*Neuroscience* 201 (2012) 199–208

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

**M. LLANSOLA,[a1] J. L. CANTERO,[b1] E. HITA-YAÑEZ,[b] M. J. MIRONES-MALDONADO,[b] B. PIEDRAFITA,[a] H. AHABRACH,[a,c] M. ERRAMI,[c] A. AGUSTI[a] AND V. FELIPO[a]***

[a]*Laboratory of Neurobiology, Centro de Investigacion Principe Felipe, Valencia, Spain*

[b]*Laboratory of Functional Neuroscience, Spanish Network of Excellence for Research on Neurodegenerative Diseases (CIBERNED), University Pablo de Olavide, Seville, Spain*

[c]*Departament de Biologie, Faculte des Sciences, Université Abdelmalek Essaadi, Tétouan, Morocco*

**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.** 

**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 with more frequent nocturnal awakenings and higher episodes 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 chronotypology, 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.

[1] These authors contributed equally to the work.

*Corresponding author. Tel: +34-963-289-680; fax: +34-963-289-701.
E-mail address: vfelipo@cipf.es (V. Felipo).

*Abbreviations:* ACTH, adrenocorticotropin hormone; EEG, electroencephalogram; EMG, electromyogram; HE, hepatic encephalopathy; LFP, local field potential; NREM, non-rapid eye movement; PCS, portacaval shunts; REM, rapid eye movement.

0306-4522/12 $36.00 
doi:10.1016/j.neuroscience.2011.11.009

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 (Ahabrach 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.

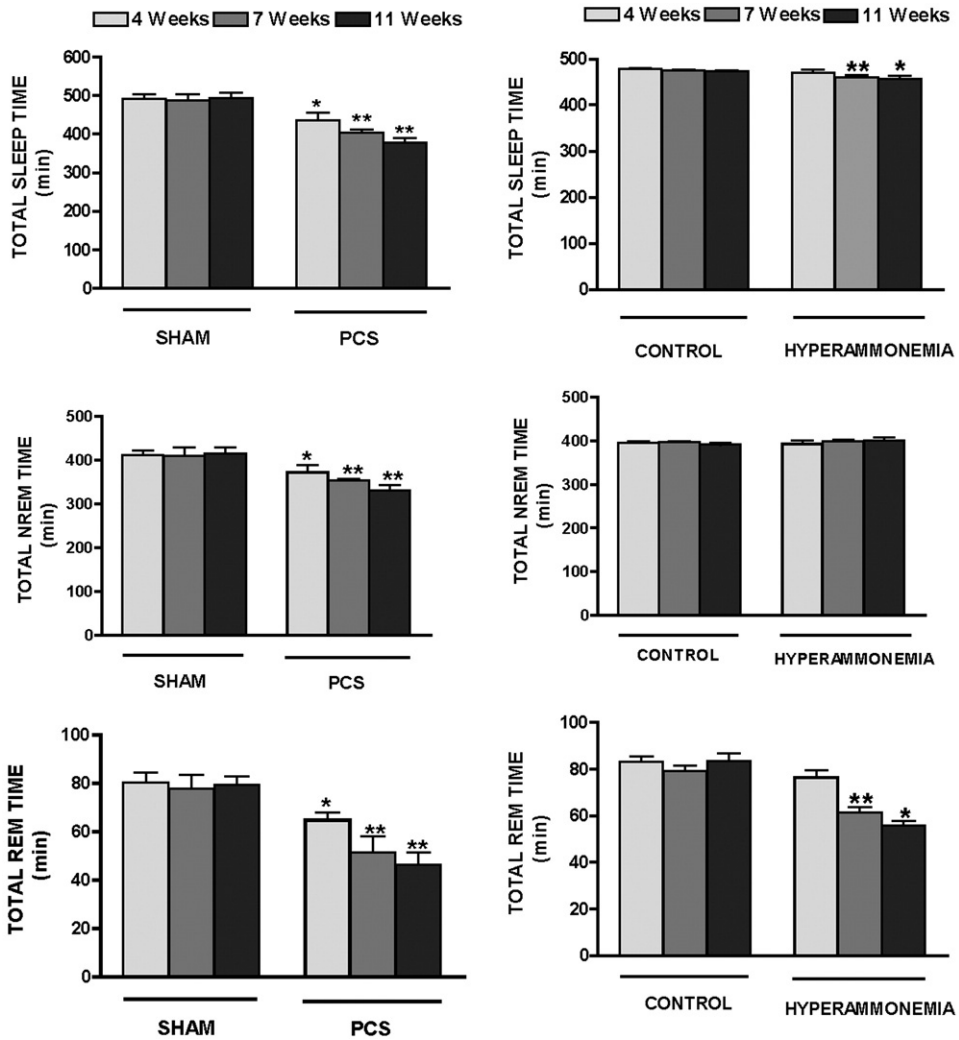


**Fig. 1.** PCS and hyperammonemic rats show a progressive reduction in sleep time. Sleep was recorded during 24 consecutive hours in control and PCS rats at 4, 7, and 11 wk after performing the PCS surgery (A), or in hyperammonemic rats at 4, 7 or 11 wk after diet (B). The total sleep time and time 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. * $P<0.05$; ** $P<0.01$.