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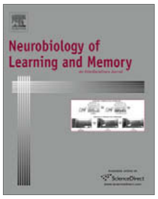

Physical exercise can reverse the deficit in fear memory induced by maternal deprivation

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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.

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

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positive 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 (±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-