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To cite this article: Hadar Segal-Gavish, Ran Barzilay, Ofri Rimoni & Daniel Offen (2017): Voluntary exercise improves cognitive deficits in female dominant-negative DISC1 transgenic mouse model of neuropsychiatric disorders, The World Journal of Biological Psychiatry, DOI: 10.1080/15622975.2017.1323118

To link to this article: http://dx.doi.org/10.1080/15622975.2017.1323118
BRIEF REPORT

Voluntary exercise improves cognitive deficits in female dominant-negative DISC1 transgenic mouse model of neuropsychiatric disorders

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ABSTRACT

Objectives: Physical exercise has gained increasing interest as a treatment modality that improves prognosis in psychiatric patients. The disrupted in schizophrenia 1 (DISC1) gene is a candidate gene for major mental illness. In this study, we aimed to determine whether voluntary wheel running can improve cognitive deficits of dominant-negative DISC1 transgenic mice (DN-DISC1).

Methods: DN-DISC1 and control mice (10-week-old male and female) were placed for 14 days in a cage with or without access to a running wheel. Two weeks later, mice underwent behavioural tests evaluating cognition and social approach and recognition.

Results: Voluntary exercise improved performance in the novel object recognition test, restored the impairment in spatial memory in the Y maze, and reversed the deficit in social recognition memory in DN-DISC1 females. DN-DISC1 males did not exhibit behavioural deficits at baseline. Tissue analysis revealed that exercise induced a significant increase in hippocampal expression of doublecortin (DCX), brain-derived neurotrophic factor (BDNF) and cannabinoid receptor type 1 (CB1R) only in DN-DISC1 females.

Conclusions: Voluntary exercise is beneficial in attenuating cognitive deficits observed in a rodent model relevant for neuropsychiatric disorders. The data add a preclinical aspect to the accumulating clinical data supporting the incorporation of physical exercise to patients’ care.

Abbreviations: DISC1: Disrupted in schizophrenia 1; CB1R: Cannabinoid receptor type1; p-TrkB: Phosphorylated tropomyosin receptor kinase B; DCX: Doublecortin; NORT: novel object recognition test

Introduction

The disrupted in schizophrenia 1 (DISC1) gene is a candidate gene for major mental illness. DISC1 was initially identified in a unique Scottish pedigree in which a balanced chromosomal translocation t(1;11)(q42.1; q14.3) disrupts this gene and segregates with major psychiatric disorders such as schizophrenia (SCZ) and affective disorders, including bipolar and major depressive disorder (St Clair et al. 1990; Blackwood et al. 2001). Since then, despite the failure of genome-wide association studies to recognise DISC1 as a candidate risk gene associated with SCZ and bipolar disorder (International Schizophrenia Consortium et al. 2009; Stefansson et al. 2009), accumulating studies have shown that genetic variations of DISC1 are linked to aberrant neurodevelopment and synaptic function (Di Giorgio et al. 2008; Carless et al. 2011; Randall et al. 2014), and associated with a variety of neurodevelopmental disorders, such as SCZ (Hodgkinson et al. 2004), and autism spectrum disorders (Kilpinen et al. 2008).

The expression pattern of DISC1 indicates that it has a critical role in neurodevelopment as it is highly expressed throughout all stages of brain development. DISC1 is also expressed in various brain areas after birth, including regions of adult neurogenesis – the olfactory bulb and hippocampus (Schurov et al. 2004). Moreover, in the adult mouse brain, the highest expression of DISC1 is detected in the dentate gyrus, the region in which newly born neurons are continuously incorporated in the hippocampus (Austin et al. 2004). This finding highlights the pivotal role of DISC1 in regulating neurogenesis (Wu et al. 2013).

Considerable evidence has accumulated in recent years highlighting the potential of physical exercise interventions as an effective adjuvant therapy for treating severe mental illnesses. Physical exercise has been shown to reduce symptoms of depression (Kvam et al. 2017).
2016; Schuch et al. 2016) and schizophrenia (Firth et al. 2015; Stubbs et al. 2015), as well as enhance cardiorespiratory fitness (Vancampfort et al. 2015) as a means of combating pharmacologically induced side effects and subsequently improving quality of life among people with mental illness (Rosenbaum et al. 2014). Notably, as current pharmacological treatments exert limited efficacy in targeting cognitive deficits, considered a core feature of SCZ and affective disorders (Barch 2009; Hugdahl & Calhoun 2010), non-pharmacological treatments such as physical activity are considered as part of a multimodal treatment programme. Indeed, recent studies have shown that physical exercise is efficient in ameliorating cognitive symptoms in SCZ and affective disorders (Knöchel et al. 2012; Malchow et al. 2013; Kandola et al. 2016; Firth et al. 2017).

Elevated neurotrophic factors, specifically brain-derived neurotrophic factor (BDNF), as well as increased adult hippocampal neurogenesis, are implicated in the beneficial effect of exercise on cognitive impairments (Vivar et al. 2013; Kandola et al. 2016), both considered to be aberrant in SCZ and affective disorders (Kempermann et al. 2008; Jun et al. 2012). Furthermore, recent reports indicate that interplay between the endocannabinoid system and BDNF signalling in the hippocampus mediates exercise-induced cognitive enhancement (Ferreira-Vieira et al. 2014).

Here, we aimed to determine whether voluntary wheel running improves cognitive deficits in a mouse model of neuropsychiatric disorders and to investigate the effect of voluntary wheel running on markers of hippocampal neuroplasticity and neurogenesis. We utilised transgenic mice with forebrain neuron-specific expression of mutant human DISC1, displaying behavioural deficits associated with major psychiatric disorders, such as cognitive and social impairments (Pletnikov et al. 2008; Kaminitz et al. 2014).

Methods

Animals

DN-DISC1 double transgenic mice were generated from breeding tetracycline response element mutant DISC1 mice and mice expressing the tetracycline-transactivator (tTA) under regulatory control of the forebrain neurons-specific calcium-calmodulin-dependent kinase II-a (CAMKIIa) promoter, established and maintained on a mixed B6;CBA genetic background (Jackson Laboratory, Sacramento, CA). Expression of both mutant DISC1 and CAMKIIa was confirmed by a standard genotyping protocol. Littermates positive for mutant DISC1 gene and negative for CAMKIIa were used as controls. All mice were kept in a 12-h light/dark cycle and had access to food and water ad libitum. All animal experiments and protocols were approved by the Committee for Animal Research at Tel Aviv University, Israel.

Experimental design

Ten-week-old male and female DN-DISC1 mice were placed for 14 days in a cage with (Running/DN-DISC1 RUN) or without (Sedentary/DN-DISC1) free access to two running wheels. Control mice were situated in the same cages but without an access to running wheels. Mice were placed in a group of three littermates per cage. We routinely monitored the cages three times a day to evaluate that the mice were using the running wheels. The monitoring reassured us that indeed the mice used the wheels to run. Two weeks later, mice underwent behavioural tests to study cognition and sociability. The behavioural experiments were carried on with two cohorts of mice (males: control $n = 8$; DN-DISC1 $n = 8$; DN-DISC1 RUN $n = 5$; females: control $n = 7$; DN-DISC1 $n = 6$; DN-DISC1 RUN $n = 6$). The second cohort of mice was sacrificed and their brains were dissected for tissue analysis (males: control $n = 11$; DN-DISC1 $n = 7$; DN-DISC1 RUN $n = 6$; females: control $n = 7$; DN-DISC1 $n = 10$; DN-DISC1 RUN $n = 12$). Results regarding male DN-DISC1 mice were included in the supplementary, rather than in the main text, as they did not differ significantly in their behavioural phenotypes compared to littermate controls, and thus did not allow evaluation of beneficial effect of the exercise intervention.

Behavioural tests

Analysis of the behavioural testing was conducted using the EthoVision XT 9 software platform (Noldus, Wageningen, Netherlands).

Open-field test

Mice were put in the arena ($47 \times 47 \times 51$ cm) and videotaped for 30 min. Total distance travelled and duration of time exploring the periphery and the centre of the arena were measured.

Novel object recognition test

The test was conducted as previously reported (Nagai et al. 2011). In brief, mice were individually habituated to the open-field arena for three consecutive days. During the training session, two identical objects were
placed in the arena and the mice were free to explore for 10 min. During the retention session, carried out 1 h following the training session, one of the now familiar objects was replaced by a novel object and the mice were put back into the arena to explore for 5 min. Sniffing durations of each object were quantified during both sessions. This test was conducted solely with the second cohort.

**Y maze spatial memory test**

The test was performed as described previously (Yau et al. 2007). Briefly, the Y maze apparatus consisted of three enclosed arms, made of white Plexiglas, in the shape of a Y. Visual cues were placed around the maze in the testing room and in the maze itself and were kept constant throughout the testing sessions. The test consisted of two trials separated by a 3-h time interval. During the first trial, mice were placed at the end of a chosen arm, the start arm, and were allowed to explore the maze for 5 min while one of the arms was closed. In the second trial, the retention trial, mice were allowed to explore freely all three arms of the maze for 5 min. The time spent in each arm was calculated during the first 2 min of the retention trial.

**Three-chambered social assay**

The three-chambered social assay was carried out according to previous reports (Moy et al. 2007; Kaminitz et al. 2014). In brief, the apparatus was a rectangular box divided into three chambers by removable partitions. After two consecutive days of habituation, the test mouse was placed into the middle chamber of the arena for 10 min. During the social approach trial, an unfamiliar mouse was introduced into a cage situated in one of the side chambers, while the cage on the other side chamber was left empty. Thereafter, the partitions were removed, allowing the test mouse to freely explore all the arena chambers for another 10 min. Lastly, during the social recognition trial, another unfamiliar mouse was introduced into the previously empty cage, permitting the test mouse to explore the arena for an additional 10 min. Sniffing durations of each cage were quantified during both trials.

**Tissue processing**

After sacrifice, brains were removed and the hippocampi of each mouse were dissected. Thereafter, tissues were cryopreserved in −80 °C.

**Synaptic and cytosolic protein extraction**

Syn-PER™ Synaptic Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA) was used for extraction of proteins expressed in the synapses of the hippocampal tissues according to the manufacturer’s instructions. The cytosolic fraction extracted during this procedure was saved for a separate analysis. The protein concentration of the synaptosomal and cytosolic fractions was quantified utilising the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific).

**Western blot**

Proteins were separated by 4–20% Mini-PROTEAN® TGX™ precast polyacrylamide gel (Bio-Rad Laboratories, CA) electrophoresis and transferred to nitrocellulose membranes. The membranes were probed overnight at 4 °C with the following primary antibodies: rabbit anti-Cannabinoid Receptor I (1:200, ab23703, abcam, Cambridge, UK), rabbit anti-BDNF (ab72439, 10 μg/ml, abcam), goat anti-Doublecortin (1:500, Santa Cruz Biotechnology, CA). Actin (mouse anti-Actin, 1:500, MAB1501, EMD Millipore, Darmstadt, Germany) and Synaptophysin (rabbit anti-Synaptophysin, 1:500, Santa Cruz Biotechnology, CA) were used as loading controls for the cytosolic and synaptosomal fractions, respectively. Thereafter, membranes were incubated with secondary antibodies: goat anti-mouse IRDye 800CW or 680CW or goat anti-rabbit IRDye 800CW or 680CW or donkey anti-goat IRDye 800CW (1:5,000, infrared dye, LI-COR Biosciences, NE) for 1 h at room temperature. The membranes were then scanned with Odyssey Infrared Imager (model 9120, LI-COR Biosciences). Densitometric analysis of western blots was performed using Image Studio Lite software (LI-COR Biosciences) to measure the area and density of protein bands.

**Statistical analysis**

We used GraphPad Prism version 7 (GraphPad Software, San Diego, CA) for statistical analysis. The effects of voluntary exercise on behaviour were analysed using one-way ANOVA, followed by Tukey’s post-hoc test, and on expression of proteins using Student’s t-test. We employed Pearson’s correlation to evaluate relationships between behavioural and biochemical data. Statistical significance was considered for $P < 0.05$ in all statistical analyses. Results are presented as mean ± standard error.
Results

Voluntary exercise does not affect locomotion and anxiety-related behaviour in DN-DISC1 mice

No difference was observed between experimental groups in locomotor activity, as measured by total distance moved during the 30 min in the open-field arena (Females: Figure 1(B), $F(2,44) = 1.059, P = 0.3554$; Males: Supplementary Figure 1A, $F(2,42) = 0.5328, P = 0.5908$). Furthermore, running DN-DISC1 mice of both sexes did not differ from sedentary DN-DISC1 or control mice in anxiety related behaviour, as observed by the percentage of time spent in the anxiety provoking centre of the open-field arena (Females: Figure 1(C), $F(2,44) = 0.6284, P = 0.5382$; Males: Supplementary Figure 1B, $F(2,42) = 0.627, P = 0.5391$).

Voluntary exercise improves object recognition memory in DN-DISC1 female mice

Female DN-DISC1 mice spent significantly less time sniffing the novel object compared to their littermate control group (Figure 2(B), Control: 72%, DN-DISC1: 55%, $F(2,25) = 7.546, P = 0.0027$; Control vs DN-DISC1: $P = 0.0039$). Voluntary wheel running resulted in increased preference towards the novel object compared to sedentary DN-DISC1 mice (Figure 2(B), DN-DISC1 RUN: 68%, DN-DISC1 vs DN-DISC1 RUN: $P = 0.0135$). In contrast, and consistent with our recent report (Kaminitz et al. 2014), sedentary DN-DISC1 male mice did not display a deficit in the NORT, as observed by sniffing the novel object 63% of total time spent sniffing both the familiar and novel objects. Voluntary exercise did not affect their preference towards the novel object as it was intact at baseline (Supplementary Figure 2).

Voluntary exercise restores spatial memory deficit in DN-DISC1 female mice

Female DN-DISC1 mice did not prefer the novel arm over the familiar arm in the Y maze task in contrast to their littermate controls (Figure 3, Control: Familiar arm vs Novel arm: $P = 0.0006$, DN-DISC1: Familiar arm vs Novel arm: $P = 0.3158$), signifying impaired spatial memory. Voluntary wheel running restored their spatial memory deficit, as indicated by spending more time exploring the novel arm compared to the familiar arm (Figure 3, DN-DISC1 RUN: Familiar arm vs Novel arm: $P = 0.0023$).

Male DN-DISC1 mice demonstrated a trend toward exploring the novel arm longer than the familiar arm, indicating a rather intact performance in the Y Maze task (Supplementary Figure 3, DN-DISC1: Familiar arm vs Novel arm: $P = 0.0940$).

Voluntary exercise improves social recognition memory in female DN-DISC1, but does not affect social approach

In the social approach trial of the three-chambered social assay (Figure 4(A)), DN-DISC1 female mice displayed a slight preference towards the social stimulus compared to an inanimate stimulus (Figure 4(B), 54%). Voluntary wheel running did not enhance their sociability in this trial (Figure 4(B), DN-DISC1 vs DN-DISC1 RUN: $P = 0.9833$). In the social recognition trial (Figure 4(C)), DN-DISC1 female mice showed impaired social
recognition, as represented by lack of preference towards a novel social stimulus over a familiar one (Figure 4D, 48%), albeit a comparison to their littermate control group did not reach statistical significance (Figure 4D, Control vs DN-DISC1: $P = 0.1950$).

Physical exercise induced a significant improvement in social recognition memory in female DN-DISC1 mice, as observed by sniffing the novel mouse 58% of the total sniffing time (Figure 4D) $F(2, 45) = 3.301, P = 0.0419$.

In our previous report, DN-DISC1 male mice did not demonstrate an impaired social approach compared with controls, but did manifest a deficit in social recognition (Kaminitz et al. 2014). Similarly, in this work we noted that DN-DISC1 male mice showed a moderate preference towards the social stimulus, indicating an intact social approach (Supplementary Figure 4A, 57%). DN-DISC1 male mice did not differ from the control mice in terms of social recognition as both experimental groups manifested lack of preference towards a novel social stimulus over a familiar one (Supplementary Figure 4B). Voluntary wheel running did not affect their performance in the social approach trial (Supplementary Figure 4A, DN-DISC1 vs DN-DISC1 RUN: $P = 0.5251$), nor did it affect their behaviour in the social recognition trial (Supplementary Figure 4B, DN-DISC1 vs DN-DISC1 RUN: $P = 0.3874$).

Voluntary exercise induced an increase in markers of hippocampal neuroplasticity and neurogenesis in DN-DISC1 female mice but not in male mice

To investigate signalling pathways associated with the voluntary exercise modulation of hippocampus-dependent recognition memory, we explored the involvement of CB1R and BDNF, markers of hippocampal neuroplasticity, and DCX, a marker of hippocampal neurogenesis, which have been suggested as mediators of the beneficial effects of exercise on cognition (Hill et al. 2010; Ferreira-Vieira et al. 2014). Western blot analysis revealed that voluntary exercise significantly increased expression levels of CB1R (Figure 5B), $t_{19} = 2.21, P = 0.04$), BDNF (Figure 5D), $t_{16} = 2.48, P = 0.02$) and DCX (Figure 4F), $t_{17} = 4.11, P = 0.0007$) in female DN-DISC1 mice.

Importantly, the levels of hippocampal DCX in DN-DISC1 female mice significantly correlated with the performance in the NORT (i.e. the percentage of time
sniffing the novel object of total time spent sniffing both the familiar and novel objects) (Figure 5(G), $R = 0.554$, $P = 0.017$). The other markers did not significantly correlate with the behavioural measures.

In contrast, in males, running DN-DISC1 mice did not demonstrate increased expression levels of CB1R (Supplementary Figure 5B, $t_{10} = 0.3$, $P = 0.77$), BDNF (Supplementary Figure 5D, $t_{11} = 0.03225$, $P = 0.97$) and DCX (Supplementary Figure 5F, $t_{10} = 0.11$, $P = 0.91$) compared to sedentary male DN-DISC1 mice.

**Discussion**

We report here that voluntary exercise induced an improvement in cognitive deficits in the DN-DISC1 rodent model of neuropsychiatric illness. Female DN-DISC1 mice having free access to a running wheel for 14 days showed a recovery in object recognition memory, spatial memory and social recognition memory compared to sedentary controls. Furthermore, the behavioural improvement was accompanied by hippocampal changes that indicate enhanced neurogenesis and neuroplasticity.

In contrast to female DN-DISC1 mice, voluntary exercise did not induce significant changes in the behaviour of DN-DISC1 males. One explanation could be that male DN-DISC1 mice did not significantly differ in their behavioural phenotypes compared to their littermate controls and therefore did not exhibit any behavioural deficits. Although exercise was previously reported to have beneficial effects on wild-type animals (Duman et al. 2008; Berchtold et al. 2010), our results indicate that the relative benefit of exercise might be more apparent when introduced to mice that demonstrate impairments. Other possible explanations could be that 14 days of voluntary running
Figure 5. Voluntary exercise induced an increase in markers of hippocampal neuroplasticity and neurogenesis in DN-DISC1 female mice. (A,C,E) Representative western blots for CB1 receptor (CB1R), brain-derived neurotrophic factor (BDNF) and Doublecortin (DCX). Synaptophysin was used as a loading control for the synaptosomal fraction (A–B,C–D) and actin was used as a loading control for the cytosol fraction (E–F). Quantitative analysis of protein levels of CB1R, BDNF and DCX in DN-DISC1 female mice (B,D,F), respectively. (G) Scatter presentation of the correlation between hippocampal DCX levels and the percentage of time sniffing the novel object of total time spent sniffing both objects of each mouse. *P < 0.05, **P < 0.001 in Student’s t-test. Data (Mean ± SEM) were obtained from 19–21 DN-DISC1 females (Sedentary n = 9–10, Running n = 9–11).
activity are not sufficient to promote favourable effects in DN-DISC1 males, or that DN-DISC1 male mice did not run an adequate daily distance.

Several studies have reported sex-specific behavioural and biochemical abnormalities in different DISC1 mouse models (Pletnikov et al. 2008; Ayhan et al. 2011; Kuroda et al. 2011; Nakai et al. 2014). Similar to our results, female mice with disruption of exons 2 and 3 of the DISC1 gene, displayed more severe deficits than male mice carrying the same mutation, in various behavioural tests, such as the prepulse inhibition test, the cliff-avoidance test and the methamphetamine-induced hyperactivity test (Kuroda et al. 2011). Neurochemical analysis of these mice revealed that female, but not male mice, exhibited alterations of GABAergic and dopaminergic systems, which may partially explain this sex-specific behavioural phenotype (Nakai et al. 2014). Notably, previous works in humans have revealed that variance in DISC1 has stronger effects in females than in males (Hennah et al. 2009; Harris et al. 2010). The mechanisms underlying gender-specific differences in DISC1 mouse models and humans are still unclear and need further investigation.

Although we could not conclude from our research that exercise differentially effected male and female DN-DISC1 mice since DN-DISC1 males were not clearly impaired in the behavioural tests employed, other studies have focussed on this question. Several animal studies showed that exercise modulates mood and cognition in a sex-dependent manner (Giménez-Llort et al. 2010; Munive et al. 2016). Furthermore, a study that included patients with mild cognitive impairment that underwent aerobic exercise showed sex-specific effects on cognition, in favour of women demonstrating improved executive control processes (Baker et al. 2010).

Our study uses a preclinical model to attempt to learn about a possible mechanism that underpins the clinical efficacy of physical exercise on cognitive capacity in human psychiatric patients (Knöchel et al. 2012; Malchow et al. 2013; Firth et al. 2017). Aerobic exercise has been widely acclaimed for its potential cognitive benefits. In particular, physical exercise has a potent impact on promoting hippocampal neuroplasticity and function (Kandola et al. 2016). The hippocampus is important for spatial memory (Broadbent et al. 2004) as well as for object and social recognition memory (Broadbent et al. 2010; Hitti & Siegelbaum 2014; Stevenson & Caldwell 2014). Physical exercise has long been known to enhance hippocampal neurogenesis (Gage et al. 1999). A previous study showed that knockdown of hippocampal neurogenesis in the dentate gyrus impairs object recognition memory in rats (Jessberger et al. 2009). We report a correlation between hippocampal DCX levels (a proxy marker for hippocampal neurogenesis) and cognitive capacity (represented by the duration of time spent sniffing the novel object in the NORT). Our findings may therefore imply that voluntary physical activity exerts its beneficial effect on object recognition memory in DN-DISC1 mice in part by enhancing hippocampal neuroplasticity and neurogenesis.

We found that increased expression of DCX was accompanied by a rise in BDNF and CB1R in the hippocampus of running female DN-DISC1 mice. These observations are consistent with previous reports demonstrating that increased endocannabinoid signalling in the hippocampus is necessary for exercise-induced hippocampal neurogenesis (Hill et al. 2010), and that the beneficial effect of exercise on spatial memory is dependent on CB1R activation, and is mediated by BDNF (Ferreira-Vieira et al. 2014). Importantly, several human studies have also shown that increased BDNF serum levels associated with aerobic exercise are directly correlated with improved performances in various memory domains including spatial memory (Piepmeier & Etnier 2015). Taken together, tissue analysis of female DN-DISC1 hippocampi suggests that exercise induces neurochemical alterations consistent with neurogenesis and plasticity that possibly explain the cognitive improvement. Notably, as we did not measure the baseline levels of DCX, CB1R and BDNF in the control mice, we suggest that our results indicate an association between the molecular changes and behavioural rescue; however, we cannot infer causality.

In conclusion, we demonstrate preclinical evidence for the therapeutic effect of physical exercise on cognitive deficits in a model of neuropsychiatric disease. Since the cognitive deficits observed in psychiatric patients are debilitating and are thought to predict the functional outcome of the patients, our study further supports incorporating physical exercise as part of a multimodal treatment programme for patients with major psychiatric disorders.

Statement of interest
None to declare.

Funding
This study was supported in part by the National Institute of Psychobiology in Israel [Grant no. 0601564281].
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