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Short communication

# Effect of low-intensity treadmill exercise on behavioural measures and hippocampal parvalbumin immunoreactivity in the rat

Jason C.D. Nguyen[a], A. Simon Killcross[b], Trisha A. Jenkins[a,*]

[a] School of Medical Sciences, Health Innovations Research Institute, RMIT University, Bundoora, Victoria 3083, Australia
[b] School of Psychology, University of New South Wales, Sydney, New South Wales 2052, Australia

## HIGHLIGHTS

- We look at treadmill running on central effects in rats.
- Exercise caused a significant increase in sociality in the rat, the first time this has ever been reported.
- In addition, exercise increased the number of parvalbumin-immunoreactive neurons in the hippocampus, demonstrating the important effect of exercise on brain plasticity.

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## ABSTRACT

Exercise has been demonstrated to have positive effects on both the body and brain. The present study aimed to determine the behavioural and morphological consequence of low-intensity running. Rats were exercised on a treadmill for a total of 30 days, 30 min/day. Social interaction, locomotor activity and behaviour on an elevated plus maze were assessed post-treatment. Exercised animals demonstrated more passive interaction and less time not interacting than control animals that were not exercised. Conversely, locomotor and anxiety measures showed no effect of exercise. Analysis of brains demonstrated an increase in expression of parvalbumin immunoreactive neurons in the hippocampus localised to the CA1 and CA2/3 regions. These results demonstrate that low-intensity exercise leads to changes in social behaviour as well as neuroplastic morphological changes within the hippocampus.



While it is well recognised that exercise has numerous beneficial effects on the body, recent research suggests that these positive effects also extend to the brain. Clinical studies indicate improvements in cognition in cognitively-normal [1] and impaired adults [2], with increases in brain volume, cerebral blood flow and functional brain plasticity observed. Much of these findings have been replicated in laboratory animals, demonstrating an important role of exercise in inducing positive brain changes in the rodent [3].

γ-Aminobutyric acid (GABA)-ergic inhibitory neurons containing the calcium binding protein, parvalbumin, are suggested to be involved in various higher brain functions including emotion, anxiety, and learning and memory [4]. These neurons synapse on the cell body or axon initial segment of glutamatergic neurons and can regulate pyramidal cell output. Indeed a strong correlation has been demonstrated between the presence of parvalbumin and the fast firing properties of hippocampal neurons [5]. Parvalbumin containing GABAergic interneurons are effective markers of hippocampal cells [6] and are demonstrated to increase in number in the hilus of dentate gyrus in rats submitted to acute physical exercise [7].

The effect of exercise on so-called mood measures is less well understood. We know that exercise increases β-endorphin levels [8] and neurotransmitter release, including serotonin and dopamine [9,10]. Behaviourally, regular moderate physical activity induces improvements in quality of life measures in normal adults and positively influences depressive symptoms in patients [11]. Positive social functioning is an important component of quality of life measures, and is often impaired in psychiatric disorders [12]. To date the social interaction test [13] is widely used by researchers to investigate animal social behaviour in response to novel interventions and treatments. The positive effects of exercise in this task are yet to be identified.

In the present study we evaluated the effect of exercise on social interaction in the rat, and assessed its effect on anxiety and locomotor measures. In addition, we determined if our exercise regime

* Corresponding author. Tel.: +61 3 99256523; fax: +61 3 99257063.
*E-mail address:* trisha.jenkins@rmit.edu.au (T.A. Jenkins).

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successfully induced morphological changes in the hippocampus, as per previous published reports [7,14].

Male Long-Evans rats ($n = 19$, Monash University, Australia), weighing 221–271 g were used for experimental behavioural testing and subsequent brain analysis. A further 10 Long-Evans rats (Monash University, Australia), weighing 239–293 g at the beginning of the study, were used as target animals for the social interaction task. Rats were housed 4–5 to a box under a 12 h light/12 h dark (lights on 6 am) photoperiod cycle with food and water *ad libutum* in the home cage (57 long × 38 cm wide × 20 cm high). Room temperature (21 ± 1 °C) and humidity (30–70%) were kept constant. The experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986 and with approval from the RMIT University Animal Ethics committee.

Exercise rats (EX, $n = 10$) were habituated to a treadmill for a total of 30 min on 3 subsequent days. Exercise rats ran for a total of 30 days at 5 m/min 5% incline for the first 5 days and then progressively trained to run 10 m/min 5% incline bi-daily (at 0900 h and 1530 h) for a cumulative total of 30 min for 5 weeks (protocol amended from [15]). The treadmill was a purpose built 8 lane exercise treadmill with dividing walls suspended over the tread surface and was cleaned with 70% alcohol after each rat's exercise session. EX rats were run in teams with their cage mates. Control rats (CON, $n = 9$) were handled daily for approximately 5 min each. At the end of this training period behavioural analysis commenced. All testing was performed during daylight hours (between 9 am and 3 pm) under 300 lx lighting in a randomised cohort order in the below test order with 24 h between tests.

In the social interaction test an EX or CON rat was placed in the test arena (black wooden box, 60 cm long × 60 cm wide × 50 cm high, rats placed nose to opposing corners) with a previously unknown 'target' rat for 10 min to observe social interaction. All sessions were videotaped for analysis of active social interaction (sniffing, grooming, following, or crawling over/under within 5 cm of the target rat), passive social interaction (defined as the experimental rat being within 2 cm of the target rat but not actively interacting) or no interaction, was carried out for the 10 min interaction period [13,16]. Experimenters were blind to treatment condition.

The elevated plus maze, elevated 70 cm above the ground, consisted of two open arms (70 × 10 cm) and two enclosed arms (70 × 10 cm) with a 25 cm high surrounding clear Perspex wall, with the arms extending from a central platform (13 × 13 cm). Rats were placed individually on the maze in the centre platform, nose facing the closed arm. Behaviour was measured for 5 min and videotaped for subsequent analysis of arm entries and time in arms. Experimenters were blind to treatment condition.

Locomotor activity was performed using a Med Associates (USA) open field test chamber (44.5 cm × 44.5 cm × 30.5 cm) as previously described [17]. Rats were placed individually in the test chamber for 10 min with analysis of total distance travelled and average velocity performed by Med Associates (USA) activity monitor software, version 4.

Rats received a lethal dose of sodium pentobarbital (1 ml/kg body weight). Brains were removed with the left hemisphere fixed in 4% paraformaldehyde in PBS and the right hemisphere frozen in isopentane (Sigma–Aldrich) cooled to −35 °C by dry ice. Serial coronal sections (30 μm) from the left hippocampus (bregma −3.3 mm) were processed for parvalbumin immunohistochemistry as previously described [16]. In brief sections were incubated in 0.6% hydrogen peroxide solution followed by 5% normal horse serum with 0.1% Triton X-100. Following a 36 h incubation with parvalbumin antibody (Swant, Switzerland; 1:10,000) and 2 h incubation in biotinylated anti-mouse IgG, sections were processed by the avidin–biotin method using a Vectastain ABC kit (Vector Laboratories, UK) and visualised using 3′,3′-diaminobenzidine (DAB) intensified with nickel chloride. Sections were mounted and allowed to dry overnight before being dehydrated and coverslipped.

For image analysis sections from each rat were captured at 40× magnification using a Nikon Eclipse 90i microscope interfaced with NIS-Elements Advanced Research 3.21.000 (Build 689) via a Nikon D-Eclipse C1 camera. Manual counting of parvalbumin-immunoreactive neurons using Image J was carried out for dentate gyrus (DG), CA1 and CA2/CA3 subregions, with the entire extent of the target region within the selected coronal sections assessed. Counts were taken from at least four alternate sections from each hemisphere, and these counts then averaged to produce a mean.

All data are presented as mean ± standard error of the mean (SEM). Social interaction, elevated plus maze data and parvalbumin levels are analysed by multivariate ANOVA (SPSS, version 19, IBM, USA) and where appropriate with simple effects. Locomotor activity was assessed by *t*-test (SPSS, version 19, IBM, USA).

Low intensity treadmill exercise had a significant effect on sociability behaviour. While there was no overall observed effect of group ($F_{(1,17)} = 2.6$, $p = 0.13$), a significant difference in the type of behaviour ($F_{(1,17)} = 60.9$, $p < 0.0001$) and group × behaviour interaction ($F_{(1,17)} = 7.4$, $p < 0.05$) was observed. Posthoc analysis showed that EX animals did not differ in their active behaviour scores ($F < 1$), but were more likely to passively interact with the target rat, that is to be in close proximity to it ($F_{(1,51)} = 4.8$, $p < 0.05$) without moving away and avoiding contact with it ($F_{(1,17)} = 13.5$, $p = 0.005$) than the CON animals who were not exercised (Fig. 1).

Exercise did not affect anxiety levels with EX and CON animals performing similarly on the elevated plus maze. Considering arm entries (open: EX 4.2 ± 0.4, CON 3.2 ± 0.4; closed: 6.8 ± 0.4, CON 6.3 ± 0.5) there was no group ($F_{(1,17)} = 2.2$, $p = 1.6$), nor group × arm interaction ($F < 1$). Both groups of rats had significantly more entries into the closed arms than the open ($F_{(1,17)} = 45.8$, $p < 0.001$) and this was not different between the EX and CON rats ($F < 1$). In addition, for time (s) spent in the arms (open: EX 82.4 ± 11.3, CON 71.0 ± 9.8; closed: 217.6 ± 11.3, CON 229.0 ± 9.8), there was no group ($F < 1$), nor group × arm interaction ($F < 1$). Both groups of rats spent significantly more time in the closed arms than the open ($F_{(1,17)} = 90.2$, $p < 0.001$) and again this was not different between the EX and CON rats ($F < 1$) (Fig. 2).

All rats exhibited equivalent basal locomotor function irrespective of being exposed to low intensity exercise (distance travelled: EX 1527 ± 132, CON 1598 ± 127; $p = 0.71$; average speed: EX 47.6 ± 2.2, CON 49.9 ± 8.6; $p = 0.52$).

Exercise significantly increased the number of parvalbumin-immunoreactive neurons in the hippocampus. Significant changes were observed between EX and CON ($F_{(1,16)} = 14.0$, $p < 0.01$); hippocampal region ($F_{(1,16)} = 197.0$, $p < 0.001$), and their interaction ($F_{(1,16)} = 9.0$, $p < 0.01$). Posthoc analysis showed that EX animals had significantly raised parvalbumin immunoreactivity in CA1

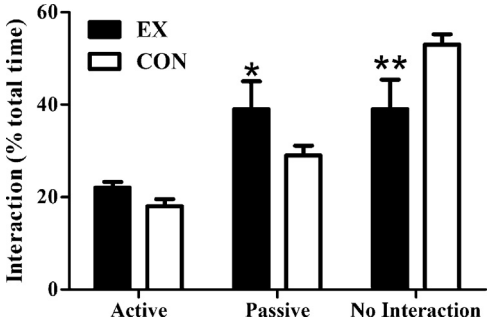


**Fig. 1.** Social interaction after low impact treadmill exercise of exercised (EX) and control (CON) rats. Data are expressed as mean interaction scores ± SEM, $n = 9$–10/group. *$p < 0.05$; **$p = 0.01$.