The effects of swimming exercise on recognition memory for objects and conditioned fear in rats

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ABSTRACT. Experiments conducted in animals have repeatedly demonstrated the ability of exercise to enhance cognitive function. This study examines the effects of chronic swimming exercise on non-spatial memory in adult rats after 12 weeks of swimming exercise in object recognition and elevated T-maze tests. In the object recognition test, repeated measures analysis of variance revealed a group effect (F₁,₄₂ = 26,093; p < 0.001), control rats had lower discrimination ratios than the exercise group. However, the swimming exercise did not affect the performance of inhibitory avoidance and escapes, when memory was tested in elevated T-maze. Analysis of variance showed a significant reduction in inhibitory avoidance 24h after the first training (F₁,₄₂ = 14,552; p < 0.001). Results indicated that regular swimming exercise significantly increased non-spatial memory in object recognition behavior, but did not affect the performance of inhibitory avoidance and escape on elevated T-maze test in adult rats. These findings suggest that the perirhinal cortex plays a role in memory consolidation and storage in addition to that of the amygdala, which could be regarded as the center of a second memory system, separate from those governed by the perirhinal cortex.

Keywords: amygdala, elevated T-maze, learning, object recognition, perirhinal cortex, physical exercise.

Introduction

It has been generally accepted that exercise produces benefits for overall health. However, only in recent years has there been an increasing interest in the scientific investigation of its effects on the brain and cognition. Exercise appears to benefit a range of cognitive abilities in animals and humans including spatial memory, working memory, executive control, and processing speed (COLCOMBE; KRAMER, 2003; VAN DER BORGHT et al., 2007). Both animal and human literature has established a number of changes in the nervous system that are correlated with exercise, some of which have been suggested to contribute to cognitive gain. These include changes in blood flow (HOLSCHNEIDER et al., 2007), concentrations of neurotransmitters (MEEUSEN; DE MEIRLEIR, 1995), growth factors (COTMAN et al., 2007), trophic factors (NEEPER et al., 1995), angiogenesis (SWAIN et al., 2003), gliogenesis (LI et al., 2005),...
and neurogenesis (VAN PRAAG et al., 1999). Any or all of these changes could contribute to enhanced performance in a given task.

Physical exercise has been reported to exert beneficial effects on different memory types, including spatial (ALAEI et al., 2008; HAJISOLTANI et al., 2011), and long-term fear memory (CHEN et al., 2007). Mello et al. (2008) detected only mild enhancing effects on spatial learning, and no effects at all in an object recognition learning task and in inhibitory avoidance. Barnes et al. (1991) were also unable to detect significant influences of physical exercise on various cognitive parameters in rats. However, exercise has been reported to reverse memory deficits caused by morphine (ALAEI et al., 2006) and aging (VAN PRAAG et al, 2005) in animals, and to reduce cognitive impairments in aged humans (FRIEDLAND et al., 2001).

Physical exercise can also improve performance in non-spatial tasks that rely on other structures, including object recognition (CRUZ, et al., 2010; FAHEY et al., 2008; GRIFFIN et al., 2009; O’CALLAGHAN et al., 2007). For example, novel object recognition is mediated by the perirhinal cortex and can be designed such that hippocampal lesions have no effect on performance (DERE et al., 2007). To date, there has been very little research on the mechanisms that underlie exercise-induced improvements in tasks that do not test spatial cognition or rely on hippocampal function. Although there is evidence that forced physical exercise also increases perirhinal brain-derived neurotrophic factor (GRIFFIN et al., 2009), it is not known whether brain-derived neurotrophic factor activity in the hippocampus, perirhinal cortex or elsewhere mediates exercise-induced improvements in object recognition. This is particularly important from a translational perspective, since exercise studies in humans have reported the greatest improvements in non-spatial learning and memory (HILLMAN et al., 2008).

Previous studies indicated that intensity level may be a determining factor in the beneficial or detrimental effects of exercise on non-spatial memory of fear conditioning (ALAEI et al., 2007; BURGHARDT et al., 2006). The amygdala is essential for several forms of fear conditioning (ROSEN, 2004). Exposure to both conditioned and unconditioned stimuli increases expression of the immediate early gene (c-fos) product Fos in a variety of brain structures, including the amygdala (ROSEN et al., 1998). Furthermore, long periods of wheel running decrease footshock-elicited Fos expression in several regions involved in learned helplessness, and decrease freezing in a shuttle box context after footshock (GREENWOOD et al., 2003). Chronic wheel running also modifies anxiety-related and defensive behaviors (BURGHARDT et al., 2004), which may involve amygdala pathways. The studies indicated that intensity level may be a determining factor in the beneficial or detrimental effects of exercise on passive avoidance learning performance. Another investigation has indicated that exposure to emotionally arousing events may facilitate the consolidation or storage of new information. The increased freezing response seen in animals suggests that chronic exercise promotes anxiety-like behaviors. Indeed, rats given prolonged access to running wheels spend less time in the open arm of the elevated plus maze or in the center of an open field (BURGHARDT et al., 2004). An alternative explanation is that chronic wheel running facilitates the learning and/or expression of an aversively-motivated adaptive behavior or the attention processing of fear associated stimuli; this interpretation would be consistent with other reports showing positive effects of exercise in a variety of different cognitive tasks - with a less explicit fear component (VAN PRAAG et al., 1999).

In this context, the present experiment studied the effect of swimming exercise on memory in two different tasks - object recognition and elevated T-maze. The object recognition task depends on the integrity of the perirhinal cortex (DERE et al., 2007); since exercise has a profound impact on the plasticity of perirhinal cortex, a facilitator modulation of object recognition memory was expected. The elevated T-maze allows the measurement of two kinds of aversively motivated behaviors, namely, inhibitory avoidance and one-way escape. Several convergent lines of evidence point to the amygdala as a key site of plasticity underlying most forms of fear conditioning (SIGURDSSON et al., 2007). The existence of multiple memory systems subserved by different neural substrates is a widely accepted view of memory organization in the mammalian brain. Thus, the general purpose of the present study was to analyze whether chronic swimming exercise promotes behavioral differentials in non-spatial learning (perirhinal cortex-dependent) and conditioned fear (amygdala-dependent).

Material and methods

Animals

Male adult Wistar rats, 90 days of age and weighing 250-300 g were used in all experiments.
Rats were reared in air-conditioned rooms (23 ± 1°C), with a 12h light-dark cycle, five rats to a cage, with food and water ad libitum. All procedures followed a protocol approved by the local Institutional Animal Care and Use Committee in accordance with institutional guidelines.

Adaptation to the water
All rats were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the animals in shallow water at 31 ± 1°C (HARRI; KUUSELA, 1986), 5 days week⁻¹, from 9:00 to 21:30h. The purpose of the adaptation was to reduce stress without, however, promoting physical training adaptations.

Exercise training
Rats were trained to swim 30 min. day⁻¹, 5 days a week, on the first day of the training period and were increased by 10 min., every 7 days. At the end of the 7th day, the animals swam continuously for 20 min., and at the end of the 14th day, they swam for 30 min. Continuous exercise (30 min.) was performed from the 14th day until the end of the period. According to previous studies, the intensity level for exercise is classified as aerobic (GOBATTO et al., 2001). Sedentary rats placed in shallow water at 31 ± 1°C, 30 min., 5 days week⁻¹, were used as controls. At the end of the training period and 48h after the last exercise bout, all rats were individually submitted to behavioral tests (object recognition and elevated T-maze).

Object recognition test
The object recognition test (ENNACEUR; DELACOUR, 1988) was carried in a circular wooden box (40 cm in diameter and 50 cm high). In the training session, two identical objects were placed near the two corners at either end of one side of the box. The rats were placed in the experimental apparatus, facing the center of the opposite wall, and were allowed to explore the two objects for 5 min. Retention was tested 1, 4 or 24h after the training trial. After a retention interval, each rat was returned to the box, which now contained a copy of the sample object and a novel object. The rats were placed in the box and allowed to explore both objects for 5 min. An exploratory behavior was defined as the subject facing the object, with its nose within 2 cm of the object. The main dependent measure was the investigation ratio of total time spent on both object-investigation during the test phase that was spent investigating the novel object $t_{novel} / (t_{novel} + t_{familiar})$.

Elevated T-maze test
The elevated T-maze test of anxiety has been used to separate in the same rat conditioned from unconditioned responses of fear/anxiety (ZANGROSSI JR.; GRAEFF, 1997). The elevated T-maze was made of wood and had three arms of equal dimensions (50 x 10 cm). One arm, the stem of the T, was enclosed by 40 cm high walls and was perpendicular to two opposite arms. To prevent the rats from falling down, the open arms were surrounded by a 1 cm high Plexiglas rim. The whole apparatus was elevated 50 cm above the floor. Each rat was placed at the end of the enclosed arm facing the intersection of the arms, and the time taken to leave this arm with all four paws was recorded (baseline latency). The animal was then immediately removed from the maze and the same measurement was repeated (60 s intervals) for as many trials as needed for the rat to stay in the enclosed arm continuously for 300 s (learning criterion for the avoidance conditioning). After another 60 s, the rat was placed at the end of the right open arm, and the time taken to leave this arm with the four paws was recorded (escape learning). Avoidance and escape latencies were measured 1, 4 or 24h later in one trial.

Data analysis
All data presented are expressed as mean ± S.E.M., and each value reflects the mean of 6-8 animals per group. The means were compared by two-way analysis of variance to reveal a difference between the two groups, sedentary control and exercise (two-way ANOVA), followed by the Newman-Keuls multiple comparisons test. A probability level of 0.05 was used to test for statistical significance.

Results
In the object recognition test, repeated measures analysis of variance revealed a group effect ($F_{1,42} = 26.093; p < 0.001$); control rats had lower discrimination ratios than the swimming exercise group (Newman-Keuls test $p < 0.01$ and $p < 0.001$; Figure 1A). A comparison of the time spent with the new objects within groups indicated that the exercise group explored the new object longer than the control group in the 4h and 24h intervals ($F_{1,42} = 9.340; p < 0.01$ and $p < 0.001$, respectively; Figure 1B). Time spent exploring the familiar
objects was not affected by swimming exercise ($F_{1,42} = 2.805; p > 0.05$; Figure 1C).

Figure 1. Object recognition performance after chronic swimming exercise. (A) Discrimination ratio in object recognition [$t_{novel}/(t_{novel} + t_{familiar})$]. Measures indicated that the control group had a lower ratio than the exercise group. (B) Time spent with the new objects within groups indicated that the swimming exercise group explored the new object longer than the control group in the 4 and 24h intervals. (C) Time spent exploring the familiar objects. Vertical lines represent ± S.E.M. Bars represent the mean of groups of 6-8 rats. **p < 0.01; ***p < 0.001; ANOVA followed by Newman-Keuls test.

Analysis of variance did not show significant changes in inhibitory avoidance along trials in the control group as well as in swimming exercise rats ($p > 0.05$). The swimming exercise did not affect the performance of inhibitory avoidance, when memory was tested. Therefore, the effect of swimming exercise on inhibitory avoidance acquisition was quantitatively similar to that of control. Analysis of variance revealed significant reduction in inhibitory avoidance latency 24h after the first training ($F_{1,42} = 14.552; p < 0.001$; Figure 2A). Analysis of escape behavior did not show such a difference between control and exercise ($F_{1,42} = 1.645; p > 0.05$; Figure 2B).

Discussion

The present results in adult rats showed that swimming exercise significantly enhanced learning and memory. The procedures used to test object recognition memory were based on the spontaneous tendency of rodents to explore novel stimuli. In the test trial of the standard procedures of novel object recognition memory tasks (ENNACEUR; DELACOUR, 1988), both novel and familiar objects are placed in one of the two positions occupied by the copies of the familiar object during the training trial (and never in different positions). Thus, the discrimination between novel and familiar objects may be based on the features of the individual objects. Comparison of the time spent with the new objects within groups indicated that the swimming exercise group explored the new object longer than the control group in the 4 and 24h delay, and control rats had lower discrimination ratios than the swimming exercise group (Figure 1), suggesting an exercise-related improvement in learning. In particular, results showed that regular swimming exercise activity significantly increased non-spatial memory on behavior measures in object recognition. Our results confirm previous research.
studies on the effects of exercise on recognition memory for objects (CRUZ et al., 2010; FAHEY et al., 2008; GRIFFIN et al., 2009; O’CALLAGHAN et al., 2007).

The elevated T-maze allows the measurement of two kinds of averesely motivated behaviors - namely, inhibitory avoidance (time the animal takes to leave the enclosed arm) and one-way escape (time taken to leave the open arm). This experimental model allows the parallel measurement of responses related to both innate and learned fear in the same subject, and permits the simultaneous assessment of memory for these behaviors (VIANA et al., 1994). Exposure to both conditioned and unconditioned stimuli increases expression of the immediate early gene (c-fos) product Fos in a variety of brain structures including the amygdala (ROSEN et al., 1998). Existing views differ on the degree of involvement of each memory structure. The effect of swimming exercise on inhibitory avoidance acquisition and escape was quantitatively similar to that of control (Figure 2). Chronic swimming exercise failed to modify T-maze behavior, which is inconsistent with previous studies showing physical exercise improved passive avoidance learning performance (SAADATI et al., 2010) and more time freezing (BURGHARDT et al., 2004, 2006). Nevertheless, no significant difference was observed between long- and short-term exercises in the passive avoidance test (SAADATI et al., 2010). It is worth noting that the exercise protocol used here did not influence behavioral performance in controls, despite many studies having shown benefits of exercise on cognitive functions (BURGHARDT et al., 2004, 2006). This controversial result might be related to many factors, such as the motivation for physical activity – either forced or voluntary, the different duration and intensity of exercise performed, as well as the age of experimental animals. In particular, results showed that regular swimming exercise does not modify anxiety-related and defensive behaviors, which may involve amygdala pathways (CRUZ et al., 2010). Interestingly, it has been suggested that the memory-enhancing effects of exercise may be directly related to its stress-reducing and anxiolytic effects (TREJO et al., 2008). That is, exercise may reduce anxiety and in turn enhance conditioned fear. Although that hypothesis has not been directly evaluated, acute pre-conditioning stress has been shown to enhance anxiety and interfere with cued fear conditioning (TODOROVIC et al., 2007).

The existence of multiple memory systems subserved by different neural substrates is a widely accepted view of memory organization in the mammalian brain. Physical activity produces numerous neurochemical and physiological adaptations and alters behavioral responding in several tests that evaluate learning. Here we note that recognition memory presents at least two qualitative different memory processes which appear to involve different areas of the brain. If memory is represented in the brain through modifications in neuronal structures, then these changes should persist for as long as the encoded experience. These non-spatial learning and physical activity-induced alterations were observed in the perirhinal cortex (CRUZ et al., 2010; DERE et al., 2007) and converging lines of evidence indicate that the amygdala is necessarily involved in the acquisition, storage and expression of conditioned fear memory (BURGHARDT et al., 2006). The methodological diversity of these studies makes it difficult to establish which other variables (from genetic differences to features of the task used in the study) interact with exercise to produce this wide range of results. Animal research could help not only to broaden knowledge of the molecular mechanisms involved in these effects but also to clarify the modulator effect of these other variables in a more controlled environment.

Conclusion

Results indicated that regular swimming exercise significantly increased non-spatial memory on behavior measures in object recognition, but did not affect the performance of inhibitory avoidance and escape in elevated T-maze test in adult rats. These findings suggest that the perirhinal cortex plays a role in consolidation and storage in addition to that of the amygdala, and would merit being viewed as the center of a second memory system, separate from those governed by the perirhinal cortex. The question is of more than just theoretical importance, inasmuch as separate systems could each have a pathology of its own and separate memory syndromes. This is not by itself sufficient to postulate anything more than a modulatory role for this structure through swimming exercise. But this role could be crucial and more necessary for some learning than for others, which might explain several of the discrepancies. This view allows the effects of exercise to be understood in terms of existing psychobiological knowledge, and it can thereby provide the theoretical base that is needed to guide future research in this area.

References

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The effects of swimming exercise on memory


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