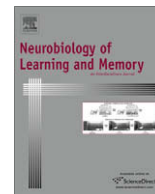




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## Physical exercise can reverse the deficit in fear memory induced by maternal deprivation

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## ABSTRACT

Maternal deprivation during the first 10 days of life induces significant behavioral alterations in rodents which persist through adulthood. Physical exercise reduces the cognitive deficits associated with pharmacologic and pathological conditions. Here we investigated whether forced physical exercise alters memory deficits caused by postnatal maternal deprivation. Male rats were divided into four groups: (1) control, (2) deprived, (3) exercised, and (4) deprived + exercised. In groups 2 and 4, pups were deprived from their mothers for 3 h/day during the first 10 days post-birth. In groups 3 and 4, from postnatal day 45 (PND-45) on, animals were submitted to forced treadmill exercise. At adulthood, animals were submitted to four different behavioral tasks: open field, Morris water maze (MWM), object recognition (OR) and inhibitory avoidance (IA). Maternal deprivation had no effect on open field behavior, but disrupted memory in the three other tasks. Physical exercise alone had no effect, except for a slight enhancement of MWM learning. Importantly, physical exercise reversed the deficit of IA and reduced the deficit of spatial memory but not that of OR seen in deprived animals. It is possible that physical exercise may counteract the influence of maternal deprivation on neurohumoral or hormonal memory modulatory systems related to stress. Indeed, the decreasing order of the effect of exercise on the memory disturbances induced by deprivation roughly follows the descending degree of stress associated with each task (IA > MWM > OR). Maternal deprivation is known to hinder hormonal mechanisms involved in coping with stress.

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## 1. Introduction

Early postnatal maternal deprivation induces cognitive deficits that persist into adulthood and senescence in rats (Benetti et al., 2009, 2007; Lehmann, Pryce, Bettschen, & Feldon, 1999; Oitzl, Workel, Fluttert, Frosch, & de Kloet, 2000; Renard, Suárez, Levin, & Rivarola, 2005). These are accompanied by neurochemical and anatomical modifications, such as reduced expression of brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate (NMDA) receptor subunits (Ang, Wong, Moochhala, & Ng, 2003; Kuma et al., 2004; Roceri, Hendriks, Racagni, Ellenbroek, & Riva, 2002), increased nerve growth factor expression (Cirulli, Micera, Alleva, & Aloe, 1998), reduced mossy fiber density (Hout, Plotsky, Lenox, & McNamara, 2002), as well as by hormonal and neurohumoral alterations, such as elevated basal pituitary–adrenal activity (Rots et al., 1996; Schmidt, Oitzl, Levine, & de Kloet, 2002), and altered responses to stress (Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Mirescu & Gould, 2006).

Physical exercise has been reported to exert beneficial effects on different memory types (Ang & Gomez-Pinilla, 2007; Winter et al., 2007), including spatial (Alaei, Moloudi, & Sarkaki, 2007; Ang, Dawe, Wong, Moochhala, & Ng, 2006), and fear long-term memory (LTM; Chen et al., 2007). We have previously detected only very mild enhancing effects on spatial learning, and no effects at all in an object recognition learning task and in inhibitory avoidance (Mello, Benetti, Cammarota, & Izquierdo, 2008). 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, Shubert, Ahao, & Gage, 2005) in animals, and to reduce cognitive impairments in aged humans (Friedland et al., 2001; Laurin, Verreault, Lindsay, Macpherson, & Rockwood, 2001).

We studied the memory deficits caused by maternal deprivation for 3 h/day during 10 days (Benetti et al., 2009; McIntosh, Anisman, & Merali, 1999) in rats, and examined whether forced physical exercise can reverse these deficits. We used forced physical exercise in a treadmill (Ang et al., 2006; Mello et al., 2008; Radak et al., 2006). Forced exercise has been shown to have positive

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effects in human learning (Winter et al., 2007). In this procedure it is easier to control the time, duration, and intensity of the running, than in free running procedures (e.g., Kennedy, Smith, & Fleshner, 2005; Van Praag et al., 2005), which permits control of training volume (Moraska, Deak, Spencer, Roth, & Fleshner, 2000); but it involves a degree of stress (Cotman & Berchtold, 2002; Dishman, 1997; Dishman et al., 1997; Mello et al., 2008).

## 2. Materials and methods

### 2.1. Animals

Pregnant female Wistar rats (age of 3–4 months, weight of 250–280 g) were obtained from the Reproduction Center of the Universidade Federal do Rio Grande do Sul (UFRGS). All animals were maintained in light/dark cycle (lights on at 07:00 AM, off at 7:00 PM). The environment temperature ( $\pm 22$  °C) and humidity (60%) were kept constant. Pregnant females were individually housed with sawdust bedding and *ad libitum* access to food and water. Litters were culled to eight pups per dam, four males and four females. The day of delivery was marked as day zero, and on postnatal day 1 (PND-1) a maternal deprivation protocol was applied to 50% of the pups from days 1–10 after birth; the other males were used as controls. Animals were weaned at the age of 21 days, and housed in regular cages 4 to a cage. Females were donated to the Reproduction Center for other research purposes. The males were used in the present experiments.

There were four experimental groups: (1) control, which received no treatment whatsoever; (2) deprived, which were submitted to maternal deprivation as described; (3) physical exercise, which from PND-45 onward were exposed to 8 weeks of forced treadmill activity as will be described below; and (4) deprived + physical exercise, which were maternally deprived from PND1-1 to PND-10 and then submitted to the treadmill from PND-45 on. Animals of the four groups were submitted to four different behavioral procedures beginning on PND-100: first, free exploration of an open field; then, spatial learning in a Morris water maze (MWM); subsequently, an object recognition task (OR), and finally aversive learning in the inhibitory avoidance task (IA). The entire battery of behavioral tests took 15 days. In all experiments the “Principles of laboratory animal care” (NIH publication No 85-23, revised 1996) were strictly followed.

### 2.2. Maternal deprivation protocol

Female Wistar rats were maintained in individual boxes until the delivery day. Deprivation was carried out for 180 min a day from PND-01 to PND-10. The deprivation protocol consisted in removing the mother from the residence box and taking her to another room. Pups were maintained in their home cage (grouped in the nest in the presence of maternal odor). We prefer this maternal deprivation protocol because it does not require manipulation of the pups (Kosten, Lee, & Kim, 2007; Todeschin et al., *in press*).

While the mothers were absent the room temperature was raised to 32 °C to compensate for the mother’s body heat (Renard et al., 2005; Benetti et al., 2009, 2007). At the end of each daily deprivation session, the mothers were returned to their home boxes; this procedure was carried out during the light part of the cycle, between 8:00 AM and 2:00 PM. Control rats remained in their resident boxes together with their mothers throughout. Only on PND-11, the boxes were cleaned normally again, according with the laboratory routine. On PND-21 the animals were weaned, and males were maintained in groups of 4 in 50 × 25 × 40 cm plastic boxes with a stainless steel lid, with food and water *ad lib.*, as all the other animals of our animal housing facility.

### 2.3. Physical exercise protocol

Animals were submitted to chronic treadmill physical exercise during 8 weeks. In the week immediately before the first week of training, all animals were placed in the training apparatus for 10 min to habituate, in order to minimize novelty-induced stress. In the first day of the second week an incremental test was carried out on an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil) to determine the physical exercise intensity that would be used in the training period. Indirect measurement of peak oxygen uptake ( $VO_2$  peak) was measured as recommended by Brooks and White (1978). Each rat ran for 25 min on the treadmill at a low initial speed followed by increases of speed of 5 m/min every 3 min, until they reached their point of exhaustion (i.e., failure to continue running). Time to fatigue (in minutes) and workload (expressed by velocity in km/h) were taken as indexes of maximum capacity for exercise, and as an indirect measurement of  $VO_2$  peak. The intensity of physical training protocol (50 min/day for 5 day per week) was kept between 60% and 75% of their respective peak oxygen uptake for 8 weeks. Each training session started with a 10 min warm-up (gradual acceleration) followed by 30 min at target intensity. The last 10 min of each session were for gradual deceleration (adapted from Scopel et al., 2006; see also Mello et al., 2008).

Running sessions on the treadmill were carried out between 10:00 AM and 2:00 PM. The treadmill had individual 10-cm wide, 50 cm-long lanes separated by transparent acrylic walls. Neither electric shock nor physical prodding was used in this study to avoid possible stress effects. The animals that refused to run were encouraged by gently tapping on their backs. Animals that were not able to perform the exercise were excluded. Control animals were transported to the experimental room and handled exactly as those in the physical exercise groups; they were placed in the running lanes for 10 min, but they were not submitted to exercise protocol.

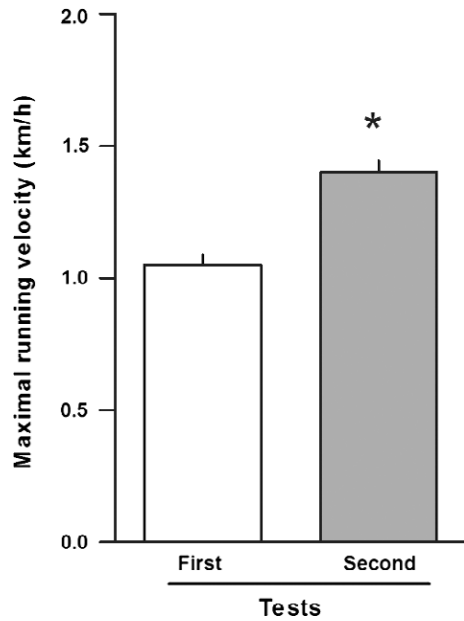
The problem of the stress associated with exercise and its influence on memory of exercised rats was addressed in a previous paper (Mello et al., 2008). It was reported there that a short but not a prolonged period of forced physical exercise produces mnemonic effects similar to those of stress induced by daily footshocks. In addition, Kennedy et al. (2005) reported that freewheel running provides sufficient exercise stimulus to produce some, but not all, physiological adaptations to training, which suggests that forced physical exercise is a more efficient protocol. The effects of chronic stress on learning and memory have been repeatedly described (Krugers et al., 1997; Mitra & Sapolsky, *in press*; Veena et al., 2007) and have been almost invariably found to be deleterious, which is the opposite of what is seen with physical exercise (Mello et al., 2008). Therefore, the effects of the latter can not be attributed to the former.

### 2.4. Second analysis of indirect maximal oxygen uptake

In the first day of the 5th week of training, the physical exercise group was submitted to a second measurement of indirect maximal oxygen uptake (Brooks & White, 1978) to analyze whether the training protocol was effective, and to verify if the forced running protocol indeed enhanced physical aerobic capacity. As can be seen in Fig. 1, this was the case. Peak oxygen uptake was not evaluated on the end of the physical exercise training, in order to avoid effects of stress on the memory tests that the animals were going to be exposed to a few days later.

### 2.5. Open field test

The animals were submitted to an open field to evaluate spontaneous locomotor and exploratory activities. The open field appa-



**Fig. 1.** Maximal running velocity (km/h) increased in physical exercise training group. The first measurement of maximal running velocity (km/h; indirect measurement of maximum oxygen uptake according Brooks and White, 1978) was carried out one day before the beginning of training, and the second one after 4 weeks of training. \* $p < 0.05$  in Student's  $t$ -test;  $n = 12$ .

ratus consisted of  $40 \times 50 \times 50$  cm open arena painted white except for the frontal wall which was of glass. The floor was divided into 12 equal rectangles by black lines; crossings of the lines were used to evaluate locomotion. Exploratory activity was monitored by counting the number of rearings performed by each animal (Benetti et al., 2009). The rats were individually placed in the arena and observed during 5 min.

### 2.6. Morris water maze (MWM) test

The MWM was a black circular pool (200 cm in diameter) conceptually divided in four equal imaginary quadrants for the purpose of data analysis. Water temperature was maintained between 21 and 23 °C. Two centimeters beneath the water surface, and hidden from the rats' view, there was a black circular platform 12 cm in diameter. It had a rough surface that allowed the rat to climb onto it easily once detected. The swimming path of the rats was recorded using a video camera mounted above the center of the pool and analyzed using a video tracking path and analysis system. The MWM was located in a well-lit white room with several posters and other distal visual stimuli hanging on the walls to provide spatial cues. A curtain separated the part of the room with the water maze from the part where the experimenter and the computer setup were installed. The MWM task was carried out during five consecutive days (Rossato et al., 2006a), which is a schedule viewed as more sensitive to different parameters of spatial learning (Rossato, Bevilaqua, Medina, & Cammarota, 2006b; Rossato, Bevilaqua, Myskiw, Medina, & Izquierdo, 2007) than; say, a 1-day protocol (Frick, Stillner, & Berger-Sweeney, 2000). In each training day/session, the rats were submitted to eight consecutive training trials, while the hidden platform was kept in a constant position. A different starting location was used for each trial, which consisted of swimming followed by a 30 s sitting on the platform. Rats that did not find the platform within 60 s were guided to platform by the experimenter. Memory retention was evaluated during a 30 s probe trial carried out 24 h after last training session in the absence of the escape platform (Rossato et al., 2006a).

### 2.7. Object recognition (OR) test

The OR test (Ennauer & Delacour, 1988) was carried out in the same arena used as an open field, following the protocol described by Myskiw et al. (2008). All animals were habituated to the experimental arena in the absence of any specific behavioral stimulus for 20 min/day during 4 days. The objects to be recognized were made of metal or glass and were fixed to arena's floor with adhesive ribbon. In the first day after habituation (training session) animals were placed in the arena containing two different objects (A and B), and left to explore them freely for 5 min. The test was repeated 24 h later to evaluate long-term memory (LTM). In the test, one of the objects was removed and a new object (C) replaced it, upon which the rat to be tested was introduced in arena for five more minutes. Positions of objects (familiar or novel) were randomly permuted for each experimental animal, with the arena being always cleaned between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on or turning around the object was not considered as an exploratory behavior. Time spent to explore each object was recorded by an observer blind to treatment and expressed as a percentage of total exploration time computed in seconds (Rossato et al., 2007).

### 2.8. Inhibitory avoidance (IA) test

The IA apparatus was a  $50 \times 25 \times 25$  cm acrylic box whose grid was a series of 3 mm caliber bronze bars spaced 3 cm apart. At the left end of floor there was a 8 cm wide, 25 cm long, 5 cm high wood platform (Cammarota, Bernabeu, Izquierdo, & Medina, 1996; Paratcha et al., 2000). During training, animals were gently placed on the platform facing the rear left corner, and when they stepped down placing their four paws on the grid they received a 3-s scrambled 0.5 mA foot-shock.

The rats were tested for retention 24 h after training. Retention test latency measurements were cut off at 300 s; i.e., scores equal to or greater than this measure were counted as 300 s. Animals received of course no footshock on the retention test.

### 2.9. Statistical analysis

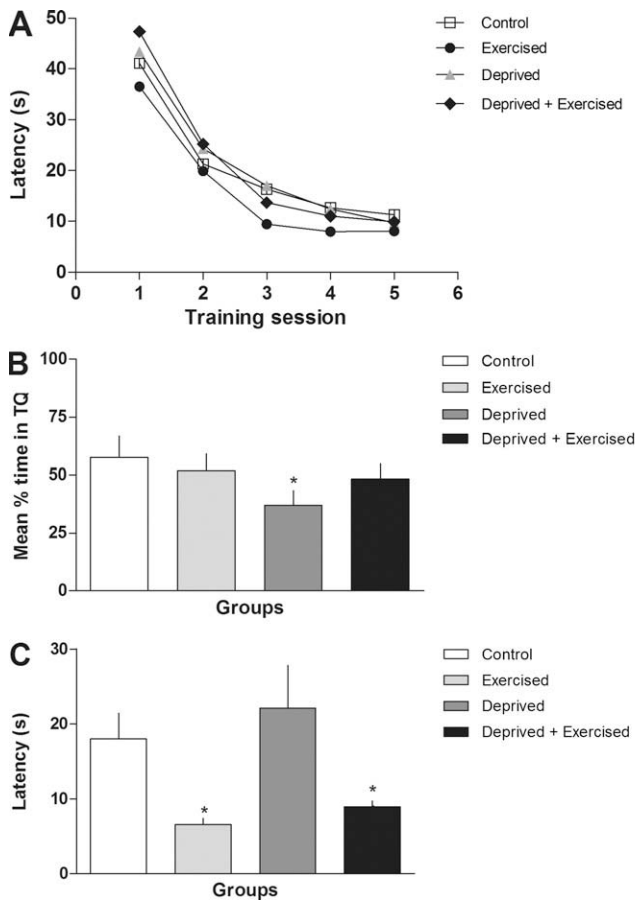
Indirect  $\text{VO}_2$  peak was analyzed using a Student  $t$ -test. The results of open field behavior were analyzed using two-way ANOVA. MWM behavior was analyzed using either two-way ANOVA followed by Newman-Keuls multiple comparison test. OR was analyzed using a one sample  $t$ -test. Since a ceiling of 300 s was imposed to step-down latencies during retention tests in the IA, retention data in this task did not have a normal distribution and are expressed as medians and interquartile ranges; comparisons among groups in this task were did using a Kruskal–Wallis non-parametric ANOVA followed by Dunn's multiple comparisons as appropriate. All other data were expressed as means  $\pm$  SEM. The sample size ( $N$ , number of animals in each group) for each experiment is stated in the figure captions. Minimum statistically significant differences were established at  $P < 0.05$ .

## 3. Results

### 3.1. Analysis of indirect maximum oxygen uptake

There was a significant increase of  $\text{VO}_2$  in the second measurement as compared to that at the beginning of the forced exercise routine ( $P < 0.05$  in Student's  $t$ -test). Thus, the forced running protocol indeed enhanced physical aerobic capacity (Fig. 1).

During the firsts four weeks of physical exercise training rats ran 650 m/day, in the last four weeks they ran around 1000 m/



**Fig. 2.** Maternal deprivation impairs and physical exercise improves the spatial memory. Animals were trained during 5 days in the spatial version of the Morris Water Maze (MWM). (A) mean  $\pm$  SEM escape latency on each training session. (B) mean  $\pm$  SEM % time spent in the target quadrant (TQ) during a 60 s probe test carried out 24 h after the 5th training session ( $*p < 0.001$  in Newman-Keuls' test). (C) mean  $\pm$  SEM latency, measured in the probe test ( $*p < 0.01$  in Newman-Keuls' test).  $N = 12$ –14 per group.

day (like in Morris et al., 2007; Silva et al., in press; Simões et al., 2008).

### 3.2. Morris water maze (MWM)

Acquisition of spatial memory in the MWM learning curves was similar among groups (Fig. 2A). However, in the probe test carried 24 h after training, a deficit was observed in the maternal deprivation group (Fig. 2B). Deprived animals spent significantly less time swimming in the target quadrant (TQ;  $P < 0.001$  in Newman-Keuls multiple comparison test), although the latency to swim over the previous location of the escape platform did not differ from controls. On the contrary, physical exercise did not affect the % of time in TQ but reduced significantly the escape latency (Fig. 2C). No significant difference in swimming speed was observed among the different experimental groups (Table 1).

**Table 1**  
Swimming velocity in MWM probe test (cm/s; data expressed as mean  $\pm$  SEM;  $n = 12$ –14 per group).

	Control	Exercise	Deprived	Deprived + exercise
Velocity (cm/s)	26.16 $\pm$ 2.96	25.67 $\pm$ 2.19	25.55 $\pm$ 1.96	27.05 $\pm$ 3.12

### 3.3. Object recognition (OR)

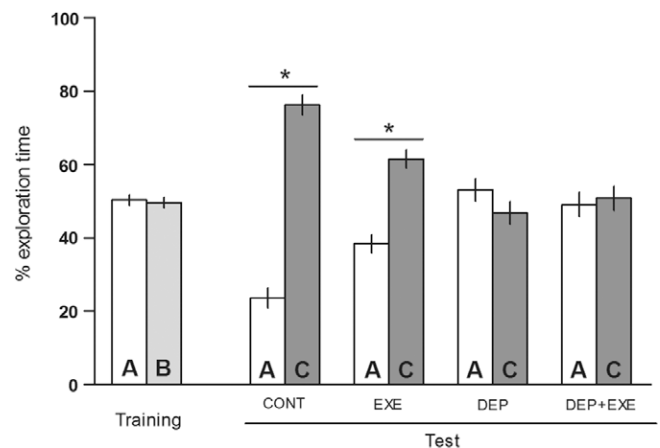
During the training phase (day 1) the animals exploring equally the two stimuli objects (Fig. 3). In the test phase (day 2), while control animals explored the novel object longer than the familiar one ( $P < 0.001$  in one sample  $t$ -test), the deprived rats spent the same amount of time exploring the two objects. Exercise did not modify the amnesic effect of maternal deprivation (Fig. 3).

### 3.4. Inhibitory avoidance (IA)

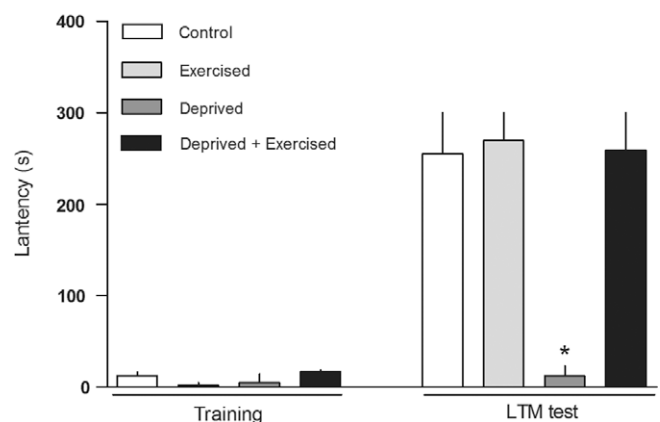
Maternal deprivation disrupted IA memory. Deprived animals had a shorter step down latency than the controls in the LTM test ( $P < 0.001$  in Dunn's multiple comparison test). No influence of exercise on the task itself was detected. However, exercise clearly reversed the deleterious effect of maternal deprivation (Fig. 4).

### 3.5. Motor and exploratory activity in the open field

Maternal deprivation and physical exercise did not affect the number of crossings and rearings during a 5-min long free-exploration session in the open field (Table 2).



**Fig. 3.** Maternal deprivation impairs and physical exercise does not affect the object recognition memory. Rats were exposed to two different objects (A and B) for 5 min in the training session. Long-term memory (LTM) was measured 24 h after training; animals were exposed to a familiar object (A) and to a novel object (C) during 5 min. Data (mean  $\pm$  SEM) are present as the percentage of total exploration time.  $*p < 0.001$  in one-sample Student's  $t$ -test;  $n = 12$ –14 per group.



**Fig. 4.** Impairment in retention of inhibitory avoidance (IA) memory caused by maternal deprivation is reversed by physical exercise. Rats were trained in IA and, 24 h after were tested (LTM test). Bars represent median  $\pm$  interquartile range of step-down latencies.  $*p < 0.001$  level in Dunns test;  $n = 12$ –14 per group.

**Table 2**

Effects of each study procedure on locomotor and exploratory activities. Maternal deprivation, physical exercise for 8 weeks or both had no effect on locomotor and exploratory activities (two-way ANOVA; data expressed for mean  $\pm$  SEM;  $n = 12$ –14 per group).

Groups	Crossings	Rearings
Control	70.00 $\pm$ 25.11	38.50 $\pm$ 10.43
Exercise	78.43 $\pm$ 21.76	40.00 $\pm$ 8.357
Maternal deprivation	60.10 $\pm$ 21.75	35.40 $\pm$ 10.42
Maternal deprivation + exercise	78.00 $\pm$ 20.94	37.60 $\pm$ 9.513

#### 4. Discussion

It is known that physical exercise can influence brain physiology and cognitive function both in humans and in laboratory animals. It has been used in the prevention or treatment of a variety of memory deficits, like those caused by natural aging (Garza, Ha, Garcia, Chen, & Russo-Neustadt, 2004; Van Praag et al., 2005), morphine (Alaei et al., 2006) and prenatal ethanol exposure (Chirstie et al., 2005). To our knowledge this is the first time that the effect of physical exercise was examined on cognitive deficits induced by maternal deprivation.

The present experiments show a clear deficit in spatial, recognition and avoidance memory in rats exposed to postnatal maternal deprivation, and a reversal or reduction of some of these effects in animals submitted to forced treadmill exercise. The reversal was complete in the IA task and partial in the MWM task. Exercise had no influence on the deprivation induced deficit of the least aversive of the three tasks, the OR test. In the present study, as in a previous paper (Mello et al., 2008), the effects of exercise *per se* on memory parameters were barely noticeable, and limited to the spatial task. This is at odds with other reports using different forms of physical exercise (Ang et al., 2006; Radak et al., 2006; Uysal et al., 2005; van Praag et al., 2005), but coincident with others reporting no effect of physical exercise on spatial memory (Barnes et al., 1991). This strengthens the finding of the effect of exercise on the cognitive deficits induced by early maternal deprivation.

It is likely that both the deleterious effect of maternal deprivation and the partially corrective effect of exercise are secondary to influences on some of the many modulatory systems that regulate both memory consolidation and retrieval (Izquierdo et al., 2006; McGaugh, 2004). In this respect, the hypothalamus–pituitary–adrenal axis is a major candidate. That system is enhanced in animals submitted to postnatal maternal deprivation (Huot et al., 2002; Plotsky et al., 2005) and this enhancement has been associated with memory deficits (Aisa, Tordera, Laceras, Del Río, & Ramírez, 2007; see McGaugh, 2004). Campbell, Rakhshani, Fediuc, Bruni, and Riddell (2008) reported that physical exercise activates the HPA axis and it is known that physical exercise reverts the hypoactivity of HPA caused by chronic administration of corticosterone in rats (Kim et al., 2008). Importantly, some authors have found that wheel running can reduce the HPA axis responses to mild stressors (Droste, Chandramohan, Hill, Linthorst, & Reul, 2007; Droste et al., 2003).

Certainly, the corrective effect of exercise on the memory deficits induced by maternal deprivation did correlate with the aversiveness and presumable stress associated with each task: more marked in IA, less marked in the MWM, inexistent in OR. An effect of either maternal deprivation or physical exercise on “core” molecular mechanisms of memory formation or retrieval (see Izquierdo et al., 2006), or on cognition itself, is by all means unlikely.

In summary, the decreasing order of the effect of exercise on the memory disturbances induced by deprivation roughly follows the descending degree of stress associated with each task (IA > MWM > OR). Maternal deprivation is known to hinder hormonal mechanisms involved in coping with stress.

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#### References

- Aisa, B., Tordera, R., Laceras, B., Del Río, J., & Ramírez, M. J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology*, *32*, 256–266.
- Alaei, H., Borjeian, L., Azizi, M., Orian, S., Pourshanzari, A., & Hanninen, O. (2006). Treadmill running reverses retention deficit induced by morphine. *European Journal of Pharmacology*, *536*, 138–141.
- Alaei, H., Moloudi, R., & Sarkaki, A. R. (2007). Effects of treadmill running on mid-term memory and swim speed in the rat with Morris water maze test. *Journal of Bodywork Movement Therapies*, *12*, 72–75.
- Ang, E. T., Dawe, G. S., Wong, P. T. H., Moochhala, S., & Ng, Y. K. (2006). Alterations in spatial learning and memory after forced exercise. *Brain Research*, *1113*, 186–193.
- Ang, E. T., & Gomez-Pinilla, F. (2007). Potential therapeutic effects of exercise to the brain. *Current Medicinal Chemistry*, *14*, 2564–2571.
- Ang, E. T., Wong, P. T. H., Moochhala, S., & Ng, Y. K. (2003). Neuroprotection associated with running: Is it a result of increased endogenous neurotrophic factors. *Neuroscience*, *118*, 335–345.
- Barnes, C. A., Forster, M. J., Fleshner, M., Ahanotu, E. N., Laudenslager, M. L., Mazzeo, R. S., et al. (1991). Exercise does not modify spatial memory, brain autoimmunity, or antibody response in aged F-344 rats. *Neurobiology of Aging*, *12*, 47–53.
- Benetti, F., Andrade de Araújo, P., Sanvitto, G. L., & Lucion, A. B. (2007). Effects of neonatal novelty exposure on sexual behavioural, fear, and stress-response in adult rats. *Developmental Psychobiology*, *49*, 258–264.
- Benetti, F., Mello, P. B., Monteiro, S. C., Bonini, J. S., Cammarota, M., & Izquierdo, I. (2009). Early postnatal maternal deprivation in rats induces cognitive impairment in adult life; the deficit is reversed by donepezil and galantamine. *International Journal of Developmental Neuroscience*, *2*, 59–64.
- Brooks, G. A., & White, T. P. (1978). Determination of metabolic and heart rate responses of rats in treadmill exercise. *Journal of Applied Physiology*, *45*, 1009–1015.
- Cammarota, M., Bernabeu, R., Izquierdo, I., & Medina, J. H. (1996). Reversible changes in hippocampal 3H-AMPA binding following inhibitory avoidance training in the rat. *Neurobiology of Learning and Memory*, *66*, 85–88.
- Campbell, J. E., Rakhshani, N., Fediuc, S., Bruni, S., & Riddell, M. C. (2008). Voluntary wheel running initially increases adrenal sensitivity to adrenocorticotrophic hormone, which is attenuated with long-term training. *Journal of Applied Physiology*, *106*, 66–72.
- Chen, H., Lin, L., Yu, L., Liu, Y., Kuo, Y., Huang, A., et al. (2007). Treadmill exercise enhances passive avoidance learning in rats: The role of down-regulated serotonin system in the limbic system. *Neurobiology of Learning and Memory*, *89*, 489–496.
- Chirstie, B. R., Swann, S. E., Fox, C. J., Froc, D., Lieblich, S. E., Redila, V., et al. (2005). Voluntary exercise rescues deficits in spatial memory and long-term potentiation in prenatal ethanol-exposed male rats. *European Journal of Neuroscience*, *21*, 1719–1726.
- Cirulli, F., Micera, A., Alleva, E., & Aloe, L. (1998). Early maternal separation increases NGF expression in the developing rat hippocampus. *Pharmacology Biochemistry and Behavior*, *59*, 853–858.
- Cotman, C. W., & Berchtold, N. C. (2002). Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends in Neuroscience*, *25*, 295–301.
- Dishman, R. K. (1997). Brain monoamines, exercise, and behavioral stress: Animals models. *Medicine and Science in Sports and Exercise*, *29*, 63–74.
- Dishman, R. K., Renner, K. J., Youngstedt, S. D., Reigle, T. G., Bunnell, B. N., Burke, K. A., et al. (1997). Activity wheel running reduces escape latency and alters monoamine levels after footshock. *Brain Research Bulletin*, *42*, 399–406.
- Droste, S. K., Chandramohan, Y., Hill, L. E., Linthorst, A. C. E., & Reul, J. M. H. M. (2007). Voluntary exercise impacts on the rat hypothalamic–pituitary–adrenocortical axis mainly at the adrenal level. *Neuroendocrinology*, *86*, 26–37.
- Droste, S. K., Gesing, A., Ulbricht, S., Müller, M. B., Linthorst, A. C., & Reul, J. M. H. M. (2003). Effects of long-term voluntary exercise on the mouse hypothalamic–pituitary–adrenocortical axis. *Endocrinology*, *144*, 3012–3023.
- Ennauer, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioral Brain Research*, *31*, 47–59.
- Frick, K. M., Stillner, E. T., & Berger-Sweeney, J. (2000). Mice are not little rats: Species differences in a one-day water maze task. *NeuroReport*, *11*, 3461–3465.
- Friedland, R. P., Fritsch, T., Smyth, K. A., Koss, E., Lerner, A. J., Chen, C. H., et al. (2001). Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 3440–3445.
- Garza, A. A., Ha, T. G., Garcia, C., Chen, M. J., & Russo-Neustadt, A. (2004). Exercise, antidepressant treatment, and BDNF mRNA expression in the aging brain. *Pharmacology Biochemistry and Behavior*, *77*, 209–220.
- Hout, R. L., Plotsky, P. M., Lenox, R. H., & McNamara, R. K. (2002). Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Research*, *950*, 52–63.

- Izquierdo, I., Bevilaqua, L. R., Rossato, J. I., Bonini, J. S., Medina, J. H., & Cammarota, M. (2006). Different molecular cascades in different sites of the brain control memory consolidation. *Trends in Neuroscience*, 29, 496–505.
- Kennedy, S. L., Smith, T. P., & Fleshner, M. (2005). Resting cellular and physiological effects of freewheel running. *Medicine and Science in Sports and Exercise*, 37, 79–83.
- Kim, H. G., Lim, E. Y., Jung, W. R., Shin, M. K., Ann, E. S., & Kim, K. L. (2008). Effects of treadmill exercise on hypoactivity of the hypothalamo–pituitary–adrenal axis induced by chronic administration of corticosterone in rats. *Neuroscience Letters*, 434, 46–49.
- Kosten, T. A., Lee, H. J., & Kim, J. J. (2007). Neonatal handling alters learning in adult male and female rats in a task-specific manner. *Brain Research*, 118, 144–153.
- Krugers, H. J., Douma, B. R., Andringa, G., Bohus, B., Korf, J., & Luiten, P. G. (1997). Exposure to chronic psychosocial stress and corticosterone in the rats: Effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus*, 7, 427–436.
- Kuma, H., Miki, T., Matsumoto, Y., Gu, H., Li, H. P., Kusaka, T., et al. (2004). Early maternal deprivation induces alterations in brain-derived neurotrophic factor expression in the developing rat hippocampus. *Neuroscience Letters*, 372, 68–73.
- Laurin, D., Verreault, R., Lindsay, J., Macpherson, K., & Rockwood, K. (2001). Physical activity and risk of cognitive impairment and dementia in elderly persons. *Archives of Neurology*, 58, 498–504.
- Lehmann, J., Pryce, C. R., Bettschen, D., & Feldon, J. (1999). The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. *Pharmacology Biochemistry and Behavior*, 64, 705–715.
- Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropic responses and norepinephrine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology*, 12, 5–12.
- McGaugh, J. L. (2004). The amygdale modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience*, 27, 1–28.
- McIntosh, J., Anisman, H., & Merali, Z. (1999). Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: Gender-dependent effects. *Brain Research*, 113, 97–106.
- Mello, P. B., Benetti, F., Cammarota, M., & Izquierdo, I. (2008). Effects of acute and chronic exercise and stress on different types of memory in rats. *Anais da Academia Brasileira de Ciências*, 80, 301–309.
- Mirescu, C., & Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus*, 16, 233–238.
- Mitra, R., & Sapolsky, R.M. (in press). Effects of enrichment predominate over those of chronic stress on fear related behavior in male rats. *Stress*.
- Moraska, A., Deak, T., Spencer, R. L., Roth, D., & Fleshner, M. (2000). Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *American Journal of Physiology – Regulatory, Integrative Comparative Physiology*, 279, 321–329.
- Morris, R. T., Fine, D. M., Lees, S. J., Booth, F. W., Link, C. D., Ferrario, C. M., et al. (2007). Exercise training prevents development of cardiac contractile dysfunction in hypertensive TG (mREN-2)27 rats. *Journal of the American Society of Hypertension*, 1, 393–399.
- Myskiw, J. C., Rossato, J. I., Bevilaqua, L. R., Medina, J. H., Izquierdo, I., & Cammarota, M. (2008). On the role of mTOR in recognition memory. *Neurobiology of Learning and Memory*, 89, 338–351.
- Oitzl, M. S., Workel, J. O., Flutterm, M., Frosch, F., & de Kloet, E. R. (2000). Maternal deprivation affects behavior from youth to senescence: Amplification of individual differences in spatial learning and memory in senescence Brown Norway rats. *European Journal of Neuroscience*, 12, 3771–3780.
- Paratcha, G., Furman, M., Bevilaqua, L., Cammarota, M., Vianna, M., de Stein, M. I., et al. (2000). Involvement of hippocampal PKCbeta isoform in the early phase of memory formation of an inhibitory avoidance learning. *Brain Research*, 855, 199–205.
- Plotsky, P. M., Thirivikraman, K. V., Nemeroff, C. B., Caldji, C., Sharma, S., & Meaney, M. J. (2005). Long-term consequences of neonatal rearing on central corticotrophin-releasing factor systems in adult male rat offspring. *Neuropsychopharmacology*, 30, 2192–2204.
- Radak, Z., Told, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., et al. (2006). The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. *Neurochemistry International*, 49, 387–392.
- Renard, G. M., Suárez, M. M., Levin, G. M., & Rivarola, M. A. (2005). Sex differences in rats: Effects of chronic stress on sympathetic system and anxiety. *Physiology and Behavior*, 85, 363–369.
- Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B. A., & Riva, M. A. (2002). Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Molecular Psychiatry*, 7, 609–616.
- Rossato, J. I., Bevilaqua, L. R. M., Lima, R. H., Medina, J. H., Izquierdo, I., & Cammarota, M. (2006a). On the participation of hippocampal p38 mitogen-activated protein kinase in extinction and reacquisition of inhibitory avoidance memory. *Neuroscience*, 143, 15–23.
- Rossato, J. I., Bevilaqua, L. R. M., Medina, J. H., & Cammarota, M. (2006b). Retrieval induces hippocampal-dependent reconsolidation of spatial memory. *Learning and Memory*, 13, 431–440.
- Rossato, J. I., Bevilaqua, L. R. M., Myskiw, J. C., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learning and Memory*, 14, 36–46.
- Rots, N. Y., de Jong, J., Workel, J. O., Levine, S., Cools, A. R., & de Kloet, E. R. (1996). Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. *Journal of Neuroendocrinology*, 8, 501–506.
- Schmidt, M. Y., Oitzl, M. S., Levine, S., & de Kloet, E. R. (2002). The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. *Brain Research Developmental Brain Research*, 139, 39–49.
- Scopel, D., Fochesatto, C., Cimarosti, H., Rabbo, M., Belló-Klein, A., Salbego, C., et al. (2006). Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. *Brain Research Bulletin*, 71, 155–159.
- Silva, S. G., Doná, F., Fernandes, M. J. S., Scorza, F. A., Cavalheiro, E. A., & Arida, R. M. (in press). Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression. *Brain and Development*.
- Simões, P. A., Zamarioli, A., Blóes, P., Mazzocato, F. C., Pereira, L. H., Volpon, J. B., et al. (2008). Effect of treadmill exercise on lumbar vertebrae in ovariectomized rats: Anthropometrical and mechanical analyses. *Acta of Bioengineering and Biomechanics*, 10, 39–41.
- Todeschin, A. S., Winkelmann-Duarte, E. C., Jacob, M. H., Aranda, B. C., Jacobs, S., & Fernandes, M. C. et al. (in press). Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior*.
- Uysal, N., Tugyan, K., Kayatekin, B. M., Acikgoz, O., Bagriyanik, H. A., Gonenc, S., et al. (2005). The effects of regular aerobic exercise in adolescent period on hippocampal neuron density, apoptosis and spatial memory. *Neuroscience Letters*, 383, 241–245.
- Van Praag, H., Shubert, T., Aha, C., & Gage, F. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *Journal of Neuroscience*, 25, 8680–8685.
- Veena, J., Srikumar, B. N., Mahati, K., Bhaga, V., Raju, T. R., & Shankaranarayana Rao, B. S. (2007). Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. *Journal of Neuroscience Research*, 87, 831–843.
- Winter, B., Breitenstein, C., Mooren, F. C., Voelker, K., Fobker, M., Lechtermann, A., et al. (2007). High impact running improves learning. *Neurobiology of Learning and Memory*, 87, 597–609.