

## Exercise influences spatial learning in the radial arm maze

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### Abstract

Previous studies indicate that the hippocampus is active during exercise, and that neurotrophin expression, receptor density, and survival of dentate gyrus granule cells in the hippocampus can be modified by moderate voluntary exercise. The present study was designed to test the consequences of voluntary exercise on a hippocampal-related behavior. Exercising and control rats were tested on the standard and delayed nonmatch-to-position (DNMTP) version of the eight-arm radial maze, both of which are sensitive to hippocampal damage. Voluntarily exercising rats ran in running wheels attached to their home cage for 7 weeks prior to and throughout testing, and took 30% fewer trials to acquire criterion performance than sedentary controls. Both groups spent the same average time per arm. Once the eight-arm maze had been learned to criterion, group differences were not apparent. Exercise can facilitate acquisition of a hippocampal-related spatial learning task, but does not affect performance following acquisition. Further work will be necessary to link these effects to hippocampal-related variables shown to be influenced by exercise. © 2000 Elsevier Science Inc. All rights reserved.

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### 1. Introduction

Accumulating evidence suggests that physical exercise can affect hippocampal neurochemistry [1–3], neuronal activity [4–7], trophic factor expression [8,9], granule cell proliferation and survival [20], and neuronal survival after ischemia [10]. These findings raise the question as to whether exercise can influence hippocampal-related behavior.

A well-known hippocampal-related behavior in rodents is spatial learning [11,12]. In the Morris water maze, exercising rats and mice relative to controls have been reported to spend a greater proportion of time in the quadrant containing the escape platform, but have equal swim speeds to controls [1,3]. Rodents in the same studies were shown to have exercise-induced changes in hippocampal variables. It is difficult to discount exercise-related differences in physical fitness and responses to stress [13] as potential confounding factors because swimming requires physical effort and is an effective stressor. For this

reason, it would be interesting to test whether or not exercise can influence spatial learning in a nonstrenuous and nonstressful task.

The present study employed the eight-arm radial maze to test for differences in acquisition and criterion level performance between an exercising group and a control group of rats. Exercising rats were given access to running wheels 7 weeks prior to and during maze testing. All rats were tested on the standard version of the radial arm maze (RAM) followed by a variant of that task, the delayed nonmatch-to-position version (DNMTP [14,15]).

### 2. Materials and methods

#### 2.1. Animals

Twenty-one Long–Evans hooded female rats obtained from Charles River were housed to 5 months of age. Three littermate sets were divided equally into two conditions: a control condition (IC,  $n = 10$ ) and a voluntary running condition (VX,  $n = 11$ ) so that both groups had the same average weight (291.35 g).

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Rats in both groups were housed individually in standard tub cages. Rats in the VX group had running wheels (34 cm diameter  $\times$  10.5 cm width) attached to their cage. Wheel rotations were recorded electronically with custom software. A LED in the wheel entryway prevented collection of rotations made by spinning the wheel with a forepaw. Rats were in their conditions for 7 weeks before the start of maze training.

Water restriction began 1 week before maze training, and was maintained throughout testing. Water was given twice a day to prevent dehydration in the VX group. To overcome the concern that VX animals would be water-deprived more than control animals, we gave the VX rats 6 min of water 4 h prior to testing, whereas the IC rats received 4 min of water. After maze testing, rats in the VX and IC conditions were given access to water bottles for 35 and 30 min, respectively. Over the 3-month period of this water restriction schedule, both groups had the same percent body weight relative to their prerestriction body weight (93%).

All rats had free access to water on weekends. VX rats were fed ad libitum. IC feeding was yoked to the VX consumption to prevent rats in the former group from overeating, which they have been shown to do [16]. The colony was maintained on a 12/12 light/dark cycle at a temperature of 22°C. Training and testing occurred during the dark phase.

## 2.2. Apparatus

Behavioral training and testing were conducted in a standard eight-arm radial maze elevated 36 in. from the floor with arms extending from a central arena with a diameter of 35 cm. Arms were 58 cm long and 9 cm wide with a small recess at the end. The positions of extramaze cues, including a wall poster, tables, cabinets, and the experimenters, remained constant throughout all phases of the experiment.

## 2.3. Behavioral training and testing

All rats were removed from their home cages and transported in identical tub cages from the colony room to the testing room 5 days a week. Each arm was baited with a drop of water. Two experimenters, blind to the treatment group of the animals, recorded arm entrances and total time for each animal in the maze. No habituation sessions were given; data collection began with the first exposure to the eight-arm maze.

There were two phases to the maze testing: (1) RAM testing and (2) DNMTTP testing. Rats were removed from the maze when they completed one of the following: (a) seven water rewards obtained from at least eight arm entries (considered a successful completion); (b) eight different arms entered with or without taking a reward; (c) 16 arms entered without entering eight different arms; or (d) 5-min duration in the maze. The total time for each

trial and total number of arms visited were recorded. Proactive errors were recorded and were defined as the number of reentries into arms previously visited. Rats reached criterion performance and were switched to DNMTTP training when they had successful completion of the task on five of six sequential trials.

During the predelay period of DNMTTP trials, four randomly selected arms were blocked with clear plastic barriers while the remaining four open arms were baited with water. After visiting each arm and returning to the center of the maze, rats were removed from the maze and placed into individual plastic cages for a 1-h delay. Correct choices, proactive errors, and time within the maze were recorded. During the postdelay period of the trials, all arms were open and only the previously blocked arms were baited. Retroactive errors, reentry into arms that were entered during the predelay period, were recorded. During the training phase, a trial ended when the rat made four correct choices. Rats reached criterion performance and were moved into the testing phase when they obtained four correct choices within the first six entries, for five of six consecutive trials. DNMTTP testing consisted of 10 testing trials in which rats were allowed only four choices in the postdelay period.

## 2.4. Statistical analyses

All dependent variables were analyzed with a general linear model analysis of variance (ANOVA; group by litter).

## 3. Results

VX rats ran an average of 6185 rotations per day (SEM = 459) prior to water restriction and 5157 rotations per day (SEM = 424) during water restriction. Over the course of the experiment, rats in both groups maintained comparable weights.

### 3.1. RAM testing

A two-way ANOVA (group by litter) revealed a main effect of group on the number of trials to reach criterion, but no main effects of litter on any measures. VX rats took 30% fewer trials to reach criterion than IC rats on the standard eight-arm radial maze task,  $F(1, 2) = 7.48$ ,  $p < 0.05$  (see Fig. 1A). The average time spent per arm during precriterion trials was equal across groups,  $F(1, 2) = 1.87$ ,  $p > 0.05$  (see Fig. 1B). During the five trials of criterion performance, the two groups had no significant differences in the number of errors,  $F(1,2) = 0.01$ ,  $p > 0.05$ , and no differences in the average amount of time spent in each arm,  $F(1, 2) = 0.003$ ,  $p > 0.05$ .

A repeated measures ANOVA on the number of entries over the first 10 trials revealed a significant group effect,  $F(1, 16) = 5.99$ ,  $p < 0.05$  (see Fig. 2) and within-subjects

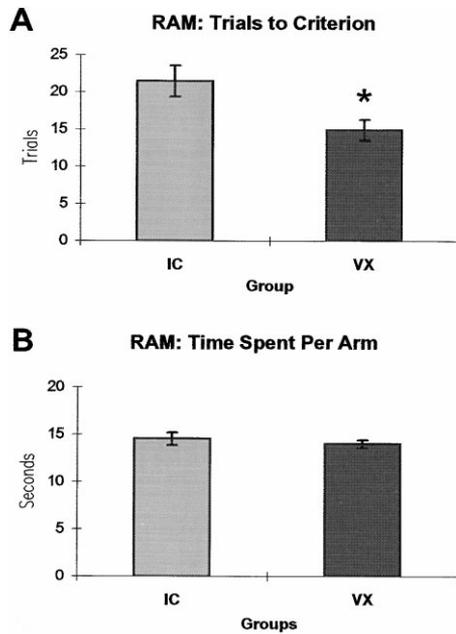


Fig. 1. Standard RAM. (A) The VX group took significantly fewer trials than the IC group to reach criterion on the RAM,  $F(1, 2) = 7.48$ ,  $p < 0.05$ . (B) Both groups of rats spent approximately the same average time per arm,  $F(1, 2) = 1.87$ ,  $p > 0.05$ . \*  $p < 0.05$ . Error bars indicate standard error of the mean.

effect over trials,  $F(9, 144) = 2.5$ ,  $p < 0.05$ , but no interaction ( $p > 0.05$ ). The VX group average indicated that they consistently entered fewer arms before reaching the daily criterion for maze completion. Analyses were not performed on data after trial 10 because trial 10 was the last trial of testing for two subjects in the VX group.

### 3.2. DNMTPT testing

No main effect of litter was revealed for any dependent measure during the DNMTPT testing. During pre-delay, no significant differences were found between the two groups in the number of proactive errors per trial,  $F(1, 2) = 2.25$ ,

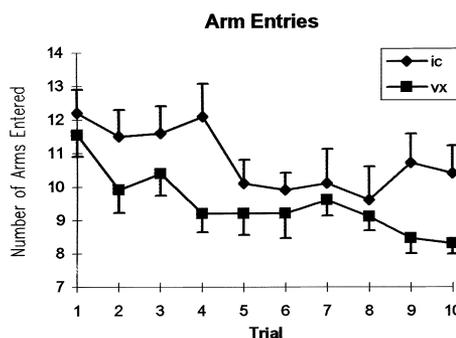


Fig. 2. Daily arm entries by group during acquisition of the RAM. The VX group entered significantly fewer arms prior to reaching the daily criterion than the IC group,  $F(1,16) = 5.99$ ,  $p < 0.05$ . Error bars indicate standard error of the mean.

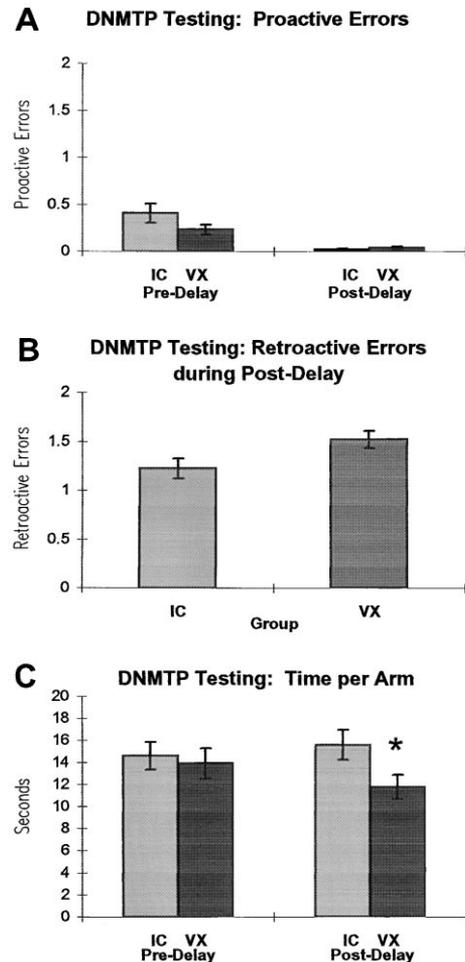


Fig. 3. DNMTPT testing. (A) The IC and VX groups did not differ significantly in the number of proactive errors made during pre-delay,  $F(1, 2) = 2.25$ ,  $p > 0.05$ , and postdelay periods,  $F(1, 2) = 0.29$ ,  $p > 0.05$ . (B) The IC and VX groups did not differ significantly in the number of retroactive errors made during postdelay,  $F(1, 2) = 4.13$ ,  $p > 0.05$ . (C) The IC and VX groups spent the same average time per arm during pre-delay periods,  $F(1, 2) = 0.19$ ,  $p > 0.05$ , but the VX group spent significantly less time per arm during postdelay periods than the IC group,  $F(1, 2) = 5.95$ ,  $p < 0.05$ . \*  $p < 0.05$ . Error bars indicate standard error of the mean.

$p > 0.05$  (see Fig. 3A), total arms entered,  $F(1, 2) = 2.25$ ,  $p > 0.05$ , and the average amount of time spent in each arm,  $F(1, 2) = 0.19$ ,  $p > 0.05$  (see Fig. 3C). Rate of DNMTPT acquisition is not reported because rate could have been influenced by condition, number of previous trials, which differed by group, or an interaction between the two factors. Therefore, difficulties would arise in interpreting such a measure.

During postdelay, a main effect of group revealed that VX rats spent less time per arm in the maze than IC rats,  $F(1, 2) = 5.95$ ,  $p < 0.05$  (see Fig. 3C). There were no significant differences in the number of retroactive errors,  $F(1, 2) = 4.13$ ,  $p > 0.05$  (see Fig. 3B) or proactive errors made during the postdelay period,  $F(1,2) = 0.29$ ,  $p > 0.05$  (see Fig. 3A).

Rats were sacrificed 1 month following the last testing session. A two-way ANOVA (group × litter) revealed significant main effects for group and litter on heart weight [group:  $F(1, 2) = 9.26$ ,  $p < 0.05$ ; litter:  $F(1, 2) = 4.59$ ,  $p < 0.05$ ]. Hearts from the VX group weighed 19% more, on average, than hearts from the IC group. We have previously observed a 12% increase in heart weight in older rats after only 1 month of voluntary exercise [17].

#### 4. Discussion

The present study tested the hypothesis that exercise influences the acquisition and performance of two versions of the eight-arm radial maze. These tasks are sensitive to hippocampal lesions, and require relatively little physical activity. The exercise (VX) group took significantly fewer trials (30%) to acquire criterion performance on the standard eight-arm radial maze, but spent the same average amount of time per arm as the nonexercising control (IC) group. Few voluntary exercise effects were seen in the DNMT version of the radial maze, the exception being that the VX group spent significantly less time per arm during the postdelay period. The noticeable effect on acquisition, but not criterion level performance, suggests that practice effects can compensate for initial group differences.

Testing required restricted access to water for two groups that differ in energy expenditure and presumably water consumption. These differences leave the results vulnerable to group differences in motivation. To control and correct for motivational differences across groups, access to food and water was monitored and controlled, with the VX group given slightly longer access to water bottles each day (a total of 7 additional min). Over the more than 3-month period of water restriction (with the exception of weekends), the average body weight for the two groups remained equal. If the greater access to water for the VX group caused them to be less motivated, they should have learned more slowly, not faster as we report. All rats eventually reached criterion performance, which suggests that all rats were sufficiently motivated to perform the task. In a follow-up study, rats with the same water access time had group acquisition rates that closely parallel the findings of this study (Anderson et al., in preparation).

The improved physical fitness of the VX group, indicated by the high number of daily wheel revolutions and greater heart weight, is unlikely to be an important factor for performance of the RAM. Rats are able to perform the RAM and DNMT tasks at their own pace. In the present study, the VX rats learned faster while spending the same average time per arm.

The effect of exercise on acquisition of the RAM can be taken to support the earlier conclusions that exercise influences spatial learning ability [1,3]. The present study adds to the previous literature by providing evidence that exercise can influence spatial learning in female rats as well as male

rats, with voluntary rather than forced exercise, and with an appetitively motivated task in addition to an aversively motivated task. In contrast to the findings in adult rats here and in earlier reports [1,3], aging rats tested on a Barnes maze did not exhibit differences in spatial ability as a consequence of exercise [18], although acquisition to a criterion was not assessed in that study. These contradictory findings could be due to age-related differences in the effect or differences in the sensitivity of the tasks.

The effect of exercise on variables ranging from heart weight to nerve growth factors necessitates further work to provide a link between exercise-induced changes in spatial learning and exercise effects on hippocampal variables such as neurotrophins [9], angiogenic factors [8,19], PKC activity [3], receptor density [1], and dentate gyrus cell proliferation and survival [20]. It is interesting to note, however, that a number of these variables have previously been shown to influence spatial learning (BDNF [21], NGF [22,23], FGF-2 [19]). It is also possible that exercise-related effects on vasculature and metabolic support might also mediate the effects on spatial learning [24–26].

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#### References

- [1] Fordyce DE, Farrar RP. Physical activity effects on hippocampal and parietal cortical cholinergic function and spatial learning in F344 rats. *Behav Brain Res* 1991;43:115–23.
- [2] Fordyce DE, Starnes JW, Farrar RP. Compensation of the age-related decline in hippocampal muscarinic receptor density through daily exercise or underfeeding. *J Gerontol B Psychol Sci Soc Sci* 1991; 46: B245–8.
- [3] Fordyce DE, Wehner JM. Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. *Brain Res* 1993;619:111–9.
- [4] Bland BH. The physiology and pharmacology of hippocampal formation theta rhythms. *Prog Neurobiol* 1986;26:1–54.
- [5] Dudar JD, Whishaw IQ, Szerb JC. Release of acetylcholine from the hippocampus of freely moving rats during sensory stimulation and running. *Neuropharmacology* 1979;18:673–8.
- [6] Vanderwolf CH. Cerebral activity and behavior: control by central cholinergic and serotonergic systems. *Int Rev Neurobiol* 1988; 30: 225–340.
- [7] Vissing J, Andersen M, Diemer NH. Exercise-induced changes in local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab* 1996;16:729–36.
- [8] Gomez-Pinilla F, Dao L, So V. Physical exercise induces FGF2 and its mRNA in the hippocampus. *Brain Res* 1997;764:1–8.
- [9] Neeper SA, Gomez-Pinilla F, Choi J, Cotman C. Physical activity

- increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 1996;726:49–56.
- [10] Stummer W, Weber K, Tranmer B, Baethmann A, Kempfski O. Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia. *Stroke* 1994;25:1862–9.
- [11] Becker JT, Walker JA, Olton DS. Neuroanatomical bases of spatial memory. *Brain Res* 1980;200:307–20.
- [12] Morris RGM, Garrud P, Rawlins JNP, O'Keefe JO. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–3.
- [13] Sothmann MS, Buckworth J, Clayton RP, Cox RH, White-Welkley JE, Dishman RK. Exercise training and the cross-stressor adaptation hypothesis. *Exercise Sport Sci Rev* 1996;24:267–87.
- [14] Chrobak JJ, Hanin I, Lorens SA, Napier TC. Within-subjects decline in delayed nonmatch-to-sample radial arm maze performance in aging Sprague–Dawley rats. *Behav Neurosci* 1995;109:241–5.
- [15] Stackman RW, Blasberg ME, Langan CJ, Clark AS. Stability of spatial working memory across the estrous cycle of Long–Evans rats. *Neurobiol Learn Mem* 1997;67:167–71.
- [16] Fiala B, Snow FM, Greenough WT. “Impoverished” rats weigh more than “enriched” rats because they eat more. *Dev Psychobiol* 1977; 10:537–41.
- [17] Isaacs KR, Anderson BJ, Alcantara AA, Black JE, Greenough WT. Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. *J Cereb Blood Flow Metab* 1992;12:110–9.
- [18] Barnes CA, Forster MJ, Fleshner M, Ahanotu EN, Laudenslager ML, Mazzeo RS, Maier SF, Lal H. Exercise does not modify spatial memory, brain autoimmunity, or antibody response in aged F344 rats. *Neurobiol Aging* 1991;12:47–53.
- [19] Gomez-Pinilla F, So V, Kesslak JP. Spatial learning and physical activity contribute to the induction of fibroblast growth factor: neural substrates for increased cognition associated with exercise. *Neuroscience* 1998;85:53–61.
- [20] van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999;2:266–70.
- [21] Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F. Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? *Behav Neurosci* 1998;112:1012–9.
- [22] Fischer W, Victorin K, Bjorkland A, Williams LR, Varon S, Gage FH. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature* 1987;329:65–8.
- [23] Markowska AL, Price D, Koliatsos VE. Selective effects of nerve growth factor on spatial recent memory as assessed by a delayed nonmatching-to-position task in the water maze. *J Neurosci* 1996;16:3541–8.
- [24] Goldman H, Berman RF, Gershon S, Murphy SL, Altman HJ. Correlation of behavioral and cerebrovascular functions in aging rat. *Neurobiol Aging* 1987;8:409–16.
- [25] Ragozzino ME, Pal SN, Unick K, Stefani MR, Gold PE. Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. *J Neurosci* 1998;18: 1595–601.
- [26] Shukitt-Hale B, Stillman MJ, Welch DI, Levy A, Devine JA, Lieberman HR. Hypobaric hypoxia impairs spatial memory in an elevation-dependent fashion. *Behav Neural Biol* 1994;62:244–52.