CHAPTER 3

Animal Models of Exercise–Brain Interactions

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INTRODUCTION

It has long been known that exercise strengthens muscles and bones, and that keeping physically active maintains a healthy cardiovascular system and a slim physique. However, only recently have both the scientific community and the public realized that regular exercise is also critical for maintaining cognitive health. Particularly as you age, it is now established that keeping physically active protects your brain from otherwise inevitable decay (Churchill et al., 2002; Erickson & Kramer, 2009; Erickson et al., 2011). Moreover, the effects of physical exercise on cognition are broad, crossing multiple domains related to learning, memory, attention, processing speed, and overall performance (Churchill et al., 2002; Colcombe & Kramer, 2003; Cotman & Berchtold, 2002; Erickson & Kramer, 2009; Erickson et al., 2011).

The effect size and broad benefits to cognition from exercise are particularly impressive. Unlike exercise, other ways known to improve cognitive performance, such as cognitive training (i.e., training subjects on crossword puzzles, memory tasks, problem solving, or other cognitively challenging computer games), typically do not produce cognitive benefits across broad domains. Rather, they improve performance on the task they were specifically practicing (Lee et al., 2012; Redick et al., 2013). Differences between the effects of exercise and cognitive training on the brain have major clinical significance, given that clinicians want to find a strategy that will transfer to other tasks. For example, if grandma does crossword puzzles she will be good at crossword puzzles, but it might not help her navigate around the neighborhood. In contrast, as will be demonstrated in this chapter, exercise will transfer to other tasks. Thus, exercise does something to the brain that is different to when you are cognitively challenged or learning something new.

Recent studies in aged or elderly humans have established that aerobic exercise enhances cognitive performance across multiple domains of cognition. For example, regularly engaging in fast walking for 45 min, three times a day for
6 months, a form of moderate intensity aerobic exercise that increases the efficiency and endurance of the cardiovascular system, enhances spatial learning, processing speed, reaction time, and executive function (Colcombe & Kramer, 2003), and also increases the volume of a specific part of the brain, known as the hippocampus (Erickson et al., 2011). In addition, strength training (four sets on the leg-press and leg-extension machines, three sets on chest press, lat pulldown, pec-dec, and vertical row machine) performed twice per week, under personal supervision, for a 24-week period improved cognitive performance on a variety of tasks (e.g., episodic memory, working memory and attention, processing speed, and executive function), many of which require hippocampus activation (van de Rest et al., 2014). Strength training or lifting weights represents anaerobic exercise, which strengthens muscles but does not necessarily enhance cardiovascular endurance. The fact that the hippocampus is the most responsive to both aerobic and anaerobic exercise and literally grows in size in response to exercise (Erickson et al., 2011) is fascinating because of the critical role that the hippocampus plays in learning and memory, stress coping, motivation, and emotion (Andersen, 2007).

Although the hippocampus is established as the center for effects of exercise in the brain, the mechanisms by which exercise enlarges the hippocampus and improves cognitive function are still unclear. This is where animal models become exceedingly useful. In the next section we will discuss three different animal models for studying effects of both aerobic and anaerobic exercise on the brain. These include two forms of aerobic exercise: voluntary wheel running, forced treadmill running, and one form of anaerobic exercise: strength training. Following a description of the animal models, we will review some recent discoveries of specific neurological mechanisms underlying procognitive effects of exercise.

**RODENT EXERCISE MODELS**

**Voluntary Wheel Running**

Voluntary wheel running is very commonly used as a way to exercise rodents in studies of the effects of exercise on the brain. The procedure is very simple. A running wheel is usually included in the cage or attached to the cage, and an automatic counter records wheel revolutions. Typically, wheel running distances are reported in km/day by multiplying the circumference of the wheel times the number of revolutions over a 24-h period. Depending on the strain, mice typically run between 2 and 10 km/day (Clark, Kohman et al., 2011). We have estimated that approximately 30% of revolutions occur without the animal actually running in the wheel (Girard, McAleer, Rhodes, & Garland, 2001). This estimate is likely to vary greatly depending on the size and resistance of the wheel. The 30% estimate occurs with mice running on rat-size wheels approximately 1 m in circumference, with very low resistance, and high momentum. Hence, the wheel continues to rotate when the mouse leaves the wheel, a very frequent occurrence. Mice run intermittently, i.e., short bouts of fast running separated by short breaks where they drink some water, eat some food, or just move around the cage a bit. We have also seen mice hold onto the wheel and ride it as it rotates (Girard et al., 2001). Hence, the equivalent horizontal distance that the mice would travel if they were walking/running on the ground is more difficult to judge. We believe it is best to think of voluntary wheel running as analogous to riding a bike rather than running on a flat surface. Nevertheless, 2–10 km is still quite a lot of exercise for a mouse. If you consider that mice move around in their standard shoe box cages between 0.1 and 0.5 km/day (Clark, Kohman et al., 2011), then by giving the animals access to a wheel, they roughly increased their level of physical activity by an order of magnitude.
Consistent with the idea that access to a running wheel constitutes a significant elevation in physical activity relative to a standard cage is that several weeks of running increase standard measures of aerobic fitness. These include increased aerobic capacity (Swallow, Garland, Carter, Zhan, & Sieck, 1998), decreased fat mass (Swallow, Koteja, Carter, & Garland, 2001), increased mitochondria in the muscles (Rowe, El-Khoury, Patten, Rustin, & Arany, 2012), and decreased oxidative stress (Alessio et al., 2005).

Advantages of the model:
1. Simple to employ.
2. Completely voluntary and hence devoid of stress from forcing the animal to do something it does not want to do.
3. Extremely reliable, meaning that knowing how much an individual animal ran on one day, greatly predicts how much the animal will run the next day. Pearson’s correlation for day-to-day variation in wheel running is usually above 80% (Rhodes, Garland, & Gammie, 2003). This is useful for establishing correlations between individual differences in levels of exercise with physiological outcomes.

Disadvantages:
1. The investigator has no control over how much the animals will run. Animals may run at levels not desired by the investigators. Within-group variation in levels of running should be expected.
2. It is difficult to study high-intensity exercise such as would only occur if the animals were forced, or had extremely high motivation.
3. Humans do not normally run for hours a day, whereas mice spend most of their dark cycle intermittently running on running wheels (Girard et al., 2001).

**Forced Treadmill Running**

Forcing animals to run on a treadmill is often used in exercise–physiological studies where the goal is to study traits such as aerobic capacity, muscle adaptations, or immune responses (Woods, Davis, Mayer, Ghaffar, & Pate, 1994). However, it is less often used in studies of effects of exercise on the brain for a few different reasons. First, it is quite time-consuming, because the animals must be trained, usually over weeks, to cooperate and run steadily on a treadmill without stopping or getting caught in the apparatus behind the wheel. Given that rodents are intermittent runners, as mentioned previously, it is not natural for them to run continuously in place, and therefore takes time to adapt. In our experience, they never really get used to it, because it is so unnatural for them to run in this way. This induces a psychological type of stress in the animals, which can complicate interpretation of the brain outcomes, because they could be due to the psychological stress of the forced running rather than the exercise per se. It is notable to mention here that usually the animals need to be constantly prodded to run continuously on a treadmill. This is usually done using an electric grid or foam pad behind the treadmill so if the animals stop running and are pushed back they either land on an electric grid, or get pushed against a foam pad. Some individuals never cooperate. One of the authors of this chapter (Rhodes) used to measure maximum oxygen consumption in mice by forcing them to run as fast as they could on a treadmill while simultaneously measuring oxygen utilization and carbon dioxide expiration (Kelly et al., 2014). This involved getting mice to run in a small box placed over the treadmill. Rhodes observed some of the mice doing a split with each hind paw touching either side of a box to avoid running. Second, typically the treadmills are used at slow speeds, less than 0.5 km/h, which even for a mouse is a walk rather than a run. This is not because of any limitations of the treadmills, but rather because at higher speeds, there are more problems getting the mice to cooperate. Typically, mice are run on a treadmill for 20 min. So at a rate of 0.5 km/h, less than 0.2 km is added to their daily movement, which at most is a doubling of their normal levels of activity in a cage. This is in comparison to wheel running, where the mice move...
an order of magnitude more than they do in their cages. On the other hand, the treadmill method of exercise training appears, at face value, more similar to how humans would train, only 20–40 min/day, 3 days/week. But the problem with this argument for the treadmill model is that mice are not humans and must be considered within the appropriate ecological context or realms of their natural behaviors. As we mentioned, mice run in short bursts, taking frequent breaks over most of the night. They do not take a break from work at the factory to take a jog.

Advantages of the model:

1. The investigator can control the amount of exercise on a treadmill. All animals in a group can experience a similar amount of work.

2. The investigator can match the pattern of aerobic exercise typically performed by humans, constant exercise over a period of 20–40 min a few times per week.

Disadvantages:

1. Because it is forced and unnatural for mice to run on a treadmill, even after extensive training, forced treadmill running induces a type of psychological stress in the animals each time it is administered (Cook et al., 2013), which confounds interpretation of brain measures as a result of exercise per se.

2. The animals can only be run at a slow speed, and the total increase in distance traveled as compared to movement in the home cage is marginal.

3. The animals can get hurt in the treadmill. For example, tails and feet can get caught, and the animals may need substantial prodding at least at the beginning of training. Some animals may never cooperate and have to be removed from the study entirely.

**Strength Training**

Resistance-based strength training regimens are more difficult to implement in rodents than in humans, because in their natural environment rodents do not typically lift heavy weights or carry heavy loads. Nonetheless, some clever solutions have been implemented to get rodents to lift weights. In one paradigm, rats stand upright restrained in an apparatus and do squats with different weights placed on their back inside a backpack (Seo et al., 2014; Tamaki, Uchiyama, & Nakano, 1992). In another paradigm, a rat pulls a weight on a treadmill (Aparicio et al., 2011). In a third paradigm, rats are trained to climb on a ladder with weights attached to their tails (Cassilhas et al., 2013). However, most studies published to date using these techniques were focused on exercise physiological changes in the muscles, not on cognitive or neurological outcomes (but see Cassilhas, Lee, Fernandes et al., 2012; Cassilhas, Lee, Venancio et al., 2012). Hence, to the best of our knowledge this is an area in the exercise–cognition field that desperately needs additional studies.

Advantages of the model:

1. The investigator can control the amount of weight an animal can carry and increase the load temporally.

2. The models have established similar exercise physiological adaptations, in the muscles of animals performing the tasks, as are seen in humans conducting strength training routines (Cassilhas et al., 2013; Tamaki et al., 1992).

Disadvantages:

1. In the squatting paradigm, the rats are restrained and forced to stand upright and squat with weights on their backs. Similarly, in the treadmill paradigm, animals are forced to pull weights. Hence, as mentioned above for the aerobic version of the forced treadmill running, psychological stress related to the restraint or punishment needed to force the animals to perform the behavior can confound interpretation of neurological results as they pertain to impacts of exercise per se.
2. These models are labor intensive because they require a great deal of training before the animal can appropriately carry out the behaviors. Some animals may react differently than others or not perform well on the tasks, and therefore will have to be removed from the study.

3. Rats have been used primarily for the squatting paradigm and treadmill running with weights. The ladder task appears to be a good choice for mice, since it takes advantage of their natural incline to want to climb up if placed on a vertical grid.

**NEUROLOGICAL EFFECTS OF EXERCISE**

Exercise has both acute and chronic neurological effects. The acute effects refer to the immediate effects of exercise on brain function soon after an individual stops exercising. Acute effects wane within a few hours. For example, right after you stop exercising, your blood pressure is still elevated, your heart rate is still increased, and your blood adrenaline is still elevated. It takes time for all the residual hormonal and biochemical changes in response to the exercise to wane. These factors can improve cognitive performance and enhance the function of cells in the brain but the short-term influences of exercise are far less interesting and useful clinically than the chronic effects. After multiple bouts of exercise over a period of weeks and months, the brain accumulates substantial changes in brain morphology and physiology. These changes take a long time to go away after exercise is stopped.

The chronic effects of exercise on the brain have substantial clinical significance. For example, chronic exercise increases the total number of granule neurons in the dentate gyrus (DG) of the hippocampus (van Praag, Christie, Sejnowski, & Gage, 1999; van Praag, Kempermann, & Gage, 1999), as will be described in more detail below. The DG is exactly the area first hit by Alzheimer’s disease (Rodriguez & Verkhratsky, 2011; Varela-Nallar, Aranguiz, Abbott, Slater, & Inestrosa, 2010), and it is a common area where epileptic seizures occur (Masukawa et al., 1997; Ribak & Dashtipour, 2002; Sloviter, 1994). If we can understand how to grow new neurons in this region, then we may be able to treat neurodegenerative disease, stroke, and brain trauma.

Chronic exercise broadly enhances performance across multiple domains of cognition, and on multiple different learning and memory tasks in humans and rodent models. In humans, exercise has been shown to enhance spatial learning, pattern separation, executive function, working memory, and processing speed, among others (Colcombe & Kramer, 2003; Voss, Vivar, Kramer, & van Praag, 2013). For example, in elderly subjects, 6 months of aerobic training mitigated age-related decline in both verbal and spatial memory (Ten Brinke et al., 2014) and a low activity, 8-week yoga intervention significantly improved performance on working memory (Gothe, Kramer, & McAuley, 2014). Further, in aged adults, a resistance-type exercise program of two sessions per week improved attention and working memory on a variety of tasks (van de Rest et al., 2014). In rodents, exercise has been found to enhance spatial learning and memory on the Morris water maze, eight-arm radial maze, Barnes maze, contextual fear conditioning, extinction of conditioned place preference for drugs, passive avoidance, and pattern separation, among others (Anderson et al., 2000; Creer, Romberg, Saksida, van Praag, & Bussey, 2010; Greenwood, Strong, Foley, & Fleshner, 2009; Jacotte-Simancas, Costa-Miserachs, Torras-Garcia, Coll-Andreu, & Portell-Cortes, 2013; van Praag, Christie et al., 1999; Samorajski et al., 1985; Thanos et al., 2010; Van der Borght, Havekes, Bos, Eggen, & Van der Zee, 2007).
Exercise is known to impact the physiology and morphology of multiple brain regions, which could account for the broad enhancements from exercise observed on multiple cognitive domains. Moderate aerobic exercise training increases hippocampal and prefrontal cortical volume (Erickson & Kramer, 2009). For example, Erickson et al. (2011) designed a randomized control trial wherein older adults were assigned to either a stretching control or an aerobic exercise group in which they walked for 40 min a day, once a week for 6 months. Aerobic exercise resulted in a 2% increase in hippocampal volume, effectively reversing the age-related decline.

In rodent models, voluntary wheel running increases the total number of neurons, synapses, dendritic complexity, and number of spines on neurons in the hippocampus, which could account for the volume differences observed in humans (Eadie, Redila, & Christie, 2005; Redila & Christie, 2006). Voluntary wheel running increases the concentration of several different growth and trophic factors in the hippocampus that likely support the morphological changes occurring in this region in response to exercise, including fibroblast growth factor 2 (Gomez-Pinilla, Dao, & So, 1997), insulin-like growth factor 1 (Ding, Vaynman, Akhavan, Ying, & Gomez-Pinilla, 2006), brain-derived neurotrophic factor (Neeper, Gomez-Pinilla, Choi, & Cotman, 1996), and vascular endothelial growth factor (Uysal et al., 2015), among others. It is currently debated as to whether these molecules are being secreted locally by neurons and glia or whether they are coming from the blood via the muscles (Fabel et al., 2003; Trejo, Carro, & Torres-Aleman, 2001; Wrann et al., 2013).

Rodent models have revealed that aerobic exercise impacts all the different cell types of the brain including microglia, oligodendrocytes, astrocytes, and neurons in multiple different brain regions. For example, voluntary wheel running decreases the proliferation and proinflammatory status of microglia in the hippocampus of aged mice. Moreover, running increases the proportion of microglia expressing a neuroprotective phenotype (Kohman, Bhattacharya, Wojcik, & Rhodes, 2013; Kohman, DeYoung, Bhattacharya, Peterson, & Rhodes, 2012; Kohman & Rhodes, 2013). In addition, voluntary running increases the density of blood vessels in brain regions involved in the voluntary control of movement, such as the dorsal striatum (Clark, Brzezinska, Puchalski, Krone, & Rhodes, 2009), cerebellum (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Isaacs, Anderson, Alcantara, Black, & Greenough, 1992), and hippocampus (Clark et al., 2009). Similarly, forced treadmill running in rats increases the proliferation of astrocytes in the striatum and frontal cortex, a result likely related to increased vascular density in these regions given the role of astrocytes in connecting blood vessels to neurons (Li et al., 2005). Recently, it was discovered that running on a complex wheel (with irregularly spaced rungs) increases the formation of new oligodendrocytes in the brain (McKenzie et al., 2014). The above-mentioned biochemical, cellular, physiological, and morphological changes illustrate the breadth of the changes induced from exercise in the brain, but certainly are not meant to represent an exhaustive list.

Of all the brain regions that change their physiology and morphology from exercise, the hippocampus is by far the brain region most impacted. This is another explanation for the far-reaching impacts of exercise on cognition, because the hippocampus plays a pivotal role in cognitive performance, and improvement in this one structure could impact many different cognitive domains (Andersen, 2007). Hence, the remainder of this chapter will focus on the impact of exercise on the hippocampus.

**EXERCISE AND THE HIPPOCAMPUS**

It is truly intriguing that the hippocampus, of all the areas of the brain, is the one most involved in exercise. The hippocampus is the first region where all sensory modalities merge together to
form unique representations and memories that bind stimuli together and, thus, it plays a critical role in learning and memory (Andersen, 2007). One of the most influential case studies ever in the history of neuroscience research that illustrates well the critical role of the hippocampus in memory is the famous case of H.M. In order to cure his epilepsy, H.M. underwent a bilateral medial temporal lobectomy, which resulted in severe anterograde amnesia and he was unable to commit new events to his explicit memory. Still, over the years, H.M. retained his short-term working memory and intellect, and he was left with residual learning capabilities (Augustinack et al., 2014). For example, he could still perform many types of motor learning tasks, though he could not remember learning them. Similarly, in rodents, hippocampal lesions impair many different forms of learning and memory (Broadbent, Gaskin, Squire, & Clark, 2010; Chen, Kim, Thompson, & Tonegawa, 1996; Cho, Friedman, & Silva, 1999; Cohen et al., 2013; Farr, Banks, La Scola, Flood, & Morley, 2000; Logue, Paylor, & Wehner, 1997). Hence, it is strange and intriguing that exercise, which presumably does not involve huge amounts of learning and memory, acutely activates the hippocampus, and results in such profound neuroanatomical, biochemical, and physiological changes in response to chronic exercise.

Anatomy of the Hippocampus

The hippocampus is located in the medial temporal lobe of the brain (Figure 1(A)). A cross-section of the brain of a macaque monkey, stained to visualize cell bodies, illustrates a key feature of the mammalian hippocampus. The mammalian hippocampus can be distinguished as a zone where the cortex narrows into a single layer of densely packed neurons, which curl into a tight U shape (Figure 1(B)). A drawing of the major cell types and their connections (Figure 1(C)) illustrates the unique circuitry, anatomy, and cellular morphological phenotypes of the hippocampus. The hippocampal circuit is unique in that its connections are unidirectional. The major cortical input to the hippocampus comes from Layers II and III of the entorhinal cortex (EC) through excitatory glutamatergic fibers, which form the perforant pathway. The perforant pathway projects mainly to the granule cells in the DG. The axons of dentate granule cells form excitatory glutaminergic mossy fibers, which then project to the proximal (closer to cell body) apical dendrites of CA3 pyramidal cells. CA3 pyramidal cells then form the glutamatergic Schaffer collaterals, which connect to both ipsilateral and contralateral CA1 neurons. Finally, axons of CA1 cells send projections to the subiculum (Sub) as well as to deep Layer V of the EC (Andersen, 2007).

Acute Effect of Exercise on Neuronal Activation of the Hippocampus

Neuronal activity in the hippocampus is tightly correlated with the speed of running or intensity of muscular contractions, such as needed for a rat to jump to different heights (Oddie & Bland, 1998). The type or pattern of neuronal activity in the hippocampus, associated with the intensity of the movements, is very different from what occurs when an animal is learning a task. Hence, there may be two separate functions for the hippocampus, one in learning and memory and another related to generating or sensing intense movements. When animals are running, large numbers of neurons in the granule layer of the DG fire in synchrony, producing rhythmic electrical activity that can be detected using electrodes placed near the region (Ahmed & Mehta, 2012; Kuo, Li, Chen, & Yang, 2011; McNaughton, Barnes, & O’Keefe, 1983; Oddie & Bland, 1998) or using immunohistochemical techniques after the animal is euthanized (Clark, Bhattacharya et al., 2011; Clark et al., 2010; Rhodes et al., 2003). When measured using electrodes placed in the brain or outside the brain, the rhythmic activity of large numbers
of cells firing in synchrony in the hippocampus produce theta and gamma oscillations. Both the amplitude and frequency of these rhythms are closely correlated with the speed of running on a treadmill in rats (Ahmed & Mehta, 2012; Kuo et al., 2011; McNaughton et al., 1983; Oddie & Bland, 1998). In addition, the force parameters exerted during a jump to different heights are closely correlated with the amplitude and frequency of theta (Oddie & Bland, 1998). These changes in theta and gamma can be explained by larger or smaller numbers of cells firing in synchrony together in a faster or slower rhythm.

The large increase in neuronal activation of the hippocampus, titrated closely to the speed or intensity of the movement, can also be observed using immunohistochemistry. When the animals are euthanized, immediately after they have been running on their running
wheels, sections of their brains can be stained to identify neurons that were recently activated. We typically stain the brain sections for a protein called c-Fos, but many other neuronal activation markers can work (Clark, Bhattacharya et al., 2011; Clark et al., 2010). C-Fos is a transcription factor, meaning it binds to other proteins that together bind to DNA and cause the transcription of many other genes. Hence, the presence of c-Fos means that the cell has recently been stimulated and is undergoing genomic changes (e.g., new proteins are being expressed because the cell is extending a process, building or removing synapses, or recycling receptors). This has been referred to as the genomic action potential (Clayton, 2000). Just as an action potential in a neuron is short lived, so is the genomic action potential, but with a slightly longer time frame. For c-Fos, concentrations reach peak concentrations within 90 min after the neuron was stimulated. As it turns out, levels of c-Fos are strongly, positively correlated with running levels within 90 min of euthanasia taken from voluntary running wheels (Clark et al., 2010) or forced treadmills (Oladehin & Waters, 2001). Although animals in sedentary cages fail to exhibit a similar correlation between distance traveled and c-Fos expression (Clark, Bhattacharya et al., 2011), the correlation reappears in animals selectively bred for high levels of physical activity that move around in their cages at distances normal animals run on running wheels (Majdak et al., 2014). Taken together, these data suggest a threshold of intensity of movement is necessary to activate the hippocampus, and that the number of neurons that are activated from exercise is strongly correlated with the intensity of the exercise.

The correlation between neuronal activation of the hippocampus and intensity of movements is incredibly strong, but that does not imply anything about causality. The leading idea in the current literature is that the hippocampus is acting as a sensory organ, integrating sensory information about the intensity of movements (Bland, 1986; Hartley, Lever, Burgess, & O’Keefe, 2014; Kuo et al., 2011). For example, the animal runs, and then the hippocampus responds to the sensory feedback from the running. However, an alternative idea is that neuronal activity in the hippocampus is the origin of the motivation required to deliver the electrical stimulation, from the motor cortex and spinal cord neurons, necessary for large rhythmic contractions of muscles (Oddie & Bland, 1998). In other words, the hippocampus controls the motor circuit at the highest level, giving it the capability to deliver the electrical activation originating from the brain, necessary to execute intense muscular contractions with the capability for doing large amounts of work. If the hippocampus is at the top of the hierarchy rather than a sensory organ, then several observations would be predicted. First is that without a hippocampus, animals should be impaired in their ability or motivation for engaging in intense physical exercise. Second, the correlation between neuronal activity in the hippocampus and movement should not occur when the level or intensity of the movement is low, such as when the animal is walking around, eating, etc. Most of these predictions appear to be true. H.M. was incredibly inactive (personal communication with Neal Cohen, Professor at the University of Illinois, who worked for several years with H.M.). Rats with their hippocampus removed can move around but they cannot run quickly or jump high (Oddie & Bland, 1998). We were only able to detect a correlation between c-Fos and distance traveled when levels of activity were high, above normal ambulation in the cage such as observed in hyperactive mice or normal mice with access to a running wheel (Clark, Bhattacharya et al., 2011; Majdak et al., 2014). Therefore, we favor the hypothesis that states that the hippocampus functions as a movement intensity generator.
Chronic Effects of Exercise on Adult Hippocampal Neurogenesis

One of the consequences of repeated activation of the hippocampus, in close association with the intensity of the exercise, is increased numbers of new granule neurons in the DG of the hippocampus. In rodents, it is clear that these new neurons add to the granule layer and make it larger (Clark et al., 2009; van Praag, Christie et al., 1999; van Praag, Kempermann et al., 1999). Human studies have confirmed that the DG grows in volume in response to exercise training in randomized controlled trials (Erickson et al., 2011). While an increase in volume could come from many changes not just increase in numbers of cells (e.g., growth of preexisting cells), the fact that neurogenesis increases in rodent models in correlation with increased dentate volume is compelling evidence of convergence across humans and rodents regarding the underlying mechanism. Hence, before we continue with a discussion of the possible functional significance of increased neurogenesis and increased volume of the granule layer of the hippocampus in response to exercise, it is necessary first to review the discovery of adult neurogenesis and its regulation by exercise.

Over 50 years ago, Joseph Altman and colleagues (Altman, 1962, 1963, 1969a,b; Altman & Das, 1965, 1966) first reported that adult neurogenesis, the continuous generation of new neurons to the adult central nervous system, occurs in the mammalian brain. However, it was not until the 1990s when new techniques to visualize new neurons became available that adult neurogenesis became a widely recognized phenomenon. Adult neurogenesis is now established to occur continuously in two regions of the mammalian brain: the subventricular zone of the anterior lateral ventricles (the site of origin for olfactory bulb neurons) and the subgranular zone of the hippocampal DG (Figure 2).

FIGURE 2  Adult mammalian neurogenesis. A schematic diagram of a rodent brain showing the two areas where adult neurogenesis occurs: the subventricular zone and the subgranular zone of the dentate gyrus. OB, olfactory bulb; RMS, rostral migratory stream; SVZ, subventricular zone; DG, dentate gyrus. (This work is licensed under a Creative Commons Attribution 2.0 Unported License. Source: 2008 Arias-Carrión.)

Stages of Adult Hippocampal Neurogenesis

Hippocampal adult neurogenesis is a process confined to the DG, which begins with the proliferation of a precursor progenitor cell and ends with the integration of a functional cell into the preexisting hippocampal network (Figure 3). Overall, the rodent DG consists of about one million granule cells (Rapp & Gallagher, 1996; West, Slomianka, & Gundersen, 1991), which are continuously being generated throughout the lifespan. It has been estimated that young adult rats have approximately 9000 new proliferating cells a day (Cameron & McKay, 2001); while older adult rats have approximately 4000 new proliferating cells a day (Rao & Shetty, 2004).

The process of hippocampal adult neurogenesis begins in the subgranular zone. It is here that progenitor cells proliferate. Progenitor cells are similar to stem cells, in that they divide, producing one cell that retains the self-renewing properties and a daughter cell that terminally differentiates into one of a variety of cell types. However, unlike true stem cells, the progenitor cells in the granule layer have limited self-renewing properties, and eventually
will differentiate as an astrocyte (Kriegstein & Alvarez-Buylla, 2009). Moreover, the daughter cells that go on to differentiate into a specific cell type are restricted to a cell fate of either a neuron, an astrocyte, or an oligodendrocyte. Approximately 60% of the newly born daughter cells will die (Dayer, Ford, Cleaver, Yassaee, & Cameron, 2003), while the remaining cells will exit the cell cycle and differentiate into immature cells. It is during this phase that cells commit to a neuronal lineage and begin to express immature neuronal markers. Approximately 80–90% of adult-born neurons differentiate into mature neurons, while a low percentage (approximately 5%) will differentiate into astrocytes and a rare percentage (less than 5%) will become oligodendrocytes (Abrous, Koehl, & Le Moal, 2005; Steiner et al., 2004). Following the cell differentiation, cells begin to migrate out of the subgranular zone into the inner granule cell layer of the DG. Immature granule cells have limited dendritic branches and are driven by inhibitory GABA interneurons. As such, they exhibit characteristics of immature neurons: hyperexcitability and enhanced synaptic plasticity (Ge, Sailor, Ming, & Song, 2008). As the neurons mature, they begin to respond solely to excitatory glutamatergic input. In addition, they form connections with inputs from the EC and send outputs to the CA3 region. Approximately 2 months after birth, adult-born neurons exhibit similar basic electrophysiological properties as mature neurons (Mongiat & Schinder, 2011), yet they do not reach a mature morphology (soma size, total dendritic length, dendritic branching, and spine density) until 4 months after their birth (Abrous et al., 2005; Song, Stevens, & Gage, 2002).

Modulation of Adult Neurogenesis from Exercise

Theoretically, exercise could increase adult neurogenesis by increasing the proliferation of progenitor cells (either by increasing the rate of cell division or by the number of cells that are dividing at a time) or by increasing the survival and neuronal differentiation of cells that have already proliferated. In 1999, Henriette van Praag hypothesized that exercise increased levels of adult neurogenesis mainly by increasing the number of proliferating cells in the DG. In fact, it was further hypothesized that exercise increased levels of adult neurogenesis mainly by increasing the number of proliferating cells through enhanced proliferation, while environmental enrichment (e.g., learning and sensory stimulation) increased levels of adult neurogenesis by supporting cell survival (Olson, Eadie, Ernst, & Christie, 2006; van Praag, Christie et al., 1999; van Praag, Kempermann et al., 1999). More recently, however, research has demonstrated that exercise increases neurogenesis mainly by
increasing cell survival rather than proliferation (Clark et al., 2010; Fuss et al., 2010; Kronenberg et al., 2006; Snyder, Glover, Sanzone, Kamhi, & Cameron, 2009; Wu et al., 2008). For example, in perhaps the clearest demonstration, C57BL/6J mice were injected with BrdU (to label dividing cells) before being placed on running wheels and showed the same doubling in neurogenesis typically observed with this strain (Snyder et al., 2009). Because the BrdU was administered before exercise, the increased neurogenesis can only be attributed to increased survival or differentiation of cells that had proliferated before the exercise began. It is important to note that in the original van Praag, Kempermann et al. (1999) study that discovered exercise-induced levels of neurogenesis, proliferation was measured by labeling cells across a 12-day period. Thus, total number of cells on day 13 reflected both proliferation and survival of cells labeled on the initial days.

Exercise has been consistently proven to increase levels of neurogenesis in a variety of strains. In CD1 mice, 6 weeks’ access to a running wheel produced a two- to threefold increase in newly born neurons (Bednarczyk, Aumont, Decary, Bergeron, & Fernandes, 2009). Further, Thuret, Toni, Aigner, Yeo, and Gage (2009) found that MRL/MpJ mice produce 75% fewer new neurons than do C57BL/6 mice; however, when given unlimited access to a running wheel this difference is abolished. Recently, our lab examined the exercise-enhanced levels of adult hippocampal neurogenesis in 12 different genetically divergent mouse strains (Clark, Kohman et al., 2011). Significant differences in levels of adult neurogenesis, across species, were apparent. Interestingly, the magnitude of exercise-induced neurogenesis was most significant in AKR/J mice and not in C57BL/6/J, the most commonly used mouse strain in studies of effects of exercise on neurological outcomes. Strain-based differences are also evident in the number of surviving cells, as 129S1/SvlmJ mice had the lowest, while both B6129SF1/J and AKR/J mice had the highest number of surviving cells. Interestingly, AKR/J mice only ran approximately 4 km/day, despite having the highest number of surviving cells. Still, it is important to acknowledge that although levels of adult neurogenesis vary in different mouse strains, all strains show a significant increase in levels of adult neurogenesis as a result of exercise exposure.

In addition, the distance that an animal runs impacts the levels of exercise-induced neurogenesis. This is evident in the previously mentioned study by Clark, Kohman et al. (2011), wherein significant differences in 12 different strains were found. It is important to recognize that in this study, the strains varied greatly with respect to running levels. For example, 129S1/SvlmJ mice ran approximately 2 km/day, whereas B6129SF1/J mice ran approximately 10 km/day. As a result, B6129SF1/J mice had a greater-fold increase in levels of adult neurogenesis than did 129S1/SvlmJ mice. In addition, it has recently been shown that the levels of neurogenesis may be influenced by individual differences in response to a complex environment (i.e., how much an animal is moving around an environmental enrichment cage), as genetically identical mice living in a nominally identical environment exhibited differing levels of adult neurogenesis that were positively correlated with their exploration of the environment (Freund et al., 2013). Thus, a large proportion of individual variation in levels of adult hippocampal neurogenesis can be explained by differential individual levels of physical activity.

The fact that levels of adult neurogenesis are strongly correlated with distance traveled on running wheels, both among individuals within a strain and across mouse strains, strongly suggests that it is the exercise per se that increases neurogenesis as opposed to the environmental enrichment component of wheel running. Nonetheless, it has been argued that increased neurogenesis in animals housed on running wheels relative to animals housed in cages without running wheels is actually a demonstration of
environmental deprivation in the rats without wheels. This is because allowing an animal to move is more natural than constraining them to live in a cage without any other stimulation (Lavenex, Lavenex, & Clayton, 2001). One way to determine whether adding the running wheel provides a type of enrichment aside from the exercise that enhances neurogenesis is to include a group that is highly enriched but unable to exercise. We and others have performed such a study where animals were reared under four different environmental conditions, standard housing, wheel running only, environmental complexity (with the addition of toys or social groups), and the combination of environmental complexity with running wheels. The observation is that only running significantly increases neurogenesis, and that adding environmental complexity does nothing further to increase neurogenesis beyond the effects of exercise (Kobilo et al., 2011; Mustroph et al., 2012). Another possibility is that mice gain enjoyment from the exercise and it is this enjoyment that drives neurogenesis. However, if this were the case then we would not expect to see increased levels of neurogenesis in the forced treadmill studies, but we do (Kim et al., 2014; Li et al., 2013; Nam et al., 2013; Shin et al., 2013). Therefore, it appears that it is the actual physical motion of running that increases levels of adult neurogenesis.

FUNCTIONAL SIGNIFICANCE OF EXERCISE-INDUCED ADULT NEUROGENESIS

Despite the fact that exercise increases hippocampal neurogenesis in every strain of rat or mouse that has been investigated using either voluntary wheel running or treadmill running paradigms, and the observation in human studies of increased volume of the DG from exercise training, the functional significance remains a mystery. Why does the hippocampus become so activated from exercise, and why does this repeated activation result in increased neurogenesis and volume of the entire structure? Our hypothesis is that the increased neurogenesis and growth of the hippocampus serves a function related to the role of the hippocampus in regulating the intensity of movement. However, ironically we have little data directly testing this hypothesis. Instead, most of our work and the work of others has focused on the hypothesis that exercise-induced neurogenesis serves a function in learning and memory (Clark et al., 2008; Gibbons et al., 2014; Luo et al., 2007; Marlatt, Potter, Lucassen, & van Praag, 2012; Merritt & Rhodes, 2015; Mustroph et al., 2012). This is because of the dominant literature on the role of the hippocampus in learning and memory as compared to regulating the intensity of movement, and also the intuitive idea that the addition of new neurons in an area of the brain critical for learning and memory might enhance learning and memory. New neurons have not yet integrated into the circuitry, and therefore are hypothesized to be more moldable to experiences than older neurons that already have most of their processes integrated in the circuitry. Also, exercise has been proven not only to promote brain plasticity but also to enhance cognitive and spatial performance on a variety of tasks (Clark et al., 2008; van Praag, Christie et al., 1999; Van der Borght et al., 2007). Whether the exercise-induced levels of adult neurogenesis play a functional role in the enhanced learning and memory performance in exercising animals remains unclear. Over the past two decades research has examined the functional significance of exercise-generated neurons by experimentally manipulating the numbers of new neurons that an animal is capable of producing. The idea behind this approach is to reduce neurogenesis in order to see what impact the lesion has on behavior. Recently, several different techniques have been developed to reduce neurogenesis in the hippocampus, such as focal irradiation, transgenic mouse models, and optogenetics. Hence, it is likely that in the
near future, we will have a better understanding of the functional role of new neurons in behavior using these new technologies.

**Role of Exercise-Induced Adult Neurogenesis in Learning and Memory**

Exercise consistently increases levels of hippocampal adult neurogenesis and also improves performance on a variety of learning and memory tasks in rodents. One of the most well-established improvements in function from exercise is enhanced spatial learning and memory, the ability to remember the location of an object relative to other objects in the environment (Clark et al., 2012; van Praag, Christie et al., 1999; Van der Borght et al., 2007). For example, rats exposed to prenatal noise stress, given an intervention of 30 min of treadmill running per day, exhibited significantly enhanced levels of neurogenesis and they completed the radial arm maze with significantly fewer errors than did their littermates not receiving the intervention (Kim et al., 2013). Further, in both young and aged mice, access to a running wheel improved performance on the Morris water maze and increased levels of adult neurogenesis when compared to aged-matched controls (Gibbons et al., 2014; Marlatt et al., 2012; van Praag, Shubert, Zhao, & Gage, 2005). Running, when administered as an intervention to corticosterone administration, increases levels of adult neurogenesis and enhances Morris water maze performance in adult male Sprague–Dawley rats (Yau et al., 2011). Recently, we found that voluntary wheel running enhanced performance on the multistrain adapted plus version of the water maze in five different mouse strains (Merritt & Rhodes, 2015). Together, these studies suggest that exercise-enhanced levels of adult neurogenesis occur in parallel with improved performance on spatial learning and memory tasks. However, they do not speak to whether the two are causally related. Improved rotarod performance is consistently correlated with increased levels of adult neurogenesis, resulting from exercise exposure (Clark et al., 2008; Marlatt et al., 2012), but no one has ever suggested that exercise-induced neurogenesis functions to support rotarod behavior. Additional work is needed to establish causality.

Several different approaches have been used to eliminate neurogenesis to see what effect that has on learning and memory tasks. Results are mixed, and the function remains unclear. When levels of adult neurogenesis are ablated through focal irradiation, the procognitive effects of exercise are no longer present on Morris water maze performance in C57BL/6J mice (Clark et al., 2008). On the one hand, these data might suggest that new neurons are required for the procognitive effects. However, on the other hand, irradiation induces inflammation, which could contribute to the impairment on the water maze observed in this study independent of whether new neurons were ablated or not. Recent studies, using transgenic methods that quite specifically reduce adult neurogenesis, have found no influence of reducing neurogenesis on learning and memory performance on a variety of tasks (for review see Groves et al., 2013). While these studies did not examine exercise-induced neurogenesis per se, the results are important because they indicate a potential limitation of the neurogenesis lesion method when studying the functional significance of new neurons. If new neurons are removed or killed, it is possible that the remaining older neurons of the granule layer can compensate for this loss by, for example, adding additional synapses, growing additional spines, or extending dendrites. Therefore, it is possible that new neurons, if present, would be preferentially recruited while an animal was learning a particular task, but if they are not present (because they are eliminated using some experimental method), then there are redundant mechanisms in the brain to compensate for their loss.

We recently obtained evidence that new neurons, in fact, are preferentially activated in the hippocampus when mice are engaged in...
multiple different behavioral tasks. We used a combination of the BrdU method for labeling newly divided cells and the c-Fos method for determining whether the cells were activated or not after the animal was performing a certain task (e.g., running on a wheel, navigating a water maze, or exploring an open field) (Clark et al., 2012). What we found was that new neurons were twice as likely as older neurons to display c-Fos regardless of the task. Even though each task activated the hippocampus to a different degree, e.g., running activated the hippocampus the greatest, followed by swimming in the water maze, and lastly normal cage activity, in each case new neurons were twice as likely to be activated as older mature neurons. Taken together, these results suggest that new neurons generated from exercise are broadly recruited into multiple functions of the hippocampus.

It is important to note here that the hypothesis that new neurons are broadly recruited into all functions of the DG is not necessarily consistent with recent studies that suggest new neurons in the DG function specifically in pattern separation (Clelland et al., 2009; Sahay et al., 2011). Pattern separation refers to the ability to distinguish and uniquely encode two very similar stimuli (e.g., patterns, objects, faces, scenes, experiences, etc.). Computational models suggest that new neurons in the DG could play a critical role in pattern separation (Aimone, Deng, & Gage, 2011). However, we are not certain how useful the concept of pattern separation is to the debate about the functional significance of adult hippocampal neurogenesis. We see no reason why it is necessary to pin a specific function to a neuron or a brain region. Rather we believe that new neurons are part of the development, function, and maintenance of a critical brain region that serves multiple brain functions including pattern separation.

One way to directly determine the extent to which new neurons play a specific role in behavior is to manipulate the activation of the cells after they are incorporated into the circuit. This could be done through an optogenetic method that would allow researchers, in theory, to inactivate cohorts of new neurons immediately while the animal is performing a task. This technology has promise for discovering the potential functional significance of new neurons in learning and memory because the new neurons can remain intact until the very moment researchers are interested in testing their function, leaving no time for compensatory mechanisms to interfere with the interpretation of their role in behavior. Previous studies, which delivered light-sensitive proteins using viral vectors to the granule cell layer, found that when the cells were inactivated, the animals displayed impaired learning and memory (Gu et al., 2012; Liu et al., 2012; Liu, Ramirez, & Tonegawa, 2014). The major limitation of these studies is that the viral vector delivery method cannot infect the entire granule cell layer. Hence, the method can only inactivate a portion of the new neurons in the hippocampus. If the new cells can be labeled using a genetic method instead of injecting viral vectors, the method may have real promise for uncovering the functional significance of exercise-induced adult hippocampal neurogenesis.

CONCLUSIONS

It is established that exercise is good for the brain and can enhance cognitive performance, both acutely and chronically, and regardless of the form (forced or voluntary or strength), though the precise differences in the neurological outcomes between the different forms of exercise still need to be worked out. One of the most robust effects of exercise in the brain is the growth of the hippocampus. This is striking because of the prominent role that the hippocampus plays in learning and memory. Why rhythmic activation of the hippocampus occurs from exercise and why this results in growth of the region is not known. The leading ideas in the literature point to a role of new
neurons in learning and memory. However, the numbers of cells in the hippocampus involved in learning and memory are sparse in comparison to the large numbers of cells that are activated when the animal is running at high speeds. When an animal is learning a task, specific cells in the DG sparsely encode a unique representation. When the animal is running, large numbers of cells fire in synchrony, with large amounts of electrical activity that can resonate throughout the entire brain. To us, this suggests the hippocampus has multiple functions, one in learning and memory and another in regulating the intensity of movements. Rhythmic activation of the hippocampus may be required to “ramp up” the motor areas of the brain in a way that is needed to generate large rhythmic muscle contractions required for intense movements. To us, it follows then that the growth of the hippocampus is related to the increased demand from this region due to being regularly engaged for motivating the behavior. Our movement intensity hypothesis for the functional significance of exercise-induced neurogenesis is perfectly consistent with a role for the new neurons in supporting learning and memory. We believe the new neurons generated from running support both the function of the hippocampus in regulating the intensity of movement and also whatever else the hippocampus does including learning and memory, motivation, stress, etc.

The bottom line is that exercise clearly can be used as a means to promote enhanced cognitive function and brain plasticity. Still, it remains unclear how exactly exercise exerts its pro-cognitive effects. It is likely that the hippocampus plays a role, and that exercise-induced neurogenesis and growth of the hippocampus contributes to the pro-cognitive effects observed. New optogenetic technologies that allow precise control over the activation of new neurons may shed light on the important question of how exercise broadly enhances cognition throughout the lifespan.

References


3. ANIMAL MODELS OF EXERCISE–BRAIN INTERACTIONS


REFERENCES


