PROGRESSIVE REDUCTION OF SLEEP TIME AND QUALITY IN RATS WITH HEPATIC ENCEPHALOPATHY CAUSED BY PORTACAVAL SHUNTS

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Abstract—Patients with liver cirrhosis show sleep disturbances. Insight into their relationship with hepatic encephalopathy (HE) can be obtained using animal models of HE. The aims of this work were to assess (1) whether rats with portacaval shunts (PCS), a model of HE, show alterations in sleep and if they are similar to those in patients with HE; (2) whether hyperammonemia plays a role in these sleep alterations; and (3) the time course of sleep alterations in these animal models. Rats were subjected to PCS to induce HE. Another group of rats was fed an ammonium-containing diet to induce hyperammonemia. Polysomnographic recordings were acquired for 24 h and sleep architecture was analyzed in control, PCS, and hyperammonemic rats at 4, 7, and 11 weeks after surgery or diet, respectively. PCS rats show a significant reduction in rapid eye movement (REM) and non-rapid eye movement (NREM) sleep time and increased sleep fragmentation, whereas reduced sleep occurs at 4 weeks and worsens at 7 and 11 weeks, sleep fragmentation appears at 7 weeks and worsens at 11 weeks. Hyperammonemic rats show decreased REM sleep, starting at 7 weeks and worsening at 11 weeks, with no changes in NREM sleep or sleep fragmentation. Therefore, PCS rats are a good model to study sleep alterations in HE, their mechanisms, and potential treatment. Mild hyperammonemia mainly impacts mechanisms involved in REM generation and/or maintenance but does not seem to be involved in sleep fragmentation. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: hepatic encephalopathy, hyperammonemia, REM sleep, NREM sleep, sleep fragmentation.

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome present in patients with liver diseases (e.g., liver cirrhosis) who may present different neurological symptoms ranging from alterations in personality, mild cognitive impairment, psychomotor slowing, and alterations in motor coordination, and may lead to coma and death. Around 50% of cirrhotic patients show sleep disturbances and unsatisfactory sleep, with increased sleep latency, reduced sleeping time, and fragmented nocturnal sleep and more frequent nocturnal awakenings and higher episodic of undesired sleepiness during the day (Córdoba et al., 1998). The authors of this work suggested that these sleep disturbances may be secondary to a malfunctioning of circadian timekeeping systems. A central circadian disruption in patients with cirrhosis has also been reported by Montagnese et al. (2010).

Unsatisfactory sleep in cirrhosis was associated with delayed bedtime, delayed wake-up time, and evening chronotropism, which could be caused by altered circadian rhythm and metabolism of melatonin (Steindl et al., 1995a; Blei and Zee, 1998). In cirrhotic patients the circadian plasma melatonin profile showed a significant delay in the onset of the melatonin increase and in its peak of nocturnal level (Steindl et al., 1995a). However, abnormalities of melatonin levels seem to be unrelated to the sleep disturbances of the patients (Montagnese et al., 2010).

Sleep disorders in cirrhosis show a poor correlation with clinical or laboratory parameters (Mostacci et al., 2008) or with performance in the psychometric tests used to evaluate the presence of minimal HE (Montagnese et al., 2009).

Kurtz et al. (1972) studied the alterations in nocturnal sleep in patients with portacaval encephalopathy. They reported a reduction in total sleep time and in slow-wave sleep, a deficit in rapid sleep, increased latency to sleep and frequent awakenings in patients at early stages of portacaval encephalopathy. With worsening of the encephalopathy successive pathological variations of physiological sleep appear, followed by a true pathological sleep corresponding to progressive breaking down of sleep function.

Bajaj et al. (2011) have recently reported in a short letter that sleep architecture is disrupted in patients with minimal HE, with absence of slow-wave sleep in 80% of patients (four out of five) and increased percentage of rapid eye movement (REM) sleep.

The mechanisms responsible for sleep alterations in cirrhosis are unclear. As a consequence there are no effective treatments to improve sleep in these patients. Spahr et al. (2007) showed a partial improvement of sleep (as measured by actigraphy) in cirrhotic patients treated with hydroxyzine, a histamine H1 blocker. However, the risk of precipitating overt HE makes its use inadvisable.

It would be of interest to have an animal model reproducing the sleep alterations seen in patients with HE. This would allow studying the underlying mechanisms and to assess new therapeutic treatments.
One of the models recommended by the International Society for Hepatic Encephalopathy to study the mechanisms and treatment of HE are rats with portacaval shunts (PCS) (Butterworth et al., 2009). PCS rats show altered levels of melatonin in the pineal gland during the day, which are associated with disruption of circadian rhythms of locomotor activity, with reduced activity during the night (the active period for rats) and increased activity during the day (the resting period for rats) (Zee et al., 1991; Coy et al., 1992; Steindl et al., 1995b; Córdoba et al., 1997). These alterations in motor activity in PCS rats are therefore similar to the alterations in sleep in cirrhotic patients: reduced activity during the active period and increased activity during the resting period. Therefore PCS rats are an appropriate model to study sleep alterations. However, polysomnographic changes in sleep structure in PCS rats have not been analyzed to date. The main aim of this work was to assess whether PCS rats show alterations in sleep structure and if they are similar to those in cirrhotic patients.

A main contributor to the neurological alterations in HE is hyperammonemia (Felipo and Butterworth, 2002). Rats with chronic moderate hyperammonemia without liver failure reproduce many alterations present in patients with HE and in PCS rats. For example, PCS rats show reduced ability to learn a Y maze task, which is mainly caused by impaired function of the glutamate-nitric oxide-cGMP pathway (Erceg et al., 2005a; Cauli et al., 2007a). Chronic hyperammonemia per se, without liver failure, has demonstrated to be enough to impair the function of the pathway (Hermenegildo et al., 1998) and the ability to learn the Y maze (Aguilar et al., 2000; Erceg et al., 2005b), supporting the role of hyperammonemia in this learning deficit in PCS rats. Hyperammonemic rats also show alterations in the circadian rhythm of locomotor activity (Ahbrach et al., 2010). It is therefore likely that hyperammonemia could also contribute to sleep alterations in HE. A second aim of this work was to assess whether hyperammonemia is responsible for the alterations in sleep in rats with HE because of PCS.

![Fig. 1. PCS and hyperammonemic rats show a progressive reduction in sleep time. Sleep was recorded during 24 consecutive hours in control and PCS rats at 4, 7, and 11 wk after performing the PCS surgery (A), or in hyperammonemic rats at 4, 7 or 11 wk after diet (B). The total sleep time and time spent in REM and NREM sleep was quantified. Values are the mean±SD of five rats per group. Values significantly different from control rats are indicated by asterisks. * P<0.05; ** P<0.01.](image-url)

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In cirrhotic patients the neurological alterations evolve with the progression of liver failure. The third aim of this study was to follow the time course of the sleep alterations in PCS and hyperammonemic rats. To reach this aim, sleep structure was determined at 4, 7, and 11 weeks after surgery for PCS or diet for hyperammonemic rats.

To assess the effects of PCS and of chronic hyperammonemia on sleep rats were implanted with electrodes and the electroencephalogram (EEG) and the electromyogram (EMG) were recorded for 24 h and sleep architecture was analyzed.

**EXPERIMENTAL PROCEDURES**

**Rats with portacaval anastomosis**

Male Wistar rats (220–240 g) (Charles River, Barcelona, Spain) were anesthetized with isofluorane, and an end-to-side portacaval shunt (PCS) was performed as described by Lee and Fisher.

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**Fig. 2.** PCS but not hyperammonemic rats show a progressive fragmentation of sleep, as revealed by increased number of wake bouts. The wake bouts of the indicated durations were quantified during the resting phase (day) after 4, 7 and 11 wk of PCS (A) or hyperammonemia (B). Values are the mean±SD of five rats per group. Values significantly different from control rats are indicated by asterisks. *P<0.05; **P<0.01.
Control rats were sham operated; they had the portal vein and inferior vena cava clamped for 10 min.

**Rats with chronic hyperammonemia without liver failure**

Male Wistar rats (120–140 g) were made hyperammonemic by feeding them an ammonium-containing diet for 4, 7, and 11 weeks as previously described (Felipo et al., 1988). These rats do not show liver dysfunction.

Adequate measures were taken to minimize pain and discomfort to the animals. The experiments were approved by the Ethics Committee of our Center and met the guidelines of the European Communities Council Directive 86/609/EEC.

**Implantation of electrodes**

Implantation of the electrodes for EEG recordings was performed 10 days before reaching 4, 7, or 11 weeks after diet or surgery for PCS. Rats were anesthetized with isofluorane. One hole overlying the hippocampus (approximately 1 mm in diameter) was drilled to permit targeting of the CA1 subfield under stereotaxic guidance. Electrode wire for hippocampal LFP (local field potential) recordings was made of stainless steel (0.125 mm diameter) and implanted over the left cerebral hemisphere (AP: +3.3 mm; ML: 1.4 mm; DV: −2.3 mm). Additionally, one stainless steel electrode (0.125 mm diameter) was implanted in the medial prefrontal cortex (AP: 3.7 mm; ML: 0.8 mm; DV: −3.5 mm). The common reference electrode was located above the cerebellum (1 mm posterior to lambda on midline). Two insulated stainless steel (0.12 mm diameter) Teflon-coated wires bilaterally placed into both trapezius muscles served as EMG electrodes. All electrodes were attached to a headset and fixed to the skull with dental cement. Rats were allowed to recover for 10 days from surgery, and habituated to the experimental settings during 24 h before the recording session.

**Electrophysiological and behavioral data acquisition**

LFPs and EMG were continuously recorded for 24 h in the animal’s home cage. Electrophysiological signals were sampled at 200 Hz with an ×10 preamp gain and ×50.78 amplifier gain using the software Sirenia Acquisition, version 1.8, from Pinnacle Technology (KS, USA). Video images of the rats were acquired synchronously with electrophysiological recording for 24 h using video cameras Model AP-5006, Pinnacle Technology, controlled by the same software. Raw signals and images were stored in a computer for off-line analysis.

**Scoring of sleep–wake states**

LFP recordings were manually scored (10-s epochs) in different behavioral states according to the following criteria (Datta and Hobson, 2000): (1) wakefulness (WK), the animal was engaged in active behaviors (locomotion, whisking, and sniffing), showing low-amplitude cortical LFPs and high theta (5–9 Hz) and gamma (30–40 Hz) power density, immobile (standing or sitting quietly) or engaged in “automatic” stereotype behaviors (eating, drinking, and grooming), with low-amplitude cortical LFPs and relatively high theta and gamma activity. (2) In NREM, the animal was lying immobile with eyes closed and slow regular respiratory movements, beginning with sleep spindles (10–14 Hz) superimposed to delta waves (1–4 Hz). As SWS deepens, delta oscillations become predominant, although isolated spindles can still be observed. (3) In REM sleep, the animal was immobile and atonic except for intermittent whisker and ear twitches, with low cortical LFP amplitude and high theta and gamma power. Scoring of sleep–wake states was performed blind to the experimental condition.

After scoring each 10-s epoch, the duration of each cerebral state (Wake, NREM, and REM) was computed for each rat. The number of transitions between cerebral states was also obtained.

![Fig. 3. PCS but not hyperammonemic rats show a progressive fragmentation of sleep, as revealed by sleep bouts of shorter duration.](image-url)
Similarly, the number of wake and sleep bouts of different duration (ranged from 10 to 2560 s) were grouped and compared among groups. Total sleep time is defined as the time (in minutes) spent in sleep over a 24-h period. Sleep and wake bouts are the number of sleep and wake periods (of different duration) either in light or dark periods. An elevated number of wake bouts indicates increased sleep fragmentation.

**Statistical analysis**

Differences in each sleep–wake parameter between experimental conditions were separately tested by using non-paired, one-way ANOVAs (Mann–Whitney U-test). In all cases, $P<0.05$ was considered as the significance threshold. Statistical analyses were performed with SPSS v. 16 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

PCS rats show alterations in sleep structure that aggravates with time. Total sleep time was significantly decreased in PCS rats 4 weeks after surgery ($437\pm19$ min/d, $P<0.03$) when compared with control rats ($493\pm11$ min/d). The magnitude of this effect increases at 7 ($405\pm7$ min/d, $P<0.01$) and 11 weeks ($378\pm12$, $P<0.01$) in PCS rats (Fig. 1A).

NREM and REM sleep followed the same trend of significant alterations in PCS when compared with control rats (Fig. 1A).

Four weeks after surgery control rats spent $412\pm11$ min/d in NREM and this time remained unchanged at 7 and 11 weeks. In contrast, PCS rats spent less in NREM: $372\pm17$ ($P<0.05$), $353\pm5$ ($P<0.01$), and $331\pm11$ ($P<0.01$) min/d at 4, 7, and 11 weeks, respectively (Fig. 1A).

Regarding REM sleep, control rats 4 weeks after surgery spent $80\pm4$ min/d, and this time remained unchanged at 7 and 11 weeks. PCS rats spent less time in REM sleep: $65\pm3$ ($P<0.03$), $51\pm7$ ($P<0.01$), and $46\pm5$ ($P<0.01$) min/d at 4, 7, and 11 weeks, respectively (Fig. 1A).

We further assessed if sleep was fragmented in PCS rats by analyzing the duration of wake bouts during the resting (light) phase.

As shown in Fig. 2, 4 weeks after surgery PCS rats did not show alterations in wake bouts compared with control rats. Sleep continuity deteriorated progressively at 7 and 11 weeks from PCS surgery. At 7 weeks PCS rats showed a significant increase in the number of wake bouts of 10–80 s of duration. At 11 weeks the number of these
wake bouts increased further and, in addition, longer wake bouts of 80–160 s also increased significantly (Fig. 2A).

Duration of sleep bouts was also analyzed during the resting (light) phase in PCS rats.

Four weeks after surgery PCS rats showed a similar distribution of sleep bouts when compared with controls (Fig. 3A).

Sleep fragmentation deteriorated progressively at 7 and 11 weeks. At 7 weeks PCS rats showed a significant increase in the number of sleep bouts of short (20–160 s) duration and a decrease in the number of longer sleep bouts (160–640 s). At 11 weeks the number of sleep bouts of short duration (10–80 s) was strongly increased, whereas the number of longer sleep bouts (160–640 s) was significantly reduced (Fig. 3A).

To get a more detailed view of sleep fragmentation, we studied the number of transitions between different cerebral states: wake, REM, and NREM sleep. As shown in Fig. 4A, the number of transitions in PCS rats was unaltered at 4 weeks. At 7 weeks there was a significant increase in the number of transitions between wake-NREM and NREM-wake, whereas transitions between the other cerebral states were not altered. The same effect, but with larger magnitude was observed at 11 weeks (Fig. 4A).

To determine if hyperammonemia and its time course contribute to changes in sleep structure, we performed similar studies in rats with chronic hyperammonemia without liver failure after 4, 7, and 11 weeks of feeding them with an ammonium-containing diet.

The effects of mild hyperammonemia alone on sleep were milder than in PCS rats. Total sleep time was similar in control rats when compared with hyperammonemic rats 4 weeks after beginning the diet. At 7 and 11 weeks, hyperammonemic rats showed less sleep time 461±4 (P<0.02) and 456±6 (P<0.01) min/d, respectively than control rats (476±1 min/d) (Fig. 1B).

![Graph](image-url)
Time spent in NREM sleep did not differ between controls and hyperammonemic rats, in any experimental condition (Fig. 1).

REM duration remained unchanged across experimental conditions in control rats, but it was significantly reduced in hyperammonemic rats at 7 weeks (61 ± 2 min/d, \( P < 0.02 \)) and 11 weeks (56 ± 2 min/d, \( P < 0.01 \)) (Fig. 1B).

Hyperammonemia did not increase sleep fragmentation at any time compared with control rats. Neither the wake bouts (Fig. 2B) or sleep bouts during the rest phase (Fig. 3B) nor the number of transitions (Fig. 4B) was different from those of control rats.

**DISCUSSION**

The data reported show that rats with PCS reproduce the sleep alterations reported in patients with liver cirrhosis. Cirrhotic patients show reduced sleeping time (Kurtz et al. (1972; Córdoba et al., 1998), and the results reported show a reduction in sleep time in PCS rats that progressively aggravated from 4 to 11 weeks. Therefore PCS rats reproduce the reduction of sleep time reported in cirrhotic patients, which was evident at early stages after performing the PCS, and progresses with time. These results agree with those reported by Kurtz et al. (1972) in nocturnal sleep in patients with portacaval encephalopathy. They reported a reduction in total sleep time at early stages and increasing pathological alterations of sleep with worsening of the encephalopathy leading to progressive breakdown of sleep function. The progression of hepatic failure over time may contribute to explain the deterioration of sleep in patients and in PCS rats.

Total sleep time reduction was accounted for a similar reduction in both NREM and REM sleep duration across time in PCS rats. These results are similar to those of Kurtz et al. (1972), who also reported a reduction in total sleep time, in slow-wave sleep and a deficit in rapid sleep. Bajaj et al. (2011) have recently reported in a short letter that sleep architecture is disrupted in patients with minimal HE, with absence of slow-wave sleep in four out of five patients and increased percentage of REM sleep. As mentioned
above, the alterations in sleep increase with progression of encephalopathy both in patients (Kurtz et al., 1972) and in PCS rats (present work). It is possible that PCS rats present a more advanced grade of HE than the four patients with MHE in the study of Bajal et al. (2011).

Cirrhotic patients also show fragmented nocturnal sleep with more frequent nocturnal awakenings (Kurtz et al., 1972; Córdoba et al., 1998). This is also reproduced in PCS rats, but at later stages after surgery. At 4 weeks sleep is not fragmented in PCS rats, which show similar wake bouts as control rats. However, at 7 weeks PCS rats show fragmented sleep, with increased number of wake bouts and sleep bouts of shorter duration. These effects are further enhanced at 11 weeks as reflected by the increased number of wake–sleep transitions.

These data show that the reduction in sleep time occurs in PCS rats before sleep fragmentation. This suggests that the mechanisms responsible for reduction of sleep time and for sleep fragmentation are different or at least that a stronger alteration is necessary to induce sleep fragmentation. This is further supported by the results obtained in rats with chronic hyperammonemia without liver failure, which show reduced REM sleep time but do not show sleep fragmentation.

This also suggests that mild hyperammonemia does not contribute to sleep fragmentation but would be a main contributor to the reduction in REM sleep time. The mechanisms by which hyperammonemia may reduce REM sleep time without affecting NREM sleep may include alterations in serotoninergic neurotransmission and/or in adrenocorticotropic hormone (ACTH).

A reduction in REM sleep with no effects on NREM sleep has been reported in rats treated with citalopram, a serotonin-specific reuptake inhibitor (Ivarsson et al., 2005). A possible role for 5-HT7 receptors in the REM sleep effects of citalopram has been suggested (Bonaventure et al., 2007). Treatment of rats with citalopram plus an antagonist of 5-HT7 receptors reduces REM sleep without affecting NREM sleep or sleep fragmentation, as occurs in hyperammonemic rats. Altered serotoninergic neurotransmission with increased serotonin turnover and regional brain serotonin receptor changes has been reported in PCS rats and in hyperammonemia (Lozeva-Thomas, 2004; Apelqvist et al., 1998). It seems therefore likely that altered serotoninergic neurotransmission could be involved in the reduced REM sleep in hyperammonemic and PCS rats.

Reduced REM sleep time with no effect on NREM sleep was also observed in rats injected with ACTH (Koo et al., 2008). ACTH is an important component of the hypothalamic–pituitary–adrenal axis, which controls the circadian rhythms of activity. ACTH is released by the anterior pituitary gland and controls the production and release of corticosteroids from the adrenal cortex. The release of corticosteroids is altered in hyperammonemic rats (Ahabrach et al., 2010), suggesting that ACTH function is also altered. This may contribute to the reduction in REM sleep in hyperammonemic and PCS rats.

The alterations in NREM sleep and the induction of sleep fragmentation in PCS are not reproduced in hyperammonemic rats. This suggests that these alterations require stronger levels of hyperammonemia or are not caused by hyperammonemia but by other alterations associated with PCS.

Ammonia levels in striatum are 0.38±0.01 μmol/g tissue in control rats, are increased in hyperammonemic rats to 0.55±0.01 μmol/g tissue, and are much more higher in PCS rats (1.8±0.3 μmol/g tissue). Ammonia levels are about three-fold higher in striatum of PCS rats than in hyperammonemic rats (Cauli et al., 2007b). Similar differences should occur in other brain areas. It is possible that reduced NREM sleep and sleep fragmentation in PCS rats could be caused by the higher levels of hyperammonemia.

Another factor that could contribute to these sleep alterations is neuroinflammation. Infection by the parasite Trypanosoma brucei induces sleeping sickness in humans and also alters sleep in rats, leading to reduced sleep and sleep fragmentation. It has been proposed that activation of microglia and increased levels of inflammatory cytokines and PGE2 would contribute to sleep alterations in rats infected with Trypanosoma brucei (Kristensson et al., 2010).

Both hyperammonemic and PCS rats show microglial activation and neuroinflammation with increased inflammatory cytokines and PGE2 (Cauli et al., 2007a,b, 2009; Rodrigo et al., 2010). Neuroinflammation contributes to the cognitive and motor alterations in both PCS and hyperammonemic rats and treatment with ibuprofen restores learning ability and motor function in both rat models (Cauli et al., 2007a,b, 2009; Rodrigo et al., 2010). It is possible that neuroinflammation also contributes to sleep alterations in hyperammonemic and PCS rats, as occurs in rats infected with Trypanosoma brucei. As is the case for the level of hyperammonemia, activation of microglia and neuroinflammation is also milder in hyperammonemic rats than in PCS rats, affecting mainly cerebellum in hyperammonemic rats and more brain areas in PCS rats. This could contribute to the stronger alterations in sleep in PCS rats than in hyperammonemic rats.

Alternatively, PCS rats can have additional alterations not present (or present in much lower grade) in hyperammonemic rats, which could be responsible for sleep fragmentation in PCS rats. One possible candidate is melatonin. Injection of a single dose of melatonin to rats induces the same alterations observed in PCS rats: it reduces sleep and increases sleep fragmentation in rats (Huber et al., 1998). Melatonin levels are altered in PCS rats (Zee et al., 1991). It has not been assessed whether hyperammonemia per se alters melatonin levels.

Vyzovskiy et al. (2011) have recently shown that during a prolonged awake state in rats, some subsets of neurons in different cortical areas can suddenly go offline, usually for short periods. The number of “off” periods increases with the duration of wakefulness, suggesting that sleep pressure may turn off transiently some subsets of neurons. The EEG is typical of an awake state and the rat appears behaviorally awake. However, the occurrence of
these “off” periods is associated with decreased performance in a sugar pellet reaching task. The authors suggest that “local sleep” in an awake brain may contribute to cognitive impairment in some situations. It is possible that PCS rats have a progressively increased number of “off” periods for some populations of cortical neurons, which may contribute to the cognitive impairment reported in these rats (Erceg et al., 2005a,b; Cauli et al., 2007a; Monfort et al., 2007).

Sleep modulates learning and memory by different mechanisms (Poe et al., 2010) and it is essential in memory consolidation (Diekelmann and Born, 2010). It is therefore likely that alterations in sleep may contribute, together with other alterations (e.g. in neurotransmission), to the cognitive alterations in rats and patients with HE.

Conclusions

The results reported here show that PCS rats show a progressive worsening in sleep with reduced sleep time and increasing sleep fragmentation. PCS rats are therefore a good model for sleep alterations in HE and may be used to study the underlying mechanisms and to test therapeutic treatments.

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