The World Journal of Biological Psychiatry, 2011; Early Online, 1–7

ORIGINAL INVESTIGATION

Voluntary exercise does not ameliorate context memory and hyperarousal in a mouse model for post-traumatic stress disorder (PTSD)

RAFFAELE CACCIAGLIA[1*], ANNEGRET KRAUSE-UTZ[2*], MIRIAM A. VOGT[3], CHRISTIAN SCHMAHL[2], HERTA FLOR[1] & PETER GASS[3]

[1]*Institute of Neuropsychology and Clinical Psychology, Central Institute of Mental Health, Mannheim, Germany,*
[2]*Department of Psychosomatic Medicine and Psychotherapy, Central Institute of Mental Health, Mannheim, Germany, and*
[3]*Department of Psychiatry, RG Animal Models in Psychiatry, Central Institute of Mental Health, Mannheim, Germany*

Abstract
Objectives. We investigated the effects of voluntary wheel running as model for intervention on the development of contextual fear and hyperarousal in a mouse model of post-traumatic stress disorder (PTSD). Physical exercise in general has been associated with improved hippocampus-dependent memory performance both in animals and humans. However, studies that have tried to link physical exercise and contextual conditioning in an animal model of PTSD, revealed mixed findings. *Methods.* Here we tested contextual fear conditioning, generalized fear response, acoustic startle response and emotionality in C57BL/6NCrl mice which had free access to a running wheel for 28 days, compared with control animals which did not run and mice which did not receive a shock during the conditioning phase. *Results.* We found no significant effects of voluntary running on the above-mentioned variables, except for enhanced anxiety levels in the Dark-Light-Box and O-Maze tests of running mice. *Conclusions*. Our results suggest that running as a model for intervention does not ameliorate contextual aversive learning but has the potency to change emotional behaviours.

Key words: *Anxiety, behaviour, acute treatment, post-traumatic stress disorder PTSD, fear conditioning*

Introduction

Contextual fear conditioning has been proposed as an animal model for Post-traumatic Stress Disorder (PTSD) (Rau et al. 2005; Vansteenwegen et al. 2005). Siegmund and Wotjak developed an animal model of PTSD, which covers both associative (context conditioning) and non-associative fear components (hyperarousal, anxiety, startle response) after trauma (Siegmund and Wotjak 2006): In this mouse model, the animals are exposed to a single short and severe stressor (e.g., a footshock). Immediately as well as 4 weeks later, the mice show increased fear memory indicated by freezing compared with non-shocked animals when replaced into the threatening context (Kim and Jung 2006; Siegmund and Wotjak 2007; Golub et al. 2009). They also exhibit increased generalized fear (hyperarousal) when exposed to a loud tone in a different context (Balogh and Wehner 2003), thus providing another appropriate parallel to PTSD symptoms (Yehuda 2002).

As an important aspect of environmental enrichment, physical exercise is known to have beneficial effects on learning and memory in animals (Cotman and Berchtold 2007; Kramer and Erickson 2007; Hillman et al. 2008; Remington 2009; van Praag 2009). For example, rodents, which undergo either voluntary or forced running protocols, show enhanced learning and memory (Van der Borght et al. 2007; van Praag 2008) and improved spatial discrimination (Leggio et al. 2005; Creer et al. 2010) demonstrated by a better performance in the Morris Watermaze or in the Radialarm-Watermaze (Vaynman et al. 2004b; van Praag et al. 2005; Nichol et al. 2007; Khabour et al. 2010). These effects persist even when retested after days of exercise offset (Vaynman et al. 2004a) and have been related to increased expression of

*Shared first authorship.
Correspondence: Dr Miriam A. Vogt , RG Animal Models in Psychiatry, Central Institute of Mental Health, J5, 68159 Mannheim, Germany. Tel: +49 621 1703 2932. Fax: +49 621 1703 6205. E-mail: miriam.vogt@zi-mannheim.de

(Received 17 January 2011; accepted 19 April 2011)

ISSN 1562-2975 print/ISSN 1814-1412 online © 2011 Informa Healthcare
DOI: 10.3109/15622975.2011.583270

hippocampal brain-derived neurotrophic factor (BDNF) (Johnson et al. 2003; Griesbach et al. 2004, 2009; O'Callaghan et al. 2007; Liu et al. 2009), hippocampal neurogenesis (Farmer et al. 2004; van Praag et al. 1999a,b), synaptic plasticity and enhanced long-term potentiation in the dentate gyrus of the hippocampus (O'Callaghan et al. 2007; Hernandez-Rabaza et al. 2009). So far, studies investigating the influence of previous physical exercise on context memory in rats and mice show enhancing effects (Baruch et al. 2004; Clark et al. 2008). In contrast, studies investigating the influence of physical exercise as a specific aspect of environmental enrichment on both associative fear (context memory) and non-associative sensitization processes (hyperarousal, startle response) – being regarded as PTSD-like symptoms – are still missing.

Therefore, the aim of this study was to investigate the influence of voluntary physical exercise as intervention after the trauma on context memory, hyperarousal, acoustic startle response and behavioural anxiety in an animal model of PTSD.

Material and methods

Animals

Six-week-old male C57BL/6NCrl mice were purchased from Charles River (Sulzfeld, Germany). Prior to and during all experiments, animals were single-housed in macrolon type III cages and kept on a reversed dark–light cycle with lights on at 19:00 h (acclimation to the dark–light cycle 2 weeks before experiments started). Mice were supplied with food and water ad libitum. Bodyweight was assessed once a week, when the cages were changed.

Behavioural testing procedure

The mice were randomly assigned to the following groups: non-shocked ($n = 6$), (shocked) sedentary controls ($n = 10$) and (shocked) runners ($n = 10$). All experiments were performed during the dark cycle, i.e. in the animals' active phase. Prior to each test, mice were acclimatized to the experimental room for at least 30 min (except the PTSD procedure and the startle experiment). The animals were tested in the following order: PTSD procedure including shock application (or corresponding handling procedure for non-shocked animals), 28 days of wheel running or inserted blocked wheel in the cage and assessment of context memory and generalization 28 and 29 days after the trauma. The startle experiment was conducted within 3 days one week after the generalization. Animals of the control and of the runner group were tested for changes of emotional behaviour (O-Maze, Dark-Light-Box, 1 day in between) 1 week after a resting period of 1 week after the startle experiments. All experiments had been approved by German animal welfare authorities (Regierungspräsidium Karlsruhe).

Shock application and test of conditioned fear and hyperarousal (PTSD procedure)

Experimental setups and procedures have been described in detail before (Kamprath and Wotjak 2004; Siegmund and Wotjak 2007). Briefly, experiments were performed in a conditioning chamber ($58 \times 30 \times 27$ cm^3, TSE, Bad Homburg, Germany), which was surrounded by 15×15 infrared light beams and illuminated with 5 lx from above. For shock application, mice were individually placed in a Plexiglas cylinder (diameter 17 cm, height 25 cm) standing on the metal shock grid of the Conditioning Box. After 198 s a single 2 s scrambled electric foot shock of 0.7 mA intensity was administered via the metal grid. Animals remained in the shock chamber for another 60 s (period of acute stress reaction) before they were returned to their home cages. For mice of the non-shocked group we followed the same protocol but disconnected the cable for the shock. Reaction to the shock was visually scored (with 0 = no reaction, 1 = running, 2 = running + screaming, 3 = jumping + screaming), and the velocity before, during and after the shock was measured with the help of the infrared light beams. To test for conditioned fear ("context memory"), animals were re-exposed to the shock chamber 28 days after the shock for 3 min without further shock application, and returned to their home cages immediately afterwards. During shock application and test for conditioned fear, the conditioning chamber was cleaned thoroughly after each mouse with 70% ethanol. To test for hyperarousal ("generalization"), animals were placed into a novel and neutral test context (macrolon type I cage with bedding within the Conditioning Box) 24 h after the tests for conditioned fear. After 3 min a neutral loud tone the animal had not heard before (80 dB, 9 kHz sine wave) was presented for 3 min. After tone presentation the animals remained in the test context for another 60 s before they were returned to their home cages. After each mouse, the bedding was changed and the context cleaned thoroughly with 1% acetic acid. Under all test conditions, freezing behaviour, defined as a complete lack of movements apart from respiration, was scored manually at intervals of 10 s in each phase.

Running procedure

One day after the shock, mice in the running group ($n = 10$) were given free access to a running wheel (diameter 11.5 cm) connected to a mechanical counter. The counting was measured by ClockLab data collection and analyzed by ClockLab data analysis (Coulburn, Whitehall, PA). Running performance was measured during the 28 days between the shock and context memory test, although the running wheel remained in the cage during the following experiments. The control animals ($n = 10$) and the animals of the non-shocked group ($n = 6$) were supplied with a blocked running wheel.

Acoustic Startle Response

Startle testing occurred in a startle chamber (SR-LAB; San Diego Instruments, San Diego, CA). A loudspeaker inside the box produced a continuous background noise of 68 dB sound pressure level (SPL) as well as the acoustic startle stimuli. A white noise pulse was used as the startle stimulus, with an intensity of 90, 105 or 115 dB SPL and duration of 20 ms. An acclimatization time of 5 min, during which the mice received no stimulus except the background noise, was followed by the presentation of startle stimuli. The test paradigm consisted of 30 stimuli of the respective intensity and 16 trials without stimuli in pseudorandomized order with an inter-trial interval of 13–17 s.

Anxiety-related behaviour

Anxiety-related behaviour was monitored in the Dark-Light-Box and the Elevated O-Maze, both inflicting an approach-avoidance conflict to enter the bright compartment (Dark-Light-Box) or to enter the unsheltered open part (O-Maze).

Dark-Light-Box

The Dark-Light-Box consisted of two plastic chambers, connected by a small tunnel. The dark chamber measured 20×15 cm^2 and was covered by a lid. The adjacent chamber, measuring 30×15 cm^2, was white and illuminated from above with 600 lx. Mice were placed into the dark compartment and latency to first exit, number of exits to and total time in the light compartment was recorded for 5 min.

O-Maze

The O-Maze consisted of a gray plastic annular runway (width 6 cm, outer diameter 46 cm, 50 cm above ground level), covered with black cardboard paper to prevent mice from slipping off the maze. Two opposing 90° sectors were protected by inner and outer walls of gray polyvinyl (height 10 cm). Animals were placed in one of the protected sectors and observed for 5 min. The maze was illuminated with 25 lx. The following parameters were analyzed: latency to first exit, number of exits to and total time spent in the open compartments.

Statistical analysis

All statistical analyses were performed using the statistical program PASW 18. Inter-group comparisons were calculated by univariate ANOVAs with Bonferroni post-hoc tests. For the bodyweight development, a repeated measurement ANOVA was calculated to evaluate dependency on time and group.

Results

Bodyweight development

Bodyweight was assessed before and during the testing procedures once per week. All mice gained weight during the testing time (repeated measurement ANOVA: factor time: $P = 0.000^{***}$) but running mice had a lower bodyweight than shocked sedentary controls (repeated measurement ANOVA: factor group: $P = 0.045^{*}$; Bonferroni post-hoc test: runners vs. controls $P = 0.043^{*}$).

Running performance

For 28 days the exercising mice ran constantly around 10 km per day, mostly during the dark phase (Table I). After returning to their home-cages after experimental sessions, mice continued running with the same intensity as before.

Context memory and hyperarousal

The mice in the runner group and the control group did not differ in their reaction to the shock regarding the subjective visual scoring and automized velocity measurements before, during and after shock exposure (see Table I). Mice of the non-shocked group did not show any changes of behaviour and performed therefore significantly different than mice of control and running group (ANOVA see Table I, Bonferroni post-hoc tests: shock scores: non-shocked vs. control or runners $P = 0.000^{***}$; velocity at shock: non-shocked vs. controls $P = 0.043^{*}$, vs. runners $P = 0.009^{**}$).

When tested in the PTSD paradigms 28 days after shock exposure, runners did not exhibit significant

Table I. Performance of non-shocked ($n = 6$), control and running mice ($n = 10$ per group) in the following tests: bodyweight development, shock reaction, context memory and hyperarousal in the animal model of PTSD, running performance, Startle reaction, Dark-Light-Box and O-Maze. Results are reported as means ± SEM. P values depend on univariate ANOVAs, P values from bodyweight development are from a repeated measurement ANOVA with the factors time and group. For post-hoc tests see text.

Test	Parameter		Non-shocked	Control	Running	P value
bodyweight	Age: 9 weeks (g)		25.50 ± 0.41	26.04 ± 0.20	25.09 ± 0.43	Time effect
	Age: 10 weeks (g)		25.53 ± 0.55	26.30 ± 0.12	25.00 ± 0.45	
	Age: 11 weeks (g)		26.50 ± 0.57	27.26 ± 0.21	25.84 ± 0.41	$P = 0.000^{***}$
	Age: 12 weeks (g)		27.02 ± 0.62	28.26 ± 0.19	26.69 ± 0.47	
	Age: 13 weeks (g)		27.88 ± 0.53	28.78 ± 0.33	27.04 ± 0.51	Group effect
	Age: 14 weeks (g)		28.73 ± 0.54	29.64 ± 0.38	28.52 ± 0.60	
	Age: 15 weeks (g)		29.28 ± 0.71	30.19 ± 0.45	28.66 ± 0.46	$P = 0.045^{*}$
PTSD	shock scores		0.00 ± 0.00	2.50 ± 0.17	2.40 ± 0.16	$P = 0.000^{***}$
	Velocity before shock (cm/s)		5.94 ± 1.19	4.74 ± 1.09	4.77 ± 0.87	$P = 0.714$
	Velocity at shock (cm/s)		4.85 ± 1.32	17.54 ± 2.84	22.33 ± 4.23	$P = 0.010^{**}$
	Velocity after shock (cm/s)		4.95 ± 1.01	2.96 ± 0.30	3.87 ± 0.73	$P = 0.161$
	Context	Freezing (%)	5.56 ± 3.80	38.89 ± 9.97	43.33 ± 3.49	$P = 0.003^{**}$
	Hyperarousal	Freezing (%)	6.48 ± 2.65	12.78 ± 3.32	20.56 ± 4.15	$P = 0.056$
Running performance	Distance (km/day)		–	–	9.72 ± 0.86	
Startle reaction	Stimulus	90 dB	77.11 ± 22.40	43.16 ± 7.81	66.99 ± 12.03	$P = 0.272$
		105 dB	179.58 ± 16.21	181.12 ± 14.03	173.14 ± 16.45	$P = 0.897$
		115 dB	190.21 ± 22.43	214.39 ± 22.30	192.10 ± 17.12	$P = 0.812$
Dark-Light-Box	Latency (s)		Not tested	43.60 ± 8.00	124.60 ± 35.30	$P = 0.040^{*}$
	Exits (n)		Not tested	10.30 ± 0.60	5.30 ± 1.25	$P = 0.002^{**}$
	Time spent in light (%)		Not tested	27.27 ± 2.86	15.17 ± 3.87	$P = 0.022^{*}$
O-Maze	Latency (s)		Not tested	48.50 ± 15.19	98.30 ± 33.01	$P = 0.187$
	Exits (n)		Not tested	6.70 ± 1.27	3.10 ± 0.81	$P = 0.028^{*}$
	Time spent on open arm (%)		Not tested	18.07 ± 4.68	12.97 ± 2.38	$P = 0.344$

differences in their freezing response compared to sedentary controls, neither in the context test nor in the generalization test (Table I). The non-shocked mice revealed significantly less freezing in the context than mice of the shocked groups (ANOVA see Table I, Bonferroni post-hoc tests: non-shocked vs. controls $P = 0.013^{*}$, vs. running mice $P = 0.004^{**}$). During generalization, mice of the non-shocked group tend to show less freezing behaviour but this did not reach significance (Table I).

Startle responses

In the startle reaction, the running mice exhibited no alteration compared to the control mice (see Table I). Also the non-shocked mice did not show alterations concerning the startle reaction (see Table I).

Anxiety-related behaviour

Runners displayed increased anxiety-related behaviour in the Dark-Light-Box test. The latency to enter the anxiety-related bright compartment was significantly higher, and the number of exits as well as the time spent in this compartment were decreased in runners (Table I). In the O-Maze the runner mice showed also significantly less exits to the anxiety-related open part, while the latency to enter the open part and the time spent on open arms were inconspicuous in runner mice in comparison to the control mice (Table I).

Discussion

The aim of the present study was to investigate the effect of 28 days of voluntary wheel running on contextual and generalized fear in mice, based on an animal model of PTSD which accounts for both associative and non-associative processes in the pathogenesis of the disorder (Siegmund and Wotjak 2006). Since physical exercise is known to increase hippocampal BDNF signaling (Johnson et al. 2003; Griesbach et al. 2004, 2009; O'Callaghan et al. 2007; Liu et al. 2009) and neurogenesis (van Praag et al. 1999a,b; Farmer et al. 2004), our suggestion was that physical exercise intervenes contextual fear, which is hippocampus-dependent (Phillips and

LeDoux 1992). We additionally assessed a possible hyperarousal state by measuring the acoustic startle response and emotional changes in particular anxiety-related behaviour, as PTSD has been related to sustained anxiety and enhanced physiological reactivity (Orr et al. 2002).

Without previous shock, mice display almost no fear response, when exposed 28 days later to the context. In contrast, during the tone presentation in the generalization test also non-shocked control mice showed a low but considerable freezing response compared to both shocked animal groups. This difference did not reach statistical significance, but a trend level ($P = 0.056$). This could be due to high individual variability especially in this group with a lower n-number ($n = 6$) in contrast to the other groups ($n = 10$ each). Moreover, the high intensity of the tone presented during the generalization (80 dB) alone obviously has the potency to induce fear and the corresponding fear response which was discovered in the non-shocked animal group. Mice exercising between the previous trauma and the consecutive investigation of freezing behaviour did not demonstrate significant alterations compared to controls concerning contextual and generalized fear as well as hyperarousal. Previous studies that directly assessed contextual fear conditioning as a function of physical exercise have revealed heterogeneous findings. For example, Baruch and colleagues (2004) reported increased memory for threatening contexts in rats which underwent free voluntary running for 4 weeks, compared to non-running controls. However, the conditioned response was measured 24 h after shock exposure, a paradigm that substantially differed from our protocol applied, as we tested freezing behaviour 28 days after shock delivery. This is a relatively long period in a mouse's life but PTSD symptoms are persistent even after months or years following trauma exposure in humans (Yehuda 2002). Another study by Clark et al. (2008) also reported increased context conditioning in mice, which voluntarily ran for 54 days, compared to sedentary controls. However, in Clark's study, the behavioural experiments were conducted after previous running and the mice were older, having been tested at the age of 5–7 months. Furthermore, fear conditioning was assessed during the light phase of the light/dark cycle, whereas our tests were performed during the dark, active phase of the animals, a feature that might also have contributed to the divergent results between Clark's and our study (Loh et al. 2010). On the other hand, our results are in agreement with Burghardt et al. (2004) who did not find significant effects of either 4 or 8 weeks of wheel running on aversive context memory in rats, and with Wojtowicz et al. (2008), who also reported no significant changes in contextual freezing after running in rats. A recent study by Greenwood et al. (2009) employed two different experimental paradigms, which either assessed fear conditioning prior to 6 weeks of running, or afterwards. Interestingly, the authors found significantly better context discrimination only in mice which underwent running before the traumatic event (i.e. shock delivery), but not afterwards, which is in accordance with our data, as we administered the shock before the beginning of the training program. In our study, voluntary wheel running was hypothesized to ameliorate the developing symptoms of PTSD after trauma exposure, however, running mice showed altered emotional behaviours but similar symptoms of PTSD as control mice. This suggests that physical exercise might have a significant effect on contextual aversive memories only if the trauma (i.e. shock) occurs when the affected individual is already extensively physically exercising, rather than starting to exercise after the trauma. A point which has also to be considered is the running-induced enhancement of corticosterone levels in exercising mice (Fuss et al. 2010b). Patients with PTSD display variable cortisol levels, sometimes found to be below normal levels, but having also reported to be increased or unchanged (Yehuda 2002). Thus, the increase in corticosterone levels in exercising mice could possibly influence or even inhibit a possible positive outcome of running as therapeutic intervention.

In the present study we reported significantly increased anxiety-like behaviour, as assessed with the Dark-Light-Box and the O-Maze, in running versus non-running mice. Similar to previous studies (Fuss et al. 2010b) running mice showed higher individual variability, in particular in the parameter latency. This could be depending on different running intensities, or on a decreased impulsivity control. Nevertheless, the overall anxious phenotype of running mice is evident and statistically significant. The decreased number of exits and increased latencies are not due to fatigue, as mice start running with the same intensity, when they are replaced in the home cages or when monitored for 24 h after removing the wheel (Fuss et al. 2010a). Our results are in agreement with several studies that reported increased anxiety in rodents after physical exercise (Binder et al. 2004; Burghardt et al. 2004; Van Hoomissen et al. 2004; Leasure and Jones 2008; Fuss et al. 2010b), but in contrast with other reports (Greenwood et al. 2003; Duman et al. 2008). In the study of Burghardt et al. (2004), increased anxiety-like/defensive response behaviour were found in mice which voluntarily ran 8 weeks, as demonstrated by reduced activity in the Openfield and diminished entries in unsheltered compartments of the Elevated

Plus-Maze. Interestingly, 8 weeks of regularized treadmill exercise in the same mice strain (45 min/ day, 5 days/week), did not yield any change in anxiety-related behaviour, suggesting that only intensive and unrestricted or voluntary running may have anxiogenic effects. Our results expand this notion, as we detected significantly increased anxiety in mice already after 28 days of running compared with sedentary controls. Also the diminished increase in bodyweight in running mice demonstrates a physical reaction to voluntary running in these animals.

Taken together, our results suggest no effect of voluntary running as intervention in the animal model of PTSD selected in this study. However, our data confirm and extend previous reports on altered emotional behaviours in running mice compared with sedentary controls, as we show that 28 days of running clearly lead to enhanced anxiety.

Acknowledgments

We thank C. Wotjak from the MPI for Psychiatry in Munich for his helpful advice concerning the establishment of the PTSD animal model. The authors further acknowledge the excellent technical support of N. Pfeiffer. This study was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) via SFB636 to CS, HF and PG. R. Cacciaglia and A. Krause-Utz held a scholarship from the Graduate Program of the SFB636.

Statement of Interest

None to declare.

References

Balogh SA, Wehner JM. 2003. Inbred mouse strain differences in the establishment of long-term fear memory. Behav Brain Res 140:97–106.

Baruch DE, Swain RA, Helmstetter FJ. 2004. Effects of exercise on Pavlovian fear conditioning. Behav Neurosci 118:1123–1127.

Binder E, Droste SK, Ohl F, Reul JM. 2004. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. Behav Brain Res 155:197–206.

Burghardt PR, Fulk LJ, Hand GA, Wilson MA. 2004. The effects of chronic treadmill and wheel running on behavior in rats. Brain Res 1019:84–96.

Clark PJ, Brzezinska WJ, Thomas MW, Ryzhenko NA, Toshkov SA, Rhodes JS. 2008. Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. Neuroscience 155:1048–1058.

Cotman CW, Berchtold NC. 2007. Physical activity and the maintenance of cognition: learning from animal models. Alzheimers Dement 3:S30–37.

Creer DJ, Romberg C, Saksida LM, van Praag H, Bussey TJ. 2010. Running enhances spatial pattern separation in mice. Proc Natl Acad Sci USA 107:2367–2372.

Duman CH, Schlesinger L, Russell DS, Duman RS. 2008. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Res 1199:148–158.

Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR. 2004. Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. Neuroscience 124:71–79.

Fuss J, Ben Abdallah NM, Hensley FW, Weber KJ, Hellweg R, Gass P. 2010a. Deletion of running-induced hippocampal neurogenesis by irradiation prevents development of an anxious phenotype in mice. PLoS One 5.

Fuss J, Ben Abdallah NM, Vogt MA, Touma C, Pacifici PG, Palme R, et al. 2010b. Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice correlating with hippocampal neurogenesis. Hippocampus 20:364–376.

Golub Y, Mauch CP, Dahlhoff M, Wotjak CT. 2009. Consequences of extinction training on associative and non-associative fear in a mouse model of Posttraumatic Stress Disorder (PTSD). Behav Brain Res 205:544–549.

Greenwood BN, Foley TE, Day HE, Campisi J, Hammack SH, Campeau S, et al. 2003. Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J Neurosci 23:2889–2898.

Greenwood BN, Strong PV, Foley TE, Fleshner M. 2009. A behavioral analysis of the impact of voluntary physical activity on hippocampus-dependent contextual conditioning. Hippocampus 19:988–1001.

Griesbach GS, Hovda DA, Molteni R, Wu A, Gomez-Pinilla F. 2004. Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. Neuroscience 125:129–139.

Griesbach GS, Hovda DA, Gomez-Pinilla F. 2009. Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. Brain Res 1288:105–115.

Hernandez-Rabaza V, Llorens-Martin M, Velazquez-Sanchez C, Ferragud A, Arcusa A, Gumus HG, et al. 2009. Inhibition of adult hippocampal neurogenesis disrupts contextual learning but spares spatial working memory, long-term conditional rule retention and spatial reversal. Neuroscience 159:59–68.

Hillman CH, Erickson KI, Kramer AF. 2008. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci 9:58–65.

Johnson RA, Rhodes JS, Jeffrey SL, Garland T Jr, Mitchell GS. 2003. Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. Neuroscience 121:1–7.

Kamprath K, Wotjak CT. 2004. Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn Mem 11:770–786.

Khabour OF, Alzoubi KH, Alomari MA, Alzubi MA. 2010. Changes in spatial memory and BDNF expression to concurrent dietary restriction and voluntary exercise. Hippocampus 20:637–645.

Kim JJ, Jung MW. 2006. Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. Neurosci Biobehav Rev 30:188–202.

Kramer AF, Erickson KI. 2007. Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function. Trends Cogn Sci 11:342–348.

Leasure JL, Jones M. 2008. Forced and voluntary exercise differentially affect brain and behavior. Neuroscience 156: 456–465.

Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L. 2005. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 163:78–90.

Liu YF, Chen HI, Wu CL, Kuo YM, Yu L, Huang AM, et al. 2009. Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. J Physiol 587:3221–3231.

Loh DH, Navarro J, Hagopian A, Wang LM, Deboer T, Colwell CS. 2010. Rapid changes in the light/dark cycle disrupt memory of conditioned fear in mice. PLoS One 5.

Nichol KE, Parachikova AI, Cotman CW. 2007. Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. Behav Brain Res 184:124–132.

O'Callaghan RM, Ohle R, Kelly AM. 2007. The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. Behav Brain Res 176:362–366.

Orr SP, Metzger LJ, Pitman RK. 2002. Psychophysiology of post-traumatic stress disorder. Psychiatr Clin North Am 25: 271–293.

Phillips RG, LeDoux JE. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285.

Rau V, DeCola JP, Fanselow MS. 2005. Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. Neurosci Biobehav Rev 29:1207–1223.

Remington G. 2009. From mice to men: What can animal models tell us about the relationship between mental health and physical activity? Ment Health Phys Activity 2:10–15.

Siegmund A, Wotjak CT. 2006. Toward an animal model of posttraumatic stress disorder. Ann NY Acad Sci 1071: 324–334.

Siegmund A, Wotjak CT. 2007. A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J Psychiatr Res 41:848–860.

Van der Borght K, Havekes R, Bos T, Eggen BJ, Van der Zee EA. 2007. Exercise improves memory acquisition and retrieval in the Y-maze task: relationship with hippocampal neurogenesis. Behav Neurosci 121:324–334.

Van Hoomissen JD, Holmes PV, Zellner AS, Poudevigne A, Dishman RK. 2004. Effects of beta-adrenoreceptor blockade during chronic exercise on contextual fear conditioning and mRNA for galanin and brain-derived neurotrophic factor. Behav Neurosci 118:1378–1390.

van Praag H. 2008. Neurogenesis and exercise: past and future directions. Neuromolecular Med 10:128–140.

van Praag H. 2009. Exercise and the brain: something to chew on. Trends Neurosci 32:283–290.

van Praag H, Christie BR, Sejnowski TJ, Gage FH. 1999a. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96:13427–13431.

van Praag H, Kempermann G, Gage FH. 1999b. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270.

van Praag H, Shubert T, Zhao C, Gage FH. 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680–8685.

Vansteenwegen D, Hermans D, Vervliet B, Francken G, Beckers T, Baeyens F, Eelen P. 2005. Return of fear in a human differential conditioning paradigm caused by a return to the original acquistion context. Behav Res Ther 43:323–336.

Vaynman S, Ying Z, Gomez-Pinilla F. 2004a. Exercise induces BDNF and synapsin I to specific hippocampal subfields. J Neurosci Res 76:356–362.

Vaynman S, Ying Z, Gomez-Pinilla F. 2004b. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 20:2580–2590.

Wojtowicz JM, Askew ML, Winocur G. 2008. The effects of running and of inhibiting adult neurogenesis on learning and memory in rats. Eur J Neurosci 27:1494–502.

Yehuda R. 2002. Post-traumatic stress disorder. New Engl J Med 346:108–114.