Published in final edited form as:

Hippocampus. 2009 October ; 19(10): 988–1001. doi:10.1002/hipo.20534.

A behavioral analysis of the impact of voluntary physical activity on hippocampus-dependent contextual conditioning

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Abstract

Voluntary physical activity induces molecular changes in the hippocampus consistent with improved hippocampal function, but few studies have explored the effects of wheel running on specific hippocampal-dependent learning and memory processes. The current studies investigated the impact of voluntary wheel running on learning and memory for context and extinction using contextual fear conditioning which is known to be dependent on the hippocampus. When conditioning occurred prior to the start of 6 weeks of wheel running, wheel running had no effect on memory for context or extinction (assessed with freezing). In contrast, when wheel running occurred for 6 weeks prior to conditioning, physical activity improved contextual memory during a retention test 24 hours later, but did not affect extinction learning or memory. Wheel running had no effect on freezing immediately after foot shock presentation during conditioning, suggesting that physical activity does not affect the acquisition of the context – shock association or alter the expression of freezing, per se. Instead, it is argued that physical activity improves the consolidation of contextual memories in the hippocampus. Consistent with improved hippocampus-dependent context learning and memory, 6 weeks of wheel running also improved context discrimination and reduced the context pre-exposure time required to form a strong contextual memory. The effect of wheel running on brain-derived neurotrophic factor (BDNF) messenger ribonucleic acid (mRNA) in hippocampal and amygdala subregions was also investigated. Wheel running increased BDNF mRNA in the dentate gyrus, CA1, and the basolateral amygdala. Results are consistent with improved hippocampal function following physical activity.

Keywords

Wheel running; amygdala; brain-derived neurotrophic factor; extinction; generalization

Introduction

Voluntary physical activity produces molecular and structural changes in the hippocampus that are consistent with improved hippocampal function (for recent reviews see Christie et al., 2008; Cotman et al., 2007; van Praag, 2008). Rats and mice allowed voluntary access to running wheels, for example, have enhanced plasticity-related gene expression in the hippocampus (Farmer et al., 2004; Gomez-Pinilla et al., 1997; Molteni et al., 2002; Vasuta et al., 2007; Vaynman et al., 2006) including increased levels of brain-derived neurotrophic factor (BDNF; Neeper et al., 1995; Neeper et al., 1996; Russo-Neustadt et al., 1999). Wheel running also promotes angiogenesis (Ekstrand et al., 2008), adult neurogenesis (van Praag et

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al., 1999; van Praag et al., 2005) and neuronal remodeling (Eadie et al., 2005; Redila and Christie, 2006) in the dentate gyrus. Improved hippocampal function could contribute to the beneficial effects of exercise on learning and memory reported in humans (Cotman and Engesser-Cesar, 2002; Kramer et al., 2006).

Although it is clear that physical activity produces molecular and structural changes in the hippocampus, investigations into the effects of physical activity on specific hippocampal-dependent learning and memory processes have only recently begun (Akhavan et al., 2008; Clark et al., 2008; van Praag et al., 2005; Vaynman et al., 2004). One method that can be used to assess hippocampal function is contextual fear conditioning. Contextual fear conditioning is a Pavlovian associative learning paradigm that is normally dependent on the hippocampus (Fendt and Fanselow, 1999; Maren, 2001; Matus-Amat et al., 2004; Myers and Gluck, 1994; Phillips and LeDoux, 1992) but see (Wiltgen et al., 2006; Zimmerman et al., 2007). A leading theory of the role of the hippocampus in contextual learning and memory processes is that the hippocampus stores a conjunctive representation of context which, during contextual fear conditioning, is associated with shock in extra-hippocampal structures such as the amygdala (LeDoux, 2000; Maren and Fanselow, 1996; Matus-Amat et al., 2004; Rudy et al., 2002; Rudy and O'Reilly, 1999; Rudy and O'Reilly, 2001). Consistent with the idea that physical activity improves hippocampal-dependent contextual learning and memory are several reports that wheel running prior to contextual fear conditioning increases conditioned freezing (a measure of context memory) to a context previously paired with shock (Burghardt et al., 2006; Clark et al., 2008; Greenwood and Fleshner, 2008; Van Hooissen et al., 2004).

The goal of the current series of experiments was to extend prior work on the effects of voluntary physical activity on hippocampus-dependent contextual learning and memory. So far, only the effects of voluntary physical activity *prior to conditioning* on contextual learning and memory have been investigated. The effects of *post-conditioning* wheel running on memory for context remain unknown. Scenarios can be envisaged in which plastic changes in the hippocampus occurring with wheel running following conditioning could facilitate cellular or systems consolidation of the memory for the conditioned context. Furthermore, the effects of wheel running on generalization have yet to be investigated. Generalization occurs when an animal responds to a new stimulus with the conditioned response (such as freezing) following conditioning to a different stimulus (Domjan, 1993). The hippocampus supports the ability to distinguish between contexts (Desmedt et al., 2003; Frankland et al., 1998; Mizumori et al., 2007), thus reduced generalization in physically active animals would be consistent with improved hippocampal function. Finally, prior studies investigating the effects of voluntary physical activity on contextual conditioning used parameters that optimized conditioning, such as long context pre-exposure times (at least 3 minutes) and multiple shock presentations. The level of freezing elicited by re-exposure to a context previously paired with shock is positively related to context pre-exposure time (Fanselow, 1986; Frankland et al., 1998; Landeira-Fernandez et al., 2006); whereby the minimal amount of time required to express a small, but significant, amount of contextually-conditioned freezing 24 hours after conditioning is about 20 sec (Fanselow, 1986; Landeira-Fernandez et al., 2006). If physical activity improves the ability of the hippocampus to learn about context, then prior wheel running should facilitate contextual conditioning under sub-optimal conditions, such as after a minimal duration of context pre-exposure time.

Extinction is a decrease in conditional responding to a previously conditioned stimulus (in this case context) that occurs following repeated presentations of the context in the absence of shock (Domjan, 1993). Extinction of conditioned responses to context is itself new learning that is supported by multiple areas including the hippocampus (Corcoran et al.,

2005; Ji and Maren, 2005; Ji and Maren, 2007), prefrontal cortex (Quirk et al., 2006; Quirk and Mueller, 2008), and amygdala (Boccia et al., 2008; Chhatwal et al., 2006; Laurent et al., 2008). The clinical analog of fear extinction is exposure therapy, a common behavioral therapy for anxiety disorders (Rothbaum and Schwartz, 2002). The effects of wheel running on extinction learning is unknown; however, exercise can reduce the incidence and severity of anxiety disorders in humans (Manger and Motta, 2005; Smits et al., 2008). Thus, it is attractive to predict that, in addition to contextual learning and memory, wheel running will improve extinction learning and memory.

Recent evidence suggests that the dorsal vs. the ventral hippocampus could have distinct functional roles in contextual learning and memory vs. emotional processing, respectively (Bannerman et al., 2004; Bertoglio et al., 2006; Esclassan et al., 2008; Hunsaker and Kesner, 2008). Despite these functional differences between the dorsal and ventral hippocampus, a characterization of the effects of physical activity on anatomically-distinct BDNF expression in the hippocampus has yet to be reported. Additionally, no studies to our knowledge have investigated the effects of physical activity on BDNF expression in the amygdala, which has been implicated in the consolidation phase of cued fear conditioning (Rattiner et al., 2004; Rattiner et al., 2005) and extinction (Chhatwal et al., 2006). A final study; therefore, examined the effects of wheel running on BDNF mRNA levels across the rostral-caudal axis of the hippocampus and amygdala. We hypothesized that increased BDNF mRNA levels would be associated with facilitated conditioning and extinction learning and memory in physically active rats.

Material and Methods

Subjects

Adult, male, Fischer 344 rats were used in the current studies because they are an inbred strain with consistent running patterns and low inter-rat variability in running distances. Rats were housed in a temperature (22°C) and humidity-controlled environment and were maintained on a 12:12 hr light/dark cycle (lights on 0600-1800). Animals acclimatized to these housing conditions and were handled for 1 week prior to any experimental manipulation. All animals were individually housed in Nalgene Plexiglas cages (45 × 25.2 × 14.7 cm). Care was taken to minimize animal discomfort during all procedures. All experimental protocols were approved by the University of Colorado Animal Care and Use Committee. Rats were weighed weekly and had *ad libitum* access to food and water.

Voluntary wheel running

Rats were randomly assigned to either remain sedentary under standard single housing conditions with no wheel (sedentary) or were allowed voluntary access to in-cage running wheels. Physically active rats either ran for 6 weeks prior to conditioning or had their wheels unlocked immediately following conditioning. Daily wheel revolutions were recorded digitally using Vital View software (Mini Mitter, Bend, OR) and distance was calculated by multiplying number of revolutions by wheel circumference (1.081 m).

Apparatus

Contextual fear conditioning took place in one half of a shuttle box (10"W × 10"D × 12"H; Coulbourn Instruments, Allentown, PA). The floor consisted of stainless steel rods spaced 2 cm apart and was attached to a shock generator (Coulbourn Instruments, Allentown, PA) for delivery of foot shock. The shuttle boxes were housed in sound-attenuating chambers and were illuminated with a 35 W bulb mounted from the shuttle box ceiling. A fan mounted on one side of each box provided ventilation and background noise. The back wall and sides of the boxes were made of silver stainless steel and the front of the shuttle boxes were clear

Plexiglas. The door separating the halves of the shuttle box remained closed, each rat was only ever exposed to 1 side of the box, and at no time were 2 rats placed into the same box simultaneously. During the conditioning procedure, rats were transported from their home cage to the conditioning room in standard rat cages (45 × 25.2 × 14.7 cm). All procedures occurred between 0800 and 1100.

Generalization tests took place in a different room in a novel context that shared no contextual features in common with the conditioned context. The generalization context was a rectangular (20"W × 12"D × 10"H) box made entirely of Plexiglas. The ceiling, back, and sides of the box were translucent black and the front door of the box was transparent. A 60 W red light bulb positioned outside of the box provided dim interior illumination and there was no background noise. The floor of the box was smooth Plexiglas covered in strips of 0.5" green tape which traversed the floor of the box in 1" intervals. Rats were transported to the generalization context in a novel carry cage which was a standard mouse cage made of translucent Plexiglas (14.5"W × 7"D × 8.5"H).

In situ hybridization

In situ hybridization for full length BDNF mRNA was performed following our previously published protocols (Greenwood et al., 2007b). Sedentary (n = 9) and physically active (n = 10) rats that were not exposed to any conditioning procedures were sacrificed by rapid decapitation. Brains were removed, frozen rapidly in isopentane and dry ice (−40 to −50°C), and stored at −80°C until sliced into 10 µm coronal sections on a cryostat. Slices through the hippocampus and amygdala were thaw-mounted directly onto polylysine-coated slides and stored at −80°C until processing. Sections were fixed in 4% PF for 1 hr, acetylated in 0.1 M triethenolamine containing 0.25% acetic anhydride (10 min), and dehydrated in graded alcohol. cRNA riboprobes (courtesy of Dr. Stanley Watson, University of Michigan, Ann Arbor) complementary to BDNF were prepared from cDNA subclones in transcription vectors and labeled with (35S)UTP (AmershamPharmaciaBiotech, Piscataway, NJ), using standard transcription methods. Riboprobes were diluted in 50% hybridization buffer containing 50% formamide, 10% dextran sulfate, 2X saline sodium citrate (SSC), 50 mM PBS (pH = 7.4), 1X Denhardt's solution, and 0.1 mg/ml yeast tRNA. Brain sections were hybridized with the probe overnight at 55°C. The next day, sections were washed in 2X SSC, treated with RNase A (200 µg/ml) for 1 hr at 37°C, and washed to a final stringency of 0.1X SSC at 65°C for 1 hr. Dehydrated, air-dried sections were exposed to x-ray film (Biomax-MR; Eastman Kodak, Rochester, NY) for 6 days.

Image analysis

Levels of BDNF mRNA were analyzed by computer-assisted optical densitometry. Brain section images were captured digitally (CCD camera, model XC-77; Sony, Tokyo, Japan), and the relative optical density of the x-ray film was determined using scion image version 4.0. A macro was written that enabled signal above background to be automatically determined. For each section, a background sample was taken over an area of white matter, and the signal threshold was calculated as mean gray value of background +3.5 SD. The section was automatically density-sliced at this value, so that only pixels with gray values above these criteria were included in the analysis. Results are expressed as mean integrated density, which reflects both the signal intensity and the number of pixels above assigned background (mean signal above background X number of pixels above background). Quantification of BDNF mRNA in the hippocampus occurred in the rostral (from −2.30mm to −3.14mm posterior to bregma), mid (from −3.3mm to −4.3mm posterior to bregma), and caudal (ventral; from −4.52mm to −6.04mm posterior to bregma) dentate gyrus, CA3, CA2, and CA1 (distances from bregma are based on the rat brain atlas by Paxinos and Watson; 1998). The caudal CA3, CA2, and CA1 are considered part of the ventral hippocampus.

Both left and right DG, CA3, CA2, and CA1 regions were analyzed independently. The only amygdala subregion containing BDNF mRNA was the basolateral amygdala (BLA). Quantification of BDNF mRNA occurred in the rostral (from -1.6mm to -1.88mm), mid (from -2.12 to -2.56), and caudal (from -2.8mm to -3.3mm) BLA. Two or three sections from each rostral-caudal level per subject were averaged to yield the integrated density value for that subject. BDNF mRNA levels were also quantified in the medial mammillary nucleus, a control region with relatively high levels of BDNF mRNA that is present on some of the same tissue slices used to quantify BDNF mRNA in the caudal hippocampus (-5.2mm to -5.3mm posterior to bregma).

Procedures

The first experiment investigated the effects of pre-conditioning wheel running on contextual conditioning and extinction. Following either sedentary housing (n = 8) or 6 weeks of wheel running (n = 8), rats were placed into the conditioning chambers for a total of 6 min. Rats were allowed to explore the conditioning chambers for 3 min prior to receiving 3, 2 sec, un-signalized foot shocks (0.8 mA) separated by an inter-trial interval of 1 min. During the 1 min intervals between each shock, and during an additional 1 min after the 3rd shock, freezing (defined by the absence of all movement except that required for respiration) was scored using a random sampling procedure whereby each subject's freezing behavior was scored every 10 seconds as either freezing or not. The freezing during the conditioning procedure was used to gauge memory acquisition. This conditioning procedure has been used previously in studies reporting enhanced conditioned freezing in physically active animals (Burghardt et al., 2006; Van Hooymissen et al., 2004). Rats were removed from the conditioning chambers 1 min after the 3rd shock and were returned to their home cages, where physically active rats were allowed access to their running wheels. Assessment of contextually-conditioned freezing occurred in a retention test 24 hours later. Rats were again placed into the conditioning chambers and freezing was scored for 5 minutes. Scoring was always performed by an experimenter blind to the treatment condition of the animals. Animals were returned to their home cages immediately after the 5 minute observation period.

Extinction trials started the day after the first retention test and were repeated daily. During each extinction trial, rats were re-exposed to the conditioning chamber for 5 minutes and freezing was scored as before. Re-exposures continued until the average freezing level for a particular group reached below 10% of the level that group displayed during the first retention test. This level of freezing was defined as extinction (Baratta et al., 2007). Two weeks after extinction was reached, during which time rats remained in their home cages, rats were again re-exposed to the conditioning context for 5 minutes. Freezing was scored as a measure of memory for extinction. Physically active rats were allowed voluntary access to their running wheels during the entire experiment except when rats were in the conditioning chambers.

A second experiment investigated the effects of post-conditioning wheel running on contextual conditioning and extinction. This experiment was performed identically to the first experiment except for the fact that the conditioning procedure occurred prior to any wheel access. Rats were returned to their home cages immediately following contextual conditioning and at this time the wheels in the cages of the rats assigned to the physically active conditions were unlocked. Separate groups of physically active rats were then allowed voluntary access to their wheels for either 1 week (n = 8) or 6 weeks (n = 8) before the first retention test. Sedentary rats (n = 8/group) remained sedentary for an equivalent amount of time following conditioning.

To determine the effect of wheel running on generalization, a third experiment was performed. Rats in this experiment either remained sedentary ($n = 8$) or had 6 weeks of voluntary access to running wheels ($n = 8$) prior to being exposed to the contextual conditioning procedure described above. A 3 min retention test was performed a day after conditioning, during which freezing was scored. Rats were returned to their home cages for 1 hour following the retention test (during which time physically active rats had voluntary access to their running wheels) before being placed into the novel context where freezing was scored for 3 min as a measure of generalization.

If wheel running facilitates the ability to learn about context, then physically active, compared to sedentary, rats should display a higher level of contextually-conditioned freezing 24 hours following the presentation of a single shock delivered following minimal context pre-exposure time. A final experiment was performed to test this prediction. Following 6 weeks of the sedentary or physically active conditions, rats were either left undisturbed in their home cages (non-shocked controls; $n = 8/\text{group}$), or were placed into the conditioning chambers for either 10 ($n = 8/\text{group}$), 20 ($n = 10/\text{group}$), or 45 ($n = 10/\text{group}$) seconds prior to administration of a single, 2 second (0.8 mA), un-signaled shock. Rats were removed from the conditioning chamber and returned to their home cages within 10 seconds following shock presentation. Rats were placed into the conditioning chamber 24 hours later and freezing was scored.

Statistical Analysis

Group differences in body weights between sedentary and physically active rats were compared using repeated measures ANOVA. Freezing data obtained during retention tests and each extinction trial were collapsed into 5, 1-minute blocks and analyzed with repeated measures ANOVA. Freezing data obtained during each extinction trial (except the extinction memory test) were also averaged and analyzed with repeated measures ANOVA across days. For the extinction memory (recovery) trials, freezing data was averaged and analyzed with ANOVA. To assess group differences in generalization, average freezing values in each context were analyzed with 2 (sedentary vs. exercise) X 2 (original context vs. novel context) ANOVA. Freezing data obtained during the retention test following minimal context pre-exposure times were analyzed with 2 (sedentary vs. exercise) X 4 (no shock, 10 sec, 20 sec, 45 sec) repeated measures ANOVA. Group differences in BDNF mRNA levels were compared with ANOVA. Fisher's protected least significant difference (Fisher's-PLSD) post hoc analyses were performed when required. Alpha was set at $p \leq .05$ for each analysis.

Results

Running distances and body weight

Physically active rats in each experiment displayed running behavior typical of Fischer 344 rats (Greenwood et al., 2005a; Greenwood et al., 2005b). In general, average running distance increased weekly until the 4th week of wheel running, after which running distance remained steady at an average of 23 ± 8.1 km / week. Average weekly running distance over 6 weeks from a representative group of physically active rats ($n = 8$) is shown in Figure 1A. Exposure to the conditioning procedure did not affect later running behavior in physically active animals.

Rats allowed voluntary access to running wheels gained less weight over time compared to sedentary rats. Figure 1B shows the effect of wheel access on body weight gain over 6 weeks. The weight data shown were taken from the animals used to investigate the effects of pre-conditioning wheel running on contextual conditioning in experiment 1; and the running

distance of these physically active rats can be found in Figure 1A. Repeated measures ANOVA revealed significant main effects of exercise ($F(1, 14) = 29.7$; $p < .0001$) and time ($F(6, 84) = 634.9$; $p < .0001$), as well as a significant exercise by time interaction ($F(6, 84) = 40.5$; $p < .0001$) on body weight. Body weights of sedentary and physically active rats did not differ prior to the start of wheel access, but physically active rats weighed less than sedentary rats every week thereafter. Body weights of rats used in other experiments followed similar patterns (data not shown).

Wheel running before conditioning improves memory for context but has no effect on extinction

The experimental design is shown in Figure 2A. Neither sedentary nor physically active rats displayed any freezing behavior when first placed into the conditioning chamber prior to the first shock (Figure 2B). Freezing levels increased in both groups following each of the 3 foot shocks ($F(2, 28) = 6.28$; $p < .05$) but did not differ between groups (Figure 2B), suggesting that wheel running affects neither the acquisition of the contextual memory nor freezing per se. In contrast, the physically active group displayed higher freezing levels than the sedentary group when re-exposed to the conditioning chamber 24 hours later (Figure 2C). This was confirmed with repeated measures ANOVA which revealed a significant main effect of exercise ($F(1, 14) = 4.9$; $p < .05$). These data suggest that wheel running facilitates the consolidation of the contextual memory that occurs during the period between acquisition and recall of the context memory.

To determine the effect of pre-conditioning wheel running on extinction, rats were placed back into the conditioning chambers once a day and freezing was measured for 5 minutes. No significant effect of wheel running was observed on within session extinction during any of the extinction trials, so each group's freezing scores for each day were averaged and analyzed with repeated measures ANOVA. Wheel running had no effect on the extinction learning or memory. Freezing to the context decreased in both groups over time ($F(11, 154) = 49.28$; $p < .0001$) and both groups reached criterion for extinction ($< 10\%$ of day 1 freezing level) on the 12th day of re-exposure (Figure 2D). Two weeks after extinction was reached, rats were placed back into the conditioning chambers for assessment of memory for extinction. Sedentary and physically active groups displayed statistically equivalent recovery of conditioned freezing, indicative of similar memory for extinction (Figure 2D).

Wheel running after conditioning has no effect on learning or memory for context or extinction

Figure 3A shows the experimental design. Rats either remained sedentary or were allowed access to running wheels for 1 or 6 weeks after contextual conditioning. The level of freezing observed prior to and following each shock during conditioning was the same between groups (not shown). Wheels in the cages of the exercise groups were unlocked immediately following conditioning. Neither 1 week (Figure 3B) nor 6 weeks (Figure 3C) of post-conditioning wheel running altered the level of freezing during the first re-exposure to the conditioning chamber, although the interaction between 1 week of exercise and time ($F(4, 56) = 2.36$; $p = .06$) on freezing just missed significance (Figure 3B). Similarly, neither 1 week (Figure 3D) nor 6 weeks (Figure 3E) of post-conditioning wheel running altered extinction learning. When rats were placed back into the conditioning chambers for extinction trials starting 1 week after conditioning, conditioned freezing displayed by both groups decreased similarly over time ($F(12, 168) = 57.02$; $p < .0001$) and both sedentary and physically active groups reached extinction on the 13th day of extinction trials (Figure 3D). When extinction trials started 6 weeks after conditioning, extinction was reached after 9 days ($F(8, 112) = 37.86$; $p < .0001$) regardless of whether or not rats had access to running wheels during the 6 weeks separating conditioning from context re-exposure (Figure 3E).

Similarly, wheel running following conditioning did not alter the level of freezing during the recovery test regardless of whether 1 week (Figure 3D) or 6 weeks (Figure 3E) separated conditioning from the first context re-exposure.

Wheel running reduces generalization of fear to a novel context

The current experiment tested the prediction that if wheel running improves hippocampal-dependent memory for context, then physically active, compared to sedentary, rats should display minimal generalization of conditioned responses to novel contexts that have never before been paired with shock. The experimental design is shown in Figure 4A. Rats remained sedentary or voluntarily ran in running wheels for 6 weeks. The day following contextual conditioning, rats were re-exposed to the conditioning context for 3 minutes, followed an hour later by a 3 minute exposure to the novel context. The results are shown in Figure 4B. As observed above, pre-conditioning wheel running did not alter freezing behavior prior to or during the conditioning procedure (not shown), but did increase freezing behavior during re-exposure to the conditioning context 24 hours later. In contrast, wheel running reduced the generalization of fear to the novel context. These observations were confirmed with ANOVA which revealed a significant main effect of context ($F(1, 28) = 61.95$; $p < .0001$) and a significant interaction between exercise and context ($F(1, 28) = 8.93$; $p < .05$). Post-hoc tests revealed that all groups differed from each other.

Wheel running facilitates the ability to learn about context

To determine if wheel running can facilitate the ability to form a representation of context following minimal context pre-exposure time, rats were placed in the conditioning chamber for either 10, 20, or 45 seconds before presentation of a single shock. The level of freezing behavior displayed by these rats during re-exposure to the context 24 hours later was compared to the level of freezing displayed by context- and shock- naïve rats. The design of the experiment is shown in Figure 5A. Exercise facilitated the ability to learn about context even following minimal context pre-exposure time (Figure 5B and C). Repeated measures ANOVA revealed main effects of exercise ($F(1, 64) = p < .05$) and pre-exposure time ($F(3, 64) = 27$; $p < .0001$) on contextual freezing. The interaction between 1-minute freezing blocks and pre-exposure time was also significant ($F(12, 256) = 4.75$; $p < .0001$), but the exercise by pre-exposure time interaction just missed significance ($p = .07$). Post-hoc tests revealed that, regardless of exercise condition, both 20 and 45 seconds of context pre-exposure resulted in significantly more conditioned freezing behavior compared to the freezing displayed by the non-shocked and 10 second pre-exposure groups, which did not differ from each other. This was true for all 5, 1-minute blocks, with the exception of the 3rd through 5th, 1-minute freezing blocks, when the sedentary, 20 second pre-exposure group did not differ from the non-shocked and 10 second pre-exposure groups. Sedentary rats given 20 and 45 seconds of pre-exposure time displayed similar freezing behavior during context re-exposure except during the 3rd, 1-minute block, when the sedentary, 45 second group froze more than the sedentary, 20 second group. Physically active rats given 20 seconds of pre-exposure to the conditioning context froze more than the sedentary, 20 second, and sedentary, 45 second, groups during the 1st, 1-minute block, and more than the sedentary, 20 second group during the 4th, 1-minute block. Physically active rats given 45 seconds of context pre-exposure time displayed more freezing behavior during all 1-minute blocks relative to all other groups. To ease visual inspection of the data, Figure 5D shows the average freezing behavior over the entire 5 minute freezing observation period.

Wheel running increases BDNF mRNA in the hippocampus and BLA

Figure 6A shows the effects of 6 weeks of wheel running on BDNF mRNA levels in the rostral-caudal axis of the hippocampus. Similar to our prior observations in F344 rats (Greenwood et al., 2007b), wheel running increased BDNF mRNA in the dentate gyrus and

CA1 only. This effect was observed in the rostral ($F(1, 17) = 6.8$; $p < .05$), mid ($F(1, 17) = 10.7$; $p < .005$), and caudal ($F(1, 17) = 14.1$; $p < .005$) dentate gyrus and the mid ($F(1, 17) = 6.4$; $p < .05$) and caudal ($F(1, 17) = 14.2$; $P < .005$) CA1. Interestingly, the greatest effect of physical activity was observed in the caudal (ventral) hippocampus, where there was also a significant effect of wheel running on BDNF mRNA in CA2 ($F(1, 17) = 8$; $p < .05$) and a trend in the CA3 ($p = .07$). Wheel running had no effect on BDNF mRNA levels in the medial mammillary nucleus (Figure 6A). Figure 6B shows representative autoradiographs illustrating group differences in levels of BDNF mRNA in the hippocampus. Wheel running also increased BDNF mRNA in the mid aspect of the BLA ($F(1, 17) = 4.25$; $p < .05$; Figure 7A). Figure 7B shows autoradiographs illustrating BDNF mRNA levels in the BLA of sedentary and physically active rats.

Discussion

The current data replicate and extend prior work on the effects of voluntary physical activity on hippocampal-dependent learning and memory. Wheel running increased BDNF mRNA in the hippocampus and BLA, suggestive of a greater potential for BDNF-related plasticity in these regions. Consistent with improved hippocampus function, physical activity facilitated contextual learning, during both optimal and sub-optimal conditions, but only if wheel access occurred prior to conditioning. Pre-conditioning wheel running also reduced generalization but had no effect on extinction learning or memory. Results are consistent with the idea that voluntary physical activity can improve the ability of the hippocampus to learn and remember contextual information.

Similar to the effects of physical activity, enhanced learning and memory processes have been reported following a period of environmental enrichment (Huang et al., 2006; Nilsson et al., 1999). Because sedentary rats in the current studies were housed in the absence of a locked wheel, it is possible that the mere presence of a running wheel contributed to the observed effects of physical activity in the current studies. Burghardt et al. (2006), however, included locked wheels in the cages of sedentary rats and reported that the access to an unlocked wheel improved contextual conditioning relative to the locked-wheel condition. Nevertheless, a role for environmental environment in the current results cannot be ruled out.

The observation that wheel running initiated after conditioning had no effect on memory for the conditioned context deserves special consideration. One explanation for the lack of improvement in context memory following post-conditioning wheel running is that the process of conditioning, during which aversive shocks are administered, somehow interfered with the plastic changes in the hippocampus or other structures that typically occur with exercise and are needed for improved memory. Indeed, stressor exposure can disrupt hippocampal-dependent learning and memory processes (Conrad et al., 1999; Diamond et al., 2006; Park et al., 2008; Roozendaal, 2002), interfere with hippocampal neuronal plasticity (Diamond et al., 2007; Foy et al., 1987; Shors et al., 1990), and reduce BDNF expression (Smith et al., 1995a; Smith et al., 1995b; Ueyama et al., 1997). This scenario is unlikely; however, because we have observed that 6 weeks of post-conditioning wheel running increases BDNF mRNA in the hippocampus and amygdala (Fleshner, 2007), indicating that at least some of the molecular changes induced by physical activity occur even when stressor exposure precedes wheel access. Thus, it is not that plastic changes don't occur when wheel running follows conditioning; rather, these changes might be insufficient to improve distant memory of context. It is possible that the molecular changes need to be in place prior to learning in order to observe the beneficial effects of wheel running on contextual conditioning.

It is of interest to speculate which aspect of contextual conditioning is affected by physical activity. Although the hippocampus and amygdala are both involved in context conditioning (Phillips and LeDoux, 1992), available data indicate that physical activity improves contextual conditioning through changes in hippocampal, but not amygdala, function. The hippocampus is critical for memory of context; whereas the amygdala stores the context-shock association during contextual fear conditioning (Huff and Rudy, 2004). Wheel running had no effect on freezing immediately prior to or following conditioning (current results and (Burghardt et al., 2006; Van Hooissen et al., 2004); nor does it affect auditory fear conditioning (Baruch et al., 2004). Auditory conditioning (Phillips and LeDoux, 1992) & freezing immediately following shock (Kim et al., 1993) are supported by the amygdala & not the hippocampus, suggesting that physical activity improves contextual conditioning by acting specifically on hippocampal-dependent consolidation of the context memory and not acquisition of the amygdala-dependent context-shock association or freezing behavior per se.

Although the mechanisms underlying improved hippocampus-dependent learning and memory following physical activity are unknown, increased BDNF likely plays a role (Liu et al., 2008; Vaynman et al., 2004; Yukako et al., 2008). Consistent with an involvement of BDNF in improved contextual memory following wheel running is the observation that chronic systemic blockade of β -adrenergic receptors with propranolol eliminates both the increase in contextually conditioned freezing and hippocampal BDNF mRNA following wheel running (Van Hooissen et al., 2004). Elevated levels of BDNF in the hippocampus of physically active rats could improve context memory by facilitating molecular processes underlying consolidation (Alonso et al., 2002; Patterson et al., 1996; Poo, 2001; Tokuyama et al., 2000; Tyler et al., 2002; Zakharenko et al., 2003).

If physical activity improves consolidation of the memory for context, then 1) the ability to discriminate between contexts should be improved by wheel running, and 2) wheel running should facilitate contextual memory processes even following sub-optimal learning conditions under which sedentary rats have difficulty forming a contextual representation. Both of these predictions proved correct. Physically active rats that displayed greater memory for the conditioned context than sedentary rats indeed showed less generalization to a novel context, indicative of improved context discrimination. Additionally, short durations of pre-exposure to the conditioning context supported minimal conditioning in sedentary rats only. Physically active rats given brief context pre-exposure still displayed robust conditioned freezing when re-exposed to the conditioned context the next day. Interestingly, 10 seconds was an insufficient duration of context pre-exposure time to support conditioning in either sedentary or physically active rats, suggesting that a minimal duration of context pre-exposure is still required to support conditioning in physically active animals. It is possible that 10 seconds is simply not enough time to gather information about contextual cues. When the minimal amount of time (20 seconds) is provided to acquire the memory trace, prior wheel running can facilitate contextual memory.

In addition to consolidation of context memory, consolidation of extinction memory requires intact BDNF signaling in the amygdala (Chhatwal et al., 2006) and the hippocampus (Heldt et al., 2007); however, whether manipulations that increase BDNF enhance extinction learning is unknown. Despite the observation of increased BDNF mRNA in the amygdala and hippocampus of physically active rats, neither pre-conditioning nor post-conditioning wheel running improved extinction learning or memory, as assessed by freezing during repeated retention tests in the conditioned context. Greenwood et al. (2007), however, reported facilitated within-session extinction of contextually conditioned freezing following 6 weeks of post-conditioning wheel running. The difference between prior work (Greenwood et al., 2007a) and the current experiments is that rats used in the Greenwood et

al. (2007) study were conditioned with 60 unpredictable shocks of a random duration between 5 – 25 seconds each, whereas only 3, 2-second shocks were used here. Given that the 60-shock conditioning paradigm is a more intense stressor than the more typical 3-shock paradigm, it is possible that the effect of wheel running on extinction depends on the emotional salience assigned to the memory trace to be extinguished. Indeed, it has recently been revealed that different neural substrates can support contextual conditioning depending on the extent of training (Zimmerman et al., 2007). Thus, it is possible that different mechanisms could also support the extinction of contextual conditioning depending on these same factors. Nevertheless, wheel running did not alter extinction learning or memory under the current experimental conditions.

The results presented here lend support to the idea that wheel running improves several aspects of hippocampal function. It is proposed that wheel running facilitates consolidation of the contextual representation in the hippocampus and therefore improves memory for context. Enhanced BDNF-related plasticity could contribute to the effect of physical activity on contextual learning and memory, but may be insufficient to alter learning and memory for extinction.

Acknowledgments

Support: Grant Sponsor: NIH Grant Number: R01-MH068283-01

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Figure 1.

(A) The mean distance (in kilometers) run each week by adult male Fischer 344 rats allowed voluntary access to running wheels for 6 weeks prior to contextual fear conditioning. (B) Mean body weight gain (in grams) across 6 weeks of sedentary housing or voluntary wheel running. Small error bars in (B) are obscured by graph symbols. Values represent group means $\pm$ SEM.

Figure 2.

Effects of 6 weeks of wheel running prior to contextual fear conditioning on conditioned freezing behavior and extinction. (A) Rats either remained sedentary or were allowed voluntary access to running wheels for 6 weeks before being exposed to contextual conditioning. The first retention test occurred one day following conditioning, followed by repeated extinction trials during which rats were re-exposed to the conditioned chamber in the absence of shock. After extinction was reached rats were not re-exposed to the conditioned chambers until 2 weeks later, when a final retention test was performed to assess memory for extinction. (B) Freezing behavior prior to and immediately following shock presentation during contextual conditioning was similar between sedentary and physically active rats. Arrows indicate when shocks were delivered. (C) Freezing behavior during the first retention trial one day following conditioning. Physically active rats displayed more conditioned freezing compared to sedentary rats. (D) Average freezing behavior during 5 minute extinction trials and a final test for extinction memory (Ext Mem). Wheel running had no effect on extinction. Data represent group means $\pm$ SEM.

Figure 3.

Effects of 1 and 6 weeks of wheel running after contextual conditioning on conditioned freezing behavior and extinction. (A) Rats exposed to contextual conditioning either remained sedentary or were allowed voluntary access to running wheels for 1 or 6 weeks. One or 6 weeks following conditioning, rats were re-exposed to the conditioning chambers and freezing was assessed. Extinction trials occurred daily until extinction was reached. Two weeks later, rats were re-exposed to the conditioned chamber for a final test of extinction memory (Ext Mem). Neither 1 week (B) nor 6 weeks (C) of wheel running altered freezing behavior during the first retention test. Sedentary and physically active rats showed similar learning and memory for extinction regardless of whether extinction trials were begun (D) 1 week or (E) 6 weeks following conditioning. Data represent group means $\pm$ SEM.

Figure 4.

Effects of 6 weeks of pre-conditioning wheel running on generalization. (A) Following 6 weeks of the sedentary or physically active conditions, rats were exposed to contextual conditioning. The day after conditioning, rats were re-exposed to the conditioning environment for a retention test. One hour later, rats were placed into a novel context and freezing was scored as a measure of generalization. (B) Freezing during exposure to the 2 contexts. Physically active rats displayed more freezing behavior in the conditioned context but less freezing behavior in the novel context. * $p < .05$ relative to all other groups. Data represent group means $\pm$ SEM.

Figure 5.

Effects of 6 weeks of pre-conditioning wheel running on contextual conditioning following brief context pre-exposure times. (A) Sedentary and physically active rats were either not exposed to shock (No Shock) or were exposed to a single shock immediately following 10 (10 sec), 20 (20 sec), or 45 (45 sec) seconds of context pre-exposure. Twenty four hours later, rats were re-exposed to the conditioning chambers and freezing was scored. Longer pre-exposure times supported greater conditioned freezing in both sedentary (B) and physically active (C) groups, although physically active rats pre-exposed to the conditioned context for 20 or 45 seconds froze more than sedentary counterparts. (D) The average freezing displayed by sedentary and physically active rats during the entire 5 minute retention test. θ $p < .05$ relative to sedentary, no shock and 10 sec groups. Φ $p < .05$ relative to sedentary, no shock, 10 sec, and 20 sec groups, and physically active, no shock and 10 sec groups. * $p < .05$ relative to all other groups. Data represent group means $\pm$ SEM.

Figure 6.

(A) Relative levels of BDNF messenger ribonucleic acid (mRNA) in the rostral – caudal axis of the hippocampus and the medial mammillary nucleus (MM) of sedentary rats and rats allowed 6 weeks of voluntary access to running wheels. (B) Representative autoradiographic coronal sections through the rostral, mid, and caudal aspects of the hippocampus of sedentary (left panels) and physically active (right panels) rats processed with in situ hybridization for BDNF mRNA. Scale bar = 1 mm. Values represent mean integrated density $\pm$ SEM. DG, dentate gyrus. * $p < .05$; ** $p < .005$ relative to sedentary group.

Figure 7.

(A) Relative levels of BDNF messenger ribonucleic acid (mRNA) in the rostral – caudal axis of the basolateral amygdala (BLA) of sedentary rats and rats allowed 6 weeks of voluntary access to running wheels. (B) Representative autoradiographic coronal sections through the mid BLA of sedentary (left) and physically active (right) rats processed with in situ hybridization for BDNF mRNA. The lines indicate the BLA. Scale bar = 1 mm. Values represent mean integrated density $\pm$ SEM. * $p < .05$ relative to sedentary group.