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THE HIPPOCAMPUS PARTICIPATES IN THE CONTROL OF LOCOMOTION SPEED

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Abstract—The hippocampus role in sensory-motor integration remains unclear. In these experiments we study its function in the locomotor control. To establish the connection between the hippocampus and the locomotor system, electrical stimulation in the CA1 region was applied and EMG recordings were obtained. We also evaluated the hindlimbs and forelimbs kinematic patterns in rats with a penetrating injury (PI) in the hippocampus as well as in a cortex-injured group (CI), which served as control. After the PI, tamoxifen a selective estrogen receptor modulator (SERM) that has been described as a neuroprotector and antiinflammatory drug, or vehicle was administered. Electrical stimulation in the hippocampus produces muscle contractions in the contralateral triceps, when 6 Hz or 8 Hz pulse trains were applied. The penetrating injury in the hippocampus reduced the EMG amplitude after the electrical stimulation. At 7DPI (days post-injury) we observed an increase in the strides speed in all four limbs of the non-treated group, decreasing the correlation percentage of the studied joints. After 15DPI the strides speed in the non-treated returned to normal. These changes did not occur in the tamoxifen group nor in cortex-injured group. After 30 days, the nontreated group presented a reduction in the number of pyramidal cell layer neurons at the injury site, in comparison to the tam-treated group. The loss of neurons, may cause the interruption of the trisynaptic circuit and changes in the locomotion speed. Tamoxifen preserves the pyramidal neurons after the injury, probably resulting in the strides speed recovery. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

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Abbreviations: CI, cortex-injured group; PI, penetrating injury; PLM, pendulum-like movement.

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INTRODUCTION

The hippocampus has long been related to memory and learning, but there is evidence suggesting that it also plays a role in locomotion control (Bland and Oddie, 2001). Previous studies describe the connections between this structure and locomotor areas such as motor cortex, cerebellum, thalamus and diencephalon (Young et al., 2011). The hippocampus possesses an intrinsic theta wave generator that receives inputs from the medial septum, which works as a node and sends glutamatergic, cholinergic and GABAergic afferents that make synapses in the hippocampal interneurons (Frotscher and Leranth, 1985; Freund and Antal, 1988; Colom et al., 2005; Lu and Henderson, 2010; Fuhrmann et al., 2015), this regulates the different parameters such as, frequency or amplitude of the theta wave (Fuhrmann et al., 2015). An injury in the hippocampus can produce theta wave alterations that may result in kinematic dysfunction; this may be due to the interruption of the intra or parahippocampal circuitry. Tamoxifen has been described as a neuroprotective and an anti-inflammatory drug in the spinal cord (Tian et al., 2009; Guptarak et al., 2014; Mosquera et al., 2014; Salgado et al., 2015) and brain damage (Hallworth and Bland, 2004; Zhang et al., 2009; Cerciat et al., 2010; Arevalo et al., 2011; Franco Rodriguez et al., 2013) models, and it restores the theta activity after a penetrating injury (PI) in the hippocampus (Franco Rodriguez et al., 2013); thus, favoring the kinematic alterations that may have occurred after the PI. In this study we analyzed the effects of CA1 electrical stimulation on contralateral triceps muscle and the four limbs locomotion kinematics, as well as the number of hippocampal pyramidal neurons after a PI in the hippocampus, in tamoxifen-treated and non-treated rats.

METHODS

The experiments were carried out in rats in accordance with the Mexican Official Norm guidelines (NOM-062-ZOO-1999) and with the National Institute of Health Guide NIH Publication No. 8023 (revised in 1996) for the Care and Use of Laboratory Animals. In addition, the animal protocols were approved by the Institutional Bioethical Committee for the Institutional Animal Care and Use (IACUC) Reference number: C.I. 074-2014.

Experimental subjects

For the electrical stimulation procedure, 60-day-old female rats were used ($n = 3$). Kinematic analysis was performed on a different set of 60-day-old female rats separated into 3 groups: cortex-injured group or CI group ($n = 3$); hippocampus-injured + vehicle group or non-treated group ($n = 5$) and hippocampus-injured + tamoxifen group or tam-treated group ($n = 5$). The histological analysis was carried on the kinematic analysis subjects, immediately after the last kinematic assay (at 30 days after the penetrating injury).

Electrical stimulation procedure

Rats were properly anesthetized with a xylazine/ketamine intraperitoneal injection (6 mg/kg and 80 mg/kg respectively), to maintain the anesthetized state, half dose injections were administered every 30 min throughout the experiment. Once the subjects did not present pain reflexes, they were placed in a stereotactic frame, an incision through the middle line was made and cavities into the skull and over the stimulation site coordinates were drilled. The stimulation electrode was placed 2 mm to the left from the middle line, 3 mm behind bregma and 2.5 mm deep. Once in place, 0.01 ms, 12v and 2–8 Hz pulse trains were applied. The EMG recordings were obtained from the contralateral triceps muscle, amplified ×1000 and bandpassed from 3 to 500 Hz. After the recordings, the subjects were euthanized.

Penetrating injury procedure

The subjects were deeply anesthetized with a xylazine/ketamine intraperitoneal injection (6 mg/kg and 80 mg/kg respectively). After verifying the absence of pain reflexes, an incision over the middle line was made and a cleft was drilled into the skull to expose the meninges. A 0.5 mm in diameter stainless steel cannula was positioned 3 mm to the left and 5 mm behind bregma, lowered to a 4-mm depth for the hippocampus-injured groups and to 2 mm in the cortex-injured group, displaced rostrally 2.5 mm (−2.5 from bregma) and finally pulled out. Bleeding was stopped by applying pressure over the injury with a gel foam sponge. After the procedure, the scalp incision was sutured and prophylactic antibiotic and analgesic measures were taken.

Kinematic recordings

Kinematic patterns were obtained before the PI and 7, 15 and 30 days post-injury (DPI). Three joints of all four limbs were studied (hip, knee and ankle in the hindlimbs; shoulder, elbow and wrist in the forelimbs) plus the pendulum-like movement (PLM) that results from drawing a line between the superior joint and the lower one on each limb (Fig. 1). To reconstruct the kinematic patters for each joint and pendulum-like movement, angular values had to be obtained, using ink marks as illustrated in the rat scheme (Fig. 1). Later on, video recordings of the animals while walking on a transparent acrylic tunnel were taken with a 120 fps/720p capable camera. The video obtained was decomposed into images and analyzed frame by frame to determine the joint coordinates. These values were introduced into a software developed in our laboratory to calculate the angle aperture of each of the studied joints and the pendulum-like movement.

Kinematic analysis

A control pattern was calculated for every joint and for the pendulum-like movement by averaging three steps from each subject in all three groups before the penetrating injury. The crossed correlation analysis was performed between the control pattern and three steps from each subject on all groups before the penetrating injury (to establish a base line) and 7, 15 and 30 DPI (Lee Rodgers et al., 1988). Stride duration and length were also determined to calculate the speed of each step in all four limbs, prior and 7, 15 and 30 DPI.

The correlation percentage is given by

$$r_{AB} = \frac{\sum_0^n (A_i * B_i)}{\sqrt{\sum_0^n A_i^2 * \sum_0^n B_i^2}} * 100$$

where "r" is the correlation percentage, "A_i" is the angular value of the control pattern at a specific time, and "B_i" is

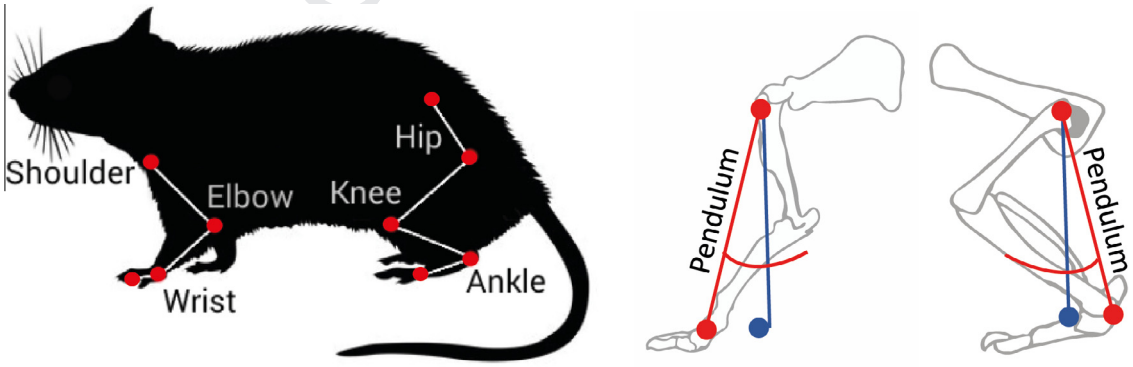

Fig. 1. Kinematic parameters. Schematic representation of the evaluated kinematic parameters. The studied joints were: shoulder, elbow, wrist, hip, knee and ankle, as well as the PLM.

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