Behavioural Brain Research 299 (2016) 27–31

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

# Ambient temperature influences the neural benefits of exercise

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## HIGHLIGHTS

- Ambient temperature influences voluntary exercise time, speed and distance.
- A limited exercise distance in heat or cold increases hippocampal cell division.
- A limited exercise distance in heat or cold increases neuronal differentiation.

## ARTICLE INFO

*Article history:*
Received 7 May 2015
Received in revised form 6 November 2015
Accepted 16 November 2015
Available online 30 November 2015

*Keywords:*
Temperature
Exercise
Neurogenesis
Hippocampus
Brain injury

## ABSTRACT

Many of the neural benefits of exercise require weeks to manifest. It would be useful to accelerate onset of exercise-driven plastic changes, such as increased hippocampal neurogenesis. Exercise represents a significant challenge to the brain because it produces heat, but brain temperature does not rise during exercise in the cold. This study tested the hypothesis that exercise in cold ambient temperature would stimulate hippocampal neurogenesis more than exercise in room or hot conditions. Adult female rats had exercise access 2 h per day for 5 days at either room (20 °C), cold (4.5 °C) or hot (37.5 °C) temperature. To label dividing hippocampal precursor cells, animals received daily injections of BrdU. Brains were immunohistochemically processed for dividing cells (Ki67+), surviving cells (BrdU+) and new neurons (doublecortin, DCX) in the hippocampal dentate gyrus. Animals exercising at room temperature ran significantly farther than animals exercising in cold or hot conditions (room 1490 ± 400 m; cold 440 ± 102 m; hot 291 ± 56 m). We therefore analyzed the number of Ki67+, BrdU+ and DCX+ cells normalized for shortest distance run. Contrary to our hypothesis, exercise in either cold or hot conditions generated significantly more Ki67+, BrdU+ and DCX+ cells compared to exercise at room temperature. Thus, a limited amount of running in either cold or hot ambient conditions generates more new cells than a much greater distance run at room temperature. Taken together, our results suggest a simple means by which to augment exercise effects, yet minimize exercise time.



Exercise provides the brain with many benefits, including enhanced hippocampal neurogenesis [1], which has been linked to improved cognition [1,2]. However, these neural benefits take time to emerge. A recent study showed that 2 weeks of exercise was necessary in order to increase neurogenesis and 8 weeks was necessary to increase synaptic efficacy [3]. It would be useful to discover ways to accelerate the neural benefits of exercise.

Exercise represents a significant challenge to the brain because it produces heat [4]. Brain functions occur in a relatively narrow temperature range, which is maintained by balancing heat production and heat loss [4,5]. Regulatory mechanisms protect the brain from being damaged by increased core temperature (for review see Refs. [6,7]), but these are challenged during exercise in the heat [6,8]. In contrast, exercise in cold ambient temperature seems to enhance physical performance [9]—both humans [9] and rats [10] are able to run longer before volitional fatigue in cold ambient temperatures versus hot. Moreover, when animals exercised in thermoneutral conditions (25 °C), brain temperature rose, but when they exercised in the cold (12 °C) the increase in brain temperature was attenuated.

The present study was conducted in order to test the hypothesis that exercise in cold ambient temperature (4.5 °C) would more effectively promote neurogenesis in the hippocampal dentate gyrus

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http://dx.doi.org/10.1016/j.bbr.2015.11.017
0166-4328/

(DG) than exercise at room temperature (20 °C). Because exercising in the heat represents a considerable challenge to the brain [6,8], we included a hot ambient temperature condition (37.5 °C) in order to test the hypothesis that running in the heat would not increase neurogenesis beyond exercise at room temperature.

Forty-nine female Long-Evans rats (Harlan Sprague Dawley, IN, USA), weighing 180–260 g were randomly divided into 6 groups in a 3 × 2 design comparing Temperature (Room, Cold, or Hot) and Activity (Sedentary or Exercise). Each group had 8 animals, with the exception of Cold Exercise, which had 9. Rats were housed in groups in the vivarium (temperature 21.7 °C) on a reversed light/dark cycle (10:00 off/22:00 on) with ad libitum food and water. Vaginal smears were taken once each day between 9:00 and 9:30 A.M.

On one occasion (three days prior to the experiment) animals were acclimated to experimental temperature conditions and exercise wheels for 2 h. During the experiment, animals in exercise groups had access to wheels at room (20 °C), cold (4.5 °C) or hot (37.5 °C) ambient temperature, 2 h a day for 5 consecutive days. Sedentary rats were placed in pairs in locked exercise wheels for two hours at their respective group's temperature. Exercisers had individual access to an exercise wheel, to determine individual running distance, speed and time. After exercise, animals were returned to group-housing. To label cells generated in response to changes in temperature and/or activity, bromodeoxyuridine (BrdU, Sigma, MO, USA, 50 mg/kg, i.p), a thymidine analog, was administered daily prior to wheel access.

Fecal samples were collected after exercise on the last day of the experiment. Fecal corticosterone is highly correlated with serum corticosterone and it has been shown that an acute stressor is reflected in elevated fecal corticosterone levels the following day [11]. Thus, if 2 h of exposure to cold or hot temperature elevated corticosterone, we would expect to detect this in fecal samples collected the following day. Also, we could detect any sustained increase in corticosterone caused by repeated exposure to the different temperature conditions. Corticosterone levels were quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, New York, USA), according to the manufacturer's instructions as we have previously described [12].

Following exercise on the fifth day, rats were perfused and brain tissue processed as previously described [12]. For BrdU immunohistochemistry, the primary antibody was sheep anti-Brdu (Exalpha Biologicals, Maynard, MA, USA; 1:400), followed by a biotinylated secondary (donkey anti-sheep, Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:250). To assess cell proliferation and neuronal differentiation, separate one-in-twelve and one-in-six series of sections were processed for Ki67 and doublecortin (DCX) respectively. The primary antibodies were rabbit anti-Ki67 (Vector Laboratories, Burlingame, CA; 1:1800) and goat anti-DCX (Santa Cruz Biotechnology, Santa Cruz, CA; 1:100). The secondary antibodies were biotinylated donkey anti-rabbit and biotinylated donkey anti-goat (both from Jackson ImmunoResearch Laboratories, 1:250). Sections were mounted onto gelatin-coated slides, counterstained with methyl-green, coverslipped and coded.

BrdU+ and DCX+ cells in the DG were quantified at 40× and 100× respectively, using the optical fractionator method applied via our automated stereology system (StereoInvestigator, MicroBrightField, VT, USA) as previously described [12]. Cells labeled with Ki67 were not abundant enough in the sedentary groups to be counted stereologically. Instead, each labeled soma in the granule cell layer or subgranular zone was counted in every twelfth section from Bregma −1.88 through Bregma −6.04, using a 40× oil objective.

One-way ANOVA's were conducted to determine the effect of Temperature on exercise distance, time and speed. Unexpectedly, temperature had a profound effect on exercise distance—animals at room temperature ran much further than those in cold or hot conditions. Therefore, we used one-way ANOVA to analyze the number of Ki67+, BrdU+ and DCX+ cells normalized for shortest distance run. We used the following formula for each exercised rat: (290 m/average distance traveled) × (cell count). Because the sedentary animals did not exercise, their cell counts could not be normalized for shortest distance run, therefore, cell count data from the sedentary groups were analyzed with a separate one-way ANOVA. All other data were analyzed with two-way factorial ANOVA with Temperature and Activity as independent variables. For all statistical analyses, an alpha level of 0.05 was set to determine significance. Bonferroni and Games Howell corrected post hoc analysis were conducted where appropriate.

To determine whether the stage of estrus affected neurogenesis, a factorial ANOVA using the variables Temperature, Activity, and Day 1–5 of the experiment were analyzed. Because stage of estrus is a categorical variable, it was necessary to dummy code by assigning a numerical value for stage of estrus (Diestrus = 0, Proestrus = 1, Estrus = 2, Metestrus = 3) for each of the five days of the experiment.

There was a significant main effect of Temperature [$F(2,22) = 7.71$, $p < .005$] on distance traveled (Fig. 1). Rats at room temperature ran significantly further than rats in cold ($p < .05$) and hot ($p < .005$) conditions, with no difference between the cold and hot conditions ($p = .891$). Analysis of average speed revealed a significant main effect of Temperature [$F(2,22) = 4.832$, $p < .05$]. Post hoc analysis showed that animals running at room temperature ran faster than animals in the hot ($p < .05$) condition. There was no difference between the cold and hot conditions ($p = .582$). Time spent running was significantly affected by Temperature [$F(2,22) = 6.737$, $p < .005$]. Post hoc analysis revealed that animals running in room temperature conditions spent more

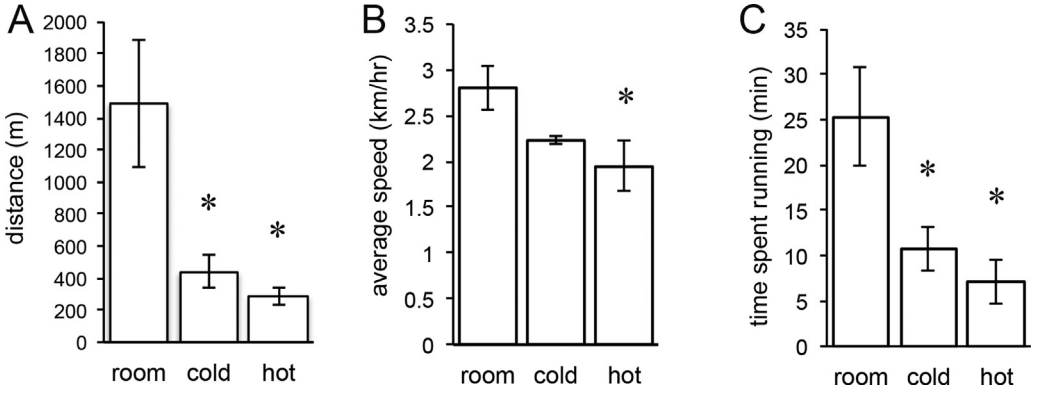


**Fig. 1.** Rats in the cold and hot ambient temperature conditions ran shorter distances (A) at slower average speeds (B) and spent less time running (C) than rats running at room temperature. *$p < 0.05$ significantly different from room temperature control.