Exercise Maintains Dendritic Complexity in an Animal Model of Posttraumatic Stress Disorder

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ABSTRACT
HOFFMAN, J. R., H. COHEN, I. OSTFELD, Z. KAPLAN, J. ZOHAR, and H. COHEN. Exercise Maintains Dendritic Complexity in an Animal Model of Posttraumatic Stress Disorder. Med. Sci. Sports Exerc., Vol. 48, No. 12, pp. 2487–2494, 2016. Introduction: This study examined the effect of endurance exercise on dendritic arborization in the dentate gyrus subregion in rodents exposed to a predator scent stress (PSS). Methods: Sprague–Dawley rats were randomly assigned to one of four treatment groups. In two of the groups, rats were exposed to PSS but either remained sedentary (SED + UNEXP) or were exercised (EX + UNEXP). In the other two groups, rats were exposed to the PSS but either remained sedentary (SED + PSS) or were exercised (EX + PSS). After 6 wk of either exercise or sedentary lifestyle, rats were exposed to either the PSS or a sham protocol. During exercise, the animals ran on a treadmill at 15 m/min, 5 min/d gradually increasing to 20 min/d, 5 d/wk for 6 wk. Eight days after exposure to either PSS or sham protocol, changes in the cytoarchitecture (dendritic number, dendritic length, and dendrite spine density) of the dentate gyrus subregion of the hippocampus were assessed. Results: No differences (P = 0.493) were noted in dendrite number between the groups. However, dendritic length and dendrite spine density for SED + PSS was significantly smaller (P < 0.001) than that observed in all other groups. In addition, neurons from animals in SED + PSS had significantly fewer (P < 0.001) dendritic intersections than all other groups. Conclusion: The results of this study indicate that 6 wk of endurance training can protect dendritic length and complexity, suggesting a degree of resiliency to stress. This provides further evidence for supporting the inclusion of an exercise regimen for reducing the risk of posttraumatic stress disorder. Key Words: POSTTRAUMATIC STRESS DISORDER, TRAINING, HIPPOCAMPUS, PHYSICAL ACTIVITY, BRAIN

It is well accepted that the brain displays persistent plasticity throughout life (16). Changes in the morphology of the brain appear to be dependent on the stimulus presented, which can modify behavior, learning, and response to a specific cue. Previous research has demonstrated that physical exercise is a potent stimulator for increasing the expression of neurotrophins within the hippocampus (2,11,15,17,27,28). For instance, both brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) suggested to promote and support neuronal health in the hippocampus (2,4,28). The increase in the expression of BDNF after endurance training is reported to stimulate an increase in dendritic spine density and dendritic arborization (7,23,25). These adaptations to neuron morphology have been associated with positive changes in memory and learning (1,6).

In contrast to the positive adaptations associated with exercise and brain health, stress may result in negative adaptations in neuronal morphology. Stress induced by 5 d of sleep disturbance in rats was associated with significant reductions in dendritic spine density in both cortical and hippocampal pyramidal neurons (3). Other investigators exposed rats to 4 wk of chronic unpredictable stress (various stimuli that included daily exposure to either cold water, vibration, restraint, overcrowding, or hot air stream) and reported significant atrophy of CA3 and CA1 apical dendrites in the dorsal hippocampus (21). These changes were also associated with cognitive deficits and increased anxiety-like behavior. The effect of stress also appears to affect certain regions of the brain differently.

In an examination of a rats/rodent model of posttraumatic stress disorder (PTSD), Cohen et al. (4) observed that changes in the morphology of the hippocampus and amygdala subregions were related to the pattern of behavioral
responses from exposure to a predator scent stress (PSS). The PSS exposes rats to well-soiled cat litter, after which the behavioral responses in several assessments are examined. On the basis of these responses, the rats in that study were classified as either exhibiting an extreme behavioral response (i.e., PTSD-like phenotype), a minimal behavioral response (well-adapted phenotype), or a partial behavioral response. Results indicated an association between the behavioral response and the pattern of morphological changes in the dendrites of hippocampal and amygdala neurons. Eight days after exposure to the stressor, significantly lower dendritic complexity, total dendritic length, and lower spine density in the hippocampal neurons of rats displaying an extreme or partial behavioral response were noted. In the rats displaying a minimal behavioral response to the stressor, changes in dendritic architecture were not observed. These results were similar to that previously reported by Zohar et al. (29).

A disruption in neuronal complexity and an increase in anxiety-like symptoms have been reported to be related to the downregulation in hippocampal BDNF expression seen in PTSD-like phenotype (14,29). Interestingly, exercise has been previously demonstrated to be a potent stimulator of both BDNF and NPY expression in the hippocampus of the stressed rat (8,11), but its ability to alter neuronal plasticity during a PTSD-like stress model is not well understood. Therefore, the purpose of this study was to examine the effectiveness of 6 wk of endurance exercise on dendritic arborization in the dentate gyrus (DG) subregion in rats exposed to a PSS.

MATERIALS AND METHODS

Animals. Adult male Sprague–Dawley rats (2.5 months of age at the onset of the study) weighing 200–250 g (n = 36) were habituated to housing conditions for at least 7 d. All animals were housed four per cage in a vivarium with stable temperature (21°C–22°C) and a reversed 12-h light–12-h dark cycle, with unlimited access to food and water. Animals were handled once daily. All testing was performed during the dark phase in dim red light conditions. This study was performed according to the principles and guidelines of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. All treatment and testing procedures were approved by the Animal Care Committee of the Ben-Gurion University of the Negev, Israel.

Experimental design. Rats were randomly assigned to one of four treatment groups:

1. Sedentary and unexposed (SED + UNEXP): rats did not exercise and were exposed to fresh, unused litter.
2. Exercise and unexposed (EX + UNEXP): rats exercised and were exposed to fresh, unused litter.
3. Sedentary and exposed to PSS (SED + PSS): rats did not exercise and were exposed to PSS.
4. Exercise and exposed to PSS (EX + PSS): rats exercised and were exposed to PSS.

After the 7-d acclimation period, rats were randomized into the four groups. After 6 wk of either exercise or a sedentary lifestyle, rats were exposed to either the PSS or sham protocol. All behavioral tests were conducted 7 d after the PSS or sham protocol, and the rats were then killed 24 h later and brains were removed. The validity of this model has been demonstrated previously (5,14).

Exercise protocol. All animals went through a 5-d adaptation period to a treadmill during which they were allowed to explore the equipment. The treadmill was turned on for only 15 min at low speeds (5.0–8.3 m·min⁻¹). This procedure had the purpose of excluding animals that were intolerant to the treadmill and refused to run, thereby providing a homogenous group of rats willing to exercise. The animals that presented problems adapting to the treadmill or refused to run were excluded. During the study, rats were run on the treadmill at a speed of 15 m·min⁻¹, 5 min·d⁻¹, gradually increasing to 20 min·d⁻¹, 5 d·wk⁻¹, for 6 wk. This exercise protocol has been previously reported (11).

PSS. After the 6-wk training program, animals were exposed to the PSS protocol. The PSS protocol consisted of placing the experimental animal on well-soiled cat litter (in use by the cat for 2 d, sifted for stools) for 10 min in a closed environment. Control animals were exposed to fresh, unused litter for the same amount of time. The situational reminder consisted of placing the animals on fresh, unused cat litter for 10 min.

Assessment schedule. Behavioral responses were assessed in the elevated plus maze, acoustic startle response, and contextual freezing. These assessments occurred 7 d after the initial exposure to the PSS. The delay in performing these measures from the PSS is based on findings that extreme behavioral changes, which remain constant after 7 d of exposure represent “chronic symptoms” and persist over a prolonged duration (5). The results of these assessment have been published elsewhere (11). After behavioral assessments, all animals were killed, and the brains were removed for analysis.

Golgi-Cox staining. All animals were euthanized 24 h after the last behavioral tests. Animals were deeply anesthetized (ketamine and xylazine mixture, 70 and 6 mg·kg⁻¹, respectively, i.p.) and perfused intracardially with 0.9% saline. The brains were immediately dissected and processed. Tissue was prepared by using the rapid Golgi kit (FD Neurotechnologies, Columbia, MD) according to the manufacturer’s instructions. Golgi-Cox staining was assessed as previously described (29). Briefly, brains were rinsed with distilled water and immersed in impregnation solution containing potassium dichromate, mercuric chloride, and potassium chromate. Brains were left undisturbed in the dark for 2.5 wk. After the 2.5 wk, brains were immersed in 30% sucrose at 4°C. Two to four days later, coronal sections (100 μm) were cut using a cryostat (Microtome HM 500 OM cryostat, kept at 22°C to 25°C) in a bath of 15% sucrose, and the slices were stored in the dark at 4°C in 15% sucrose solution until mounting. Sections were mounted on gelatin-coated slides and firmly pressed using moist filter paper to prevent the slices from falling off the slide during development (9). Slides were placed in a humidity chamber in
the dark and were stored at 4°C overnight. For development, slides were rinsed with distilled water twice for 2 min and then placed in developing solutions provided in the FD GolgiStain Kit, dehydrated via a graded ethanol series (50%-100%, 4 min each rinse), cleared with xylene for 8 min, and coverslipped with Permount (Fisher Scientific, The Netherlands).

Neuronal reconstruction and morphometric analysis. The hippocampal DG was chosen as a target in this study for several reasons. The DG is highly sensitive to stress (12) and has a critical role in the function of the entorhinal–hippocampal circuitry in health and disease by regulating the flow of information into the hippocampus. To obtain accurate measurements of dendritic parameters, strict criteria that have been previously identified were used for the selection of the filled neurons before quantitative analysis (4). In brief, only well-impregnated neurons were chosen for the histological analysis. In addition, granule cells were included in this analysis only if the cell body and primary dendrites were clearly stained and easily distinguishable from those of neighboring cell bodies and their dendrites. Granule cells were sampled from the suprapyramidal blades of the DG, in both the right and the left sides of the brain. Granule cells from the inner granule zone were included in this analysis because the dendritic morphology of hippocampal DG cells varies with their position in the granule cell layer (GCL) (10). A cell was classified as belonging to the inner granule zone if the entire soma was positioned in the inner half of the GCL. Granule cells whose soma was intersected by the midline of the GCL, in the outer granule zone, or in the subgranular zone were not included in the analysis. Figure 1 contains photomicrographs of representative examples of the Golgi–Cox-stained DG granule cell in the DG subregion of the hippocampus in animals that exercised (a) and animals that were sedentary and exposed to the PSS (b).

We performed an analysis to characterize the extent that dendrites branched out from both somal and dendritic sites. Primary dendrites were defined as direct extensions from the soma of at least 10 μm in length. Only DG regions with at least one primary dendrite >10 μm in total length were analyzed. When a primary dendrite bifurcated at a branch point, the dendrites extending from that branch point were classified as secondary dendrites. We extended this analysis to include tertiary (3), quaternary (4), quinary (5), and senary (6) order dendrites. This procedure provides an additional measure of the pattern of dendritic arborization, allowing a more comprehensive analysis of differences in the branch patterns of the dendrites themselves. To evaluate the detailed distribution of dendritic processes across the DG regions, we also performed a Sholl analysis (24) in which summed dendritic length is expressed as a function of distance from the soma in radial bins of 15–20 μm. A series of concentric rings, spaced 15–25 μm apart, was placed over the neuron and centered on the cell body. The number of dendrite crossings as a function of distance was recorded.

All slides were coded and the analysis was performed with the experimenter blinded as to the origin of the slides. Dendritic morphology was observed by epifluorescent microscopy (Leica). A 0.5-mm interval z-series was captured throughout the extent of the dendritic arbor of the DG with a CCD camera (Leica) controlled by LAS software.

The spines on the dendrites were counted using a 100× oil immersion objective lens (NA, 0.8; working distance, 0.66 mm). To count the spines, straight branches that provided clear resolution of spines were preferred, and spine density was calculated as the number of spines per 10 μm of dendrite for six segments per cell and five cells per animal. For each animal, cell values were averaged to derive a single value per variable.

Statistical analyses. The statistical analyses of total dendritic number and length were performed using a one-way ANOVA. Sholl analysis was analyzed using a two-way (groups × Sholl intersections) ANOVA. In the event of a significant F-ratio, a Bonferroni post hoc analysis was used for pairwise comparisons. To gain additional understanding between the relationship between DG dendritic morphology and behavioral responses, linear regression between dendritic number, length, and dendritic spine density in the DG, and anxiety index, mean startle response, and startle

FIGURE 1—A photomicrograph of a Golgi-stained dentate granule cell of the dorsal hippocampus. A, Animal unexposed to PSS. B, Animal exposed to PSS.
RESULTS

No statistical difference was found between the soma areas of randomly chosen Golgi-stained pyramidal/granular cells. This indicates that the analyzed cells represented an unbiased, random sampling of the neuron population and that there was no cell death in the PSS-exposed groups.

No statistical differences ($F_{3.32} = 0.818, P = 0.493$) were noted in dendritic number (see Fig. 2) between the groups. However, the average dendrite number for animals that were sedentary and exposed to PSS were 13.0%, 11.0%, and 13.5% lower ($P > 0.05$) than rats who were sedentary and unexposed, rats who exercised and were unexposed, and rats who exercised and were exposed to PSS, respectively. A significant difference ($F_{3.32} = 12.16, P < 0.001$) was seen in dendritic length between the groups (see Fig. 3). Dendrite length for rats that were sedentary and exposed to PSS was significantly smaller ($P < 0.019$) than that observed in all other groups.

A significant difference ($F_{1.32} = 12.60, P < 0.001$) was seen in dendritic spine density between the groups (see Fig. 4A). Quantitative analysis of spine density per 10 $\mu$m of dendrite indicated that there were significantly fewer spines ($P < 0.001$) in animals that were sedentary and exposed to PSS compared with all other groups (see Fig. 4B). In addition, the spine density per 10 $\mu$m of dendrite in the animals that exercised but were exposed to PSS were significantly greater than those animals that were sedentary and either exposed ($P < 0.001$) or not exposed to the stressor ($P = 0.047$).

The number of Sholl intersections, points where dendrites cross the virtual Sholl shells, is a measure that reflects the complexity of the dendritic tree in response to a stress. A significant main effect ($F_{3.25} = 10.98, P < 0.001$) was noted for neurons across radii from 10 to 355 $\mu$m. Neurons from animals that were sedentary and exposed to PSS had significantly fewer dendritic intersections than all other groups (see Fig. 5). No other between group differences were noted.

Results depicting the linear regression analysis of DG total dendritic number, DG dendritic length, and DG dendritic spine density and the behavioral responses (anxiety index, mean startle response, and startle habituation) are depicted in Figures 6A–I. A significant negative correlation was noted between DG total dendritic number and anxiety index ($r = -0.41, F_{1.34} = 6.83, P = 0.013$) (Fig. 6A), but the correlations between DG total dendritic number and startle response (Fig. 6B) or startle habituation (Fig. 6C) were not significant ($r = -0.18, F_{1.34} = 1.16, P = 0.288$, and $r = -0.09, F_{1.34} = 0.31, P = 0.584$, respectively). Significant correlations were noted between DG total dendritic length and anxiety index ($r = -0.43, F_{1.34} = 7.69, P = 0.009$) (Fig. 6D), mean startle response ($r = -0.59, F_{1.34} = 17.99, P < 0.001$) (Fig. 6E), and startle habituation ($r = 0.37, F_{1.34} = 5.37, P = 0.026$) (Fig. 6F). Significant correlations were also observed between DG dendritic spine density and anxiety index ($r = -0.54, F_{1.34} = 13.88, P < 0.001$) (Fig. 6G), mean startle response ($r = -0.57, F_{1.34} = 16.72, P < 0.001$) (Fig. 6H), and startle habituation ($r = 0.438, F_{1.34} = 8.05, P = 0.008$) (Fig. 6I).

DISCUSSION

The results of this study indicate that 6 wk of endurance exercise provided a degree of resiliency to the negative morphological changes observed in sedentary rats that were exposed to the PSS. In animals that remained sedentary and were exposed to PSS, dendritic length and dendrite spine density were significantly reduced, indicating negative...
adaptations of neuronal architecture to the PTSD model. These results are consistent with previous investigations indicating that the PSS model for PTSD stimulates negative behavioral and morphological adaptations (4,11,14,29). We have previously reported that 6 wk of endurance training was able to stimulate both BDNF and NPY expression in the hippocampus after exposure to PSS (11). The present results provide further understanding of the benefits of exercise by demonstrating that endurance training can also increase the resiliency of the cytoarchitecture of neurons in the DG subregion of the hippocampus. Whether it is related to the maintenance of BDNF expression in this area of the brain

FIGURE 4—A, Computer-generated plots of reconstructions of the dendritic trees from DG granule cells of animals that remained sedentary and were not exposed to the PSS. B, Dendritic spine density. *Significantly different than all other groups. #Significantly different than SED + UNEXP and SED + PSS. SED + UNEXP, rodents that remained sedentary and were not exposed to the PSS; EX + UNEXP, rodents that were trained and were not exposed to the PSS; SED + PSS, rodents that remained sedentary and were exposed to the PSS; EX + PSS, rodents that were trained and were exposed to the PSS. All data are reported as mean ± SD.

FIGURE 5—Sholl analysis for intersections per 15 μm radial unit distance. Neurons from animals that were sedentary and exposed to PSS (SED + PSS) had significantly fewer dendritic intersections than all other groups. *Dendritic complexity was significantly less in SED + PSS than SED + UNEXP, EX + UNEXP, and EX + PSS. #Dendritic complexity was significantly less in SED + PSS than SED + UNEXP and EX + UNEXP. ^Dendritic complexity was significantly less in SED + PSS than SED + UNEXP. §Dendritic complexity was significantly less in SED + PSS than EX + UNEXP. SED + UNEXP, rodents that remained sedentary and were not exposed to the PSS; EX + UNEXP, rodents that were trained and were not exposed to the PSS; SED + PSS, rodents that remained sedentary and were exposed to the PSS; EX + PSS, rodents that were trained and were exposed to the PSS. All data are reported as mean ± SD.
can only be speculated; however, previous studies have demonstrated the important role that neurotrophins have on neurogenesis (7,23,25).

Comparisons between sedentary and trained rats not exposed to PSS in this study revealed significant differences in spine density of the dendrites in the DG region of the hippocampus. However, no other differences (e.g., dendrite number, length, or complexity) were noted. The benefits of exercise on dendritic cytoarchitecture is consistent with other investigations demonstrating the potent effects of exercise on stimulating morphological adaptations to dendrites (18,22,23,25). However, these adaptations were not consistent in all measures of dendritic complexity. It is possible that the effectiveness of the exercise stimulus may be intensity specific. Shih et al. (23) reported that rats running on a treadmill at a low intensity (8 m·min⁻¹) resulted in significantly greater dendritic complexity in the DG and CA1 subregions of the hippocampus compared with rats running at a higher intensity (20 m·min⁻¹). Interestingly, the animals in the present study exercised at a rate of 15 m·min⁻¹. Although this exercise intensity was sufficient to provide resilience to PSS exposure, it may be a potential explanation for the partial adaptation seen between rats that were sedentary and not exposed to PSS compared with rats that exercised and were unexposed. In addition, the duration of the exercise protocol may also have limited dendritic adaptation to training. Patten et al. (20) evaluated various periods (3–56 d) of endurance activity (running wheel) in rats and reported significant increases in cell proliferation in the DG after only 3 d of running, but an increase in the capacity of synaptic efficiency was not observed until the rats had been running for 56 d. Adaptations in dendritic arborization appear to occur relatively quickly, which may provide some degree of protection against stress. However, the incomplete adaptation observed in the morphological profiles of dendrites in the DG between exercised and sedentary rats that were unexposed to

FIGURE 6—Linear correlations between DG dendritic morphology versus behavioral responses parameters such as anxiety index, mean startle response, and startle habituation. DG total dendritic number versus anxiety index (A), startle response (B), and startle habituation (C). DG total dendritic length versus anxiety index (D), startle response (E), and startle habituation (F). DG dendritic spine density versus anxiety index (G), startle response (H), and startle habituation (I). *Statistically significant.
PSS suggests that adaptation may not have been complete by the end of the 6-wk exercise program. This may provide some explanation for improvements seen in dendrite length and spine density, but not in dendritic number. Although speculative, it is possible that changes in dendritic number are adaptations seen in longer duration endurance training programs.

The 6-wk endurance training program appeared to increase dendrite spine density and maintain dendritic length in rats exposed to PSS. As previously discussed, this is consistent with other studies that have demonstrated the beneficial role that exercise has in alleviating anxiety- and depression-like behavioral responses and cognitive deficits associated with exposure to a PTSD causing stressor (11,13,19). Studies by Kim and Seo (13) and Patki et al. (19) initiated their exercise protocol after the delivery of the stressor to the animal, whereas a previous study from our research team (11) exercised the rodents before the initiation of the stress protocol. The mechanism associated with improving behavioral outcomes when exercise is used therapeutically (enhanced cell proliferation and neurogenesis in the DG subregion of the hippocampus) (13) is similar to that reported in the present study. On the basis of these investigations, it appears that exercise training performed either before or after stress exposure may prevent or reverse stress-induced symptoms using a similar mechanism of supporting neurogenesis in the DG of the hippocampus. Several studies have suggested that exercise is associated with maintaining or elevating BDNF and NPY expression (11). Fang et al. (8) have suggested that the increased expression of BDNF in the hippocampus stimulated by exercise may be related to elevations in the mTOR signaling pathway. An upregulation of BDNF and its binding to its receptor (p-tropomyosin-related kinase B) has been reported to enhance the phosphorylation of mTOR signaling activity in the hippocampus (26). This may have important implications for subsequent neurogenesis. Future research may wish to provide further insight into the relationship between BDNF, mTOR signaling, and neurogenesis.

The present study also examined the association between DG dendritic morphology and behavioral parameters with a high degree of resolution. These findings provide evidence implying a possible relationship between the morphological findings and the psychopathological processes, which result in altered behavior. In summary, the results of this study indicate that 6 wk of endurance training can provide a degree of resiliency to stress, which allows for protection of dendritic complexity. This provides further evidence for supporting the inclusion of nonpharmacological interventions for the treatment of PTSD. Whether these protective effects are a function of endurance training only is not well understood, and further study examining a different mode of exercise (i.e., resistance training) is warranted.

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REFERENCES


EXERCISE AND DENDRITIC COMPLEXITY


