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Aversive memory in sepsis survivor rats

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Abstract Sepsis is an infectious insult resulting in disturbances in the normal regulation of many organic systems, including the central nervous system. This study aims to evaluate aversive memory as well as its variances—posttraumatic memory and memory of extinction—in survivor rats submitted to sepsis by cecal ligation and perforation (CLP) at 10, 30, and 60 days after CLP, utilizing the inhibitory avoidance (IA) task paradigm. Male Wistar rats underwent either CLP or sham surgery under anesthesia. Sepsis group received antibiotics and fluid support, whereas sham group received only fluid replacement. The rats were divided in four different tasks: (1) aversive memory after 10, 30, and 60 days after CLP; (2) memory of extinction 60 days after CLP; (3) aversive memory–two trainings paradigm 10 days after CLP; and (4) posttraumatic memory 10 days after CLP. The aversive memory was impaired at 10, 30, but not 60 days after CLP, However, no damage was found in aversive memory after two training sessions. Additionally, there was no damage to the memory of extinction 60 days after CLP. Posttraumatic memory impairment was also observed. In this regard, we believe that our results provide relevant insights into the mechanisms involved in the cognitive deficits associated with sepsis.

Keywords Sepsis · Brain · Aversive memory · Rat

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Introduction

Sepsis is a frequent, severe condition accounting for approximately 750,000 cases per year in North America (Angus et al. 2001). It is a stressful condition resulting in disturbances in the normal regulation of the neuroendocrine, metabolic, behavioral, and immunologic systems. Central nervous system (CNS) dysfunction secondary to sepsis can occur in 8–70% of septic patients (Sprung et al. 1990). Thus, survivors from the intensive care unit (ICU) presented long-term cognitive impairment, including alterations in memory, which was associated with a decrease in quality of life (Rothenhausler et al. 2001). In animal model of sepsis, rat survivors also presented long-term cognitive impairment, mainly in aversive memory, after 10 and at least up to 30 days of sepsis induction by cecal ligation and perforation (CLP) (Barichello et al. 2005, 2007; Tuon et al. 2008).

The aversive memory is evaluated through step-down inhibitory avoidance (IA) task paradigm. This task represents one of the major determinants of survival behavior in all species (Gold 1986), corresponding to many important examples of learning in humans. It is with the use of IA and its variations that we can evaluate posttraumatic memory and the memory of extinction. Aversively motivated learning is influenced by neuromodulators and hormones related to emotional aspects of the training experience (Izquierdo et al. 2006). In this context, this study aimed to evaluate the aversive memory 10, 30, and 60 days after

sepsis induction and aversive memory with two training sessions paradigm after 10 days. Also 60 days after CLP, aversive memory variances—the posttraumatic aversive memory and memory of extinction—were assessed.

Materials and methods

Under anesthesia (ketamine, 80 mg/kg; xylazine, 10 mg/kg), male Wistar rats (300–350 g) underwent either CLP (sepsis group) and sham operation (control group) as previously described (Comim et al. 2008, 2010). The animals were housed five each in a cage with food and water available ad libitum and were maintained on a 12-h light/dark cycle (lights on at 7:00 am). After surgery, the sepsis group received "basic support" (saline at 50 mL/kg immediately and 12 h after CLP plus ceftriaxone at 30 mg/kg and clindamycin at 25 mg/kg every 6 h over a total of 3 days). The sham-operated group received only saline, 50 mL/kg, immediately and 12 h after surgery and the volume of saline corresponded to antibiotic administration. Survival in the sham group was 100%, and in the sepsis group, 60%. The rats were divided in four different experimental protocols to evaluate: (1) aversive memory after 10, 30, and 60 days after surgery; (2) memory of extinction 60 days after surgery; (3) aversive memory–two trainings paradigm 10 days after surgery; and (4) posttraumatic memory 10 days after surgery. The behavioral tests were realized utilizing as base the step-down IA and performed by the same person blind to the groups (sham or CLP). All experimental procedures involving animals were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and with the approval of the local ethics committee.

The step-down IA task evaluates aversive memory. Briefly, the training apparatus is a 50 × 25 × 25 cm acrylic box whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform is placed on the floor of the box against the left wall. In the training session, 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 stopwatch. Immediately after stepping down on the grid, the animals received a 0.4-mA, 2.0-s foot shock and were returned to their home cage. A retention test session was performed 24 h after training. The retention test session was procedurally identical to training, except that no foot shock was presented. The retention test step-down latency (maximum, 180 s) was used as a measure of inhibitory avoidance retention.

Extinction of IA memory is realized to extinguish the avoidance response (involves the aversive memory). The rats were submitted to five non-reinforced IA test sessions (24, 48, 72, 96, 120 h) after training. To this purpose, IA-trained animals were put back on the training box platform until they stepped down to the grid. No foot shock was given and the animals were allowed to freely explore the apparatus for 30 s after they had stepped down. During this period, they stepped up onto the platform and down again several times. A ceiling of 180 s was imposed on step-down latency during the five non-reinforced test sessions (Cammarota et al. 2003).

In the step-down IA task–two trainings paradigm, the animals were submitted to two training sessions with a 24 h break between them. During the trainings, they received a 0.2-mA, 1-s foot shock. After 24 h of the last training, the animals were submitted to the test session. This memory involves exposure to fear in two stages mimicking what happens in everyday life. To evaluate posttraumatic memory, the training was 2 days after CLP, and the test was performed 8 days after training (10 days after CLP). This memory involves the fear that people experience after major events.

Paired *t* tests were applied when comparing same groups exposed to two different conditions, such as training and test scores. Comparisons between different groups were done using two-way analysis of variance (ANOVA) using condition (sham or sepsis) and time as factors. Mean differences were assessed using one-way ANOVA followed by Tukey multiple-comparison procedure that was derived from the interaction error term for the mixed-model ANOVA. A p value <0.05 was considered to indicate nominal statistical significance. The data were presented using mean + SEM.

Results

Inhibitory avoidance memory

During the training, two-way ANOVA revealed that condition ($F = 0.5836$; $p = 0.4513$) and time ($F = 1.372$; $p = 0.1558$) were not significant factors. Applying one-way ANOVA, there were no statistical difference between condition (sham or sepsis) and time ($F = 2.562$; $p = 0.053$). During the test, two-way ANOVA revealed that the relation between time and condition is statistically significant ($F = 5.90$; $p = 0.0047$) and that condition ($F = 22.46$; $p = 0.0001$) and time ($F = 11.90$; $p = 0.0001$) affect the results of the test. Applying one-way ANOVA there were statistical difference between Sham 10d versus Sepsis 10d ($F = 11.54$; $p = 0.0001$), Sham 10d versus Sepsis 30d ($F = 11.54$; $p = 0.0001$), Sham 30d versus Sepsis 10d ($F = 11.54$; $p = 0.0.021$), Sham 30d versus Sepsis 30d ($F = 11.54$; $p = 0.005$), Sham 60d versus Sepsis 10d ($F = 11.54$; $p = 0.0001$), Sham 60d versus Sepsis 30d

($F = 11.54$; $p = 0.0001$), Sepsis 10d versus Sepsis 60d ($F = 11.54$; $p = 0.0001$) and Sepsis 30d versus Sepsis 60d ($F = 11.54$; $p = 0.0001$). Applying the paired t test to evaluate training versus test, there is a significant difference in the groups Sham 10d ($t = -5.429$; $df = 14$; $p = 0.000$), Sham 30d ($t = -3.716$; $df = 14$; $p = 0.002$), Sham 60d ($t = -7.333$; $df = 14$; $p = 0.0001$) and Sepsis 60d ($t = -6.969$; $df = 14$; $p = 0.0001$). The groups Sepsis 10d ($t = -0.581$; $df = 14$; $p = 0.571$) and Sepsis 30d ($t = -0.964$; $df = 14$; $p = 0.352$). These results showed the sepsis group present aversive memory impairment up to 10 and 30 days, but not up to 60 days (Fig. 1a).

Memory of extinction

Two-way ANOVA revealed that the relation between time and condition is not statistically significant ($F = 1.42$; $p = 0.2217$) and the condition ($F = 4.01$; $p = 0.0551$) does not affect the results, but the time ($F = 363.34$; $p = 0.0001$) affects the results in both groups when analyzed together. Applying Student's t test, there were no significant difference between the condition (sham and sepsis) during the training ($t = 0.282$; $df = 28$; $p = 0.772$); test 24 h ($t = 1.677$; $df = 25.604$; $p = 0.106$), test 48 h ($t = 1.505$; $df = 23.733$; $p = 145$), test 72 h ($t = 0.124$; $df = 28$; $p = 902$), test 96 h ($t = 1.145$; $df = 28$; $p = 0.258$) and test 120 h ($t = 1.172$; $df = 28$; $p = 0.249$). Using the paired t test to compare training and test, in the sham group there were a significant difference between training and test 24 h ($t = -25.619$; $df = 14$; $p = 0.0001$), training and test 48 h ($t = -14.698$; $df = 14$; $p = 0.0001$), training and test 72 h ($t = -21.423$; $df = 14$; $p = 0.0001$), training and test 96 h ($t = -11.147$; $df = 14$; $p = 0.0001$) and training and test 120 h ($t = -7.854$; $df = 14$; $p = 0.0001$). When compared test 24 h versus test 120 h there is a statistical difference ($t = -20.107$; $df = 14$; $p = 0.0001$) showed that the extension of memory is normal; because there is a decrease of latency time. In the sepsis group there was a significant difference between training and test 24 h ($t = -16.974$; $df = 14$; $p = 0.0001$) training and test 48 h ($t = -20.333$; $df = 14$; $p = 0.0001$), training and test 72 h ($t = -24.686$; $df = 14$; $p = 0.0001$), training and test 96 h ($t = -11.514$; $df = 14$; $p = 0.0001$) and training and test 120 h ($t = -9.093$; $df = 14$; $p = 0.0001$). When compared test 24 h versus test 120 h there is a statistical difference ($t = -16.154$; $df = 14$; $p = 0.0001$) showed that the extension of memory is normal because there is a decrease of latency time (Fig. 1b).

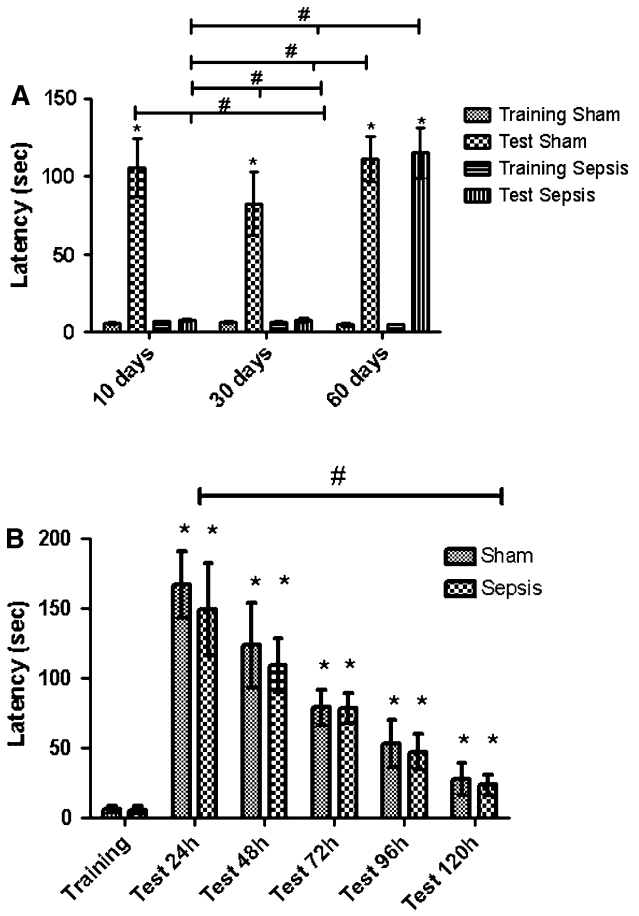

Fig. 1 **a** The step-down latency in the IA after 10, 30, and 60 days after surgery. **b** Extinction of IA memory 60 days after of surgery. Data are presented as mean and SEM, $n = 15$ rats per group. $^{*}p < 0.05$ versus training, $^{\#}p < 0.05$ versus other groups during the test

Inhibitory avoidance–two trainings paradigm

Two-way ANOVA revealed that the relation between time and condition is not statistically significant ($F = 2.22$; $p = 0.1178$) and the condition ($F = 2.74$; $p = 0.1089$) do not affect the results, but the time ($F = 546.12$; $p = 0.0001$) affect the results in both groups when analyzed together. When the t test was applied there were no statistical difference between groups during the first training ($t = 0.858$; $df = 28$; $p = 0.398$), second training ($t = 0.124$; $df = 28$; 0.902) and test ($t = 1.677$; $df = 25.604$; $p = 0.106$). Applying the paired t test, there was statistical difference in the sham group between first training and second training ($t = -21.696$; $df = 14$; $p = 0.0001$), first training and test ($t = -25.737$; $df-14$; 0.0001) and second training and test ($t = -11.352$; $df = 14$; $p = 0.0001$). In the sepsis group there was statistical difference between first training and second training ($t = -24.611$; $df = 14$; $p = 0.0001$), first training and test ($t = -16.709$; $df = 14$; $p = 0.0001$) and second training and test ($t = -8.968$; $df = 14$; $p = 0.0001$). These results showed that when

administrated two consecutive low-foot shock, the sepsis groups did not present aversive memory impairment.

Posttraumatic memory

When the groups were analyzed together applying two-way ANOVA, they revealed that the relation between time and condition is statistically significant ($F = 159.49$; $p = 0.0001$) and the condition ($F = 146.85$; $p = 0.0001$) and time ($F = 166.48$; $p = 0.0001$) affect the results. Applying the t test to evaluate difference between groups (sham and sepsis), there were no difference during the training ($t = -2.236$; $df = 28$; $p = 0.815$), but there were difference during the test ($t = 12.396$; $df = 14.207$; $p = 0.0001$). Using the paired t test, there were statistical difference between training and test in the sham group ($t = -12.815$; $df = 14$; $p = 0.0001$), but there were no difference in the sepsis groups ($t = -1.570$; $df = 14$; $p = 0.139$). The results showed that sepsis causes traumatic memory up to 10 days after surgery in the animal model (Fig. 2).

Discussion

Several studies have found alterations in neurocognitive function following critical illnesses (Hopkins and Jackson 2006; Granja et al. 2004; Comim et al. 2008), and the recognition of these long-term sequelae in survivors from critical illnesses (Rubenfeld et al. 1999). However, nowadays, the mechanisms associated with these alterations are still not fully elucidated; thus, animal models can perhaps be used to address these limitations. The present study demonstrated that sepsis survivor rats presented aversive memory impairment after 10, 30, but not 60 days after sepsis induction by CLP. This observation is consistent with our previous results that demonstrated a time-dependent recuperation of memory deficits in sepsis survivor rats (Tuon et al. 2008). In animals, explicitly or implicitly learning tasks involve the performance or the inhibition of some form of movement in response to sensory or other cues. The IA task has been the most popular test in the past few years and produces various forms of fear conditioning, all of which closely mimic human situations of daily life. This task and its variations not only rely heavily on the dorsal hippocampus, but also depend on the entorhinal and parietal cortex and are modulated by the amygdala (Izquierdo and Medina 1995).

Post-traumatic memory is characterized by the following features: re-experiencing the traumatic event, avoidance of stimuli associated with the trauma, and hyper-arousal (Yehuda 2002). These re-experiencing symptoms result from excessive retrieval of traumatic memories, which often retain their vividness and power to evoke distress.

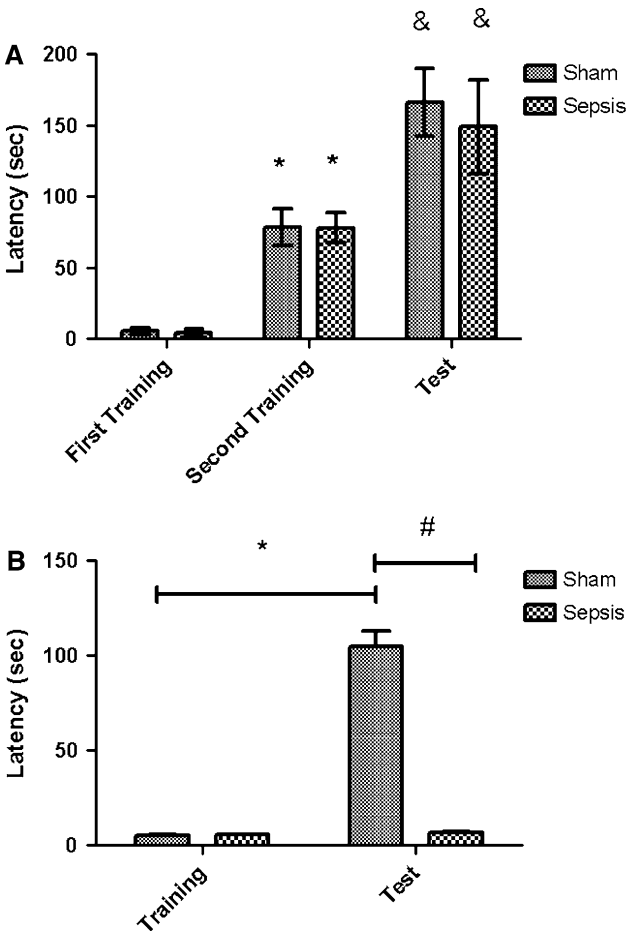

Fig. 2 The step-down IA task–two trainings paradigm (**a**) and posttraumatic memory (**b**) 10 days after surgery. Data are presented as mean and SEM, $n = 15$ rats per group. $^{*}p < 0.05$ versus training, $^{\#}p < 0.05$ versus other groups during the test, $^{\&}p < 0.05$ versus first and second training

Importantly, traumatic re-experiencing phenomena are again consolidated (reconsolidated) into memory, which cements the traumatic memory trace. Persistent retrieval, re-experiencing and reconsolidation of traumatic memories are a process that keeps these memories vivid (Yehuda 2002). Other results that we've obtained were that sepsis survivor rats presented impairment in the posttraumatic memory during the initial period after recovery.

In this context, like other types of learning, extinction learning occurs in three phases: acquisition, consolidation, and retrieval. Acquisition of extinction is the initial learning that occurs when conditioned responses are declining within an extinction training session. This is followed by a consolidation phase in which physiological and molecular processes stabilize a long-term memory for extinction and, subsequent presentation of the extinguished stimulus triggers retrieval of extinction. Poor retrieval of extinction could be due to uncovering phenomena or to a pathological process that prevents consolidation or recall of extinction

(Quirk and Mueller 2008). However, we demonstrated that the memory of extinction and IA with two training sessions is not affected, showing that the molecular mechanisms associated with affective memory formation are preserved in sepsis survivors after the initial period.

But, these results observed here are limited to aversive (affective) memory, which has several characteristics that are very different from declarative, procedural, or instrumental memory (LeDoux 2000). In this regard, we believe that our results provide relevant insights into the mechanisms involved in the cognitive deficits associated with sepsis.

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