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Sleep deprivation has a neuroprotective role in a traumatic brain injury of the rat

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HIGHLIGHTS

- Rats were subjected to traumatic brain injury (TBI).
- TBI produced morphological damage and impairment in a neurobiological test.
- 24 h of total sleep deprivation after TBI reduced the damage.

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ABSTRACT

During the process of a brain injury, responses to produce damage and cell death are activated, but self-protective responses that attempt to maintain the integrity and functionality of the brain are also activated. We have previously reported that the recovery from a traumatic brain injury (TBI) is better in rats if it occurs during the dark phase of the diurnal cycle when rats are in the waking period. This suggests that wakefulness causes a neuroprotective role in this type of injury. Here we report that 24 h of total sleep deprivation after a TBI reduces the morphological damage and enhances the recovery of the rats, as seen on a neurobiological scale.

1. Introduction

Trauma to the brain is one of the most important health problems in the world today. In the USA each year an estimated 1.7 million people sustain a traumatic brain injury (TBI) and this is a contributing factor to a third of all injury-related deaths in the United States [10].

A TBI triggers pathological pathways that may potentially harm brain cells, including excitotoxicity, formation of free radicals, inflammation, and apoptosis. However, self-protective mechanisms are also activated by a brain injury. These mediators possess damage-reducing properties and are endogenous efforts to counteract traumatic damage and improve neuronal repair. The balance between harmful and protective mechanisms will ultimately determine the fate of the injured brain [20]. We have shown that this balance depends on diurnal variations, because we have documented that the recovery of rats subjected to a TBI is better if damage is caused during the dark phase of the day, which is in the period of increased wakefulness [22].

There are few data in the literature supporting the neuroprotective role of wakefulness. When a child falls and hits its head, a popular recommendation states "Do not let it sleep", but there is no solid data in the literature that support that sleep deprivation will have some protective effect. More informed recommendations state that if the child is sleepy let it sleep, but awaken the child every 2 h to verify that speech is unaffected, it can move all four limbs, and it is oriented [9]. However there is some evidence that sleep deprivation can be a neuroprotective factor. For example, several reports in the literature suggest that total sleep deprivation (TSD) for relatively short periods (6–12 h) can produce neurogenesis in the hippocampus [13,17] and increases the expression of neurotrophins, BDNF, and NGF in the cerebral cortex [3]. TSD for 24 h reduces neuronal death caused by TNFα in the cerebral cortex of rats [24], suggesting a neuronal protection. We have documented that the rebound after deprivation of rapid eye-movement sleep

Abbreviations: CTRL, control; non-REMS, sleep without rapid eye movement; REMS, rapid eye movement sleep; REMSD, rapid eye-movement sleep deprivation; TBI, traumatic brain injury; TBI + REMSD, traumatic brain injury and REM sleep deprivation; TBI + TSD, traumatic brain injury and total sleep deprivation; TSD, total sleep deprivation.

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(REMSD) increases the expression of the CB1 cannabinoid receptors in the rat pons [21], which could also have a neuroprotective effect. Sleep deprivation and the rebound modify the release of both glutamate and GABA. The TSD increases the level of glutamate [6] and of GABA, but reduces the ratio glutamate/GABA [34], whereas the rebound decreases the level of glutamate [6] and increased the GABA [2].

With all these data, we decided to analyze the effect of REMSD and TSD on the recovery of rats subjected to a TBI.

2. Methods

2.1. Subjects

Male Wistar rats (250–300 g) were maintained under a controlled dark–light cycle (12 h:12 h, lights on at 08:00 h) with food and water ad libitum. All animal experiments were made according to guidelines and approval of the local ethical committee.

Rats were housed individually, divided into 5 groups, and a measured amount of food and water was delivered daily. The subjects of group 1 or control were only anesthetized; those of group 2 or sham, besides being anesthetized, their skulls were exposed. Those of group 3 were subjected to a TBI while anesthetized. Those of group 4, after a TBI and the recovery from anesthesia, were subjected to REMD for 24 h, and those of group 5, after a TBI and recovery from anesthesia, were TSD for 24 h. We quantified mortality and bleeding immediately after the TBI, and the daily intake of food and water, body weight, and neurological damage for 8 days. Rats were anesthetized and transcardially perfused with 4% paraformaldehyde. The brains were removed, frozen, and sectioned (thickness, 20 μm) in a cryostat. The brain sections were collected serially from the lateral 4 mm of the ipsi lateral hemisphere to the lateral 4 mm of the contralateral hemisphere, and stained with cresyl violet.

TBI: The rats were anesthetized with chloral hydrate (350 mg/kg) and a moderate head injury was produced using a calibrated pneumatic piston on the exposed skull to impact the motor cortex (coordinates $P = -2$ and $L = 1.4$) previously determined by a stereotaxic device.

A moderate-head injury is defined as an injury resulting in a mortality rate of less than 40%. All experiments were done at 13:00 h, during the light phase of the cycle.

The REMSD was done by using the inverted-flower-pot technique for 24 h. Deprivation began approximately at 13:30 h, when rats were recovered from anesthesia. The inverted flower pot was a small acrylic platform (diameter, 6 cm; height, 6 cm) surrounded by water 1-cm deep. Rats were provided with food and water during the sleep deprivation. When the animals entered REMS, which is characterized by loss of postural tone, they fell from the platform and woke up [4].

TSD: For the total sleep deprivation, rats were placed in slowly rotating wheels. The wheel (33-cm diameter) rotates slowly with a speed of one revolution/3 min. The wheel is subdivided into four compartments by concentric plates, allowing placement of four rats simultaneously and individually in each compartment. Such rotating drums force rats to stay awake and to move slowly, it does not require from them effortful movements and it allows them to eat and drink freely. Food and a small bottle with water hang from a concentric middle tube and were available ad libitum. This technique was standardized in Dr. Escobar's laboratory [27].

We evaluated the external hemorrhage produced by the TBI by weighing the blood drained after the TBI. In brief, the blood was drained and collected by pipette and then deposited into micro tubes and weighed [22].

We used a 21-point behavioral–neurological scale reported by Hunter et al. [16] to evaluate neurological damage at days 1, 4,

Table 1
Statistical analysis.

	Groups	Days	Interaction
Food intake[a]	CTRL > SHAM = TBI = TBI + REMSD TBI = TBI + REMSD = TBI + TSD $F_{4,98} = 7.786$, $P < 0.001$	PRE > 7 = 4 > 1 $F_{4,98} = 36.243$, $P < 0.001$	$F_{12,98} = 5.191$, $P < 0.001$
Water intake[a]	CTRL > SHAM = TBI = TBI + REMSD = TBI + TSD $F_{4,82} = 3.425$, $P < 0.012$	PRE = 4 = 7 > 1 $F_{4,82} = 26.158$, $P < 0.001$	$F_{12,82} = 5.675$, $P < 0.001$
Body weight[a]	CTRL > SHAM = TBI = TBI + REMSD = TBI + TSD $F_{4,109} = 7.58$, $P < 0.001$	PRE = 7 > 4 > 1 $F_{3,109} = 11.908$, $P < 0.001$	$F_{12,109} = 0.906$; NS
Bleeding[a]	TBI = TBI + REMSD = TBI + TSD $F_{2,62} = 1.28$, NS		
Neurobiological score[b]	Day 1 CTRL = SHAM SHAM = TBI + TSD > TBI = TBI + REMSD	Day 4 and 7 CTRL = SHAM = TBI + TSD > TBI = TBI + REMSD	

[a] Two-way ANOVA and a Duncan post hoc.
[b] Kruskall–Wallis test and Kolmogorov–Smirnov as post hoc.