Hippocampal Morphology in a Rat Model of Depression: The Effects of Physical Activity

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Abstract: Accumulating in vivo and ex vivo evidences show that humans suffering from depression have decreased hippocampal volume and altered spine density. Moreover, physical activity has an antidepressant effect in humans and in animal models, but to what extent physical activity can affect hippocampal volume and spine numbers in a model for depression is not known.

In this study we analyzed whether physical activity affects hippocampal volume and spine density by analyzing a rodent genetic model of depression, Flinders Sensitive Line Rats (FSL), with Magnetic Resonance Imaging (MRI) and ex vivo Golgi staining.

We found that physical activity in the form of voluntary wheel running during 5 weeks increased hippocampal volume. Moreover, runners also had larger numbers of thin spines in the dentate gyrus. Our findings support that voluntary wheel running, which is antidepressive in FSL rats, is associated with increased hippocampal volume and spine numbers.

Keywords: Animal model, dendrite morphology, flinders sensitive line rats, hippocampus, major depression, MRI, volume based morphometry.

INTRODUCTION

Imaging studies have demonstrated structural brain changes in depression in hippocampus, amygdala, striatum, frontal cortex and ventricles. Consistently, a volume loss of the hippocampal formation is detected using MRI [1-3] and it appears that it is the time with depression that determines the extent of shrinkage of hippocampus [4]. Moreover, depression is associated with cognitive deficits and postmortem analyses show hippocampal atrophy [5, 6]. The total number of neurons is not altered but it is the size of the neuropil and glial cell numbers that are reduced [7].

Chronic stress or sustained high levels of glucocorticoids leads to reduction and retraction of dendritic spines of hippocampal pyramidal neurons in animal models [8]. Similarly, post mortem analysis of depressed patients also show reduction of hippocampal volume and retraction of dendritic spines of hippocampal pyramidal neurons [7].

A non-pharmacological intervention suggested to treat depression is physical training. Indeed, it is now widely accepted that physical training has antidepressant properties in both humans [8-12] and in animal models including the FSL rats [13-18]. The FSL rat is a selectively bred rat model of depression that displays several depressed behaviors [19]. It is even noted that many of the effects observed in FSL rats after chronic treatment with selective serotonin reuptake inhibitors (SSRIs), such as decreased immobility time in the forced swim test, increased hippocampal neurogenesis and levels of the neuropeptide Y (NPY), are also observed after long-term wheel running in FSL rats [20-22]. In other models of voluntary exercise, in rodent models, it has also been noted that the cellular as well as the dendritic structure of the hippocampus can be altered [23-30].

However, even though rats respond similarly to for example SSRI and wheel running, the underlying mechanism for antidepressant pharmacotherapy and physical activity is under investigation [31].

Interestingly, physical activity increases hippocampal volume in both humans and rodents [32-34]. However, to the best of our knowledge it is only in the preadolescent...
(developing) human brain that the hippocampus increased by exercise [33]. Whether exercise can alter hippocampal volume in a rat model of depression in post adolescent individuals is not known.

Others have studied dendritic spines in the hippocampus, and the relation between stress and exercise. It has been noted, in different rodent models, that voluntary exercise increases the number of spines in the hippocampus [28, 29, 35, 36].

In this study, we tested whether voluntary wheel running could alter hippocampal volume and synaptic spines in FSL rats.

The animals were group housed and bred at Karolinska Institutet in standard cages until the start of the experiment. All animal experiments were approved by the Ethical committee for animal research in Stockholm, Sweden. The average age of the animals at the start of the experiment was three months. The animals had free access to food and water and were housed in a controlled environment of 12-h light/dark cycle.

During the whole experiment, male FSL rats were single housed in cages (43 cm x 22 cm x 20 cm) with and without free access to a running wheel (34 cm in diameter) (n = 30 for both groups) during a period of five weeks. After the five week period the animals were used for either an in vivo MRI experiment or for an ex vivo golgi staining experiment. Running distance was recorded and stored every tenth minute by a computer-based data system with customized software, which registers revolutions. Similarly as in previous studies the rats gradually increased daily running distance during the first week and then leveled out the daily running during the rest of the experiment [14, 16, 22, 37]. On average over the whole 5 week period the runners covered a daily distance of 1250 ± 146 m (n=30) per day (Fig. 1). Previously we have shown that wheel running is neurogenic and antidepressive [14, 16, 22, 37]. Now we tested if voluntary wheel running also can induce volumetric changes of hippocampus and ventricles as well as spine numbers in dentate gyrus (DG) in the depressed rat strain.

Animals (n = 7 runners and n = 8 controls) were subjected to MRI scanning within 2 days after blocking access to the running wheels. The in vivo MRI experiments were conducted using a horizontal 9.4 T Varian magnet equipped with a 12 cm inner diameter gradient system with maximum gradient strength of 600 mT/m. The gradient coils have capacity for 8 shim channels (Z, X, Y, Z², XZ, YZ, XY and X²Y²). An actively tuned, circularly polarized birdcage resonator with an inner diameter of 72 mm and a resonator length of 100 mm was used for excitation (Rapid Biomedical, Wurzburg, Germany). A linearly polarized rat brain 4-channel phased array surface coil with mean coil diameter 39 mm, resonator length 26.5 mm (Rapid Biomedical, Wurzburg, Germany) served as receiving coil.

Animals were anaesthetized with isoflurane (5% for induction and maintained at 1.75% during scanning) in a 1:9 mixture of O₂ and air. Rats were positioned in a prone position in a MR compatible animal holder with the head firmly fixed. The receiving surface coil was secured to the animal holder above the head of the rat.

The volumetric 3D-data were acquired using an inversion recovery 3d fast spin echo sequence, with TR 1500 ms, TI 500 ms, ETL 8, kzero 1, matrix size 512x128x128 field of view (FOV) 51.2x22x19.2 mm³. Three saturation bands were employed to suppress signal from tissue outside of the FOV. Total acquisition time was 1h 25min. Temperature was maintained at 37±0.2 °C throughout the MRI experiment with a feedback controlled air-heater system. In addition, respiration rate, pulse and oxygen saturation was monitored (SA Instruments) during scanning. At the end of the imaging experiment the animals were sacrificed with an i.p. overdose of sodium pentobarbital.

The left hippocampus and the whole brain for each rat was manually segmented on the 3D dataset using AMIRA software (Amira 3.1, Template Graphics Software, Inc., San Diego, California) by a blinded experimenter. The volume was quantified based on the size of the voxel and the numbers of voxels comprising the hippocampus or the brain. To adjust for possible differences in body and brain size in runners versus non-runners, the ratio hippocampus: whole brain volume was calculated.
brain was calculated. Data was subsequently analyzed with Student’s t-test. 3-D image segmentation of the ventricles was performed with ITK-SNAP version 2.2 (http://www.itksnap.org), an open source segmentation software [38].

Volumetric measurements on 3D MRI images of left hippocampus and whole brain revealed that voluntary running during five weeks significantly increased the hippocampus/whole brain ratio (0.0194±0.0003, non-runners (n=8) versus 0.0209±0.0002, runners (n=7); t= 3.61, d.f.=13, **p<0.01, 2-tailed unpaired Student’s t-test) but had no effect on the ventricular/brain ratio (0.00671±0.000217, non-runners (n=8) versus 0.00650±0.000150, runners (n=7); t=0.79, d.f.=13, p= 0.44, 2-tailed unpaired Student’s t-test) or on the total brain volume (2109.6±12.1 mm$^3$, non-runners (n=8) versus 2113.7±50.1 mm$^3$, runners (n=7); t=0.11, d.f.=13, p=0.913 2-tailed Student’s t-test) (Fig. 2D). Thus the increased size of hippocampus (Fig. 2A-C) appears to be independent of changes in total brain and ventrical volume (Fig. 2C-D).

Dendritic spine numbers was analyzed in hippocampal granule cells in the dentate gyrus after five weeks of wheel running (runners, n = 7) and compared to the non-runners (n = 8).

Animals were sacrificed and the brains were rapidly removed, and placed in 5ml of Golgi-solution (A+B solution, FD Rapid Golgi kit, MTR Scientific) for 19 days. The brains were then put in solution C (FD Rapid Golgi kit, MTR Scientific) for 24h where after the brains were cut in 200µm sections using a vibratome. These sections were stained according to the manufacturers protocol (FD Rapid Golgi kit, MTR Scientific) and the neurons were traced using the NeuroLucida program (MBF Bioscience). The neurons traced (6 neurons/animal) were all at the same level (coordinates: -2.76mm from Bregma) and all around the same location in the dentate gyrus. Number and type of spine were counted and analyzed on 20µm from the very end of a dendrite (in average 3.7 sections à 20µm/neuron), as indicated in Fig. (3), using a Zeiss microscope and a 100 x objective with a numerical aperture (NA) = 1.4. Spines were defined as headless (thin) or headed protrusions (mushroom). Long thin headless protrusions (> twice the length of a thin spine) were defined as filopodia spines. Protrusions
connected in the same base were defined as branched spines. Stubby spines were defined as low (<1/2 thin spine) and wide protrusion. The analysis was performed by using the NeuroLucida explorer software (MBF Bioscience). Student’s t-test was applied to treat differences in number of spines between the two groups: runners vs non-runners. Running induced an increase in spine numbers, which was visualized and outlined in Golgi impregnated sections (Fig. 3A-C), in dentate gyrus granule cell dendrites (20.1 ± 1.73 spines/20µm; n=6 for runners versus 15.8±0.92 spines/20µm; non-runners (n=6); t= 2.20, d.f.=10, *p<0.05 2-tailed Student’s t-test) (Fig. 3). Five different types of spines were detected (thin, mushroom, filopodia, stubby and branched spines), however, only thin spines increased in number (Fig. 3D) (13.4±0.90 spines/20µm in runners (n=6) versus 10.1±0.50 spines/20µm in non-runners (n=6); t=3.18, d.f.=10, **p<0.01 2-tailed unpaired Student’s t-test).

Our data supports a previous study where the dendritic spines of dentate gyrus in Sprague-Dawley rats (weight 200-250g.) are induced by voluntary exercise. Non-runners had approximately 7.7 spines/10µm where as runners had approximately 9.9 spines/10µm, which is similar to the FSL rats in this study, where non-runners had 7.9 ± 0.46 spines/10µm versus runners that had 10.05 ± 0.87 spines/10µm [35].

As with most other treatments for depression, the understanding of the underlying biology for the positive effects of physical activity, are still under debate. Here we show, by using MRI and volumetric analysis that wheel running in single housed FSL rats increases volume as well as spine numbers of hippocampus. Our findings are also in line with a recent report that used post mortem analysis on histological sections to show that electro convulsive shock treatment (ECT), which has shown to be antidepressive in FSL rats, increases hippocampal volume [39].

Based on our findings we hypothesize that the beneficial effects of physical activity on mood could involve structural reorganization of hippocampal circuits. Moreover, tissue-level of glutamate is decreased in adult C57BL/6J mice that exercised [34]. The astrocytic involvement in glutamate metabolism and signaling has been shown to be altered in the FSL rat [40]. Interestingly, there is a strong link between glutamate, learning, memory, synaptic plasticity (LTP) and spine dynamics in the hippocampus and cerebral cortex. The generation and turnover of new spines is an ongoing process and it is the small new spines that are the preferential site for the LTP-induction [41]. Interestingly, Gómez-Galán [40] recently showed that there is a dysfunctional astrocytic glutamate transmission in the hippocampus of the FSL rats.
It has also been suggested that exercise is associated with cell proliferation, changes of vasculature, and cellular structure that could influence the volume of the hippocampus [25]. This might be a contributing mechanism to the expressed antidepressant effects in the FSL rats.

CONCLUSION

To summarize, depressed patients have alterations in brain structures, which are progressive with the development of the disease. Here we show that wheel running, which is antidepressive and neurogenic, also increases hippocampal volume and spine numbers. Our data is in line with the hypothesis of exercise induced synaptic remodeling in hippocampus.

CONFLICT OF INTEREST

Authors Adam Sierakowiak, Anna Mattsson, Marta Gómez-Galan, Teresa Femenia Canto, Lisette Graae, Sahar Nikko Aski, Peter Damberg, Mia Lindskog, and Stefan Brené declares no conflict of interest. Elin Åberg has financial ties to the following companies: E. Å. does not consider that this creates any conflict of interest with the subject-matter of this paper.

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ABBREVIATIONS

| Acronym | Description |
|---------|-------------|
| FSL    | Flinders Sensitive Line Rats |
| MRI    | Magnetic Resonance Imaging |
| SSRI   | Selective Serotonin Reuptake Inhibitors |
| NPY    | Neuro Peptide Y |
| DG     | Dante Gyrus |
| FOV    | Field of View |
| TR     | Repetition Time |
| TI     | Inversion Time |
| ETL    | Echo Train Length |
| NA     | Numerical Aperture |
| ECT    | Electro Convulsive shock Treatment |
| LTP    | Synaptic Plasticity |

REFERENCES

[1] Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. Am J Psychiatry 2000; 157(1): 115-8.
[2] Sheline YI. 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. Biol Psychiatry 2000; 48(8): 791-800.
[3] Kempton MJ, Salvador Z, Munafò MR, et al. Structural neuroimaging studies in major depressive disorder: Meta-analysis and comparison with bipolar disorder. Arch Gen Psychiatry 2011; 68(7): 675-90.
[4] Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999; 19(12): 5034-43.
[5] Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 1999; 45(9): 1085-98.
[6] Zakianz KK, Leach L, Kaplan E. On the nature and pattern of neurocognitive function in major depressive disorder. Neuropsychiatry Neuropsychol Behav Neurol 1998; 11(3): 111-9.
[7] Stockmeier CA, Mahajan GJ, Konick LC, et al. Cellular changes in the postmortem hippocampus in major depression. Biol Psychiatry 2004; 56(9): 640-50.
[8] Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000; 57(10): 925-35.
[9] Babyak M, Blumenthal JA, Herman S, et al. Exercise treatment for major depression: Maintenance of therapeutic benefit at 10 months. Psychosom Med 2000; 62(5): 633-8.
[10] Churchill JD, Galvez R, Colcombe S, Swain RA, Kramer AF, Greenough WT. Exercise, experience and the aging brain. Neurobiol Aging 2002; 23(5): 941-55.
[11] Farmer ME, Locke BZ, Moscicki EK, Dannenberg AL, Larson DB, Radloff LS. Physical activity and depressive symptoms: The NHANES I epidemiologic follow-up study. Am J Epidemiol 1988; 128(6): 1340-51.
[12] Camacho TC, Roberts RE, Lazarus NB, Kaplan GA, Cohen RD. Physical activity and depression: Evidence from the Alameda County study. Am J Epidemiol 1991; 134(2): 220-31.
[13] Greenwood BN, Foley TE, Day HE, et al. Freewheel running prevents learned helplessness/behavioral depression: Role of dorsal raphe serotonergic neurons. J Neurosci 2003; 23(7): 2889-98.
[14] Bjornebekk A, Mathe AA, Brene S. The antidepressant effect of running is associated with increased hippocampal cell proliferation. Int J Neuropsychopharmacol 2005; 8(3): 357-68.
[15] Ernst C, Olson AK, Pinel JP, Lam RW, Christie BR. Antidepressant effects of exercise: Evidence for an adult-neurogenesis hypothesis? J Psychiatry Neurosci 2006; 31(2): 84-92.
[16] Brene S, Bjornebekk A, Aberg E, Mathe AA, Olson L, Werne M. Running is rewarding and antidepressive. Physiol Behav 2007; 92(1-2): 136-40.
[17] Duman CH, Schlesinger L, Russell DS, Duman RS. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Res 2008; 1199: 148-58.
[18] Greenwood BN, Flesner M. Exercise, learned helplessness, and the stress-resistant brain. Neuronumol Med 2008; 10(2): 81-98.
[19] Overstreet DH, Friedman E, Mathe AA, Yadid G. The Flinders Sensitive Line rat: A selectively bred putative animal model of depression. Neurosci Biobehav Rev 2005; 29(4-5): 739-59.
[20] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000; 20(24): 9104-10.
[21] Bjornebekk A, Mathe AA, Brene S. Running has differential effects on NPY, opiates, and cell proliferation in an animal model of depression and controls. Neuropsychopharmacology 2006; 31(2): 256-64.
[22] Bjornebekk A, Mathe AA, Brene S. The antidepressant effects of running and escitalopram are associated with levels of hippocampal NPY and Y1 receptor but not cell proliferation in a rat model of depression. Hippocampus 2010; 20(7): 820-8.
[23] Dong HX, Yueue CM, Coughlan CA, Murphy KM, Csermiansky JG. Effects of donepezil on amyloid-beta and synapse density in the Tg2576 mouse model of Alzheimer's disease. Brain Res 2009; 1303: 169-78.
[24] Lin TW, Chen SJ, Huang TY, et al. Different types of exercise induce differential effects on neuronal adaptations and memory performance. Neurobiol Learn Mem 2012; 97(1): 140-7.
[25] Olson AK, Eadie BD, Ernst C, Christie BR. Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus 2006; 16(3): 250-60.

[26] Pereira AC, Huddleston DE, Brickman AM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci USA 2007; 104(13): 5638-43.

[27] Redila VA, Christie BR. Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus. Neuroscience 2006; 137(4): 1299-307.

[28] Stranahan AM, Khalil D, Gould E. Running induces widespread structural alterations in the hippocampus and Entorhinal cortex. Hippocampus 2007; 17(11): 1017-22.

[29] Yau SY, Lau BWM, Tong JB, et al. Hippocampal neurogenesis and dendritic plasticity support running-improved spatial learning and depression-like behaviour in stressed rats. PLoS One 2011; 6(9): 15.

[30] Yi SS, Hwang IK, Yoo KY, et al. Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. Neurochem Res 2009; 34(6): 1039-46.

[31] Covington HE, 3rd, Vialou V, Nestler EJ. From synapse to nucleus: Novel targets for treating depression. Neuropharmacology 2010; 58(4-5): 683-93.

[32] Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci USA 2011; 108(7): 3017-22.

[33] Chaddock L, Erickson KI, Prakash RS, et al. A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. Brain Res 2010; 1358: 172-83.

[34] Biedermann S, Fuss J, Zheng L, et al. In vivo voxel based morphometry: Detection of increased hippocampal volume and decreased glutamate levels in exercising mice. Neuroimage 2012; 61(4): 1206-12.

[35] Eadie BD, Redila VA, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. J Comp Neurol 2005; 486(1): 39-47.

[36] Ota KT, Duman RS. Environmental and pharmacological modulations of cellular plasticity: Role in the pathophysiology and treatment of depression. Neurobiol Dis 2013; 57: 28-37.

[37] Bjornebekk A, Mathe AA, Gruber SH, Brenes S. Housing conditions modulate escitalopram effects on antidepressive-like behaviour and brain neurochemistry. Int J Neuropsychopharmacol 2008; 11(8): 1135-47.

[38] Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. Neuroimage 2006; 31(3): 1116-28.

[39] Kaae SS, Chen F, Wegener G, Madsen TM, Nyengaard JR. Quantitative hippocampal structural changes following electroconvulsive seizure treatment in a rat model of depression. Synapse 2012; 66(8): 667-76.

[40] Gomez-Galan M, De Bundel D, Van Eeckhaut A, Smolders I, Lindskog M. Dysfunctional astrocytic regulation of glutamate transmission in a rat model of depression. Mol Psychiatry 2013; 18(5): 582-94.

[41] Kasai H, Hayama T, Ishikawa M, Watanabe S, Yagishita S, Noguchi J. Learning rules and persistence of dendritic spines. Eur J Neurosci 2010; 32(2): 241-9.