogenesis in the adult hippocampus, primarily if not exclusively through an increase in cell proliferation (van Praag et al., 1999; Kobilo et al., 2011). The current lab recently examined the effect of exercise alone on cell survival and noted no change in cell number (Curlik et al., 2013). As in the current experiment, BrdU was injected once in adult rats. One week later, one group was trained on the accelerating rotarod and another group was given free access to running wheels. Even though animals that had access to the running wheels traveled approximately twenty times farther than the animals trained on the rotarod, their number of surviving cells did not increase, whereas numbers did increase in animals that were trained on the accelerating rotarod task. The newly born BrdU-labeled cells were already present when the exercise and/or training began, thereby indicating that exercise alone does not rescue new neurons from death, although it would presumably increase the numbers that are generated. Based on these findings, we would propose that the increase in cell number reported in the present study, in response to training on the motirod, reflected an increase in cell survival and not in proliferation and that the increase only occurred in response to learning the new physical skill.

**Fate of Adult-born Hippocampal Cells**

The results from the experiments presented here demonstrated that successful acquisition of these motor skills tasks rescued newly-born adult
hippocampal cells from death. While it is beyond the scope of this study, previous research has provided evidence that these adult born cells in the DG mature into functional neurons in the mammalian brain. Early studies implementing double labeling with neuronal markers reported that newly generated cells in the adult mouse hippocampus have a similar morphology to mature hippocampal neurons and display similar properties to these neurons (Gould et al., 1999; van Praag et al., 2002). These adult born cells in the DG are capable of generating action potentials and receive synaptic inputs from the perforant path (van Praag et al., 2002). Based on these and other studies it is widely accepted that these new adult cells in the DG become functional neurons that are integrated into the hippocampal circuitry (Vivar & van Praag, 2013). Most studies did not specifically target cells that are rescued from death by learning and it is possible that the fate could change as a result, although it is unlikely. After learning on a trace eyeblink conditioning task, more than 80% of the new cells that survived in the GCL were double labeled with BrdU and neuron specific markers class III beta-tubulin (TuJ1) and neuron specific enolase (NeuN). Moreover, these cells remain in the hippocampus more than two months after the learning experience, thereby indicating that they have been incorporated and are now part of the existing hippocampal circuitry (Leuner et al., 2004a).

**Sex Differences in Mental Illness and Health**

Women are especially vulnerable to mental illnesses that are induced or exaggerated by stressful life events. These illnesses include depression, post-traumatic stress disorder and social phobias (Kessler, 2003; Parker & Brotchie,
However, most neuroscientific experiments have been conducted exclusively in males. Because of this historical behavior, we know relatively little about sex differences in the brain, especially as they relate to the high incidence of stress-related mental illness in women. This is changing, thanks to the Office of Research on Women’s Health (ORWH) and the NIH strategic plan to include studies on sex and gender differences in biomedical and basic science research (Clayton & Collins, 2014). With these new guidelines, many such reports will be produced and it will be up to us, as scientists, to fully understand and translate the meaning and the meaningfulness of sex differences in learning into practical applications for women and men. Indeed, the results presented here were recently translated into a clinical intervention known as MAP Training, for mental and physical skill training (Shors, Olson, Bates, Selby, & Alderman, 2014). The idea behind this intervention is to increase the number of new neurons with aerobic exercise and then keep them alive through mental training with focused attention meditation. In a proof-of-concept study, we provided MAP Training to young traumatized women in the local community, who were recently homeless and taking care of their infants. To facilitate participation, we adopted an exercise routine similar to the popular Zumba dance program, which was developed for women. Eight weeks of training (twice a week) significantly increased their overall physical health (oxygen consumption) as well as decreasing measures of anxiety and depression. This is just one example of how we can capitalize on sex differences in performance and motivation to develop novel interventions, which enhance mental and physical health in humans.
Here, we have examined the presence of sex differences in learning on two forms of a gross motor skill task, the standard accelerating rotarod and the more motivating motirod task. Based on the previous literature on operant conditioning, we hypothesized that we would discover a difference between the sexes and that the females would outperform the males on both tasks, likely due to the fact that they are generally more active than males. Additionally, we hypothesized that performance for both sexes would be enhanced on the motirod as compared to the rotarod, because the cold water would serve as a motivating factor to remain on the rod. However, this was not the case; both males and females learn the rotarod task similarly and only the females showed an enhancement in learning performance on the motirod task. The sex difference reported here could be because the females found the cold water under the motirod more aversive, and thus motivated them to learn to remain on the rod for a longer period of time than did the males. In addition to sex differences in learning, we also tested whether successful learning of these motor skill tasks would be capable of rescuing newly born adult hippocampal DG neurons. We previously reported that successful learning of the rotarod task does in fact increase the number of surviving DG cells in adult males, and we hypothesized that learning on the motirod would significantly increase the number of rescued cells, because it is a more effortful and motivating task. Moreover, we hypothesized that learning on both tasks would increase the number of surviving cells as compared to untrained animals for both sexes. The present study demonstrated that the sex differences in learning these tasks led to significant
consequences for cell rescue in the adult DG of the hippocampus. The males and females that were trained (rotarod and motirod) rescued significantly more BrdU-labeled cells than compared to the control groups of untrained males and females. These findings are in agreement with the literature that reports an increase in the number of surviving cells in the DG through successful learning of a task that occurs at the time when these cells would typically begin to undergo apoptosis, which is approximately 1-2 weeks after being born. Furthermore, the females trained on the motirod rescued significantly more cells than both untrained animals and those trained on the rotarod. This is in agreement with existing studies that demonstrated that better performance on a learning task and also a task that is more difficult to master will result in a greater number of surviving DG cells. The females performed better on the motirod than on the rotarod, thus it makes sense that they would show a greater number of rescued cells as we had hypothesized. The males trained on the motirod and rotarod performed similarly, therefore it is expected that they would not display a difference in the number of surviving cells.

Our results from the present study are important for various reasons. We have developed a novel motor skill learning task, the motirod, that we have demonstrated to express sex differences in learning performance that ultimately produced consequences on neurogenesis in the adult DG. Specifically, we have discovered that females outperform males on this particular task and as a result rescued a significantly greater number of newly born adult hippocampal cells. It is not only important to discover different types of learning tasks that are capable of
increasing the number of surviving cells in adults, but when we revealed that males and females displayed a difference in performance on a particular learning task, it provided us with an avenue through which we can further investigate the underlying mechanism behind such differences. Thus, we can gain a better understanding of how the male and female brain differ in their neurobiology, which we can then translate to human research to develop critical sex-specific interventions and therapies for pathologies of the brain.
CHAPTER 2

Introduction

Most young adults, especially adolescents, enjoy sports and learning new skills that require physical effort and training. It is assumed that engaging in these activities is beneficial for their normal growth and development, but the benefits of these activities for the brain are less well described. In previous studies (Beylin et al., 2001; Curlik & Shors, 2011; Shors, Anderson, Curlik, & Nokia, 2012), we determined that physical skill training in a rodent model of learning increased the survival of newly-generated neurons in the hippocampus, a brain region necessary for many types of learning. Laboratory rodents were trained to maintain their balance on a large rod that rotated 360°. During each trial, the rod would rotate faster and faster, enhancing the effort (presumably both physical and mental) necessary to remain on the rod. Animals that were trained on this task expressed evidence of learning because the time that they remained on the rod increased over trials and days of training (Curlik et al., 2013). Most of the animals did not remain on the rod for the maximum length of the trial, again suggesting that learning continued to occur, even after several days of training. However, many of the animals would simply step off the rod once they learned that there was no consequence for doing so. Therefore, in a follow-up study, we increased the motivation to remain on the rod by placing cold water underneath the rotating rod. When the animals stepped off the rod, they would drop into the water, which became a major consequence. As expected, the animals were more likely to remain on the rod when the water was present than when it wasn’t (DiFeo, Curlik, & Shors, 2015). We have since termed this task the "motirod"
task, because it increases motivation to learn. In both cases, training with this new physical skill increased the survival of new neurons in the hippocampus. In the absence of training, most of the new cells died within weeks.

The pubescent brain produces significantly more new hippocampal neurons than an adult brain does (Curlik, DiFeo, & Shors, 2014). But like the adult brain, many of these new cells are subject to programmed cell death. In one study, approximately 40% of newly generated cells in the granule cell layer (GCL) and 80% of those in the hilus were no longer present three weeks after they were generated. However, animals that engaged in an effortful learning experience retained most of the cells that would have otherwise died. These findings are consistent with widely reported studies on the positive impact of learning on cell survival during adulthood (Curlik et al., 2013; Curlik & Shors, 2011; Shors et al., 2012). Because so many more cells are generated during adolescence, the effects of learning appear to have an even more profound consequence for cellular integrity in the hippocampus.

As noted, teenagers and pubescent children are motivated to and generally inclined toward learning new physical skills in school and elsewhere. Therefore, we thought it was important to determine whether learning new physical skills, which model those physical activities, would have a positive impact on neurogenesis during puberty. Specifically, we hypothesized that physical skill training in both sexes would increase the survival of newly generated cells in the pubescent hippocampus.

**Methods**
Subjects

Male and female Sprague-Dawley rats were bred at Rutgers University in the Department of Psychology. At 28 days, animals were weaned and housed in groups in standard plastic shoebox style cages (44.5 cm long by 21.59 cm wide by 23.32 cm high). Animals were given access to food and water *ad libitum* and maintained on a 12:12 hr light-dark cycle; the light cycle began at 7 a.m. and ended at 7 p.m. All handling and experiments were carried out in the light portion of the cycle. Experiments were conducted with full compliance with the rules and regulation specified by the PHC Policy on Human Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals. The Rutgers University Animal Care and Facilities Committee approved all procedures. An independent samples t-test revealed no significant differences in weight between the pubescent males (mean= 92.55 g) and pubescent females (mean= 87.06 g) ($t_{1,25} = 2.08, p > 0.05$).

Physical skill training

The rotarod (Four-station Rotarod for rat, Model #ENV575, MED Associates Inc., Georgia, VT, USA) is a cylindrical rod that is elevated 26.75 cm above a platform. The rod can either accelerate or maintain a constant velocity over a five-minute period. To further enhance motivation to perform the task, we modified the standard version of the rotarod by placing cold water directly under the rod (approximately 55 mm deep). Animals were removed from the water as soon as they fell off the rod and immediately dried off with a towel. As noted, this version of the task is referred to as the “motirod”. Groups of animals were trained
on either the standard accelerating rotarod (“rotarod”) or the modified motirod task. Rotarod and motirod training consisted of four trials per day over four consecutive days. Animals in both training conditions were placed on the rotarod while it was stationary, facing in the opposite direction in which it moved. Thus, animals had to move forward in order to remain on the rotating rod. Each trial began the animal was placed on the rod in the correct orientation. The rod linearly accelerated from 1.47 cm/sec to 14.74 cm/sec over a five-minute period. After the first five minutes of a trial, the rod no longer accelerated and remained at a constant maximum velocity of 14.74 cm/sec. The latency to fall from the rod (in seconds) was the recorded behavioral measure of the task. Animals were allowed to remain on the rod until they fell off or until 10 minutes had passed.

**Experimental timeline**

Four groups of pubescent animals were trained as followed: males trained on the rotarod (n=6), males trained on the motirod (n=6), females trained on the rotarod (n=6), and females trained on the motirod (n=6). All groups received one single intraperitoneal injection of 5-bromo-2-deoxyuridine (BrdU; 200 mg/kg) at 28 days of age (PND 28). Additional groups of pubescent male (n=6) and female (n=6) rodents were injected with BrdU injection at the same time point but not trained (Fig. 9).
Twenty-one days after the BrdU injection, animals were deeply anaesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were extracted and post-fixed in 4% paraformaldehyde at 4 degrees Celsius for 24-48h to preserve the tissue structure before being transferred to phosphate buffered saline (PBS). A vibratome was used to obtain 40 µm coronal sections through the entire rostral-caudal extent of the DG in one hemisphere. This is the standard practice in our laboratory, as no differences in neurogenesis have been observed in the DG of the right or left hemisphere (Anderson et al., 2011; Dalla et al., 2009). Every twelfth slice was mounted onto a superfrost glass slide (Fisher, Suwane, GA, USA) and allowed to air dry. Once dry, the tissue was then stained using standard peroxidase methods to visualize the cells that incorporated BrdU as described previously (Curlik & Shors, 2011). Tissue was pretreated with heated 0.1 M citric acid (pH 6.0), rinsed with 0.1 M PBS, incubated in trypsin for...
10 minutes, and denatured in 2N HCl for 30 minutes with PBS rinses in between. The tissue was incubated overnight in primary mouse anti-BrdU (1: 200, Becton-Dickinson, Franklin Lakes, NJ, USA) and 0.5% Tween-20 (Vector Laboratories, Burlingame, CA, USA). The next day, tissue was rinsed and incubated in in biotinylated anti-mouse antibody (1: 200, Vector Laboratories) for 60 minutes and placed in avidin-biotin-horseradish peroxidase (1: 100, Vectastain ABC Kit, Vector Laboratories) for 60 minutes. The tissue was placed in diaminobenzidine (DAB SigmaFrost tablets, Sigma, Atlanta, GA, USA) for four minutes, rinsed, counterstained with 0.1% cresyl violet, dehydrated, cleared, and coverslipped with Permount glue (Fisher Scientific, Fair Lawn, NJ, USA).

**Data analyses**

Quantitative analysis was performed blind to the experimental condition by coding each slide. Estimates of the total number of BrdU-positive cells were determined using a modified unbiased stereology protocol (Gould et al., 1999; West et al., 1991). The number of BrdU-positive cells in the DG of each slice (granule cell layer and hilus) were counted by hand at 1000X on a Nikon Eclipse 80i light microscope. The number of cells was multiplied by 24 to obtain an estimate of the total number of BrdU-positive cells in the entire DG of both hemispheres.

**Results**

The time spent on the rod during each trial was used as a dependent measure of performance. There were no interactions among the independent variables of sex (male versus female) or the type of physical skill training (rotarod
versus motirod) (p>0.05). However, as expected, there was a main effect of trial 
($F_{(15,210)}= 8.53, p= 0.001$), suggesting the animals were learning to remain on the 
rod longer over trials. Separate repeated-measures analysis of variance revealed 
that the latency to fall from the rod increased as training progressed over the 16 
trials in the four groups: male rotarod ($F_{(15,60)}= 2.47, p= 0.01$; Fig. 10a), male 
omotirod ($F_{(15,30)}= 4.15, p= 0.001$; Fig. 10b), female rotarod ($F_{(15,75)}= 2.49, p= 0.01$; 
Fig. 10a) and female motirod ($F_{(15,45)}= 3.70, p= 0.0001$; Fig. 10b). These results 
indicate that each of the four groups successfully learned either skill task and that 
the pubescent males and females performed similarly on both tasks.

**Figure 10: Physical skill learning on the rotarod and motirod tasks in pubescent 
males and females.** Males and females readily learned to remain on the rotarod (A) or 
the motirod (B) during puberty. There was no sex difference in performance, probably 
because they all learned quickly to remain on the rod throughout each trial.

The number of BrdU-labeled cells in the DG was used as a dependent 
measure and the type of training as the independent measure. There were no 
interactions between the type of training or sex on the number of BrdU-labeled 
cells (p>0.05). However, there was an overall effect of physical skill training on
the number of BrdU-labeled cells. The trained pubescent males and females retained significantly more BrdU+ cells in the total DG than the untrained pubescent males and females (n=9) \( (F_{(1,26)} = 6.03, p = 0.02; \) Fig. 11c). These effects were observed in both the GCL \( (F_{(1,26)} = 4.53, p = 0.04; \) Fig. 11d) and the hilus \( (F_{(1,26)} = 4.71, p = 0.04; \) Fig. 11e).

Discussion

Both males and females quickly and successfully learned the two motor skill tasks and their performances on either task were similar. Overall, training
increased the number of cells previously labeled with BrdU, a marker of cell mitosis. Therefore, training with these physical skill tasks was sufficient to increase the number of newly-generated cells, as has been reported in adults (DiFeo et al., 2015). We did not directly compare performance in the pubescent animals to adults in this study, but have reported that adult rats do not learn this task very well, particularly males (DiFeo et al., 2015). In contrast, most of the pubescent animals in the present study reached the maximal level of performance within just a day or two of training, which indicated that most of the learning occurred very quickly in pubescent rats. It is presumed that the young animals retained a large number of the new cells because they learned so well and so quickly, as well as the fact that they already produced more new cells than do adults (Curlik et al., 2013).

**Sex differences in performance**

Sex differences in learning and performance are reported in the literature, but many of these differences depend on performance variables, such as activity or weight (Shors, 2016). There were no significant differences in performance here but as noted, both sexes were performing near the maximum within just a few days of training, especially during training on the motorod task. It is not surprising that the animals learned this task so well; by remaining on the rod, they avoided falling into the cold water. It is nonetheless surprising that the pubescent animals learned this task so much better than do adults. Pubescent rats tend to be more active than adults and weigh less, both factors of which may contribute to the maximal performance during puberty. In a previous study
(Shors, 2016) with adults, the females learned these tasks faster than males. Because adult females weigh less than their age-matched males, they are better able to stay on the rod as it rotated. This revealed a sex difference in performance that is probably not attributable to inherent differences in learning processes, but rather one that is evoked by differences in physical characteristics. In the present study, the young males and females weighed the same, which likely explains why there were no sex difference in performance.

**Learning and cell survival**

Adolescence is an important developmental stage, during which the brain is undergoing extensive maturation and morphological changes (Romeo & McEwen, 2006), many of which occur within the hippocampus. An overproduction of axons and synapses during early pubescence is followed by a rapid increase in dendritic pruning by the end of adolescence (Anderson et al., 2000; Giedd et al., 1999). Some measures of plasticity decrease within the adolescent period (He & Crews, 2007), but those related to neurogenesis remains relatively high, at least as compared to levels in adulthood (Curlik et al., 2013). As a consequence, learning has a disproportionate effect on the cells and other substrates within the brain of the pubescent animal. We reported that many more cells were produced in the adolescent hippocampus and, although many of them died, many more survived in animals that were trained to learn a new task. In this study, animals were trained to learn an associative task during which animals learned to associate stimuli across time. This task depends on the hippocampus for learning and increased the survival of newly generated neurons.
in the hippocampus in adult (Leuner et al., 2006) and pubescent rats (Curlik et al., 2013).

Not all types of learning, such as delay eyeblink conditioning and a cued version of the Morris water maze, increased the survival of new neurons in the hippocampus in adulthood. Both of these types of learning were found to be hippocampal-independent tasks and do not rescue these cells from death (Beylin et al., 2001; Gould et al., 1999). However, not all hippocampal dependent tasks are capable of rescuing cells either. Learning to associate two stimuli across a very short trace interval (250 ms) required the hippocampus but did not enhance cell survival (Waddell et al., 2011). Moreover, even non-hippocampal dependent tasks, such as contiguous trace eyeblink conditioning, during which the animal learned to associate the two stimuli better and faster, because the first stimulus (the conditioned stimulus) was presented again with an unconditioned stimulus (US), did not rescue newly born adult DG cells from apoptosis (Dalla et al., 2007). Therefore, the types of tasks that increased cell survival are not necessarily dependent on the hippocampus, which almost are, but rather are tasks that were effortful to learn, meaning more trials of training were necessary to achieve an optimal level of performance (Curlik & Shors, 2013; Dalla et al., 2007; Leuner et al., 2004; 2006; Waddell & Shors, 2008). Importantly, learning the accelerating rotarod did not require an intact hippocampus (Curlik et al., 2013), even though more cells remained in the hippocampus as a consequence of learning. These reported findings were similar to those discussed above, in which the task did not depend on the hippocampus, but training nonetheless
increased the number of BrdU-labeled cells that remained. That being said, there are some important differences that will be discussed below.

First, to be discussed, aerobic exercise increased cell proliferation in the hippocampus and some studies (Hamilton and Rhodes, 2015; Kobilo et al., 2011; van Praag, Christie, Sejnowski, & Gage, 1999) reported an increase in cell survival as well. Previously, we examined the potential effects of exercise versus rotarod training on cell survival. Animals were injected with BrdU once and then either given the opportunity to exercise in a running wheel or trained on the rotarod. Only the animals trained on the rotarod retained more new cells when compared to animals that weren’t trained. We concluded from these data that the effect of rotarod training on neurogenesis was not attributable to exercise, per se, and that exercise itself did not increase cell survival (Curlik et al., 2014).

However, in this same previous study, animals were adults and they did not perform nearly as well as pubescents and not nearly as well as animals trained on the motirod task. Because young animals performed so well on the rotarod and motirod tasks, they exerted more physical effort than the adults did. Therefore, it is possible that some of the increase in cell number reported here is due to an increase in physical exertion and activity. Because training on the tasks involves both exercise and learning, it may be that the two manipulations together are especially effective at increasing neurogenesis under these conditions.

In other studies, we have proposed that the combination of mental and physical training, referred to as MAP Training increased the number of new cells
more than either activity alone (Curlik & Shors, 2013) and others have reported similar effects by combining exercise with environmental enrichment (Kempermann et al., 1997). Based on these rodent studies, we developed a clinical intervention for humans, also known as MAP Training. During the training, humans engage in twice weekly sessions of mental training with meditation and physical training with aerobic exercise. We have documented significant changes in brain activity and mental health outcomes with this combined training intervention in otherwise healthy young adults, depressed individuals, and young mothers, who were recently homeless and suffering untold traumas (Alderman et al., 2016; Shors et al., 2014). After just 8 weeks of training, the young adults with depression were less depressed and ruminated less about the past. They also expressed increased synchronized brain activity that were evoked during conditions that required cognitive control. Similarly, positive changes were observed in otherwise healthy individuals. Because neurogenesis cannot be measured in live humans, the connection to neurogenesis is only theoretical. But as one might expect, lifestyles that combine effortful training with physical activity are associated with greater brain health and overall well-being in young and older adults (Herzog et al., 2009).

The present data indicate that training on the rotarod and motirod tasks increased the number of BrdU-labeled cells in the pubescent DG. We did not double label these cells, therefore we cannot confirm that they are, in fact, neurons. However, newly generated cells in the hippocampus of young animals have matured into neurons with similar morphology and functional properties
(Ambrogini et al., 2004; Toni & Sultan, 2011) and in adults, the vast majority of BrdU-labeled cells differentiated into functional neurons and incorporated themselves into the existing hippocampal circuitry (Vivar & van Praag, 2013). In our previous studies (Leuner et al., 2004), more than 80% of new cells in the GCL double labeled with BrdU and neuron specific markers TuJ1 and NeuN after learning. Similarly, double-labeled cells were present in the GCL of the hippocampus more than two months after training, suggesting that they were relatively permanent and had integrated themselves into the circuitry of the hippocampus. That said, cells in the hilus of the DG could be less well characterized and some may not differentiate into neurons. In adult rodents, we observed very few new cells in the hilus and learning did not appear to increase their survival (Gould et al., 1999). But in adolescents, the effects of physical skill training did extend to these cells. Interestingly, we reported similar increases in adolescent hilar cells after training with trace eyeblink conditioning (Curlik et al., 2014). It would be important to further characterize these new cells and their incorporation into hippocampal circuitry, as well as their potential function in processed related to learning and memory during puberty.

**Conclusion**

The positive benefits of exercise on the cardiovascular, hormonal and muscular systems of the body are well established in adolescents and young adults. Increased cardiovascular fitness in teenagers is associated with decreased obesity and risk factors that predict cardiovascular disease later in life (Andersen, Bugge, Dencker, Eiberg, & El-Naaman, 2011; Tanha et al., 2011).
Studies of adolescent children consistently reported positive relationships between cardiovascular fitness and academic performance (Castelli, Hillman, Buck, & Erwin, 2007; Chaddock et al., 2012; Van Dusen, Kelder, Kohl, Ranjit, & Perry, 2011). More generally, aerobic exercise and physical activity improved and maintained cardiovascular fitness, which in turn positively impacted brain plasticity and function (Hillman, Erickson, & Kramer, 2008). Some studies (Berchtold, Castello, & Cotman, 2010; Hillman et al., 2009; Hillman et al., 2008) suggested that exercise can directly enhance cognition, with improvements in memory and executive control. However, aerobic exercise, on its own and without a mental skill training component, may not be as effective in improving cognitive function as combining mental and physical activities together (Alderman et al., 2016; Diamond and Ling, 2015; Sacco et al., 2015; Shors et al., 2014).

One of the primary ways in which adolescents and teens combined mental and physical activity is through their participation in organized and/or competitive sports. Over the past decade participation in youth sports in the United States increased more than 20%, with ~30-40 million young adults engaging in organized activities (Myer et al., 2011). The type of training procedures used in this laboratory study models the types of activities considered as athletic sports and/or physical training, at least to the extent that both require physical exertion, endurance and strength, along with gross motor skill training for agility, balance and coordination. Because of budget and time constraints, many schools have reduced or even eliminated their sports programs. Perhaps these laboratory data
and similar ones in pubescent animals, if applicable to young teenagers, could incentivize the institution of physical activity and sports programs for our youth.
CHAPTER 3

Introduction

Adolescence is a critical developmental period during which the brain undergoes extensive neurobiological changes, such as the maturation of the HPA axis along with overall structural remodeling of the central nervous system. In humans, numerous studies (Durston, Mulder, Casey, Ziermans, & van Engeland, 2006; Giedd et al., 1999; Romeo & McEwen, 2006) have reported during adolescence the brain is in the process of crucial changes, both structurally and functionally. It is during this developmental period that structures, most important to learning and memory, have undergone significant remodeling. Most notably and relevant structure to the present studies is the hippocampus, where the volume and complexity of this structure increased dramatically (Giedd et al., 1999; Gogtay et al., 2006). This critical period of development is surprisingly one of the least studied in animal research although studies (McCormick & Mathews, 2010) have shown that, similar to humans, adolescent rodents also showed significant changes in the brain regions important for learning and memory. Previous research (He & Crews, 2007; Koshibu, Levitt, & Ahrens, 2004; Yildirim et al., 2008) has also revealed that during adolescence the volume of the hippocampus increased drastically and there were significant decreases in neurogenesis and in the density of dendritic spines in the hippocampus from the start of pubescence to adulthood.

In addition, the hippocampus and other brain regions important for learning and memory, like the medial prefrontal cortex (mPFC) and amygdala, are involved in regulating the brain’s main stress response system, which is
known as the hypothalamic-pituitary-adrenal (HPA) axis (Herman, McKlveen, Solomon, Carvalho-Netto, & Myers, 2012). These structures, especially the hippocampus, have a high expression of glucocorticoid receptors (GR), which mediate the action of corticosterone, therefore the function of these brain regions are in turn influenced by glucocorticoids (Oitzl, Champagne, van der Veen, & de Kloet, 2010). In addition to the high plasticity of the hippocampus during adolescence, this is also a time in which the brain is highly sensitive to the activation of the HPA axis. Thus, exposure to stressors, during this time, will undoubtedly affect the development of brain stress systems and lead to long lasting changes in these systems and consequently alter the structure and function of brain regions associated with such systems. It is surprising that there are so few studies examining the long-term consequences of stress during adolescence.

There are various ways in which we can study early life stress and oftentimes it is difficult to compare between studies because of this. These features include, but are not limited to, duration, and number of stressor exposures, which vary between studies. Another important factor is the age at which the stress occurs, because throughout development stress has been demonstrated to affect the brain differently at different stages of life. Most of the research thus far has focused on the effects of stress in early postnatal life, typically within 1 to 2 weeks of birth. However, as discussed, adolescence is a sensitive time period for stress to exert any negative impacts. While much of the research on stress typically involves either acute or chronic physical stressors,
these do not necessarily mimic common human experiences. Arguably, a more ethologically relevant form of stress is one that involves a stressor consisting of a change in the animals’ social environment. One such stressor is social instability stress (SS) (Herzog et al., 2009; Oines et al., 2012), which is a stress paradigm used to study early life stress and involves the changing of cage mates, crowding and isolation. Another variation of social stress is the resident-intruder paradigm (Buwalda et al., 2012), which is the possibility of physical contact between two animals. This form of stressor is more relevant to the typical form of aggression that occurs in adolescent humans and results in various learning and behavioral deficits both immediately following the introduction of the stressor as well as later in life. Studies that have used different types of manipulations, in which there was a change in the animals environment, such as including the addition of another animal, has revealed that this type of stressor is capable of activating a more complex response involving multiple neurotransmitter and hormonal systems (Isgor et al., 2004; Yuen et al., 2012). These results would not be possible using the more traditional methods, such as footshock or swim stress. It would be important to develop an animal model that similarly acts upon these systems in order to be considered a valid and ethologically relevant stressor. This relevant stressor can then assess the long lasting consequences on the animals being exposed to this type of stress procedure.

To be gender specific, women are especially vulnerable to mental illnesses that are induced by stressful life events, and these disorders usually begin to emerge during puberty. These mental illnesses include, but are not
limited to, depression and anxiety disorders, such as post-traumatic stress disorder and social phobias (Kessler, 2003; Parker & Brotchie, 2010). One of the most stressful life experiences during adolescence and early adulthood is sexual trauma and abuse, which occurs more than three times as often in women than it does in men (Gault-Sherman, Silver, & Sigfúsdóttir, 2009) and is associated with the onset and diagnosis of a broad spectrum of mental and physical disorders during adulthood (Scott et al., 2011). Various human studies (Bremmer, Ilg, Thiele, Distler, & Hoffmann, 1997; Carrion et al., 2001; De Bellis et al., 2000; De Bellis, Keshavan, & Harenski, 2001; Stein, Yehuda, Koverola, & Hanna, 1997; Teicher et al., 1997; Vythilingam et al., 2002), on early exposure to physical and/or sexual abuse, have demonstrated associations between the resulting adulthood psychopathology and a reduction in the size of the corpus callosum, lowered hippocampal volume, and significant alterations of the frontal cortex. In a study (Anderson, LaPorte, & Crawford, 2000), which is most relevant to the present set of experiments, adult human subjects, who experienced repeated childhood sexual abuse, showed a significant reduction of hippocampal volume when the abuse occurred between the ages of 3-5 years old and between 11-13 years old. Trauma and aggression that occurred during early life experience has also been shown to dramatically increase the risk of depression and can result in a variety of disruptions in females (Chen, Epstein, & Stern, 2010). In addition to depression, there is also a high likelihood for women to develop PTSD after experiencing sexual abuse instances. This disorder has been associated with a decreased volume in both the amygdala and hippocampus and typically is
associated with deficits in various forms of learning, such as deficits in shuttle avoidance tasks and fear conditioning. (Weniger, Lange, Sachsse, & Irle, 2008).

As mentioned, much of the animal research on early childhood abuse and aggression has been performed on male rodents, therefore we know very little from preclinical research on how sexual trauma and aggression can alter the brain of adolescent females. To date, there has not been an animal model established in female rodents that mimics the traumatic experience of early life sexual trauma and aggression. In order to meet this need, the present study developed an animal model of early trauma during pubescence called Sexual Conspecific Aggressive Response (SCAR). In this model (SCAR), pubescent females are exposed to a sexually experience adult male conspecific for 30 minutes. It was devised to model select aspects of sexual trauma, such as fearful responses to an aggressive male, which would be observable by changes in affect and behavior. In the present experiment, females were repeatedly exposed to an aggressive adult male for 30 minutes every third day throughout pubescence in order to mimic chronic stress and examine how this impacted future learning on a motivating physical skill learning task, the motirod. It was hypothesized that females exposed to an aggressive adult male for 30 minutes, every three days throughout puberty, would be impaired in their performance on this task compared to animals that were not exposed to a male conspecific. The present study then examined the effects of motirod training on cell survival in the DG of the hippocampus in these same female animals, because past research (Dalla et al., 2009; DiFeo et al., 2015; Shors, 2014) revealed that the cells in this
region are known to be rescued from death when learning occurs. Both human and animal research (Jordan, Campbell, & Follingstad, 2010; Scott et al., 2011; Vythilingam et al., 2002) has demonstrated that sexual abuse during the critical stage of adolescent brain development is associated with a negative impact on the animals' cognitive measures, such as emotional processing and learning deficits. Due to these findings, the current research study hypothesized that the no SCAR group would outperform the SCAR group. We also hypothesized that if the no SCAR animals outperformed the SCAR animals on motoric training then they would thus retain a greater number of newly generated cells in the DG of the hippocampus. This hypothesis was driven by a plethora of research (Curlik et al., 2014, 2013; Curlik & Shors, 2011; Shors, 2014) from our as well as other laboratories, which reported successful learning positively impacted the survival of cells born between 1-2 weeks prior to the training experience.

**EXPERIMENT 3A:**

**Methods**

**Sexual Conspecific Aggressive Response (“SCAR”) Model**

The onset of puberty in rats begins at approximately PND 35 and ends at PND 56 (Ojeda & Urbanski, 1994). Therefore pubescent females at PND 35 began the SCAR procedure, and were exposed to an adult male conspecific for 30 minutes every 3 days for a total of 8 exposures. The SCAR exposures began when the pubescent female was at PND35 while the age of the male breeders varied in age from approximately 120-160 days old. In addition to age, there was a significant difference in the size of the adult males compared to the pubescent
females; pubescent females ranged between 120-220 grams, whereas the adult males ranged between 400-700 grams. All interactions were video recorded and behaviors were hand scored by two independent experimenters. The behaviors we investigated were ano-genital tracking events, pinning, mounting, and intromission attempts. An ano-genital tracking event consisted of the adult male following and sniffing the ano-genital region of the female and a tracking event was operationalized as the snout of the male touching or close to touching this region of the female for a time of approximately 1 second or more. A pin consisted of the adult male effectively restraining the female by either sitting on top of or holding her down with his paws. Of these behaviors, the two that were consistently observed during these interactions were ano-genital trackings and pins, which were analyzed and presented. In addition to these two measures, we calculated the number of escape behaviors made by the pubescent female, as this could be used as a behavioral measure of fear or stress. During an escape behavior, the female stood up on her hind paws and reached towards the top of the cage, as if trying to escape. These escape behaviors were the third measure used in statistical analyses. These three behaviors were counted in 10-min intervals across the 30 min SCAR exposure interval. These behaviors were compared between the pubescent female paired with the adult male (SCAR) and a separate group of control pubescent females paired with an adult female (female/female). We chose this control group in order to determine the sex specific effects of our SCAR model in terms of stress effects on the pubescent female. These behavioral measures were analyzed during the first SCAR
exposure and the last (8\textsuperscript{th}) SCAR exposure to examine whether the animals
habituated over time and the experience became less stressful for the pubescent female.

\textbf{Corticosterone assay}

The HPA axis is the brain’s main stress response system and is highly involved in the effect of stressors on cognitive function. In response to stress the paraventricular nucleus (PVN) of the hypothalamus releases corticotropin releasing hormone (CRH) and arginine vasopressin (AVP). This then stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) in the brain. The presence of ACTH then stimulates the release of the glucocorticoid corticosterone (CORT) by the adrenal cortex in the rodent brain, which is similar to cortisol release in humans. Corticosterone is therefore the endpoint of the brains stress response system and is a commonly used measure of stress levels in rodent models. Peak levels of CORT concentrations typically occurs approximately 10-20 mins after exposure to a stressor (Sapolsky, Romero, & Munck, 2000). Therefore, here we examined corticosterone levels following the SCAR exposures at 2 different time points and compared these to control groups in order to determine whether the SCAR experience is in fact stressful to the pubescent female.

Pubescent females were exposed to either the adult male breeder or an adult female (PND 60-120) for 30 minutes and trunk blood was collected 30 minutes after a single exposure. Animals were given a lethal dose of pentobarbital i.p. injection, quickly decapitated and trunk blood was collected.
Blood was collected in heparin tubes (BD Biosciences, Franklin Lakes, NJ), centrifuged at 2500 RPM for 20 minutes and stored at -20C. Corticosterone immunoassay was performed according to the manufacturer’s protocol (Arbor Assays, Ann Arbor, MI). Corticosterone concentrations were also analyzed for the pubescent females 2 hours after either a single 30-minute exposure of SCAR (adult male/pubescent female) or placement in a novel cage for 30 minutes (No SCAR).

Experiment 3A

Results

Behavioral measures

During the SCAR interactions, the adult male often chased and pinned down the pubescent female, while she often tried to escape from and avoid the adult male. Very few of these behaviors were observed between a pubescent and an adult female. Instead, two females more often engaged in face-to-face contact and whole body sniffs (not ano-genital) and the young female did not try to escape. During the first SCAR exposure, an independent samples t-test revealed that the number of ano-genital trackings was significantly greater in the SCAR (adult male/pubescent female) group compared to the female/female (adult female/pubescent female) group ($t_{(18)} = -6.07, p < .001$; Fig. 12a). During this first exposure there is a greater number of escape behaviors displayed in the SCAR group compared to the female/female group ($t_{(18)} = -6.94, p < .001$; Fig. 12b), as well as a greater number of pins by the adult in the SCAR group compared to the female/female group ($t_{(18)} = -5.77, p < .001$; Fig. 12c). The
aggressive behaviors toward the SCAR females did not habituate over days. Instead, they remained the same or increased over repeated SCAR exposures. An independent samples t-test revealed that during the 8\textsuperscript{th} SCAR exposure the SCAR group had a significantly greater number of ano-genital trackings ($t_{(18)} = -10.51$, $p < .001$; Fig 12d), escape behaviors ($t_{(18)} = -6.09$, $p < .001$; Fig 12e), and number of pins ($t_{(18)} = -5.57$, $p < .001$; Fig. 12f) compared to the female/female group. These results suggest that exposing the pubescent female to an adult male is significantly more stressful, as examined through behavioral measures, than being exposed to an adult female. These data also suggest that this experience remains stressful over time, even after repeated consecutive exposures, thus making the SCAR model a valid chronic stressor.
The SCAR experience is stressful for the female, as indicated by elevated concentrations of the stress hormone corticosterone. An independent samples t-
test revealed that thirty minutes after a single 30-minute exposure, corticosterone concentrations were significantly elevated in the SCAR group compared to the female/female group ($t_{(9)} = -3.07, p < .05$; Fig. 13a). A separate independent samples t-test revealed that corticosterone concentrations remained elevated 2 hours after a single SCAR exposure compared to a control group of pubescent females that were not exposed to an adult animal ($t_{(13)} = -2.59, p < .05$; Fig. 13b). These data indicate that social interaction with the opposite sex is more stressful than with the same sex, at least in female rodents during puberty.

Figure 13: Effect of SCAR on corticosterone levels. (A) Corticosterone concentrations were significantly elevated in pubescent females thirty minutes after they were exposed to the adult male when compared to concentrations in pubescent females that were paired with an adult female. (B) Concentrations were elevated two hours later in pubescent females that were paired with an adult male when compared to concentrations in pubescent females that were placed in novel context.
Experiment 3B

Methods

In the first experiment we wanted to examine the effect of the SCAR exposures on performance on the motirod physical skill training task and compare this performance to a control group of pubescent females that did not undergo the SCAR experience. We wanted to examine the effects of SCAR on motirod training immediately following the 21 day long period during which the pubescent females were exposed to the adult male every third day throughout pubescence. Pubescent females were injected with a single i.p. injection of BrdU at PND 50, and began motirod training at PND 57, the day following their last SCAR exposure. The cells labeled with BrdU at PND 50 would begin to die off a week later, therefore we began training at PND 57 to determine how motirod training would impact the survival of these newly born DG cells of the hippocampus. A control group of females was also trained on the motirod at this time point; this control group is referred to as “No SCAR” because they were not exposed to the aggressive adult male. Motirod training consisted of 4 trials per day over the course of 4 consecutive days. In order to determine whether motirod learning rescues newly born cells in the DG, both groups of animals were perfused 3 weeks following the BrdU injection. This timeline for experimental procedures was chosen because the majority of newly born hippocampal cells die within 1 and 3 weeks of birth unless they are rescued from death during that time through new and effortful learning. (Fig. 14: experimental timeline for experiment 3B).
Experiment 3B

Results

Motor skill learning

A within-subjects repeated measures ANOVA revealed a significant main effect of trial ($F_{(15,150)} = 17.567, p = .000$) and an interaction between trial and condition ($F_{(15,150)} = 2.067, p = .014$) for learning of the motirod task by the No SCAR (n=7) and SCAR (n=5) animals (Fig. 15). These data suggest that both the No SCAR and SCAR animals successfully learned to perform the motirod physical skill training task. A between-subjects repeated measures ANOVA did not indicate any significant differences between the No SCAR and SCAR animals on their performance on the motirod task ($F_{(1,10)} = .560, p > 0.05$). These data suggest that both the No SCAR and SCAR animals successfully learn to perform the motirod task over the 16 trials of training and that there are no differences
between the two groups on learning this task. These results were unexpected in that we hypothesized that the SCAR exposures would result in impairment on motor skill learning in the pubescent females repeatedly exposed to the adult males prior to training. This hypothesis was based on previous research demonstrating that early life stress commonly results in impairments in a number of cognitive tasks.

![Motirod training](image)

**Figure 15: Effect of SCAR on learning the motirod task.** No SCAR versus SCAR motirod training. A between-subjects repeated measures ANOVA did not indicate any significant differences between the No SCAR and SCAR animals on their performance on the motirod task \(F_{(1,10)} = .560, p > 0.05\). These data suggest that both the No SCAR and SCAR animals successfully learn to perform the motirod task over the 16 trials of training and that there are no differences between the two groups on learning.

**Neurogenesis: Cell survival**

In order to determine whether training on the motirod motor skill task was capable of rescuing newly born cells from death, the No SCAR and SCAR
animals were perfused 3 weeks following the single BrdU injection and brains were processed for neurogenesis. An independent samples t-test revealed that the No SCAR animals rescued a significantly greater number of BrdU+ cells in the DG compared to the SCAR group ($t_{(10)} = 2.797$, $p = .019$; Fig. 16a). We also observed a greater number of BrdU+ cells in the No SCAR group compared to the SCAR group in the GCL ($t_{(10)} = 3.292$, $p = .008$; Fig. 16b); but no differences were observed in the hilus between the 2 groups ($t_{(10)} = 1.725$, $p > 0.05$; Fig. 16c). Results from this first experiment suggest that the SCAR group may have a fewer number of cells at the beginning of training compared to the No SCAR group or that they have higher rates of cell death; both of these instances would result in a lower number of cells when we examined the effect of training on cell survival. The following two experiments described below will test both of these hypotheses.

**Figure 16: Experiment 3B. Neurogenesis: effect of motirod training on cell survival.** (A) An independent samples t-test revealed that the No SCAR animals rescued a significantly greater number of BrdU+ cells in the dentate gyrus compared to the SCAR group ($p < 0.05$). We also observed a greater number of BrdU+ cells in the No SCAR group compared to the SCAR group in the GCL ($p < 0.01$) (B); but no differences were observed in the hilus between the 2 groups ($p > 0.05$) (C).
Experiment 3C

Methods

In experiment 1 we determined that both the No SCAR and SCAR groups successfully learned the motirod task, yet only the No SCAR group retained a significant number of cells born one week prior to the training task. It is therefore necessary to determine how many cells were present at the beginning of training because it is possible that the SCAR and No SCAR groups differed in this baseline number. It is possible that the SCAR exposures had a negative impact on the number of proliferating cells in the SCAR group of pubescent females. In order to determine how many cells the animals have at the start of motirod training, the second experiment was performed to examine what effects the SCAR exposures had on cell proliferation. One group of pubescent females underwent SCAR exposures beginning at PND 35, while a control group was not exposed to the adult male. Again, both groups received a single i.p. injection of BrdU at PND 50, but these animals were perfused 1 week following the BrdU injection at PND 57 (Fig. 17). This experiment determined whether the SCAR exposures have an effect on cell proliferation and also determined the baseline number of cells that the two groups of animals from the first experiment would have had at the start of motirod training.
Experiment 3C

Results

To determine what effect the SCAR exposures had on 1-week cell proliferation in the DG of the hippocampus, and more specifically the number of cells that both groups began with at the start of motirod training, 2 separate groups of animals either underwent the SCAR exposures as described above, SCAR (n=11), or were not exposed to the adult male, No SCAR (n=9). Both groups were sacrificed 1 week after a single BrdU injection at PND 57, the time point in which animals began motirod training in the first experiment. We performed an independent samples t-test to analyze the number of BrdU+ cells and did not find any significant differences between the No SCAR and SCAR groups in the DG \( (t_{18} = .182, p > 0.05; \text{Fig. 18a}) \). There also were no significant differences between the two groups in either the GCL only \( (t_{18} = .629, p > 0.05; \text{Fig. 18a}) \).
Fig. 18b) or hilus only ($t_{(18)} = -0.764, p > 0.05$; Fig. 18c). These results reveal that the exposures to the adult male did not alter cell proliferation, at least the 1 week cell proliferation that we examined. Moreover, the results suggest that the 2 groups of animals in experiment 1 likely would have started their motor training with a similar number of cells capable of being rescued by learning this motor skill learning task. Therefore, a third experiment was necessary to determine whether animals undergoing SCAR exposures have more newly born DG cells dying than the No SCAR animals, which would explain why we did not determine an effect of training on cell survival.

Figure 18: Experiment 3C. Neurogenesis: effect of SCAR exposures on 1-week cell proliferation. (A) An independent samples t-test did not find any significant differences between the No SCAR and SCAR groups on the number of BrdU+ cells in the total dentate gyrus ($p > 0.05$). There also were no significant differences between the two groups in either the GCL only ($p > 0.05$) (B) or in hilus only ($p > 0.05$)(C). These results reveal that the exposures to the adult male did not alter cell proliferation, at least the 1 week cell proliferation that we examined. Moreover, the results suggest that the 2 groups of animals in experiment 3B likely would have started their motor training with a similar number of cells capable of being rescued by learning this motor skill learning task.
EXPERIMENT 3D

Methods

We have demonstrated that the SCAR exposures are stressful to the pubescent females as evidenced by the analyses of behaviors during the SCAR exposures compared with the pubescent female exposed to an adult female, as well as the elevation in corticosterone levels both 30 min and 2 hrs following the SCAR exposures. However, the experience was not sufficiently stressful enough to decrease one week cell proliferation nor did it disrupt learning on the motirod task. Because both the SCAR and No SCAR groups began training with a similar baseline number of cells and learned the motirod task similarly we still needed to determine whether differences in cell survival are due to an overall decrease in cell survival in the SCAR animals or if the SCAR exposures somehow rendered the newly born cells as unresponsive to learning. Therefore, we performed a third experiment in which both SCAR and No SCAR groups were perfused 3 weeks following the BrdU injection, but were not trained on the motirod (Fig.19). The results from this extra measure were compared to the results from the cell proliferation and cell survival studies. This experiment’s results are important in explaining the results from the study on cell survival due to learning because they will allow us to determine whether the SCAR exposures lead to an increase in cell death compared to the pubescent females not exposed to the adult male aggressor. It is possible that we did not see a positive effect of motirod training on cell survival in the hippocampus only as a result of an abnormal decrease in
the number of newly born cells dying off as a result of the stressful experience of the SCAR exposures.

**Figure 19: Experimental timeline for Experiment 3D.** One group of pubescent females underwent SCAR exposures beginning at PND 35, while a control group was not exposed to the adult male. Both groups received a single i.p. injection of BrdU at PND 50, but these animals were perfused 3 weeks following the BrdU injection at PND 71. These animals did not undergo any form of training between the time of BrdU injection and their sacrifice 3 weeks later. This timeline allowed us to determine whether the SCAR exposures led to an increase in cell death compared to the pubescent females not exposed to the adult male aggressor and assessed 3 week cell survival with no training intervention.

**Experiment 3D**

**Results**

In order to determine what effects the SCAR exposures had on cell survival, two additional groups of No SCAR (n=7) and SCAR (n=7) animals were given a single BrdU injection at PND50 as in the first two experiments, but were sacrificed 3 weeks later (PND71) without any motirod training. We analyzed the number of surviving cells at this time for both groups using an independent samples t-test and did not observe any significant differences between the 2 groups in the total DG ($t_{(12)} = .315, p > 0.05$; Fig. 20a). There were also no significant differences between the two groups in either the GCL only ($t_{(12)} = -.112, p > 0.05$; Fig. 20b) or the hilus only ($t_{(12)} = 1.079, p > 0.05$; Fig. 20c). These
results demonstrate that the stress of the SCAR exposures did not cause an increase in the number of newly born cells dying as a result of the stressful experience of being exposed to the adult male repeatedly throughout pubescence. This means that the findings from the first experiment in which the SCAR animals did not rescue a similar number of cells due to motirold learning cannot be explained by the fact that they inherently more newly born DG cells dying off than the No SCAR animals.

![Image of graphs](image)

**Figure 20: Experiment 3D. Neurogenesis: effect of SCAR exposures on 3-week cell survival with no training intervention.** We analyzed the number of surviving BrdU+ cells at this time for both groups using an independent samples t-test and did not observe any significant differences between the 2 groups in the total dentate gyrus ($p > 0.05$) (A). There were also no significant differences between the two groups in either the GCL only ($p > 0.05$) (B) or the hilus only ($p > 0.05$) (C). These results demonstrate that the stress of the SCAR exposures did not result in a lower number of surviving BrdU+ cells compared to No SCAR animals 3-weeks following the injection.

**Experiments 3B-D**

**General Results**

Another way we analyzed these results was by performing a one-way ANOVA with experimental time point as our between groups variable (proliferation at start of training, survival with no training, and survival with...
motirod training) and the number of BrdU+ cells as our dependent variable. For the No SCAR animals there was a significant main effect in the total DG ($F_{(2,22)} = 4.932, p = .018$; Fig. 21). Post-hoc Tukey tests revealed significant differences between the number of cells at the start of training and the number of surviving cells without motirod training ($p = .031$). There was also a significant difference between the number of surviving cells with and without motirod training ($p = .033$). There were no significant differences between the number of cells at the start of training and the number of cells surviving with motirod training ($p = .988$) in the No SCAR group, suggesting that motirod training was capable of rescuing most of the newly born cells from death in this group. In the groups of SCAR animals, a one-way ANOVA revealed a main effect experimental time point for the number of total BrdU+ cells in the total DG ($F_{(2,22)} = 9.611, p = .001$; Fig. 21b). The post hoc Tukey tests revealed a significant difference between the number of cells at the start of training and survival with no training ($p = .010$) and survival with motirod training ($p = .003$). There was no significant difference between the number of cells rescued with and without motirod training ($p = .670$) in the SCAR animals. What these findings suggest is that even though the SCAR animals successfully learn the motor skill task, they do not rescue cells as a result of this training and have a similar number of surviving BrdU+ cells as do the SCAR animals that did not undergo any training.
Figure 21: Neurogenesis: effect of SCAR model on proliferation and 3-week cell survival with or without motirod training. We analyzed the results from experiments 3B, 3C and 3D by performing a one-way ANOVA with experimental time point as our between groups variable (proliferation at start of training, survival with no training, and survival with motirod training) and the number of BrdU+ cells as our dependent variable. (A) For the No SCAR animals there was a significant main effect in the total dentate gyrus ($p < 0.05$). Post-hoc Tukey tests revealed significant differences between the number of cells at the start of training and the number of surviving cells without motirod training ($p < 0.05$). There was also a significant difference between the number of surviving cells with and without motirod training ($p < 0.05$). There were no significant differences between the number of cells at the start of training and the number of cells surviving with motirod training ($p > 0.05$) in the No SCAR group, suggesting that motirod training was capable of rescuing most of the newly born cells from death in this group. (B) In the SCAR animals, a one-way ANOVA revealed a main effect experimental time point for the number of total BrdU+ cells in the total dentate gyrus ($p < 0.001$). The post hoc Tukey tests revealed a significant difference between the number of cells at the start of training and survival with no training ($p < 0.05$) and survival with motirod training ($p < 0.01$). There was no significant difference between the number of cells rescued with and without motirod training ($p > 0.05$) in the SCAR animals. What these findings suggest is that even though the SCAR animals successfully learn the motor skill task, they do not rescue cells as a result of this training and have a similar number of surviving BrdU+ cells as do the SCAR animals that did not undergo any training.
Experiments 3A-D

Discussion

The current study developed a novel animal model, Sexual Conspecific Aggressive Response (SCAR), of sexual aggression towards pubescent female rodents. This model was established to better understand the neuronal and behavioral consequences on the pubescent female brain when repeatedly exposed to an aggressive and sexually experienced adult male rodent. The present data, provided by our newly developed SCAR model, indicated that it is stressful to the pubescent female to be exposed to the adult male conspecific and might serve as a useful model for chronic early life adversity in female rodents. We have demonstrated that the SCAR experience resulted in the adult male tracking, chasing, and pinning down the pubescent female, which then led to a significant amount of escape behaviors by the young female during the 30-minute exposure period. Furthermore, we did not observe as many of these behavioral measures of stress when the pubescent female was exposed to an adult female, demonstrating the sex specificity of our model induced stress in the pubescent females. This finding is important because sexual aggression and abuse during puberty in humans is one of the most traumatic of life experiences and occurs more commonly in young females than young males. We have also demonstrated that the SCAR experience is stressful for the pubescent female, as indicated by elevated concentrations of the stress hormone corticosterone. After one 30-minute exposure to an adult male, the current research revealed that corticosterone concentrations continued to be elevated in the pubescent female
for 30-minutes after the initial exposure, when compared to pubescent females that were exposed to an adult female. These findings indicated that interaction with the opposite sex is significantly more stressful to a young female. In addition, the corticosterone levels remained elevated for two hours after the first exposure to an adult male when compared to females that were placed alone in a novel setting.

    Using the same animals from the single SCAR exposure (or No SCAR – novel setting), of which we performed the two hour corticosterone analysis on, we also examined, two hours after the experience, the impact the single exposure to the male had on the number of proliferating cells in the pubescent females. Evaluating this analysis, it revealed that while the behavioral measures and corticosterone results certainly suggested that the SCAR exposures were stressful, it did not have any impact on two hour proliferation. The results showed no difference between the No SCAR and SCAR animals, which displayed a similar number of BrdU+ cells. (Fig. 22a). In a separate experiment, this study also examined the impact of daily 30-minute SCAR exposures beginning at PND35 for 8 consecutive days on 1 week cell proliferation in the DG. Similar to the findings from the two hour post single SCAR exposure, there were no significant differences between the No SCAR and SCAR animals (Fig. 22b). These experiments have supported our finding that although the SCAR exposures are stressful, as indicated by our behavioral and corticosterone results, the stress did not impact the proliferation of cells in the DG of the hippocampus.
This study also investigated the effects of repeated exposures to the adult male throughout pubescence on the ability to perform on the motirod, a newly designed motor skill learning task. The SCAR and No SCAR animals had similar performance on the motirod and both groups successfully learned the task. We have previously demonstrated in our laboratory (DiFeo et al., 2015) that the motirod task is capable of rescuing newly born cells from death in adult male and female rodents. In the present experiment, it was determined that motirod training only rescued cells in the No SCAR females but not in SCAR females, even though both of the groups successfully learned the task. These results led

Figure 22: Neurogenesis: effect of SCAR on 2-hr and 1-wk cell proliferation in the dentate gyrus of the hippocampus. (A) A single 30 minute SCAR exposure at PND 35 did not have any impact on 2 hour proliferation, as the No SCAR and SCAR animals displayed a similar number of BrdU+ cells. (B) Daily 30 min SCAR exposures beginning at PND 35 for 8 consecutive days on 1 week cell proliferation in the dentate gyrus. Similar to the finding from 2 hrs after a single SCAR exposure, there were no significant differences between the No SCAR and SCAR animals.
us to investigate the possibility that the SCAR animals may have begun training with fewer cells capable of being rescued which could be due to a decrease in cell proliferation caused by the stress of repeated exposures to the adult male during pubescence. However, this was not the case; it was determined that both the No SCAR and SCAR females began training with no difference in the number of BrdU+ cells in the DG of their hippocampus. This led to the possibility that although the SCAR exposures did not affect proliferation of neurogenesis, it might have an impact on cell survival in SCAR animals. We, therefore, examined the number of surviving cells 3 weeks following a single BrdU injection without any training in the No SCAR and SCAR females. The results indicated that there were no differences on cell survival in the No SCAR and SCAR females. These data suggested that the differences found between the No SCAR and SCAR animals in cell survival, which were initially thought to be due to motirod training, were not the result of differences in cell proliferation or cell survival due to the stress of exposures to the adult male during pubescence. The newly born cells in the SCAR animals were not susceptible to the positive effects on cell survival due to successful learning as they are in the No SCAR animals. The authors of this study know that adult neurogenesis is only one factor contributing to the functional role of the hippocampus. Therefore, it is possible that other factors, such as the LTP, synaptic density, or dendritic length may have been altered by the SCAR exposures, thus leading to the attenuated effects of learning on the number of surviving BrdU+ cells in the SCAR females.
The present and other studies have highlighted the fact that pubescence is a sensitive period of brain development, which is particularly vulnerable to stress. This area needs more research to better understand the mechanisms underlying the processes by which stress exerts its effect and impact on cognitive functioning in adulthood. This study has demonstrated that our newly designed SCAR model might serve as a valid measure for studying the effects of chronic early life stress in the pubescent female. The SCAR model could also be beneficial in investigating the damaging effects of sexual trauma and aggression imposed on females during this important developmental period.

Most of the research over the past few decades on early life stress has focused on the effects of stress in early postnatal life, typically within 1 to 2 weeks of birth. During the first two weeks of birth the hippocampus, and especially the DG, are in the process of developing and differentiating. Studies that employed adverse manipulations were reported to have their most prominent and lasting effects during this time period. Even though adolescence is a sensitive time period for stress to exert any negative impacts, it is one of the least studied of the developmental stages of life. Adolescence is especially important because the HPA axis and brain regions, which influence and are influenced by this system, such as the hippocampus, continue to mature over this developmental period. Due to this lack of research and attention, the current study developed our SCAR model that examined the effects of stress during this critical time period. Our SCAR model would be a useful laboratory model for the
study of potential consequences of sexual trauma and aggression on the pubescent female brain.

**Adolescent stress and impact on learning in adulthood**

In the present study, we have demonstrated that although the SCAR experience appeared to be stressful to the adolescent female, it did not impact learning performance on the motirod task, as indicated by the research comparison of the SCAR animals performing similarly to the No SCAR animals. The current laboratory and study developed a novel animal model of stress in pubescent females that involved the repeated exposure to an aggressive adult male. In addition to factors, such as type of stressor, duration of stressor, number of exposures, and the exact age at which the stress occurs, past studies differ in the timing of the administration of testing to collect the effect of stress on learning. So, not only are the effects on cognition stressor-specific, these effects may also differ if the subjects were tested immediately after the stress occurs or at a later time in adulthood. In contrast to the present findings, some forms of chronic stress, during adolescence, appear to affect some forms of learning in adulthood. A previous study (Isgor et al., 2004) examined the effects of either a daily variable, which were either a physical or social stress regimen, in adolescent male rats from PND 28- PND 56 on spatial navigation of the MWM. The animals, in both stress groups as well as a control group, were tested on the MWM 24 hours after the last stress exposure and different set of animals were tested 3 weeks following the last exposure. Both, the physical and social stress, groups performed better than the controls when tested 24 hours later. However,
at the 3 week testing the physical stress group expressed impaired performance compared to the other two groups along with significantly decreased hippocampal volume. These results suggested that the physically stressful experience during adolescence alters the ongoing maturation of the hippocampal circuitry. The delayed deficit in performance on the MWM was also demonstrated using a brief stressor, 30-minutes on an elevated platform, from PND 28-PND 30 (Avital & Richter-Levin, 2005). The animals, which were stressed during adolescence, were impaired on the MWM in adulthood when compared to control animals. Interestingly, if the stressed animals were given an acute stressor just prior to the testing in adulthood, their performance was enhanced compared to controls and animals that were not exposed to an acute stressor. The study’s authors interpreted the findings as the animals, which experienced stress during adolescence, having a better coping ability to stress later in life. Therefore, in the present study, it is possible that, although we did not see impairments in performance on the motirod in the SCAR animals immediately following the exposures, we might have observed a similar finding to the Avital & Richter-Levin (2005) study, but only if we trained the animals on the motirod at a later time during adulthood.

**Adolescent stress and neurogenesis: cell proliferation and survival**

The present study utilized our novel SCAR model to demonstrate that there were no differences in learning the motirod motor skill training task between pubescent females exposed to an adult male during pubescence and those that were not. In addition to learning the motirod task, there were also no differences
discovered between the SCAR and No SCAR females in either cell proliferation or cell survival in the DG of the hippocampus. Based on our behavioral and hormone analyses, the SCAR experience was indeed stressful to the females that were exposed to the adult males during adolescence; therefore we would have expected to observe some form of negative impact on some measure of learning or neurogenic process. However, this was not the case.

One of the earliest studies (Barha, Brummelte, Lieblich, & Galea, 2011) that examined the influence of stress during adolescence and the impact on neurogenesis, used a chronic intermittent restraint stress paradigm. This study exposed adolescent male and female rats to one hour of restraint stress every three days from PND 30 – PND 52, a very similar timeline to the present experiment. The study investigated basal corticosterone levels as well as cell proliferation and cell survival rates in the males and females in adulthood. They determined that the males, but not the females, had elevated CORT levels 60-minutes the followed the stressful exposures and that neither of the sexes habituated to the stressor over the 3 week exposure period. The present study’s findings have supported these data, because we also determined that the pubescent females did not habituate to the SCAR exposures over the course of our study based on our behavioral measure. Importantly, Barha and colleagues (2011) also showed that only the females, and not the males, when compared to controls, had elevated basal CORT levels into adulthood. These findings indicated that although the males were more responsive to the stressor during adolescence, the females showed long-term differences in stress hormone
concentrations as adults. These data suggested that the females suffered longer lasting consequences as a result of adolescent stress. Furthermore, only the females showed decreased cell proliferation and cell survival, when compared to controls, while the males displayed no differences in cell proliferation and a slight increase in cell survival as adults. These findings are important because they demonstrated that neurogenesis is differentially influenced by adolescent stress in male and female rats and that the females appear to display more detrimental consequences. However, these findings, in the females, are in contrast to our own, with regards to cell proliferation and survival as a result of the SCAR exposures. It could be concluded that these opposing results were due to the fact that our SCAR procedure was not as inherently stressful as the restraint stress and therefore did not significantly impact the long term effects on cell proliferation and survival.

This study’s neurogenesis findings were also in contrast to another study (McCormick et al., 2010) that employed a social instability stressor in adolescence. McCormick and others (2010) exposed pubescent female rats daily to social instability stress, which consisted of isolation and a change of cage partners, from PND 30 – PND 45, and also examined spatial memory as well as cell proliferation and cell survival. The results revealed that the stressed females had reduced rates of cell proliferation and cell survival, when compared to controls, which also conflicted with our present findings. In the present study, the SCAR exposure had no impact on either cell proliferation or survival When McCormick and others (2011) tested the animals on the object spatial location
test during adolescence, which was one day after the stress procedure, there were no differences in performance between the stressed and control animals. However, when tested during adulthood, the stressed animals expressed deficits in performance, when compared to the no stress control group. These results supported the findings that chronic stress during adolescence caused deficits in performance on spatial memory in adulthood. However, when tested shortly after the stress procedure, there was no performance deficits observed, which, again, supported the results from our findings on motirod learning, in which no differences were observed between the No SCAR and SCAR animals. The findings raised the possibility, again, that we might have observed deficits in the SCAR animals, but only if the animals were trained later in adulthood rather than immediately following the last SCAR exposure. The impact of the stress from SCAR might not be immediate and may instead cause alterations in the development of the hippocampus that would influence learning at a later time.

The present study demonstrated that the stressful SCAR exposures during adolescence, in females, did not negatively impact motor skill learning or cell proliferation and cell survival. While these results are in contrast to many findings in the research on stress during adolescence, it seems likely that this is related to a number of factors that challenge any investigation involving stress and the effect on learning and neurogenesis. Our findings that the SCAR animals were not susceptible to the positive effects of learning on cell survival, are particularly interesting because the majority of research which included data from our own laboratory, has demonstrated that successful learning does increase the
retention of newly born cells in the DG and rescued them from cell death, when compared to untrained animals. It is likely that the stress of the SCAR procedures interacted in a complex manner with the effects of learning and cell survival. Stress, in general, is known to adversely affect the hippocampus in different ways, such as increasing the excitability of hippocampal neurons, altering the production of neurons, and modifying the dendritic morphology of the hippocampus. Therefore, it is possible that the adverse effects of stress influenced adult neurogenesis and behavior independently and through different pathways. Conversely, these adverse effects could act in competition with one another and therefore, stress, could prevent some type of mechanism and by learning could have influenced the survival of hippocampal cells. There have been a number of past studies (Oomen et al., 2010; Oomen, Mayer, de Kloet, Joëls, & Lucassen, 2007) that examined adolescent stress and learning in adulthood that involve an acute stressor prior to training and the results indicated that early life stress affects the responsiveness of hippocampal plasticity to the surrounding environment.

Future research on the interplay among stress, neurogenesis and learning will be necessary to elucidate our findings as well as designing experiments that include both males and females in order to better understand the impact early life stress and vulnerability to certain psychological disorders. Our SCAR model, which this laboratory developed will serve as a useful tool in order to better understand how the female brain responds to sexual trauma and aggression that occurs during pubescence. Our model could ultimately lead to clinical
interventions for young women who have experienced the severely detrimental trauma caused by sexual abuse and aggression.

**General Conclusions**

The results from the first experiment demonstrated that adult male and female rats that successfully learn to perform physical skill learning tasks are able to rescue significantly more newly born DG cells in their hippocampi than animals not trained on these tasks. We have developed a novel motor skill learning task, termed the motirod, which is a more motivating task than the standard accelerating rotarod and observed a sex difference between adult male and female rodents. Specifically, the adult female rats performed better on the motirod than they did on the rotarod task and, as a result, rescued significantly more newly born DG cells than both rotarod trained and untrained adult females. This difference in performance was not observed in the adult males; they performed similarly on both the motirod and rotarod tasks. This sex difference was not observed in the pubescent males and females in experiment 2. The pubescent males and females successfully learned to perform both physical skill tasks similarly and this resulted in a greater number of newly born DG cells being retained compared to untrained pubescent males and females. Therefore, we have demonstrated here for the first time that there is a sex difference in performance on these particular motor skill tasks and this difference only occurs in adult animals.
These data are in agreement with a previous finding from our laboratory that sex differences in eyeblink conditioning only emerge after pubescence (Hodes and Shors, 2005). Adult females outperform adult males in a classical eyeblink conditioning task, and this difference was not observed during or before pubescence. This finding and the present data from experiments 1 and 2 suggest that the observed sex differences in learning are likely influenced and/or mediated by sex hormones. The findings here on sex differences in learning are important given that they appear to have a significant impact on the survival of newly born DG cells in the hippocampus. While it is beyond the scope of the present studies, the research has provided evidence that these newly born DG cells do in fact mature into functional neurons in the brain and become integrated into the existing hippocampal circuitry (Gould et al, 1999; van Praag et al, 2002; Vivar and van Praag, 2013).

It is important for biomedical and basic scientific research to include studies such as the present ones on sex differences in order to better understand and translate such sex differences into practical applications for men and women. We conducted the third set of experiments to do just this. Most of the research involving the neuroscience of learning and behavior thus far has exclusively involved the use of male animals, thus little is known about sex differences in the brain. This is troublesome, especially due to the fact that women tend to be more vulnerable to mental illnesses induced by stressful life events, and most importantly, when these events occur during the critical developmental period of pubescence.
It is important for biomedical and basic scientific research to include studies such as the present ones on sex differences in order for us to better understand and translate such differences into practical applications for men and women. We conducted the third set of experiments in order to do just this. Much of the research on the neuroscience on learning and behavior thus far has exclusively involved the use of male animals, thus little is known about sex differences in the brain. This is troublesome, especially due to the fact that women tend to be more vulnerable to mental illnesses induced by stressful life events in early life, and most importantly, when these events occur during the critical developmental period of pubescence. Sexual aggression and abuse is one of the most stressful of life experiences for a woman to be exposed to and oftentimes this trauma leads to the emergence of mental health disorders such as anxiety, negative affect, depression, and cognitive deficits in adulthood.

To better understand how these negative experiences affect and/or alter the female brain we developed a novel animal model coined Sexual Conspecific Aggressive Response, or SCAR. In this SCAR model, we expose a pubescent female rat at PND 35 to a much larger sexually experienced adult male rat for 30 minutes a day every 3 days throughout pubescence for a total of 8 exposures. The encounters were video taped in order to record behaviors related to aggression; specifically we counted and analyzed anogenital trackings and pins by the adult male and escape behaviors by the pubescent female. We analyzed these 3 behaviors on the first and last day of the SCAR exposures and compared them to a similar timeline of exposures that consisted of pairing the pubescent
female with an adult female rat. These behaviors were expressed to a greater extent when the pubescent female was exposed to the adult male compared to the adult female, which suggests that our SCAR model is significantly stressful for the pubescent female. Furthermore, the numbers of these behaviors did not change between the first and eighth exposures to the adult male, suggesting that the animals did not habituate with continued social interaction and remained stressful throughout pubescence. In addition to these behavioral measures, our SCAR model was determined to be significantly stressful to the pubescent female as indicated by the high elevations of blood corticosterone levels both 30 mins and 2 hrs following a single exposure. These behavioral and hormonal data suggest that our newly developed SCAR model is indeed a useful animal model to study the consequences of a stressful aggressive experience during pubescence in a female rodent.

Although the SCAR experience is stressful to the pubescent female, it did not impact learning performance on the motirod physical skill training task; SCAR animals performed similar than did a control group of no SCAR females. However, we have determined here that animals exposed to the SCAR model did not retain a significant number of newly born DG cells as a result of this training experience, although the no SCAR animals did rescue a significant number of these cells due to motirod training. This was not due to the SCAR exposures having an impact on the number of cells that were present at the start of training, because both the SCAR and no SCAR animals began training with a similar number of cells. Furthermore, we analyzed cell survival with no training
intervention 3 weeks following an injection of BrdU, and discovered that the SCAR and no SCAR animals had a similar number of newly born cells remaining in their hippocampi. This suggests that SCAR does not affect the neurogenic processes of 1 week cell proliferation or the 3 week survival of those newly born cells.

We determined in the first two experiments here that motirod training is capable of rescuing newly born hippocampal DG cells from death in both adult and pubescent animals. The third set of experiments presented here reveal that this was not the case when pubescent females are exposed repeatedly to an adult male throughout pubescence. It is likely then that the stress of the SCAR experience interacts with the effects of learning on cell survival in a complex manner in these animals. Our newly developed SCAR model will therefore serve as a useful laboratory tool to study the complex interactions within the female brain resulting from early life experiences involving sexual trauma and aggression.
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Figure 1

Figure 1: Diagram of the hippocampus.
Figure 2: Physical skill training for Experiment 1. (A) The standard accelerating rotarod apparatus. (B) The “motirod: apparatus, which was similar to the standard rotarod, however, cold water was placed below the rod to motivate animals to remain on the rod. Groups of animals were trained on either the standard accelerating rotarod or motirod task. Training consisted of 4 trials per day over 4 consecutive days. Animals were allowed to remain on the rod for each trial until they fell off the rod or until 10 minutes had passed. The time from the start of one trial to the start of the next trial was 20 minutes, meaning there was a 10-minute intertrial interval (ITI) between each of the trials.
Figure 3: Experimental timeline for Experiment 1. All rats received one single intraperitoneal injection of BrdU at approximately PND 60. Training began exactly one week after the BrdU injection. All groups (untrained and trained) were sacrificed three weeks after the BrdU injection.
Figure 4

Figure 4: Physical skill learning on the rotarod and motirod tasks in adult males and females. (A) Females trained on the motirod outperformed females trained on the rotarod ($p < 0.0001$). (B) Male rats trained on the motirod performed comparably to male rats trained on the rotarod ($p > 0.05$)
Figure 5: Neurogenesis: Effect of training condition on cell survival in adult females. (A) Females trained on the motirod (n=10) retained significantly more newly-generated cells than females that were not trained (n= 10; p < 0.001) and females trained on the rotarod (n=11; p < 0.05). (B) In females, animals that were trained (on either rotarod or motirod) rescued significantly more cells in the dentate gyrus than untrained females (p< .001).
Figure 6: Neurogenesis: Effect of training condition on cell survival in adult males. (A) In males no significant differences were observed between training condition (no training, rotarod, motirod) and number of surviving cells in the dentate gyrus (p’s > 0.05). (B) In males, those that were trained (on either the rotarod or motirod) rescued significantly more cells than males that were not trained (p < 0.05).
Figure 7: Correlation between average performance on physical skill task and the number of BrdU+ cells retained in adult females. In females trained on either the rotarod or motirod, the average latency to fall from the rod across the 4 days of training positively correlated with the number of cells retained in the dentate gyrus ($r = 0.56$, $p < 0.01$).
Figure 8: Correlation between average performance on physical skill task and the number of BrdU+ cells retained in adult males. In males, a significant positive correlation was observed between average latency to fall across the four days of training and the number of surviving cells in the dentate gyrus (r= 0.49, p< 0.05).
Figure 9: Experimental timeline for Experiment 2. Male and female rats were injected once with BrdU (200 mg/kg) at approximately PND28. Animals were trained on one of two tasks beginning one week later. Trained and untrained groups were sacrificed three weeks after injection to assess the numbers of remaining BrdU-labeled cells in the dentate gyrus.
Figure 10: Physical skill learning on the rotarod and motirod tasks in pubescent males and females. Males and females readily learned to remain on the rotarod (A) or the motirod (B) during puberty. There was no sex difference in performance, probably because they all learned quickly to remain on the rod throughout each trial.
Figure 11: Neurogenesis: effect of physical skill training on number of surviving BrdU+ cells in pubescent males and females. Representative photomicrographs of immunohistochemistry for BrdU at 1000X from the dentate gyrus of an untrained pubescent animal (A) and a trained animal (B). Training with the physical tasks increased the number of BrdU-labeled cells in the hippocampus of pubescent male and female rats (C). These effects were observed in both the GCL (D) and the hilus (E).
Figure 12: Behavioral measures of SCAR exposures. (A) During the first SCAR exposure, the number of anogenital sniffs was significantly greater in the SCAR (adult male/pubescent female) group than in females paired with another female (female/female). (B) During the first exposure, the female made a greater number of escape behaviors when paired with an adult male than when paired with an adult female. (C) The adult male also pinned the pubescent female down more times than did the adult female. (D–F) These behavioral results were similar during the eighth exposure. The SCAR group expressed more anogenital sniffs, escape behaviors, pins when compared to similar behaviors expressed when a pubescent was paired with an adult female.
Figure 13: Effect of SCAR on corticosterone levels. (A) Corticosterone concentrations were significantly elevated in pubescent females thirty minutes after they were exposed to the adult male when compared to concentrations in pubescent females that were paired with an adult female. (B) Concentrations were elevated two hours later in pubescent females that were paired with an adult male when compared to concentrations in pubescent females that were placed in novel context.
Figure 14: Experimental timeline for Experiment 3B. Pubescent females at PND 35 began the SCAR procedure (SCAR), and were exposed to an adult male conspecific for 30 minutes every 3 days for a total of 8 exposures. A control group of pubescent females (No SCAR) were not exposed to an adult male during this same time. We wanted to examine the effects of SCAR on motirod training immediately following the 21 day long period. Pubescent females were injected with a single i.p. injection of BrdU at PND 50, and began motirod training at PND 57, the day following their last SCAR exposure. The cells labeled with BrdU at PND 50 would begin to die off a week later, therefore we began training at PND 57 to determine how motirod training would impact the survival of these newly born dentate gyrus cells of the hippocampus. The No SCAR group were also injected and trained at these same time points. In order to determine whether motirod learning rescues newly born cells in the dentate gyrus, both groups of animals were perfused 3 weeks following the BrdU injection.
Figure 15: Effect of SCAR on learning the motirod task. No SCAR versus SCAR motirod training. A between-subjects repeated measures ANOVA did not indicate any significant differences between the No SCAR and SCAR animals on their performance on the motirod task ($F_{(1,10)} = .560$, $p > 0.05$). These data suggest that both the No SCAR and SCAR animals successfully learn to perform the motirod task over the 16 trials of training and that there are no differences between the two groups on learning this task.
Figure 16

Figure 16: Experiment 3B. Neurogenesis: effect of motirod training on cell survival. (A) An independent samples t-test revealed that the No SCAR animals rescued a significantly greater number of BrdU+ cells in the dentate gyrus compared to the SCAR group ($p < 0.05$). We also observed a greater number of BrdU+ cells in the No SCAR group compared to the SCAR group in the GCL ($p < 0.01$) (B); but no differences were observed in the hilus between the 2 groups ($p > 0.05$) (C).
Figure 17: **Experimental timeline for Experiment 3C.** One group of pubescent females underwent SCAR exposures beginning at PND 35, while a control group was not exposed to the adult male. Both groups received a single i.p. injection of BrdU at PND 50, but these animals were perfused 1 week following the BrdU injection at PND 57. This experiment determined whether the SCAR exposures have an effect on 1-week cell proliferation and also determined the baseline number of cells that the two groups of animals from experiment 3B would have had at the start of motirod training.
Figure 18: Experiment 3C. Neurogenesis: effect of SCAR exposures on 1-week cell proliferation. (A) An independent samples t-test did not find any significant differences between the No SCAR and SCAR groups on the number of BrdU+ cells in the total dentate gyrus ($p > 0.05$). There also were no significant differences between the two groups in either the GCL only ($p > 0.05$) (B) or in hilus only ($p > 0.05$)(C). These results reveal that the exposures to the adult male did not alter cell proliferation, at least the 1 week cell proliferation that we examined. Moreover, the results suggest that the 2 groups of animals in experiment 3B likely would have started their motirod training with a similar number of cells capable of being rescued by learning this motor skill learning task.
Figure 19: Experimental timeline for Experiment 3D. One group of pubescent females underwent SCAR exposures beginning at PND 35, while a control group was not exposed to the adult male. Both groups received a single i.p. injection of BrdU at PND 50, but these animals were perfused 3 weeks following the BrdU injection at PND 71. These animals did not undergo any form of training between the time of BrdU injection and their sacrifice 3 weeks later. This timeline allowed us to determine whether the SCAR exposures led to an increase in cell death compared to the pubescent females not exposed to the adult male aggressor and assessed 3 week cell survival with no training intervention.
Figure 20: Experiment 3D. Neurogenesis: effect of SCAR exposures on 3-week cell survival with no training intervention. We analyzed the number of surviving BrdU+ cells at this time for both groups using an independent samples t-test and did not observe any significant differences between the 2 groups in the total dentate gyrus ($p > 0.05$) (A). There were also no significant differences between the two groups in either the GCL only ($p > 0.05$) (B) or the hilus only ($p > 0.05$) (C). These results demonstrate that the stress of the SCAR exposures did not result in a lower number of surviving BrdU+ cells compared to No SCAR animals 3-weeks following the injection.
Figure 21: Neurogenesis: effect of SCAR model on proliferation and 3-week cell survival with or without motirod training. We analyzed the results from experiments 3B, 3C and 3D by performing a one-way ANOVA with experimental timepoint as our between groups variable (proliferation at start of training, survival with no training, and survival with motirod training) and the number of BrdU+ cells as our dependent variable. (A) For the No SCAR animals there was a significant main effect in the total dentate gyrus \( (p < 0.05) \). Post-hoc Tukey tests revealed significant differences between the number of cells at the start of training and the number of surviving cells without motirod training \( (p < 0.05) \). There was also a significant difference between the number of surviving cells with and without motirod training \( (p < 0.05) \). There were no significant differences between the number of cells at the start of training and the number of cells surviving with motirod training \( (p > 0.05) \) in the No SCAR group, suggesting that motirod training was capable of rescuing most of the newly born cells from death in this group. (B) In the SCAR animals, a one-way ANOVA revealed a main effect experimental timepoint for the number of total BrdU+ cells in the total dentate gyrus \( (p < 0.001) \). The post hoc Tukey tests revealed a significant difference between the number of cells at the start of training and survival with no training \( (p < 0.05) \) and survival with motirod training \( (p < 0.01) \). There was no significant difference between the number of cells rescued with and without motirod training \( (p > 0.05) \) in the SCAR animals. What these findings suggest is that even though the SCAR animals successfully learn the motor skill task, they do not rescue cells as a result of this training and have a similar number of surviving BrdU+ cells as do the SCAR animals that did not undergo any training.
Figure 22: Neurogenesis: effect of SCAR on 2-hr and 1-wk cell proliferation in the dentate gyrus of the hippocampus. (A) A single 30 minute SCAR exposure at PND 35 did not have any impact on 2 hour proliferation, as the No SCAR and SCAR animals displayed a similar number of BrdU+ cells. (B) Daily 30 min SCAR exposures beginning at PND 35 for 8 consecutive days on 1 week cell proliferation in the dentate gyrus. Similar to the finding from 2 hrs after a single SCAR exposure, there were no significant differences between the No SCAR and SCAR animals.