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BENEFICIAL INFLUENCE OF PHYSICAL EXERCISE FOLLOWING STATUS EPILEPTICUS IN THE IMMATURE BRAIN OF RATS

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Abstract—Studies in adult animals have demonstrated a beneficial effect of physical exercise on epileptic insults. Although the effects of physical exercise on the mature nervous system are well documented, its influence on the developing nervous system subjected to injuries in childhood has been little explored. The purpose of our study was to investigate whether a physical exercise program applied during brain development could influence the hippocampal plasticity of rats submitted to *status epilepticus* (SE) induced by pilocarpine model at two different ages of the postnatal period. Male Wistar rats aged 18 (P18) and 28 (P28) days were randomly divided into four groups: Control (CTRL), Exercise (EX), SE (SE) and SE Exercise (SE/EX) (n = 17 per group). After the aerobic exercise program, histological and behavioral (water maze) analyses were performed. Our results showed that only animals subjected to pilocarpine-induced SE at P28 presented spontaneous seizures during the observational period. A significant reduction in seizure frequency was observed in the SE/EX group compared to the SE group. In adulthood, animals submitted to early-life SE displayed impairment in long-term memory in the water maze task, while the exercise program reversed this deficit. Reduced mossy fiber sprouting in the dentate gyrus was noted in animals that presented spontaneous seizures (SE/EX vs SE). Exercise increased cell proliferation (Ki-67 staining) and anti-apoptotic response (bcl-2 staining) and reduced pro-apoptotic response (Bax staining) in animals of both ages of SE induction (P18/28). Exercise also modified the brain-derived neurotrophic factor (BDNF) levels in EX and SE/EX animals. Our findings indicate that in animals subjected to SE in the postnatal period a physical exercise program brings about beneficial effects on seizure frequency and hippocampal plasticity in later stages of life.

Keywords: physical exercise, status epilepticus, immature brain, brain development, epilepsy, neuroplasticity.

INTRODUCTION

Epilepsy is a neurological condition that occurs more frequently in children than in adults (Macleod and Appleton, 2007). Retrospective studies indicate a correlation between temporal lobe epilepsy in adult patients and the occurrence of seizures and *status epilepticus* (SE) in their childhood (Falconer, 1971; Sagar and Oxbury, 1987). Clinical (Lowenstein and Alldredge, 1998) and experimental (Sankar et al., 2000) investigations have shown that SE can lead to brain damage and negative effects on cognitive capacity later in life and to this end strategies to reduce the pathophysiological processes that occur after SE are needed. Although several pharmacological agents following SE (Wasterlain and Chen, 2008) have been investigated, non-pharmacological and experimental approaches such as physical exercise have been extensively explored for preventing and treating epilepsy (Acharya et al., 2008).

Data from animal and human studies, controlled trials, and epidemiological surveys suggest a role for exercise as a complementary form of therapy for epilepsy (for a review see Arida et al., 2008, 2013). Human studies have generally shown that regular physical exercise can decrease seizure frequency and improve cardiovascular and psychological health in people with epilepsy (Nakken et al., 1990, 1997; Eriksen et al., 1994; McAuley, 2001). Animal studies have been carried out to elucidate the mechanisms by which exercise can lead to positive effects in epilepsy. For instance, physical exercise reduces the development of amygdala-kindling (Arida et al., 1998) and the frequency of seizures in the pilocarpine model of epilepsy (Arida et al., 1999); increases interictal local cerebral metabolic rates for glucose in some brain regions related to vigilance and alertness (Arida et al., 2003); decreases CA1 hyperresponsiveness (Arida et al., 2004); and induces positive plastic changes in the hippocampal formation of rats with epilepsy (Arida et al., 2007a,b). Although beneficial effects of physical exercise in the mature epileptic brain have been reported, little is known about the impact of

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Abbreviations: BDNF, brain-derived neurotrophic factor; EDTA, ethylenediaminetetraacetic acid; MB, Millonigs's buffer; PBS, phosphate-buffered saline; SE, status epilepticus; SG, supragranular.

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an exercise program after SE early in life. Information about the subject is lacking and enriched environment has usually been employed to explore this issue. For instance, environmental enrichment improved cognitive function in rats submitted to SE at the time of weaning (Faverjon et al., 2002; Wang et al., 2007) or reduced spontaneous seizures and neuronal damage in the Q54 model of temporal lobe epilepsy (Manno et al., 2011). To our knowledge, in the only study that addressed this question treadmill running reversed the impairment of long-term memory in an object recognition task by rats subjected to early-life SE (Córdova et al., 2013). Therefore, the aim of this study was to investigate the potential benefits of an exercise program on the behavioral, morphological and functional hippocampal changes in adult rats subjected to SE early in life. To address this question we examined the influence of an aerobic exercise program following pilocarpine-induced SE at postnatal days 18 and 28 (P18 and P28 respectively).

EXPERIMENTAL PROCEDURES

Animals

Developing Wistar male rats aged P18 and P28 were used in this study. The colony room was maintained at 21 ± 2 °C with a 12-h light/dark schedule, and *ad libitum* food and water throughout the experiments. Animals were bred in our laboratory and the date of birth was considered day 0. The pups were housed with their mother in individual cages until weaning at postnatal day 21 (P21). The animals were selected randomly for the experimental (SE) or saline (control) groups. All experimental protocols were approved by the Ethics Committee of the Universidade Federal de São Paulo (protocol #0332/12), and all efforts were made to minimize animal suffering in accordance with the proposals of the International Ethical Guidelines for Biomedical Research (CIOMS, 1985).

Induction of SE

SE (n = 105; 57 animals at P18 and 48 animals at P28) was induced through the administration of pilocarpine (Sigma, St. Louis, MO, USA) (170 and 260 mg/kg, i.p. at P18 and P28 respectively), preceded 30 min by scopolamine methylnitrate (Sigma; 1 mg/kg, s.c.) to limit peripheral cholinergic effects (Turski et al., 1983). The latency (time elapsed from pilocarpine injection to the first behavioral epileptic manifestations), and duration of SE were observed. At P18, 30 of the 57 animals died during SE. The mortality rate 24 h after SE induction was 29.62% (eight of 27 animals died). At P28, 26 of 48 animals died during SE. The mortality rate 24 h after SE induction was 22.72% (five of 22 animals died). Therefore, 19 animals at P18 and 17 animals at P28 survived 48 h after SE. As demonstrated in previous study (Priel et al., 1996) the latency for the appearance of seizures in rats treated with pilocarpine after the 18th day of life is longer in younger animals when compared to adult rats. Therefore, following the SE period, we chose to monitor continuously the surviving animals from both ages of SE induction from P45 to P90 over 24 h for detection of spontaneous seizures, using a video system. Infrared emitting lights were used for video-recording animal activity during the dark periods. Spontaneous seizures ranging from stages 3 to 5 (according to Racine) were considered for statistical analysis. Control rats (n = 34) were injected at P18 (n = 17) and P28 (n = 17) with the same volume of 0.9% saline instead of pilocarpine preceded 30 min by scopolamine methylnitrate.

Physical exercise protocol

Animals in the exercise group were familiarized with the treadmill (Columbus Instruments, Columbus, Ohio, USA) over 3 days for 5 min/day at a speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly during running familiarization. To provide a measure of trainability, animals were rated on treadmill performance on a scale of 1–5 according to previous studies (Dishman et al., 1988; Arida et al., 2007a,b). Animals with a mean rating of 3 or higher were included in the exercise group. Animal excluded from the exercise group were not included to the control group. This procedure was used to avoid differences in stress levels between animals. Animals were submitted to a physical exercise program during their adolescent period, as previously described by Gomes da Silva et al. (2010). In brief, each exercise session started with a 5-min warm-up at 8–10 m/min. Running time and speed were gradually increased until 18 m/min for 60 min. Animals from the control group were moved to the experimental room and handled in the same way as animals from the exercise group (privation of water and food during treadmill exercise). Two animals submitted to SE at P18 were excluded for failing to adapt to the physical exercise program. Therefore, animals submitted to SE at P18 and P28 were randomly divided into four groups: control (CTRL), exercise (EX), SE and SE exercise (SE/EX) (n = 17 for each group). Animals submitted to SE at P18 were subjected to the exercise program between P21 and P90 and animals submitted to SE at P28 were subjected to the exercise program between P31 and P90. Behavioral analyses to determine seizure frequency were performed between P45 and P90. Briefly, of 17 animals in each group, six were used for the neo-Timm staining and 11 were used in a water maze task. Six of these 11 animals were used for histological analysis (Ki-67, Bax and Bcl-2) and five for brain-derived neurotrophic factor (BDNF) analysis.

Water maze test

To determine the learning and memory of animals from all groups (n = 11 from each group) the water maze test (between P57 and P60), similar to that described by Morris (1984) was performed. Animals were placed in the water maze for 120 s in order to find a platform hidden 1 cm below the surface of a pool (200 cm in diameter). Over 4 days (P56-P60), animals performed two trials per day (with a 10-min interval between trials). In each trial, animals began the test at different points (labeled N, S, E and W). A video-camera (Sony, Tokio, Japan) fixed above the water maze recorded all the experiments.