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Correlation between behavioral deficits and decreased brain-derived neurotrofic factor in neonatal meningitis

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ABSTRACT

We investigated the correlation between memory impairment and hippocampal brain-derived neurotrophic factor (BDNF) levels in adult rats submitted to experimental meningitis (*Streptococcus pneumoniae*) in the neonatal period. Sixty days after inoculation the animals were submitted to the behavior tasks and hippocampal BDNF protein were evaluated. In the meningitis group, there was impairment in habituation and avoidance memory and a decrease in the BDNF levels. The decrease in hippocampal BDNF levels correlated to impairment in memory in adult animals submitted to experimental meningitis in the neonatal period.

1. Introduction

Neonatal bacterial meningitis, characterized by an intense inflammation of the subarachnoid and ventricular spaces, particularly when caused by *Streptococcus pneumoniae*, is associated with substantial mortality and severe morbidity (Harvey et al., 1999; Bellac et al., 2007). Therapy with highly active antibiotics is only partially effective in preventing death in patients with bacterial meningitis (Daoud et al., 1995; Goetghebuer et al., 2000). Even with optimal treatment up to 40% of survivors suffer from neurologic sequelae after meningitis, including sensory-motor deficits, cerebral palsy, seizure disorders, mental retardation, and learning impairments (Grimwood et al., 2000; Merkelbach et al., 2000; Schmidt et al., 2006). In the meningitis animal model, adult survivors mice presented learning and memory impairment after 7 days of induction and persisted after 180 days (Wellmer et al., 2000).

Neuropsychological evidence in humans and animal models suggests that the damage to the hippocampus contributes for impairing learning and memory (Izquierdo and Medina, 1997). However, dying from bacterial meningitis, necrotic and apoptotic neuronal cell death, preferentially located in the hippocampal formation, are regularly observed at autopsy and in animal models of bacterial meningitis (Leib et al., 2001, 2003; Nau et al., 1999). This damage tissue may be caused by direct toxicity of bacterial compounds and by the inflammatory reaction of the host, and mediated by free radicals, excitatory amino acids, and caspases [4, 9, 12, 20]. In infant rats infected with *S. pneumoniae,* histopathological examination of the neuronal injury in the dentate gyrus of the hippocampus showed that *S. pneumoniae* caused predominantly classical apoptotic cell death (Bifrare et al., 2003).

Neurotrophin brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and the most widespread growth factor in the brain. Thus, BDNF regulates the neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity and it is highly expressed in the hippocampus. Recently, it was demonstrated that in adulthood rats the hippocampal BDNF levels were positively correlated with the ability to learn (Francia et al., 2006). In this context, the aim of this study was to evaluate habituation and aversive memory correlating with BDNF levels in the hippocampus of adult rats submitted to experimental meningitis by inoculation of *S. pneumoniae* during the neonatal period.

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2. Materials and methods

Wistar rats, 3–4 days-old, used for the experiments were obtained from our breeding colony. The animals were housed with food and water available *ad libitum*, and maintained on a 12-h light/dark cycle (lights on at 7 a.m.). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and approved by the Animal Care and Experimentation Committee of UNESC, Brazil.

On experimental day 1, thirty one rats divided in two groups (meningitis group: 19 animals; sham group: 12 animals) underwent a magna cistern tap with a 23-gauge needle. The position of the needle was verified by the free flow of clear cerebral spinal fluid (CSF). *S. pneumoniae* (ATCC 6303) was cultured overnight in 10 mL Todd Hewitt broth medium, diluted in fresh medium, and grown for another 6 h (35 °C, CO_2 5.5%) to the logarithmic phase. The culture was centrifuged for 10 min at 5000×*g*, and resuspended in sterile saline to the desired concentration and used for intracisternal injection (Irazuzta et al., 2000; Grandgirard et al., 2007), containing 1×10^6 cfu/mL (Trampuz et al., 2007). CSF was withdrawn and the animals received either 10 µL of sterile saline as a placebo or an equivalent volume of the *S. pneumoniae* (Grandgirard et al., 2007). At the time of inoculation or not, both groups received fluid replacement (Irazuzta et al., 2000). Meningitis (meningitis group) or not (sham group) was documented by a quantitative culture of 5 µL of CSF obtained by puncture of the cisterna magna at 16 h after infection or not, followed by the initiation of the antibiotic treatment in both groups (ceftriaxone, 100 mg/kg body weight, s.c., bid) for 7 days (Grandgirard et al., 2007). Mortality in the sham group was 16.6% (2 animals) and in the meningitis group 47.3% (9 animals). Sixty days after inoculation the animals were submitted to the open field and the step-down inhibitory avoidance tasks ($n=10$ per group).

The habituation to the open-field task was carried out in a 40×60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 equal rectangles by black lines. The animals were gently placed on the left rear quadrant and left to explore the arena for 5 min (training session), and 24 h later were submitted again to a similar open-field session (test session). The crossings of the black lines and the rearings performed in both sessions were counted (Tuon et al., 2008; Comim et al., 2009). The step-down inhibitory avoidance task was carried out in the apparatus with a 50×25×25 cm acrylic box with the floor consisting of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7 cm-wide by 2.5 cm-high platform was placed on the floor of the box against the left wall. In the training trial, the animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, the animals received a 0.4 mA, 2.0 s foot shock. A retention test trial was performed 24 h after training. The retention test trial was procedurally identical to training, except that no foot shock was performed (Tuon et al., 2008). Immediately after the behavioral test, the rats were killed and the hippocampi were removed and stored at −80 °C. BDNF levels in the hippocampus were quantified using enzyme-linked immunosorbent assay (ELISA) and standard protocols (ChemiKime Brain Derived Neurotrophic Factor, Sandwich ELISA, Chemicon, USA), according to Comim et al. (2010).

Data from the open-field task were analyzed with Student's *t* test and expressed as mean ± S.D. Data from the inhibitory avoidance task are reported as median and interquartile ranges, and comparisons among groups were performed using Mann–Whitney *U* tests. Data within individual groups were analyzed by Wilcoxon tests. In all comparisons, $p<0.05$ indicated statistical significance. The correlation coefficient was performed using the Pearson and Spearman correlation coefficient. All analyses were realized with SPSS version 15.0.

3. Results

Fig. 1 shows the open field (Fig. 1A) and the step-down inhibitory avoidance (Fig. 1B) tasks. In Fig. 1A, there were no differences between training and test sessions in the number of crossings ($t=-3.981$; $df=120$; $p=0.0612$) and rearings ($t=-2.801$; $df=20$; $p=0.0692$) in the meningitis group. The sham group presented significant training-test differences in crossings ($t=10.113$; $df=10$; $p=0.0001$) and rearings ($t=5.581$; $df=10$; $p=0.0001$). We also observed that there were no alterations in the motor and exploratory activity during the training session, between groups evaluated. Fig. 1B demonstrates that the sham group presented significant training-test differences in the step-down latency ($Z=-3.409$; $p=0.001$). In addition, there were no differences between training and test sessions in the step-down latency ($Z=-1.769$; $p=0.077$) in the meningitis group.

Fig. 2 shows that there was a decrease in the BDNF levels in the hippocampus of rats submitted to meningitis ($t=2.921$; $df=18$; $p=0.009$) compared with sham. In Fig. 3, we demonstrate that the Pearson correlation coefficient between crossings/BDNF levels was −0.864 (Fig. 3A) with $p=0.0001$. The Spearman correlation coefficient between latency/BDNF was 0.883 with $p=0.0001$ (Fig. 3B).

4. Discussion

We demonstrated that adult meningitis survivors rats, when submitted to bacterial inoculation in the neonatal period, presented

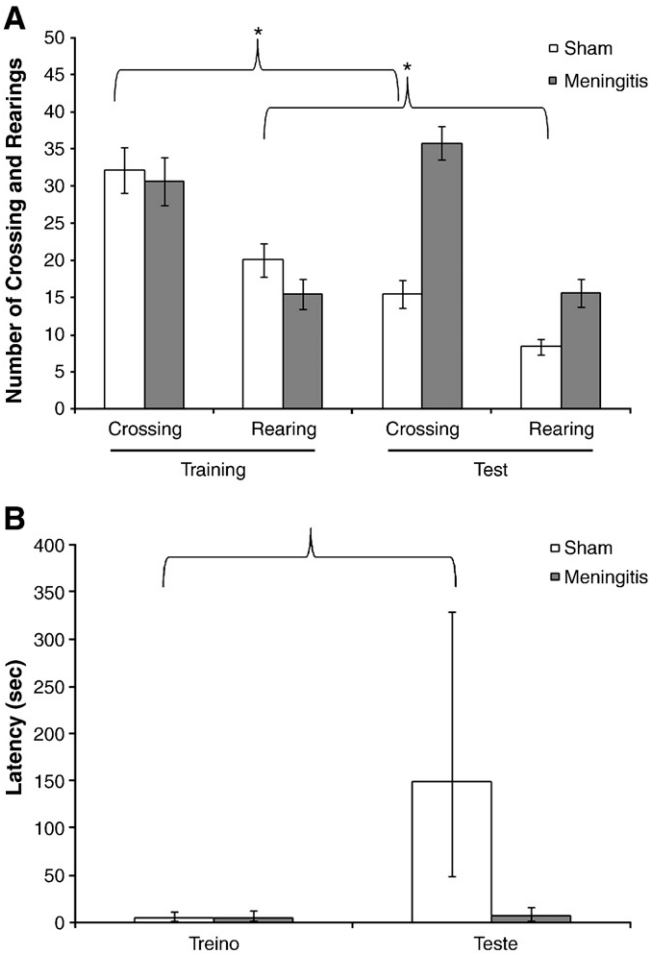

Fig. 1. Effects of neonatal meningitis in the number of crossings and rearings of survivor adult rats subjected to the open field test (A) and the step-down inhibitory avoidance test (B). Bars represent means ± S.D. in the habituation in the open field test and median and interquartile ranges in the inhibitory avoidancy test. *$p<0.05$ vs. training ($n=10$).