Int. J. Devl Neuroscience 31 (2013) 196–201

Contents lists available at SciVerse ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Low-intensity physical training recovers object recognition memory impairment in rats after early-life induced *Status epilepticus*

Sandro Daniel Córdova*, Cássio Morais Loss, Diogo Losch de Oliveira

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form 2 January 2013
Accepted 3 January 2013

Keywords:
Status epilepticus
Pilocarpine
Physical training
Learning
Memory

ABSTRACT

When it occurs early in life, *Status epilepticus* (SE) can cause behavioural and cognitive impairments in adulthood. Here, we evaluated the putative benefits of low-intensity treadmill training on long-standing cognitive impairment in rats submitted to SE early in life. Wistar rats were submitted to LiCl-pilocarpine-induced SE at P16. Animals from the trained group underwent a low-intensity treadmill protocol for 5 days per week for 4 weeks. At adulthood, rats subjected to early-life SE displayed impairment in long-term memory in an object recognition task, while the training protocol completely reversed this deficit. This result was associated with neither locomotor alterations nor changes in emotional behaviour; there were no differences between groups in the distance travelled, grooming or rearing in the open field test; there were also no differences between groups in the number of risk assessment, time spent in open arms in an elevated plus maze and number of entries into the open arms. These data suggest that physical exercise can ameliorate the long-standing recognition memory deficit induced by early-life SE, suggesting that it may be useful as a putative intervention for patients who suffered SE during infancy.

1. Introduction

Status epilepticus (SE) is a common childhood neurological emergency characterised as a seizure or repeated seizures that last more than 30 min (Chen and Wasterlain, 2006). Although SE can occur at all ages, the highest incidence is observed before 2 years of age (Shinnar et al., 1997; Singh et al., 2010). When it occurs early in life, SE can be potentially harmful to the brain (Wasterlain et al., 1993, 2002) and is associated with a higher incidence of neurological disorders later in life (Kwong et al., 2004).

The most frequent neurological dysfunctions associated with early-life SE include alterations in sociolinguistic and psychomotor development, cognitive impairments and alterations in emotional behaviour (Roy et al., 2011). Neurological disabilities observed in humans are very similar to those found in animal models of SE. Male rats that suffered SE at 16–20 days old showed long-term deficits in spatial learning in the water maze task (Cilio et al., 2003) and showed an impairment in aversive memory learning in an inhibitory avoidance task (de Oliveira et al., 2008). Furthermore, animals submitted to LiCl-pilocarpine-induced SE between the 2nd and 3rd weeks of life presented increased levels of anxiety in the elevated plus maze, as well as impaired performance in the water maze task 3 months after the insult (Kubová et al., 2004).

Animal studies and clinical trials have demonstrated that treatment of SE with traditional or new antiepileptic drugs (AEDs), such as phenytoin and levetiracetam, may stop seizure activity but fails to prevent the above-mentioned SE-induced cognitive and behavioural alterations later in life (Brandt et al., 2007; Temkin, 2001).It has been demonstrated that physical training may have benefits for the brain and can be used as a potential therapeutic strategy for different degenerative brain diseases (Ahlskog et al., 2011; Cotman and Berchtold, 2002; Lafenêtre et al., 2010; O'Callaghan et al., 2009). Aerobic training promoted reduction in the fall risk, increased mobility, and improved the quality of life in Parkinson's disease (PD) patients (Fisher et al., 2008; Herman et al., 2007). In addition, PD patients who participated in a training programme exhibited cognitive benefits in frontal lobe-dependent tasks (Cruise et al., 2011). For patients with Alzheimer's disease, 12 weeks of physical training led to an improvement in the performance of daily activities (Santana-Sosa et al., 2008). Benefits were also observed in several animal models, with improvements in cognitive function, elevation of hippocampal BDNF (Ke et al., 2011b; Sartori et al., 2009; Vaynman et al., 2004), and increased BrdU-labelled cells in the dentate gyrus of the hippocampus (Chae et al., 2009).

Although studies have demonstrated the beneficial effects of physical training on SNC, little is known about the effects of an exercise programme on cognitive changes in individuals who

* Corresponding author at: Departamento de Bioquímica, ICBS, UFRGS, Rua Ramiro Barcelos 2600-Anexo, CEP 90035-003, Porto Alegre, RS, Brazil. Tel.: +55 51 33085555, fax: +55 51 33085540.
E-mail address: sandrocordova@gmail.com (S.D. Córdova).

0736-5748/$36.00 © 2013 ISDN. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijdevneu.2013.01.002

suffered SE early in life. The aim of this study was to investigate the potential benefits of a low-intensity treadmill exercise protocol on behavioural alterations in adult rats subjected to LiCl-pilocarpine-induced SE early in life.

2. Experimental procedure

2.1. Materials

Pilocarpine hydrochloride was purchased from Sigma–Aldrich (USA). Fluoro-Jade B was purchased from Chemicon, Inc. (USA). Other chemicals were purchased from Nuclear (Brazil).

2.2. Subjects

Male Wistar rats (15 days postnatal) were obtained from a local breeding facility. The litters were culled to 8 pups. The day of birth was defined as day 0, and the animals were weaned on postnatal day 21. After weaning, animals were housed in standard polypropylene cages (4–5 animals per cage) with food and water *ad libitum* (21 ± 1 °C room temperature). Rats were kept under a 12:12 h light/dark cycle (lights on at 7:00 a.m.). The handling and care of the animals was conducted according to the Guide for Care and Use of Laboratory Animals from the National Institutes of Health (NIH Publications No. 80-23, revised 1996). All procedures in the present study were approved by the Committee of Ethics of the Universidade Federal do Rio Grande do Sul.

2.3. Induction of S. epilepticus

Rat pups were injected with a solution of LiCl (3 mEq/kg, i.p.) 12–18 h prior to pilocarpine hydrochloride administration (60 mg/kg, i.p.) on postnatal day 16 (Hirsch et al., 1992). Control animals were handled and housed in the same manner and received an equal volume of saline solution (0.9% NaCl). Rats were kept in individual plastic cages at nest temperature for seizure observation. The duration of SE was evaluated only by behavioural measures where SE was defined, according to Hirsch et al. (1992), as sustained orofacial automatisms, salivation, chewing, forelimb clonus, loss of the righting reflex and falling. The rats were allowed to spontaneously recover from SE. Each experimental group contained pups from several litters. According to Priel et al. (1996), the induction of SE at this age does not induce spontaneous seizures in adulthood.

2.4. Exercise protocol

Animals were divided into 4 groups: control (C) ($n = 10$), trained (T) ($n = 10$), *S. epilepticus* (SE) ($n = 11$) and *S. epilepticus*+trained (SE+T) ($n = 11$). Seven days after SE induction, animals from the T and SE+T groups were submitted to a low-intensity exercise protocol over 4 weeks, starting the familiarisation at P25 and the training programme at P27. Rats ran 30 min daily on one four-line treadmill for 5 days a week. The warm-up consisted of 2 m/min for the first 5 min, followed by 5 m/min for the next 5 min and 8 m/min in the last 20 min (Kim et al., 2003). Control and SE animals were left on the treadmill for 30 min, 5 days a week, with the treadmill stopped. Stimulation was not used to motivate rats to run; animals that refused to run were removed from the experiment (only 1 animal in the T group was excluded).

2.5. Behavioural tasks

After the end of the exercise protocol, animals were subjected to behavioural tasks. Open-field, object recognition and elevated plus maze tasks were conducted on P56, P57-58 and P60, respectively. For all behavioural procedures, animals were placed in the testing room (temperature 21 ± 2° C) 1 h before the beginning of the task to allow them to habituate to the environment and the researchers. Because rats are nocturnal, all tasks were performed between 6:00 p.m. and 10:00 p.m. The behavioural profile was recorded and analysed using the ANY-Maze® video-tracking system (Stoelting, CO). Between every trial, all apparatuses were cleaned with a 70% ethanol solution.

2.6. Open field

The test consisted of a circular wooden black arena with the dimensions of 60 cm × 50 cm (diameter × height). The floor was divided virtually into 28 squares (12 central and 16 peripheral). The testing room was illuminated by two lamps directed towards the ceiling. The light intensity was 15 lx in the centre of the arena. Each animal was individually placed in the periphery of the arena and was left to explore it freely for 15 min. Based on the findings of Eilam (2003), the following behavioural parameters were quantified:

(a) Levels of locomotor and exploratory activities: number of rearing and whole-body grooming behaviours, total distance travelled, distance travelled across time and total time spent in locomotion.
(b) Temporal organisation of locomotion: time in home-base (the square where animal remained for more time during the test), number of trips (departures from the home-base), trip length (distance/number of trips) and stops/trip (number of stops/number of trips).
(c) Spatial distribution of locomotion: distance travelled along the vicinity and locomoting time spent along the vicinity of the arena walls.

2.7. Object recognition task

The arena for the object recognition test was the same used in the open field task. Two glass objects of comparable size (10 cm high) were fixed to the apparatus 10 cm from the wall and 15 cm from each other. In the training session, animals were allowed to explore two identical objects (object A and A') for 5 min. Object exploration was considered to be any investigative behaviour (head orientation or sniffing), deliberate contact with the object at a distance of ≤2 cm, or when the animal touched the object with its nose. To evaluate short-term memory (STM), animals were allowed to explore one familiar and one novel object (object B) for 5 min at 3 h after the training session. Twenty-four hours after the training session, long-term memory (LTM) was assessed by changing object B for a new form (object C) and allowing the animals to explore them for 5 min (Bevins and Besheer, 2006). Memory was operationally defined through the discrimination index, which was calculated in absolute values as follows: (A/A + A')-(A'/A + A') for training session, (B/A + B) − (A/A + B) for STM, and (C/A + C) − (A/A + C) for LTM. Average speed, total time exploring objects and the distance travelled in the apparatus were also recorded.

2.8. Elevated plus-maze

The maze consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm), with arms of each type opposite to each other (Pellow et al., 1985). The maze was elevated to a height of 50 cm from the floor. The experiment was conducted in a room illuminated by red light. The light intensity in the centre of apparatus was 5 lx. Rats were placed into the centre of the maze facing a closed arm and were left free to explore it for 5 min. Number of arm entries, time spent in the arms, number of risk assessment behaviours (when the rodent is motionless in the centre or closed zone, but is stretched forward into the open arms with some but not all paws, returning then to the initial position) and total distance travelled were registered.

2.9. Data analysis

All data were expressed as the mean ± SEM and analysed by two-way ANOVA followed by Bonferroni *post hoc* test for unequal samples. For all parameters, $p < 0.05$ was considered significant.

3. Results

3.1. Characterisation of S. epilepticus

Within 2–5 min after pilocarpine administration, all LiCl-pilocarpine-treated animals started having behavioural changes consisting of defecation, salivation, body tremor, and scratching. This behavioural pattern progressed within 8–12 min to increased levels of motor activity and culminated in a convulsive SE in all LiCl-pilocarpine-treated animals. Convulsive SE consisted of sustained head nodding, orofacial automatisms, hyperextension of tail, elevated levels of salivation, chewing, and repetitive forelimb and hindlimb clonus. Two hours after SE onset, animals cycled through periods of forelimb clonus and periods of loss of the righting reflex with falling. All behavioural manifestations reported here for convulsive SE are in agreement with previous descriptions from Hirsch et al. (1992) and de Oliveira et al. (2008).

3.2. Effects of S. epilepticus and treadmill exercise on open field task

There was no significant effect of exercise, as well as SE, in all evaluated parameters of locomotor and exploratory activities (Fig. 1A). All groups travelled similar distances ($F(1,39) = 0.1059$; $p < 0.7466$) and performed similar numbers of whole-body grooming behaviours ($F(1,39) = 0.006574$; $p < 0.9358$) and rearing ($F(1,39) = 0.006574$; $p < 0.9658$). Moreover, there were no differences in the distance travelled across time ($F(42,280) = 1.018$;