Original Paper

Aerobic exercise improves memory and prevents cognitive deficits of relevance to schizophrenia in an animal model

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Abstract

Introduction and objectives: Cognitive impairment associated with schizophrenia (CIAS) greatly reduces patients' functionality, and remains an unmet clinical need. The sub-chronic phencyclidine (scPCP) rat model is commonly employed in studying CIAS. We have previously shown that voluntary exercise reverses impairments in novel object recognition (NOR) induced by scPCP. However, there has not been a longitudinal study investigating the potential protective effects of exercise in a model of CIAS. This study aimed to investigate the pro-cognitive and protective effects of exercise on CIAS using the translational NOR and attentional set-shifting tasks (ASST).

Methods: Female Lister Hooded rats were either exercised (wheel running for one hour per day, five days per week, for six weeks; n=20) or not (n=20) and then tested in a natural-forgetting NOR test. Rats in each group were then administered either PCP (2 mg/kg intraperitoneally (i.p.)) or saline solution (1 mL/kg i.p.) for seven days, followed by seven days washout. Three NOR tests were conducted immediately and two and nine weeks after washout, and a natural-forgetting NOR test was carried out again eight weeks post washout. Rats were trained and tested in ASST from week 6 to week 10 post washout.

Results: Non-exercised rats displayed a deficit in both of the natural-forgetting NOR tests, whereas exercised rats did not. The scPCP exercise group did not show the expected deficit in NOR at any time point, and had a significantly ameliorated deficit in the ASST compared to the scPCP control group.

Conclusion: Voluntary exercise has long-lasting pro-cognitive and protective effects in two cognitive domains. Exercise improves cognition and could provide protection against CIAS.

Keywords

Exercise, running, cognition, schizophrenia, rat, sub-chronic phencyclidine model

Introduction

Cognitive dysfunction – specifically deficits in executive function and working, verbal and visual memory – occurs in schizophrenia patients prior to the onset of positive symptoms (Addington and Barbato, 2012), and is consistent throughout the course of the illness (Heaton et al., 2001). Cognitive deficit and negative symptoms contribute greatly to reduced functionality and quality of life, in addition to having a large socio-economic burden (Walker et al., 2017). Currently approved antipsychotics primarily treat the positive symptoms, with little impact on cognitive or negative symptoms (Green, 2016). Therefore, developing therapeutic strategies for this unmet clinical need in schizophrenia is of the utmost importance.

GABAergic inhibitory interneurons (IINs) and gamma oscillations play an integral role in the pathophysiology of cognitive deficits in schizophrenia (for a review, see Glausier and Lewis, 2017). Brain oscillations play an essential role in encoding and storing information by forming neuronal assemblies, and this establishes the basis for cognitive processing (Buzsáki, 2006; Uhlhaas and Singer, 2010). For instance, processes such as executive function in the prefrontal cortex (PFC) and visual memory in the hippocampal formation depend on gamma oscillations generated through rhythmic electrical activity of a network of glutamatergic excitatory pyramidal cells (Fries et al., 2007; Grosse-Wentrup et al., 2011). This synchronized output is regulated by temporally precise inputs from IINs (Chen et al., 2014; Wang, 2010), which also receive recurrent excitation from pyramidal cells via NMDA receptors (NMDARs; Zheng et al., 2011). Within the hippocampal formation, the generation of gamma oscillations relies on IINs expressing a calcium-binding protein called parvalbumin (PV; Gonzalez-Burgos and Lewis, 2012; Sohal et al., 2009). Within the PFC, gamma oscillations depend on both somatostatin (SST)-positive and PV-positive IINs in order to regulate the output of pyramidal cells (Du et al., 2018; Gonzalez-Burgos et al., 2015).

In schizophrenia patients, reduced mRNA and protein levels of SST and PV are observed in PFC and hippocampal IINs (Enwright et al., 2016; Fung et al., 2014; Konradi et al., 2011), as well as reductions in mRNA and protein expression of an enzyme involved in GABA synthesis: glutamate decarboxylase enzyme

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67 (GAD67; Chung et al., 2016; Curley et al., 2011; Konradi et al., 2011). These reductions predict a decline in the synthesis and release of GABA from the synapses, thus leading to the disinhibition of excitatory pyramidal cells and therefore generation of impaired gamma oscillations (Pittman-Polletta et al., 2015). Indeed, patients with schizophrenia show compromised gamma oscillations in both the PFC and hippocampal formation (Chen et al., 2014; Grech et al., 2018; Minzenberg et al., 2010; Volk et al., 2016). Impairments in gamma oscillations worsen as the cognitive load increases (Cho et al., 2006), and lower cortical GABA levels are linked to poorer cognitive performance (Rowland et al., 2016).

The blockade of NMDARs found on GABAergic IINs by NMDAR antagonists such as phencyclidine (PCP) induces schizophrenia-like symptomatology in humans (Jones et al., 2011; Krystal et al., 1994), and exacerbates symptoms of schizophrenia in patients (Lahti et al., 1995a, 1995b). We (and others) have repeatedly shown that sub-chronic PCP (scPCP) induces aspects of both negative and cognitive symptoms of schizophrenia in rats (Neill et al., 2010, 2014), as well as exhibiting pathological changes relevant to the disorder such as reduced PV in both PFC and hippocampal IINs (Abdul-Monim et al., 2007; Amitai et al., 2012; Cadinu et al., 2018; Cochran et al., 2003; Jenkins et al., 2010). Our work uses female Lister Hooded (LH) rats, as females have been reported to be more sensitive to the scPCP-induced effects (Snigdha et al., 2011) and are well suited to certain behavioural tasks (Sutcliffe et al., 2007) and social behaviour studies. We focus on performance in the novel object recognition (NOR) test and attentional set-shifting task (ASST), which measure visual recognition memory and executive function, respectively (Janhunen et al., 2015; Tait et al., 2018). These cognitive processes are impaired in both patients with schizophrenia (McGuire et al., 2013; Orellana and Slachevsky, 2013) and scPCP-treated female LH rats (Idris et al., 2010; Le Cozannet et al., 2010; McLean et al., 2012). Most recently, we have also shown reduced object recognition memory, PV and postsynaptic density 95 in the frontal cortex and hippocampus in a mouse scPCP model (Gigg et al., 2019), supporting the robust nature of the model for schizophrenia pathophysiology in rodents.

Aerobic exercise can be beneficial for cognitive function in schizophrenia patients (Falkai et al., 2017; Firth et al., 2017; Lin et al., 2015; Oertel-Knöchel et al., 2014; Pajonk et al., 2010; Subramaniapillai et al., 2016). However, it is challenging to identify the optimal intensity, duration and frequency of exercise in human studies, as there are many differences between the methods employed in studies (e.g. type and duration of exercise) and individual patients (e.g. different antipsychotic treatments, age and duration of treatment), in addition to methodological limitations such as low adherence to the exercise regimen and lack of follow-up assessments (Cooney et al., 2013; Wang et al., 2018). Furthermore, human studies often lack an adequate control group due to small sample size or a suboptimal control group (e.g. either exercised healthy or non-exercised schizophrenia patients), with the caveat of differences in the amount of attention given to the groups (Van Der Stouwe et al., 2018). The latter could lead to changes in subjects' behaviour due to the awareness of being observed, known as the Hawthorne effect (Merrett, 2006). The scPCP rat model not only controls for these variables, but also allows for the brain region-specific analysis of proteins influenced by exercise beyond the examination of serum levels. The application of voluntary aerobic exercise, in the form of wheel running, has produced robust improvements in the cognitive performance of healthy rodents (Bhattacharya et al., 2015; Hopkins and Bucci, 2010; Hopkins et al., 2011; Lin et al., 2012) and, in our laboratory, of scPCP-treated rats (Gonzalez et al., 2017).

We previously reported that voluntary wheel running reverses scPCP-induced NOR deficits in our female LH rat model for schizophrenia – an effect that persisted up to two weeks post exercise (Gonzalez et al., 2017). Many studies have investigated the possible therapeutic effects of exercise in various animal disease models. However, very few have studied the protective and pro-cognitive effects of voluntary exercise in healthy and in scPCP-treated rats over time in a well-controlled longitudinal study. Hence, in this study, we examined the pro-cognitive effects of voluntary wheel running in naïve (prior to scPCP or vehicle treatment) rats, and the potential protective effects of voluntary exercise in scPCP-treated rats in two behavioural tasks of relevance to the deficits observed in schizophrenia patients. We test two hypotheses: (a) that exercise will improve cognitive performance in healthy animals, and (b) that exercise can prevent cognitive dysfunction that occurs in schizophrenia. In support of our hypotheses, a recent large-scale study revealed a positive correlation between physical fitness (PF) in healthy people, assessed by high levels of walking endurance, enhanced cognitive performance and white-matter microstructure (Opel et al., 2019). This study found beneficial effects of high PF on almost all domains of cognition, supporting the idea that exercise can improve cognitive function over a range of tests.

Methods

Animals

Female LH rats (n=40; Charles River Laboratories, Margate, UK), weighing a mean of 231.78±16.94 g at the beginning of the studies, were housed in groups of five in individually ventilated, two-tiered plastic cages (38 cm×59 cm×24 cm; GR1800 Double-Decker Cage; Tecniplast, Kettering, UK). These cages contained paper sizzle nest, sawdust and cardboard tunnels (Datesand Group, Manchester, UK). The rats had access to water and standard rat chow (Special Diet Services) ad libitum within the home cage. The environment was kept constant at 20±2°C, 55±5% humidity, under a reversed 12-hour/12-hour light/dark cycle (lights off at 22:00). All procedures took place at the University of Manchester. Drug administration and wheel running were carried out during the dark phase under red light, and the behavioural testing was carried out under normal lighting (100 lux). A blackout curtain was placed around the entrance to the room containing the home cages, and the cages were covered using blackout blankets during transportation for behavioural tasks in order to minimize light exposure. A seven-day acclimatisation period was given prior to the start of this study to minimise effects of transportation stress on the results. All procedures were performed in compliance with the Home Office (Scientific Procedures) Act 1986, and approved by the University of Manchester's Animal Welfare and Ethical Review Body.

Wheel-running regimen

An aerobic voluntary wheel-running regimen, developed in-house, was employed in this study (Gonzalez et al., 2017). The cages that

Figure 1. Set-up used in the wheel-running regimen. Exercise rats (n=20) were placed in these cages for one hour per day, five days per week, for six consecutive weeks, where they voluntarily exercised. Total distance covered per day was recorded with a pedometer, which detected a magnet attached to the rotating wheel. Non-exercised rats were also placed in these cages for the same duration of time, but the wheels were locked.

contained the wheels had the same design as the home cages except that the second floor was removed in order to house the plastic running wheel, which had a diameter of 30 cm (Figure 1). Wheel-running cages did not contain any food or water, and were cleaned once a week by renewing the bedding and wiping the cage and the wheel with 70% ethanol solution. Prior to the start of the wheel-running regimen, all rats were placed in cages containing the running-wheels for one hour a day for five days. The total distance run by each rat per day was recorded using the AS 11G pedometer (Wilko, Worksop, UK), which worked by detecting a small magnet attached to the wheel. The average distance run by each rat during this five-day period was calculated, and 20 rats, each with an average distance ⩾0.1 km, were selected to form the exercise group. It is important to note that we selected the best runners for the running group. The remaining 20 rats, which contained one non-runner, formed the non-running control group. Rats were regrouped within the home cages at this stage in order to have exercising rats and non-exercising rats in separate cages for ease of experimentation. Following this, the exercise group had access to running wheels for one hour a day, five days a week, for six consecutive weeks, following the same protocol as in our post-scPCP dosing exercise regimen study (Gonzalez et al., 2017). The control group had access to immobilized wheels for the same duration of time. Rats were randomly assigned to cages containing the wheels using the Latin square method, and were given access between 13:00 and 16:00 in the dark phase, as rats are more active during this period (Clemens et al., 2014).

scPCP administration

PCP hydrochloride, purchased from Sigma–Aldrich (P3029; Gillingham, UK), was administered at a dose of 2 mg/kg to half of the rats in the exercise and control groups (n=10 per group). The remaining rats received vehicle solution (0.9% saline; n=10 per group). The scPCP treatment protocol has been very well validated in our laboratory and elsewhere, and consisted of intraperitoneal (i.p.) injections that were administered for seven days, twice a day (at approximately 10:00 and 16:00; Barnes et al., 2014; Cadinu et al., 2018; Neill et al., 2010, 2014). After administration of the final injection, the rats were given a seven-day washout period, and during this 14-day period, no behavioural testing was carried out. It is important to state that this was an unbiased procedure, that is, rats in both groups were randomly assigned to receive either the PCP or the vehicle treatment. In addition, the drug treatment was carried out blind to the wheel-running regimen. This randomisation was performed at cage level. Hence, all rats in one cage received the same treatment.

NOR apparatus and testing

NOR testing was conducted in square Plexiglas boxes (w=52 cm, l=52 cm, h=30 cm), which consisted of a white floor divided into nine sectors (17.3 cm×17.3 cm each) and black walls. As previously detailed by us, the objects used were brown glass medicine bottles and Cola cans (Figure 2; Grayson et al., 2007). One day prior to the first NOR test, all rats were placed into the NOR test chambers with their cage mates for 15 minutes. On the day of testing, rats were individually placed into the test chambers to explore two identical objects (objects A and B) for three minutes (acquisition phase). Rats were then removed from the chamber and returned to their home cage for the six-hour inter-trial interval (ITI) or kept individually in rectangular Plexiglas boxes (w=24 cm, l=44 cm, h=19 cm) for the one-minute ITI. After this, rats were returned to the NOR test chamber, where they explored an object identical to that used in the acquisition phase (familiar object) and a novel object for three minutes (retention phase). At the end of each testing session, the objects and the NOR test chambers were wiped clean with 70% ethanol solution in an attempt to eliminate olfactory trails. The performance of all rats was video recorded for subsequent experimenter-blinded scoring. Object exploration time for each object in both phases of the NOR test was scored using an online stopwatch (https://jackrrivers.com/program/). Discrimination indices (DIs) in retention were also obtained using the formula: (time exploring novel

object (s)–time exploring familiar object (s))/(time exploring novel object (s)+time exploring familiar object (s)). The NOR test videos were also employed in measuring locomotor activity (LMA). This was carried out by counting the total number of lines each rat crossed in both the acquisition phase and the retention phase.

ASST apparatus and testing

Habituation paradigm. Habituation and training were conducted as previously described (McLean et al., 2008). Briefly, specific aspects of the testing paradigm commenced six weeks after the seven-day scPCP washout period. Habituation to the testing box was conducted for one hour on three consecutive days prior to training. Two smooth ceramic circular digging pots (measuring 9 cm in diameter by 5 cm internal depth) identical to those used during the testing phases were introduced to each home cage. Each bowl was filled with grade 5 sawdust (also covering the home-cage floor) and baited with food reward in the form of several 1/4 honey nut loops (Kellogg's, Manchester, UK). Digging bowls were continuously re-baited during this habituation period and remained in the home cage for the duration of the study. Following habituation, all rats had to complete the entire training regime successfully in order to proceed to testing in the test apparatus (see Figure 3(a) and (b)). Rats were first trained to dig quickly and reliably in both bowls by progressively covering a single food reward per trial with incrementally thicker layers of digging media (from one third full, to one half full and then full pots). Once a rat had repeatedly demonstrated that it had acquired the digging procedure, rats were placed into the test arena and given four discovery trials to retrieve a food reward from both media-filled pots with the dividing panels in place. Digging itself was defined as a vigorous movement of the front paws to displace digging media and obtain the food reinforcement buried 2–2.5 cm below the surface level. The second phase of training introduced the concept of simple discrimination (SD) between first medium and then odour. Rats were presented with identical digging bowls that had been anointed (smearing a few drops of oil around the rim of the bowl using a tissue) with two aromatic oils (The Body Shop, London, UK), only one of which was baited with a food reward. Placement of the bowls in either the left or right compartment was randomised with the aid of an adapted pseudorandom Gellerman schedule (Gellerman, 1933). Rats were permitted to explore both bowls for the first four trials, irrespective of which bowl they dug in first, thereby acting as an opportunity to associate the food reward with the positive predictor. Subsequent incorrect selections ended a trial without the opportunity to explore the correct bowl. A criterion for successful learning of each discrimination was set at six consecutive correct trials. All exemplars used in training were not used during testing or at any other point in the study.

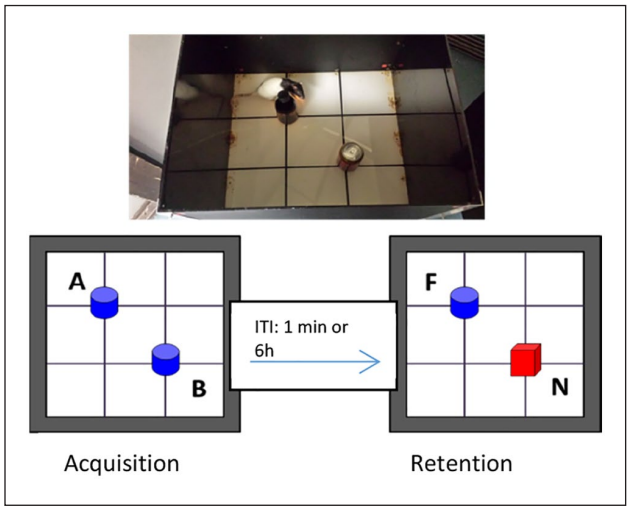

Figure 2. Novel object recognition (NOR) test apparatus. Rats were placed into the NOR chamber with objects located as shown, and allowed to explore for three minutes in both the acquisition and the retention phase. During the inter-trial interval (ITI), rats were housed elsewhere between the test phases for a variable period of time (i.e. one minute or six hours).

Testing paradigm. In all cases, rats were tested in the attentional set-shifting procedure 24 hours after training. A trial was initiated by raising both dividers to give access to both digging bowls, only one of which was baited. The first stage was the SD, which was identical to the SD in the training session on the previous day, except new exemplars were used. Testing continued until the rat reached a criterion of six consecutive correct

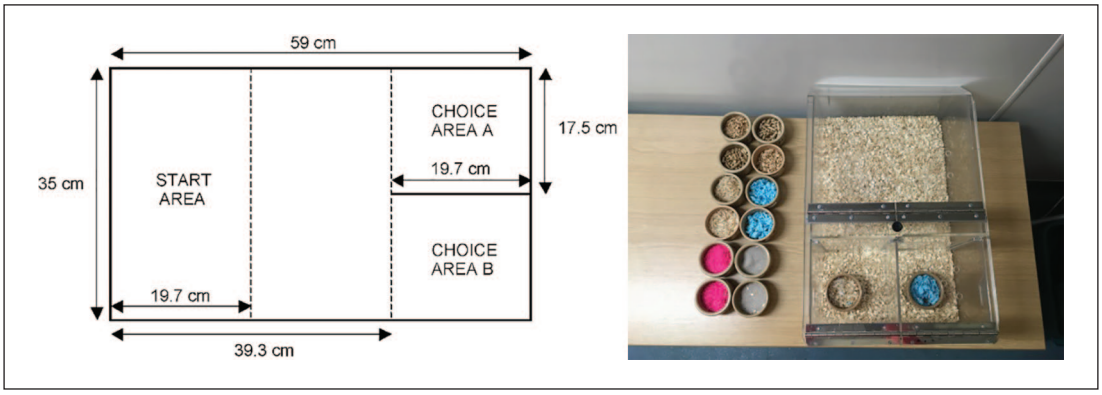

Figure 3. Attentional set-shifting task (ASST) apparatus. Plan view of the ASST box with dimensions shown (a), where unbroken lines illustrate fixed panels and walls, and dotted lines illustrate removable Plexiglas dividers. (b) An actual ASST box with digging pots containing different media.

Table 1. Specific exemplars used (presented in pairs).

Dimension	Pairing 1 (CD)	Pairing 2 (IDS)	Pairing 3 (EDS)
Odour	Rose=O1	Pomegranate=O3	Orange=O5
	White musk=O2	Cinnamon=O4	Almond=O6
Medium	Wood shaving=M1	Small pebbles=M3	Fine sawdust=M5
	Cat litter=M2	Aspen=M4	Wood blocks=M6

Specific exemplars used in each phase of the attentional set-shifting task. Odours were applied around the rims of digging pots, which were filled with various digging media, depending upon the phase being tested. The significance of pairing within a test phase ensures that, for example, rose is always accompanied with white musk within a test trial (O: odour; M: medium).
CD: compound discrimination; EDS: extra-dimensional shift; IDS: intra-dimensional shift.

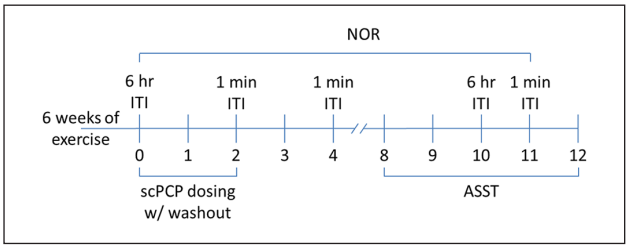

Figure 4. Timeline of the experimental procedures and behavioural tests, with the end of the wheel-running regimen as the starting point. The numbers on the axis represent weeks.
scPCP: sub-chronic phencyclidine.

responses. In a test session, rats performed a series of discriminations (detailed description in McLean et al., 2008). For the compound discrimination (CD), a second dimension was introduced (odour), but the correct and incorrect exemplars remained the same (digging medium). For the reversals, the exemplars and relevant dimensions remained the same (medium), but the rats had to learn that the previously baited odour was now incorrect, and the other odour was now the correct one. New exemplars were used for the intra- and extra-dimensional shifts (IDS and EDS, respectively). The specific exemplars used are shown in Table 1. For the EDS, the previously irrelevant parameter (i.e. media) was now relevant. It has been shown that rats find the difficulty of each discrimination change (i.e. medium to odour or odour to medium) equivalent (McLean et al., 2008). Therefore, in the SD, digging medium was the relevant parameter for all rats.

Experimental design

Forty-eight hours after the six-week wheel-running regimen, a NOR test with a six-hour ITI was conducted, and a second NOR test with a six-hour ITI was conducted 10 weeks post exercise. Following the first of these NOR tests (i.e. 48 hours post exercise), rats underwent seven days of treatment with either PCP or saline. Three NOR tests with a one-minute ITI were conducted after the seven-day washout period: one immediately after the washout period, another at week 4 (i.e. two weeks after the washout period) and a final one at week 11 (i.e. nine weeks after the washout period). The training and testing for ASST was carried out from week 8 to week 12. The experimental design is shown in Figure 4.

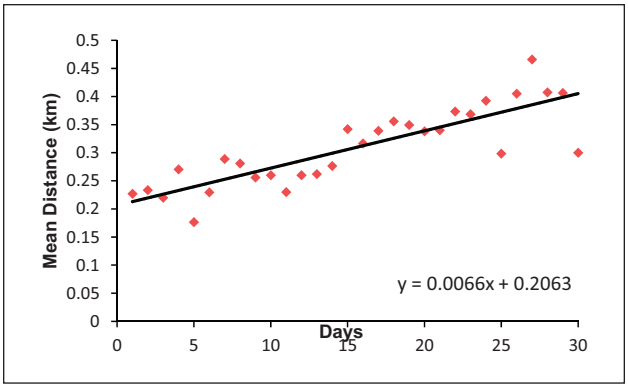

Figure 5. Mean distance (km) run by rats (n=20) during the voluntary wheel-running regimen (one hour per day, five days per week, for six weeks). The scatter plot, the corresponding regression line and the regression equation are shown for the relationship between dependent variable mean distance and independent variable time (days). There was a significant increase in the distance covered over time (linear regression analysis; R^2=0.707; $F(1, 28)$=67.56; $p<0.001$).

Statistical analysis

All statistical analyses were performed using GraphPad Prism v7.04 (GraphPad Software, Inc., La Jolla, CA). Shapiro–Wilk's normality test was used to confirm that all data sets were normally distributed. Running distances throughout the wheel-running regimen were analysed by linear regression. The relationship between mean distance run by each rat and the corresponding rat's DIs from the NOR tests 48 hours and 10 weeks post exercise was analysed using Pearson's correlation analysis. For NOR tests, differences within a group were assessed using a two-tailed Student's paired t-test, with object exploration time as the dependent variable. DIs from NOR tests post dosing were taken as the dependent variable, and were compared within each treatment group across different time points using one-way repeated measures analysis of variance (ANOVA). DIs from NOR tests with a six-hour ITI were taken as the dependent variable, and were compared within each treatment group across the two different time points using a two-tailed Student's paired t-test. For ASST, differences between groups were assessed using two-way ANOVA with post hoc least significant difference t-test (α=0.05), with treatment as independent and total trials to reach the criterion as dependent variables for each task phase. Total trials to reach criterion in two different phases of ASST for the same treatment group were compared using a two-tailed Student's paired t-test.

Results

Wheel-running activity increases over time in female LH rats

Throughout the 30-day wheel-running regimen, there was a significant increase in the running distance (linear regression analysis; R^2=0.707; $F(1, 28)$=67.56; $p<0.001$; Figure 5). The rats covered an average distance of 0.23 km in the first five days, which increased to an average distance of 0.4 km in the last five days (Figure 5).

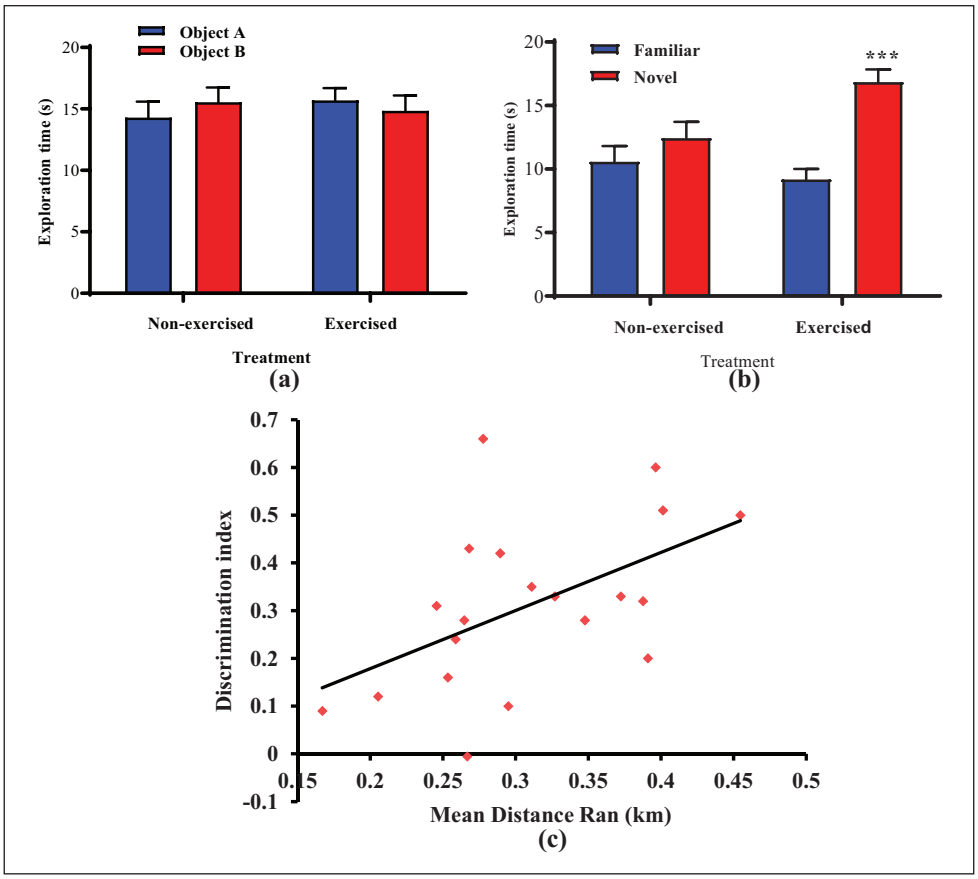

Figure 6. The effects of voluntary exercise (wheel running for one hour per day, five days per week, for six weeks) on the exploration times (s) of identical objects A and B, 48 hours post cessation of exercise in the three-minute acquisition trial (a), and the familiar and novel object in the three-minute retention phase following a six-hour ITI (b). The relationship between the mean running distance (km) and the discrimination index (DI) (c). Data are expressed as the mean±standard error of the mean (SEM; n=20 per group). Data for the exploration times were analysed by two-tailed Student's paired t-test.

***$p<0.001$. Significant difference between the time spent exploring the novel and familiar objects within the same treatment group. The association between the mean running distance (km) and DI was examined using Pearson's correlation analysis, and a positive correlation was found (r=0.514; $p<0.05$; n=20).

Exercise enhances performance in the six-hour ITI NOR test prior to scPCP treatment

Both the exercise and the control (no exercise) group spent similar times exploring the two objects in the acquisition phase (Figure 6(a)). There were no significant differences in the time spent exploring object A compared to the time spent exploring object B in either group. In the retention phase, which was carried out following a six-hour ITI, the control group displayed a delay-induced deficit. Thus, the time spent exploring the familiar object and the novel object was similar (Figure 6(b)). In contrast, the exercised rats spent significantly more time exploring the novel object ($t(19)$=8.27; $p<0.001$). Hence, the exercise group did not exhibit any delay-induced deficits.

Significant correlation between mean distance run and DIs from NOR test 48 hours post exercise

There was a positive correlation ($p<0.05$) between the mean distance run (km) throughout the 30-days of wheel running and the DI scores obtained from the NOR test with a six-hour ITI at 48 hours post exercise (r=0.514; $p<0.05$; Figure 6(c)). This indicates that the rats which ran furthest during the wheel-running regimen were able to discriminate the novel from the familiar object best (i.e. they had the best recall of the familiar object).

Exercise still enhances vehicle-treated rats' performance in the six-hour ITI NOR test 10 weeks post exercise

In the NOR test with a six-hour ITI 10 weeks post exercise, again, both the vehicle control (no exercise) and vehicle exercise group spent a similar amount of time exploring the objects in the acquisition phase (Figure 7(a)). There were no significant differences in the time spent exploring object A compared to the time spent exploring object B in either group. In the retention phase, the vehicle control group showed a delayed-induced deficit, whereas the vehicle exercise group spent significantly more time exploring the novel object ($p<0.01$; Figure 7(b)). In order to examine changes in the extent of the exercise-induced pro-cognitive effects on the NOR test performance over time, DIs of the NOR test 48 hours post exercise and 10 weeks post exercise were

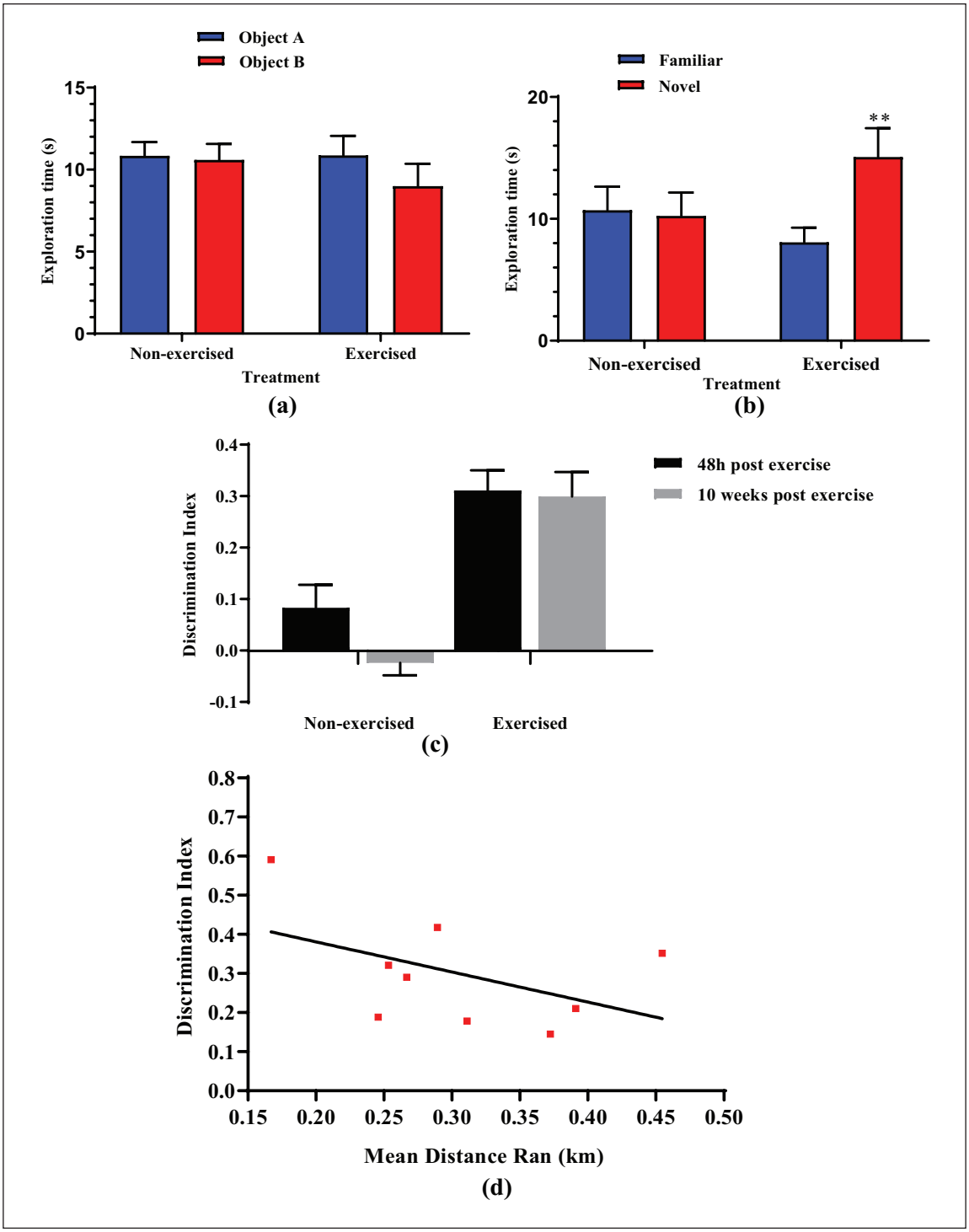

Figure 7. The effects of voluntary exercise (wheel running for one hour per day, five days per week, for six weeks) on the exploration times (s) of identical objects A and B, 10 weeks post cessation of exercise in the three-minute acquisition trial (a), and the familiar and novel object in the three-minute retention phase following a six-hour ITI (b). DIs from the two NOR tests with a six-hour ITI were compared within the same treatment groups (c). The relationship between the mean running distance (km) and the discrimination index (DI) was again examined (d). The data are expressed as the mean±SEM (n=9 for the exercise group, n=10 for the non-exercise group). Data for the exploration times were analysed by two-tailed Student's paired t-test.

**$p<0.01$. Significant difference between the time spent exploring the novel and familiar objects within the same treatment group. The association between the mean running distance (km) and DI was examined using Pearson's correlation analysis, and no correlation was found (r=–0.48; n=9). The two DI values for each rat from different time points (i.e. different NOR tests) were compared using two-tailed Student's paired t-test, and no significant differences were found.

compared within the same treatment groups. Both DI values were significantly increased following exercise ($p<0.001$). However, there were no significant changes in the DI scores between 48 hours and 10 weeks post exercise (Figure 7(c)).

No significant correlation between mean distance run and DIs from NOR test 10 weeks post exercise

There was no correlation between the mean distance run (km) throughout the 30 days of wheel running and DI scores obtained from the NOR test using a six-hour ITI 10 weeks post exercise ($r=-0.48$; Figure 7(d)).

Exercise protects against scPCP-induced NOR test impairments – an effect that persists over nine weeks post washout

In the acquisition phase of all three NOR tests with a one-minute ITI post-scPCP washout, all four treatment groups spent similar times exploring objects A and B (Figure 8(a) immediately post washout, Figure 8(c) two weeks post washout and Figure 8(e) nine weeks post washout). In the retention phase, the scPCP-treated control group displayed a deficit, that is, these rats did not spend more time exploring the novel object in any of these tests (Figure 8(b) immediately post washout, Figure 8(d) two weeks post washout and Figure 8(f) nine weeks post washout). In contrast, vehicle control, vehicle exercise and the scPCP-treated exercise group spent significantly more time exploring the novel object in the NOR test immediately post washout ($t(9)=4.91$, $p<0.001$; $t(9)=2.8$, $p<0.05$; $t(9)=4.48$, $p<0.01$, respectively), two weeks post washout ($t(9)=2.95$, $p<0.05$; $t(9)=2.51$, $p<0.05$; $t(9)=4.07$, $p<0.01$, respectively) and nine weeks post washout ($t(9)=4.78$, $p<0.01$; $t(9)=4.07$, $p<0.01$; $t(9)=2.65$, $p<0.05$, respectively). In order to examine the changes in the extent of exercise-induced protective effects on the NOR test performance, DIs of NOR tests from different time points were compared within the same treatment groups. There were no significant changes in DIs for any of the groups (Figure 8(g)).

Line crossing and total exploratory activity of different treatment groups

In the NOR test with six-hour ITI 48 hours post exercise, there was no significant difference in line crossings between exercised and non-exercised rats. Thus, the two groups displayed similar activity levels (Table 2). Similarly, the exercise and control groups showed similar total object exploration times. For all three NOR tests with a one-minute ITI, there were no significant differences in line crossings (see Table 2) or total object exploration times between any of the treatment groups. The object exploration times can be seen in the NOR figures.

Exercise protects against scPCP-induced ASST deficits

A paired Student's *t*-test demonstrated that both vehicle control groups (exercised and non-exercised) required significantly more trials to reach the criterion in the EDS than the in IDS stage of the task ($t_{(9)}=4.8$, $p<0.001$), indicating that they had formed an attentional set towards the relevant dimension before the EDS discrimination (Figure 9). A two-way ANOVA (treatment: vehicle or scPCP, exercised and non-exercised×set-shifting phase (EDS)) showed a significant effect of treatment ($F(3, 39)=11.3$; $p<0.001$). There was no effect of treatment in other phases of the task (SD, CD, Rev1, IDS, Rev2 or Rev3). Post hoc analysis within the EDS phase revealed that scPCP treatment in the control (non-exercised) group significantly increased the number of trials to reach criterion when compared to the vehicle control group ($p<0.001$; from 12.50 ± 1.53 to 18.90 ± 0.78). This deficit was significantly reduced by the exercise regimen ($p<0.05$; 15.60 ± 0.67). However, the scPCP exercise group still had an increase in trials to criterion in the EDS phase that was significantly greater than the vehicle control group ($p<0.05$; see Figure 9). Thus, exercise protection of the scPCP-induced deficit was not complete in this task, unlike in the NOR test.

Discussion

The aims of the present study were to investigate the pro-cognitive effects of voluntary exercise in naïve female LH rats using the NOR test, and to investigate possible preventative effects of exercise in scPCP-treated rats in the NOR and ASST. Our findings show that exercise, in the form of voluntary wheel running for six weeks, enhanced the performance of naïve rats in the NOR test with a six-hour ITI, which lasted for at least 10 weeks. A positive correlation was observed between the average distance run and the DI scores (a measure of novel object preference) in the first NOR test (i.e. 48 hours post exercise) but not at 10 weeks post exercise. Exercise protected against scPCP-induced deficits in the NOR test with a one-minute ITI, and this protection was sustained for at least nine weeks. Lastly, the scPCP exercise group exhibited significantly improved performance in the ASST compared to the scPCP control group. We have previously shown that exercise reverses the scPCP-induced deficit in NOR (Gonzalez et al., 2017), and this is now the first study to show that exercise can *prevent* that deficit from occurring, and to have explored the longitudinal nature of this effect.

Our aerobic exercise regimen consisted of voluntary wheel running for one hour per day, five days per week, for six weeks. The rats increased their running distance over time, which is consistent with the findings of Greenwood et al. (2011) who observed an increase in the running activity of male Fischer rats when they had access to the wheels every other night for six weeks. However, in studies where rats had unrestricted access to running wheels, running activity stabilized after three to four weeks at around 5.3 km for female Long-Evans rats (Smith et al., 2008) and around 7.7 km for male Sprague Dawley rats (Burghardt et al., 2006). This difference could be explained by rats reaching their maximal running capacity in a shorter period of time due to having free access to the wheels, as well as sex and strain potentially affecting the running activity of the rats.

We focused on scPCP-induced deficits in the NOR test and in the ASST. The NOR test is a measure of visual recognition memory, dependent on the hippocampus and perirhinal cortex (Antunes and Biala, 2012). Patients with schizophrenia also exhibit deficits in tasks that measure visual recognition memory such as the recurring figures test (McGuire et al., 2013). In

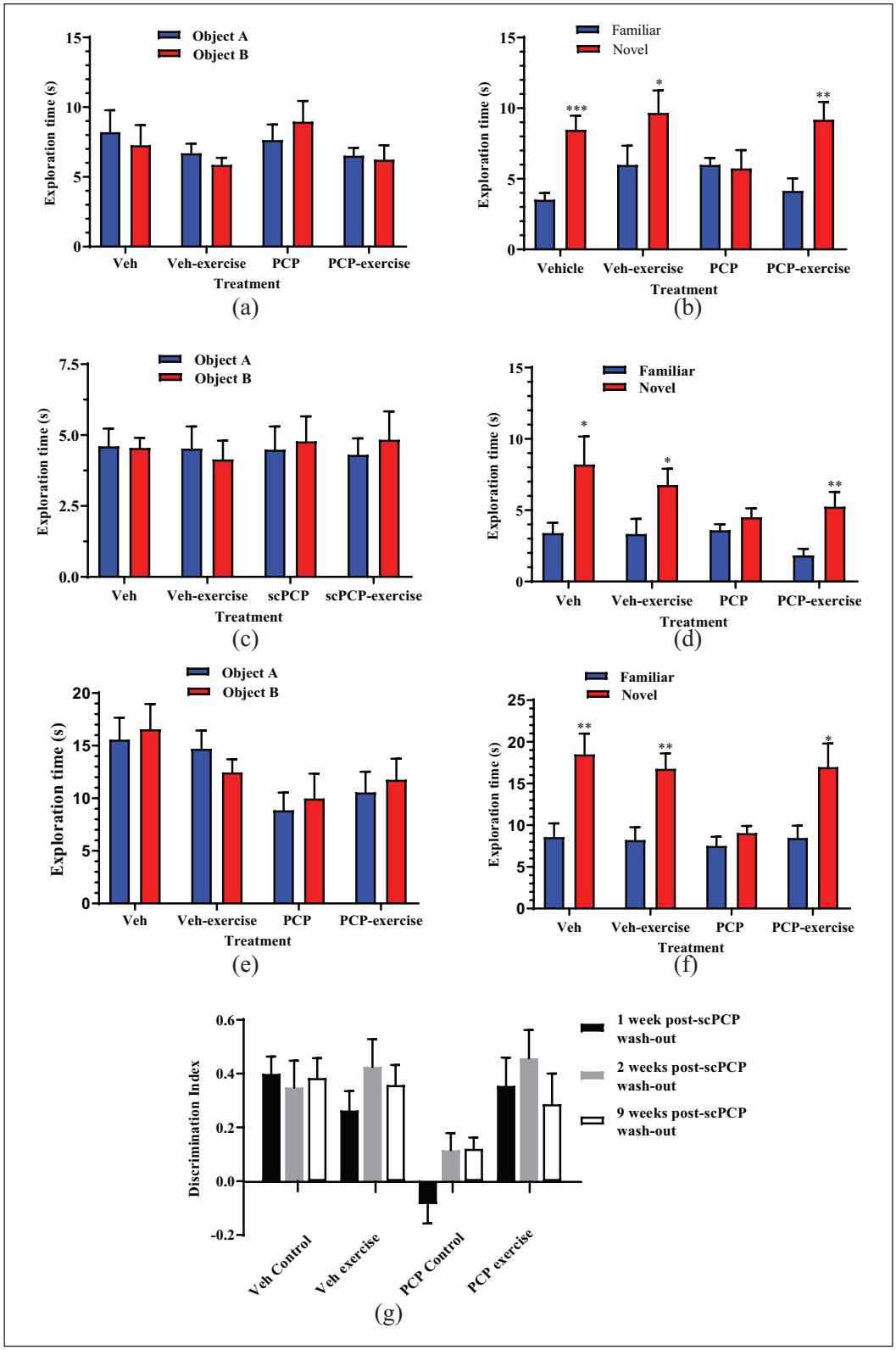

Figure 8. The effects of voluntary exercise (wheel running for one hour per day, five days per week, for six weeks) on the exploration times (s) of identical objects A and B, at one, two and nine weeks post-scPCP washout in the three-minute acquisition trial ((a), (c), and (e), respectively), and the familiar and novel object in the three-minute retention phase following a one-minute ITI ((b), (d), and (f), respectively). The DIs for all three NOR experiments are also shown (g). Data are expressed as the mean±SEM (n=10 per group, except n=9 for PCP control group in the NOR test nine weeks post-scPCP washout). Veh: vehicle. Data for the exploration times was analysed by two-tailed Student's paired t-test.
*$p<0.05$; **$p<0.01$; ***$p<0.001$. Significant difference between the time spent exploring the novel and familiar objects within the same treatment group. The DIs were analysed by one-way repeated measures analysis of variance (ANOVA), and there were no significant differences found.

addition, the NOR test in rodents excludes the confounding effects of stress due to food restriction and is ethologically relevant, as rats have a natural preference for novelty (Engelmann et al., 2006; Ennaceur and Delacour, 1988). In rodents, NOR deficits are found in a range of disorders (for a rewview, see Grayson et al., 2015), including in an animal model for diabetes recently by us (Kassab et al., 2019). We routinely find NOR deficits in the scPCP model (for our most recent review, see Cadinu et al., 2018). In the ASST, rats initially learn a rule and form an attentional set. Then, they are required to switch the attentional set towards a new dimension, which is a display of cognitive flexibility, processed in the PFC (Tait et al., 2018) A human-equivalent version of the ASST is the Wisconsin card sorting test, where schizophrenia patients perform poorly compared to controls (Wobrock et al., 2009). Patients have difficulties in shifting their attentional set (i.e. unlearning the old rule and learning a new rule; Prentice et al., 2008), which is also the deficit reliably shown by scPCP-treated female LH rats (McLean et al., 2012). Hence, the cognitive performance of scPCP-treated rats in both the NOR test and in the ASST is relevant to cognitive impairments in schizophrenia.

Table 2. Mean total line crossings±standard error of the mean for NOR test pre dosing and zero, two and nine weeks post PCP washout are illustrated from top to bottom.

Treatment groups	Total line crossings (acquisition+retention phase)
Control	114±3
Exercise	108±3
Vehicle control	74±5
Vehicle exercise	74±4
PCP control	77±4
PCP exercise	75±4
Vehicle control	69±5
Vehicle exercise	64±2
PCP control	65±4
PCP exercise	66±5
Vehicle control	90±6
Vehicle exercise	94±5
PCP control	90±4
PCP exercise	104±4

The NOR test pre dosing shows the total line crossings (acquisition+retention) for exercised and non-exercised rat groups (n=20 per group). The NOR tests post dosing show the total line crossings for all four treatment groups (n=10 per group). Data for the NOR test pre dosing were analysed by two-tailed Student's unpaired t–test, and data for NOR tests post dosing were analysed using one-way analysis of variance. No significant differences were found.
NOR: novel object recognition; PCP: phencyclidine.

Regarding the pro-cognitive effects of exercise, in the NOR test 48 hours post exercise, non-exercised rats displayed a delay-induced deficit, as they failed to discriminate the novel from familiar object during the retention phase, shown as no significant difference in exploration times of the novel object and the familiar object. This is consistent with studies employing a natural-forgetting NOR test model (McLean et al., 2010), which is a validated method for assessing pro-cognitive effects in a non-disease model (McLean et al., 2016). In contrast, exercised rats spent significantly more time exploring the novel object compared to the familiar one. Hence, voluntary wheel running enhanced the ability of drug-naïve rats to retain information about the object experienced in the initial trial six hours previously. Hopkins and Bucci (2010) showed that voluntary exercise improves NOR test performance in rats, and that the expression of a well-documented exercise-influenced protein, brain-derived neurotrophic factor (BDNF) within the perirhinal cortex, is positively correlated with NOR test performance. In our study, higher mean distances run throughout the six-week exercise regimen were positively linked to better task performance (i.e. higher DI

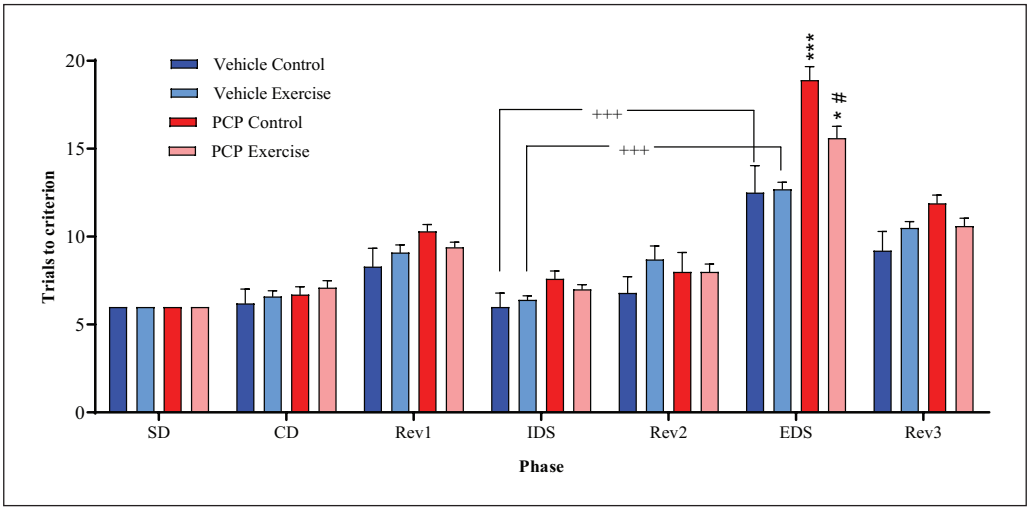

Figure 9. The effect of voluntary exercise (wheel running for one hour per day, five days per week for six weeks) on total trials to reach criterion in the ASST. All data are expressed as the mean±SEM (n=10 per group) and were analysed by a two-way ANOVA and post hoc least significant difference t-test. *$p<0.05$ and ***$p<0.001$ compared to vehicle control group in the extra-dimensional shift (EDS) phase; #$p<0.05$ compared to the scPCP control group in the EDS phase. Total trials to reach criterion in EDS and intra-dimensional shift (IDS) phases for the vehicle control group were compared using a two-tailed Student's paired t-test. +++$p<0.001$ compared to stage of task.
CD: compound discrimination; Rev1: reversal 1; Rev2: reversal 2; Rev3, reversal 3; SD: simple discrimination.

scores). Therefore, it is likely that a greater amount of exercise induces a greater increase in BDNF expression, which in turn leads to greater task performance – a hypothesis we aim to test. In the second NOR test with a six-hour ITI (i.e. 10 weeks post exercise), the results were similar to those of the NOR test 48 hours post exercise. The vehicle control group spent a similar amount of time exploring the novel object and the familiar object, whereas the vehicle exercise group spent significantly more time exploring the novel object compared to the familiar one. This is rather extraordinary and shows that the beneficial effects of exercise persist for 10 weeks after the exercise regime has finished. Examination of the DI values shows that there was no significant difference between the task performances of either of the treatment groups in the NOR test 48 hours post exercise and the NOR test 10 weeks post exercise. Hence, the degree of overall enhancement induced by exercise did not change. Furthermore, there was no relationship between the amount of mean distance run throughout the exercise regimen and the DI values of NOR test 10 weeks post exercise. This could indicate that the short-term effects of exercise depend on the amount of exercise performed, whereas the long-term effects are regulated through a mechanism that is independent of the level of exercise. A study by Berchtold et al. (2005) showed that BDNF levels of male Sprague Dawley rats are significantly increased above baseline (obtained from sedentary rats) following free access to running wheels for 28 days. However, BDNF levels gradually declined back to baseline 14 days after the cessation of exercise activity.

Regarding the protective effects of voluntary exercise, in the NOR test with one-minute ITI at week 2 (i.e. immediately after the end of the seven-day washout period), the scPCP control group had the expected deficit in the retention phase, as rats failed to discriminate between novel and familiar object (Grayson et al., 2007). In contrast, the scPCP exercise group, like the vehicle groups, successfully discriminated between the novel object and the familiar object. The same outcome was observed in NOR tests at 4 and 11 weeks post exercise. The scPCP control group could not discriminate between the novel object and the familiar object, whereas the vehicle control, vehicle exercise and scPCP exercise groups spent significantly more time exploring the novel object compared to the familiar object in the retention phase. Upon examination of the DI scores, there was no significant change in the task performances of any of the treatment groups throughout the course of testing. Therefore, voluntary exercise protected against the scPCP-induced deficits in the NOR test. This protection did not show a significant change over time and lasted for at least nine weeks. It will be important to determine how long this effect does persist. Again, it seems extraordinary that it lasted so long.

In the ASST, the scPCP control group displayed the expected deficit in the ability to switch from one attentional set to another, shown as significantly more trials were needed to reach criterion in the EDS stage compared to the vehicle control group (McLean et al., 2012). In the same stage of the ASST, the scPCP exercise group required significantly fewer trials to reach criterion compared to the scPCP control group, but required significantly more trials compared to the vehicle control. Therefore, exercise significantly reduced the scPCP-induced deficit in the ASST, but did not fully prevent a deficit from occurring. Lack of full protection against the scPCP-induced deficit in this task is perhaps not unexpected, as this task is lengthy and considerably more complex than the NOR test and may therefore require a more intensive exercise regimen for full improvement, or reduced time between exercise and testing. Our future analysis of exercise effects in hippocampus and PFC will be of considerable importance in understanding different effects in both of these tasks. In all stages of the ASST, the vehicle exercise group exhibited a similar performance to that of the vehicle control group. Both the vehicle control and vehicle exercise groups formed an attentional set, shown as a significant difference in the number of trials needed to reach criterion between the ID and ED stage, whereas the scPCP exercise and scPCP control groups did not. The formation of an attentional set before shifting this attentional set is important and shows that the rats can maintain their attention (Tait et al., 2018). Brockett et al. (2015) demonstrated that exercised male Sprague Dawley rats exhibited a better task performance in ASST compared to sedentary rats. However, sedentary rats formed an attentional set, and exercised rats did not. This might be reflective of exercised and scPCP-treated rats completing the task successfully by adopting a different strategy.

Other studies showed that the effects of exercise diminish two weeks following the cessation of exercise (Bocalini et al., 2010; Greenwood et al., 2012; Hopkins and Bucci, 2010). However, we found exercise-induced effects lasted for at least 10 weeks post exercise. Hopkins et al. (2011) showed that exercise-induced effects diminish after two weeks in adult male Long Evans rats, but last up to four weeks in rats that were adolescents during the time period that the exercise regimen was carried out. The rat strain, age and sex used by Hopkins et al. (2011) were different from our study, and the duration of their exercise regimen was four weeks, whereas we employed an exercise regimen that lasted for six weeks in adult female LH rats. In a previous study, we employed the same six-week exercise regimen *after* administering scPCP or vehicle to adult female LH rats, and tested their performance in NOR tests (Gonzalez et al., 2017). We observed a reversal of the scPCP-induced deficit that lasted for two weeks. In the NOR test four weeks post exercise, the scPCP exercise group did not spend significantly more time exploring the novel compared to the familiar object. However, the sample size was small (n=5), and we are repeating this study with larger numbers. These key methodological differences certainly influence the duration of exercise-induced effects, and further investigation is required to assess *how* the duration of exercise in different strains, age and sex of rats produce their effects.

There was no difference in the number of line crossings or total exploration times between any of the treatment groups in any of our NOR tests (data not shown). This indicates that neither scPCP application nor voluntary wheel running influences LMA or exploratory activity of rats, which is in agreement with other studies that found no exercise-induced changes in LMA (Hopkins et al., 2011; Rajizadeh et al., 2018) or exploratory activity (Hopkins et al., 2011), or scPCP-induced changes in LMA (Grayson et al., 2014) or exploratory activity (Neill et al., 2016). Therefore, the differences observed in NOR performance are unlikely to be due to any changes in exploratory activity. There was a noticeable reduction in the exploration times of the acquisition phase but not the retention phase for all treatment groups in the NOR test at week 4 compared to the NOR test immediately after the washout period. This decrease was no longer present in the NOR test at week 11, and there were no changes in the exploration times between the two NOR tests with six-hour ITI.

Taken together, our data in the NOR and ASST tests suggest that voluntary aerobic exercise has pro-cognitive effects in a task known to be mediated via activation of the hippocampal formation, and protective effects in tests known to be mediated via activation of both the hippocampal formation and the PFC. Therefore, it is possible that exercise has global beneficial effects on brain function, although no analysis of the effects of exercise on this has been conducted by us yet. Our future studies will focus on protein analyses to unravel the mechanism behind these exciting and very promising effects of exercise. As mentioned, one protein of particular importance is BDNF, which is a growth factor with various roles, including stabilization of neuronal survival and enhancement of synaptic plasticity and memory (Woo and Lu, 2006; Yoshii and Paton, 2010). BDNF triggers these actions by binding to and activating tyrosine kinase receptor B (TrkB) that is abundantly present on PV-positive IINs (Swanwick et al., 2004; Zheng et al., 2011). Activation of the BDNF-TrkB signalling pathway has been shown to trigger proliferation and differentiation, as well as supporting maturation and survival of GABAergic IINs (Waterhouse et al., 2012), whereas studies in BDNF knockout animals resulted in decreased PV and SST protein levels (Guilloux et al., 2012; Xenos et al., 2018). We have shown robust deficits in PV protein in our rat model and most recently in a mouse scPCP model (Abdul-Monim et al., 2007; for commentary, see Reynolds and Neill, 2016; Gigg et al. 2019) and mRNA for BDNF (Snigdha et al. 2011). Exercised healthy rodents have been shown to have increased expression of BDNF within both PFC and hippocampal formation (Bechara and Kelly, 2013; Marlatt et al., 2012; Uysal et al., 2015). It is therefore a reasonable hypothesis that this is the mechanism behind the effects we observed here.

Conclusion

Voluntary aerobic exercise, in the form of wheel running, enhances visual recognition memory of naïve female LH rats and protects against recognition memory and executive function deficits induced by scPCP treatment, with exercise-induced benefits lasting up to 10 weeks following the cessation of exercise. The influence of rat strain, age and sex used and the duration of exercise on these exercise-induced effects needs to be further investigated. However, it is clear that exercise has beneficial effects on potentially all domains of cognition affected in psychiatric illness which has important implications for improving the lives of patients and their carers. Furthermore, the protective effects induced by exercise shown here could provide the basis for early-intervention therapy for people at high risk of developing psychiatric illnesses. Future studies in our laboratory will focus on BDNF, PV, SST and neuroinflammatory marker analyses in the hippocampal formation and PFC in order to determine the mechanism(s) by which exercise produces its beneficial effects.

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