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SHORT REPORT

Ion concentrations in cerebrospinal fluid in wakefulness, sleep and sleep deprivation in healthy humans

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Summary

Sleep is controlled by a circadian rhythmicity, via a reduction of arousal-promoting neuromodulatory activity, and by accumulation of somnogenic factors in the interstitial fluid of the brain. Recent experiments in mice suggest that a reduced neuronal excitability caused by a reduced concentration of potassium in the brain, concomitant with an increased concentration of calcium and magnesium, constitutes an important mediator of sleep. In the present study, we examined whether such changes in ion concentrations could be detected in the cerebrospinal fluid of healthy humans. Each subject underwent cerebrospinal fluid collection at three occasions in a randomized order: at 15:00 hours–17:00 hours during waking, at 06:00 hours–07:00 hours immediately following 1 night of sleep, and at 06:00 hours–07:00 hours following 1 night of sleep deprivation. When compared with wakefulness, both sleep and sleep deprivation produced the same effect of a small (0.1 mM, about 3%), but robust and highly significant, reduction in potassium concentration. Calcium and magnesium concentrations were unchanged. Our results support a circadian modulation of neuronal excitability in the brain mediated via changes of the interstitial potassium concentration.

KEYWORDS
calcium, magnesium, potassium, sleep, sodium, wakefulness

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1 | INTRODUCTION

The regulation of sleep and wakefulness is of vital importance for wellbeing, as demonstrated by conditions like narcolepsy, hypersomnia, insomnia or various parasomnias. Sleep and wakefulness represent brain states characterized by different firing patterns of cortical neurons (Steriade, McCormick, & Sejnowski, 1993; Steriade, Timofeev, & Grenier, 2001). The overarching regulation of sleep and wakefulness is mediated via an interaction of two processes. First, a circadian rhythmicity leading to distinct activity changes in arousal-promoting neuromodulators such as norepinephrine, serotonin, histamine and acetylcholine (for review, see Saper, Scammell, & Lu, 2005). Second, accumulation of somnogenic factors, including adenosine, in the interstitial fluid (ISF) of the brain increases the propensity for sleep (Portas, Thakkar, Rainnie, Greene, & McCarley, 1997; Rainnie, Grunze, McCarley, & Greene, 1994). Neuromodulators and somnogenic factors act to change the activity of various ion channels, which, in turn, changes the firing pattern of neurons.

The extracellular concentration of potassium has been shown *in vivo* to vary with the brain states sleep and wakefulness (Amzica, 2002; Ding et al., 2016), as well as quiescence and locomotion (Rasmussen et al., 2019). However, because $[K^+]_E$ is affected by neuronal activity it remains unclear whether this variation reflects a cause or a consequence. In 2016, Ding et al. (2016) provided a new perspective when they showed that a reduced $[K^+]_E$, together with an increased concentration of magnesium and calcium, in the ISF correlated strongly with and was sufficient to facilitate the transition from wakefulness to sleep in mice. Altered extracellular concentration of these ions would facilitate switching to a bursting type of neuronal firing characteristic of slow-wave sleep (Rasmussen, O'Donnell, Ding, & Nedergaard, 2020). The aim of this study was to examine whether such changes in ion concentrations could be detected and reflected in the cerebrospinal fluid (CSF) of healthy humans.

2 | METHODS

2.1 | Participants

Twelve healthy volunteers (seven females, five males) were recruited to the study after completing a screening procedure for general health and sleep habits. During screening, nine additional subjects were excluded due to abnormal sleep (5), medication (3) or withdrawal of consent (1). Criteria for sufficient sleep were normal sleep duration of 6–9 hr and sleep in the window 21:00 hours–08:00 hours, latency to sleep < 30 min, and the absence of the following; nocturnal awakenings, restless legs syndrome, excessive daytime sleepiness (evaluated with Epworth Sleepiness Scale < 10), morning headache and dryness of mouth (Boulos et al., 2019; Watson et al., 2015). Participants were also excluded if they had worked a nightshift or travelled across more than two time zones within 2 months of the CSF collection. One of the 12 participants was excluded after inclusion before analysis due to loss of data.

2.2 | Ethics

This study was approved by a local ethics committee (Regionala etikprövningsnämnden i Göteborg, #492-18). Each participant gave oral and written informed consent to participation in the study.

2.3 | Design

Each participant spent 3 nights at the sleep laboratory, with sampling of blood and CSF in the morning (sleep-CSF and sleep deprivation-CSF) or in the afternoon (awake-CSF). The order of the procedures was randomized, and study nights were separated by at least 4 weeks. The sample of 10 ml of lumbar CSF was sampled by an experienced neurologist. Nights before the sampling of sleep-CSF and awake-CSF the participants were monitored by polysomnography (PSG) to document sleep. In this study, we used a reduced set of electroencephalography (EEG) sites (A1, A2, C3, C4, O1 and F4), along with electrooculography and electromyography recorded over the masseter muscle bilaterally. Signals were sampled at 50 Hz and scored according to the AASM Scoring Manual (Berry et al., 2020) by a registered PSG technologist, blinded to allocation. Awake-CSF was collected in the afternoon (15:00 hours–17:00 hours) after a night of normal, monitored sleep. The participants lived freely during the day up to the sampling in the afternoon. For sleep-CSF, the participants were awakened between 06:00 hours and 07:00 hours, and maintained in bed until the lumbar puncture. The sleep deprivation-CSF was also collected between 06:00 hours and 07:00 hours, but after a night of complete sleep deprivation. During sleep deprivation, the participants spent the night with the lights on playing games, studying, talking and walking around, but refrained from more strenuous physical activity. The participants carried actigraphs during 24 hr prior to arriving in the sleep laboratory at each occasion as well as during the entire sleep-deprivation night to ensure that there were not sustained sleep periods. They were also instructed to refrain from excessive sleep in preparation for the nights in the sleep laboratory, and to avoid intake of any drugs that might have affected sleep (such as caffeine, nicotine or alcohol) for at least 24 hr before the experiment.

2.4 | Ion measurements

The measurements of ion concentrations were carried out by board-certified laboratory technicians at the Clinical Chemistry Laboratory at Sahlgrenska University Hospital using accredited methods with inter-assay coefficients of variation below 2%. CSF concentrations of sodium, potassium and chloride were measured using ion-selective electrodes (ISEs), integrated into the cobas c 501 instrument, which have been approved for clinical use without clinically relevant interferences (Roche Diagnostics). ISE Standards Low (S1) and High (S2) are used to calibrate the methods at least once per day (Roche Diagnostics). CSF calcium and magnesium concentrations were measured by colorimetric *o*-cresolphthaleion and chlorophonazo III methods, respectively, a cobas c 501 instrument, according to instructions from the manufacturer (Roche Diagnostics). The methods are calibrated after each reagent lot change, using Standards Low (S1) and High (S2) for calcium and magnesium, respectively (Roche Diagnostics).

2.5 | Statistical analysis

The data were analysed and graphs created using the software GraphPad Prism® (GraphPad Software). Statistical significance was determined by repeated measurements one-way ANOVA with post hoc paired *t*-tests with Bonferroni correction, and data are presented as average ± SEM.

3 | RESULTS

Sleep was monitored with PSG the night before the sampling of sleep- or awake-CSF. Participants were requested to stay in bed with the lights out in individual rooms for 8 hr. The mean total sleep time was 435 ± 4.8 min (7 hr 15 min), with a mean latency to sleep onset of 17 ± 2.3 min. Analysed sleep parameters, all in the normal range, included total sleep time, sleep efficiency, wake time after sleep onset, % rapid eye movement (REM) of total sleep and sleep-onset latency (Boulos et al., 2019). Mean values for all recorded parameters, as well as the mean for groups divided by gender, age, study night order and whether CSF sampling was planned for the subsequent morning (which might have provided anticipation stress), are shown in Table 1.

The $[K^+]_E$ was significantly reduced in sleep-CSF compared with awake-CSF (2.85 ± 0.01 mM versus 2.94 ± 0.02 mM; Figure 1a). The $[K^+]_E$ in CSF collected after sleep-deprivation was 2.79 ± 0.02 mM, significantly lower than in awake-CSF, but not significantly different from the sleep-CSF.

The extracellular concentrations of sodium ($[Na^+]_E$) followed the same pattern, with a decrease from 149.4 ± 0.7 mM in awake-CSF to 147.6 ± 0.6 mM in sleep-CSF (Figure 1b). In the CSF from sleep-deprived subjects, the $[Na^+]_E$ was 148.0 ± 0.7 mM, which did not differ significantly from either sleep-CSF or awake-CSF. The extracellular concentration of chloride ($[Cl^-]_E$) showed a similar pattern, with a significantly higher concentration in awake-CSF (127.4 ± 0.5 mM) compared with sleep-CSF (125.5 ± 0.5 mM; Figure 1c). In the sleep deprivation-CSF the $[Cl^-]_E$ was 126.5 ± 0.7 mM, not significantly different from either sleep-CSF or awake-CSF. Calcium ($[Ca^{2+}]_E$) and magnesium ($[Mg^{2+}]_E$) showed trends similar to that of $[K^+]_E$, with the highest levels during wakefulness. These changes were, however, smaller and not statistically significant (Figure 1d,e).

Because all ions followed the same general trend with lower concentration during sleep than during wakefulness, we investigated whether the findings might be explained by a general dilution of the CSF during sleep or due to circadian changes. However, the relative decrease in sleep-CSF concentrations compared with those in the

TABLE 1 Polysomnography parameters for the participants

	TST (min)	SE (%)	WASO (min)	%REMoTST	SOL (min)
Total sample					
n = 11	435 ± 4.8	90 ± 0.9	33 ± 4.4	20 ± 0.9	17 ± 2.3
Age (years old)					
20–23 (*n* = 7)	439 ± 5.7	90 ± 1.1	27 ± 4	20 ± 1.1	19 ± 3.0
24–28 (*n* = 4)	428 ± 8.6	88 ± 1.8	43 ± 9.2	21 ± 1.6	13 ± 3.3
Sex					
Females (*n* = 7)	433 ± 6.8	89 ± 1.4	34 ± 6.6	20 ± 0.8	17 ± 3.3
Males (*n* = 4)	438 ± 6.0	90 ± 1.0	31 ± 4.0	21 ± 2.1	17 ± 2.7
Night of sleep					
First night	433 ± 5.9	90 ± 1.1	33 ± 5.8	20 ± 0.8	18 ± 2.7
Second night	437 ± 7.8	90 ± 1.6	33 ± 6.9	20 ± 1.6	16 ± 3.8
Morning LP	439 ± 5.6	91 ± 1.2	31 ± 6.2	18 ± 1.2	12 ± 2.2
Afternoon LP	432 ± 8.0	88 ± 1.4	35 ± 6.4	22 ± 1.1	22 ± 3.5

LP, lumbar puncture; REM, rapid eye movement; SE, sleep efficiency; SOL, sleep-onset latency; TST, total sleep time; WASO, wake after sleep onset. (*n*=11), data presented as mean ± SEM.

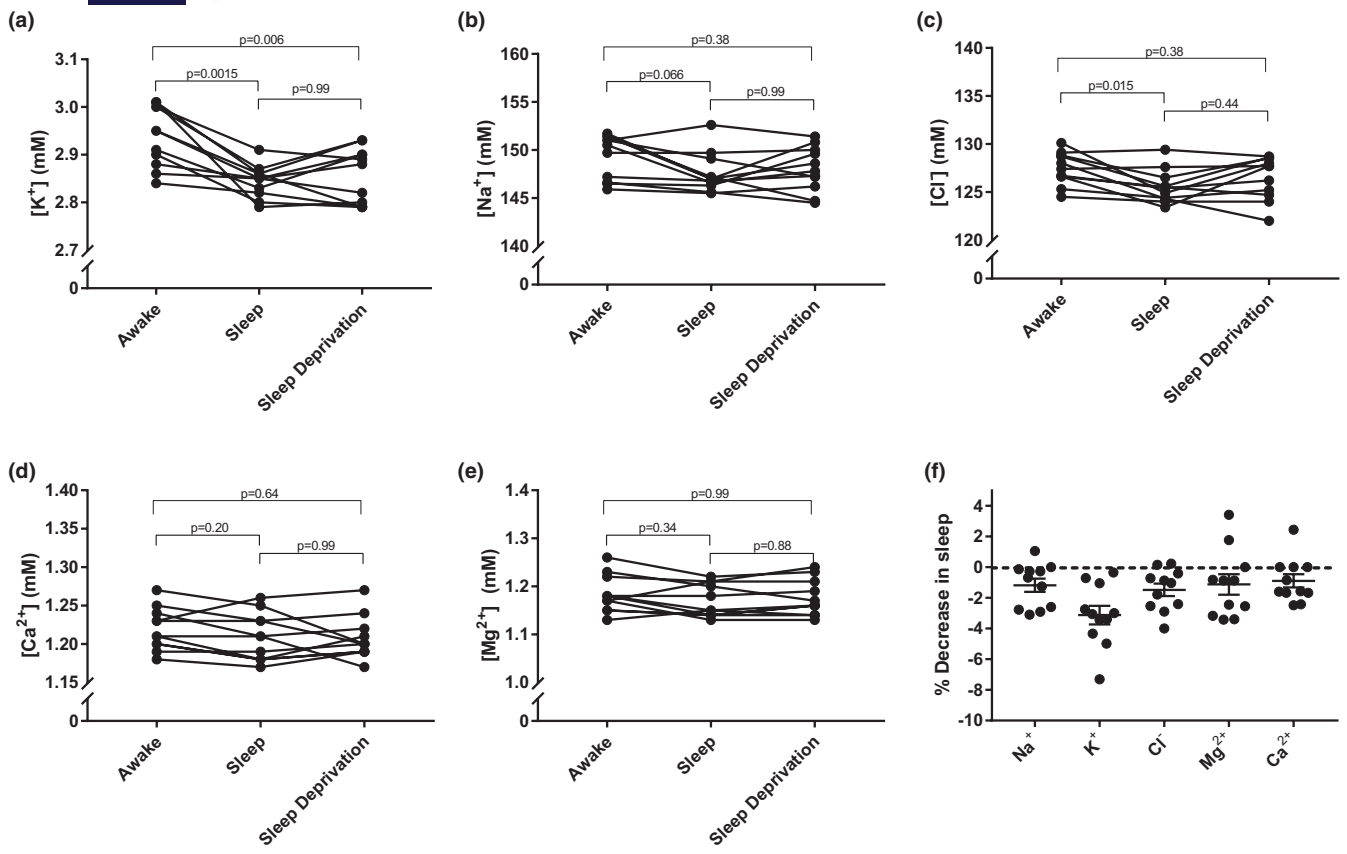

FIGURE 1 The concentrations of ions in awake-cerebrospinal fluid (CSF), sleep-CSF and sleep deprivation-CSF, obtained from three separate occasions in randomized order. (a–e) Absolute changes in potassium, sodium, chloride, calcium and magnesium, respectively. (f) Relative changes in sleep-CSF versus awake-CSF ($n = 11$). Lines connect paired data (study participants)

awake-CSF including sodium, chloride, magnesium and calcium were all of a similar magnitude, 0.9%–1.5%, whereas the decrease in potassium was considerably larger, on average 3.1% (Figure 1f).

The findings led us to examine the overall osmolality of the various CSF samples as well as the albumin content. The osmolality was similar in all samples (323 ± 4 mOsm kg^{-1} in awake-CSF versus 327 ± 10 mOsm kg^{-1} in sleep-CSF and 324 ± 3 mOsm kg^{-1} in sleep deprivation-CSF; Figure 2a). Somewhat to our surprise, albumin in CSF showed the opposite pattern to the ion concentrations, being significantly higher in sleep-CSF compared with awake-CSF (182.5 ± 19.3 mg L^{-1} and 161 ± 16.8 mg L^{-1}; Figure 2d). In CSF obtained after a night of sleep deprivation, values were 157.2 ± 16.4 mg L^{-1} albumin, non-significantly lower than that in sleep-CSF but similar to the concentration in awake-CSF. In order to determine if the change in albumin concentration was specific to the central nervous system, we compared with levels in serum collected at the same time as the CSF. The serum concentration of albumin was 44.5 ± 0.5 g L^{-1} in sleep-serum, 43.3 ± 1.0 g L^{-1} in awake-serum and 44.9 ± 1.4 g L^{-1} in serum from sleep-deprived subjects (Figure 2e). There were no significant differences between the groups. Similarly, the serum osmolality did not differ significantly between the three groups (297 ± 1 mOsm kg^{-1} in awake-serum, 294 ± 2 mOsm kg^{-1} in sleep-serum and 294 ± 1 mOsm kg^{-1} in sleep deprivation, respectively; Figure 2b).

4 | DISCUSSION

In this study we have, for the first time, examined ion concentrations in human CSF during a setting of sleep, sleep deprivation and awake conditions. There was a rather modest, but robust and highly significant reduction in $[K^+]_E$ following sleep as well as following sleep deprivation at the same time of the day. Although this study was not specifically designed to investigate circadian rhythmicity, our result suggests a circadian regulation of $[K^+]_E$ in the CSF, rather than a change in $[K^+]_E$ as a consequence of sleep pressure. A specific circadian regulation of $[K^+]_E$ might involve Kir4.1 channel activity in astrocytes (Rasmussen et al., 2020), perhaps involving post-translational regulation as has been shown for potassium channels in red blood cells (Henslee et al., 2017). Other ions measured, Na^+, Cl^-, Ca^{2+} and Mg^{2+}, all followed a similar pattern as K^+ with the highest levels during wakefulness. However, these changes were smaller and inconsistent. We do not think that the change in $[K^+]_E$ could be explained by a circadian regulation of the water content in the CSF (Hablitz et al., 2020), because we found no significant change in osmolality and an increase, rather than a decrease, in the albumin concentration in sleep-CSF.

Ding et al. (2016), who were the first to suggest that sleep–wake changes in neuronal excitability are mediated via changes in the extracellular concentration of ions, showed that $[K^+]_E$ was decreased, while $[Ca^{2+}]_E$ and $[Mg^{2+}]_E$ were increased in brain ISF during sleep

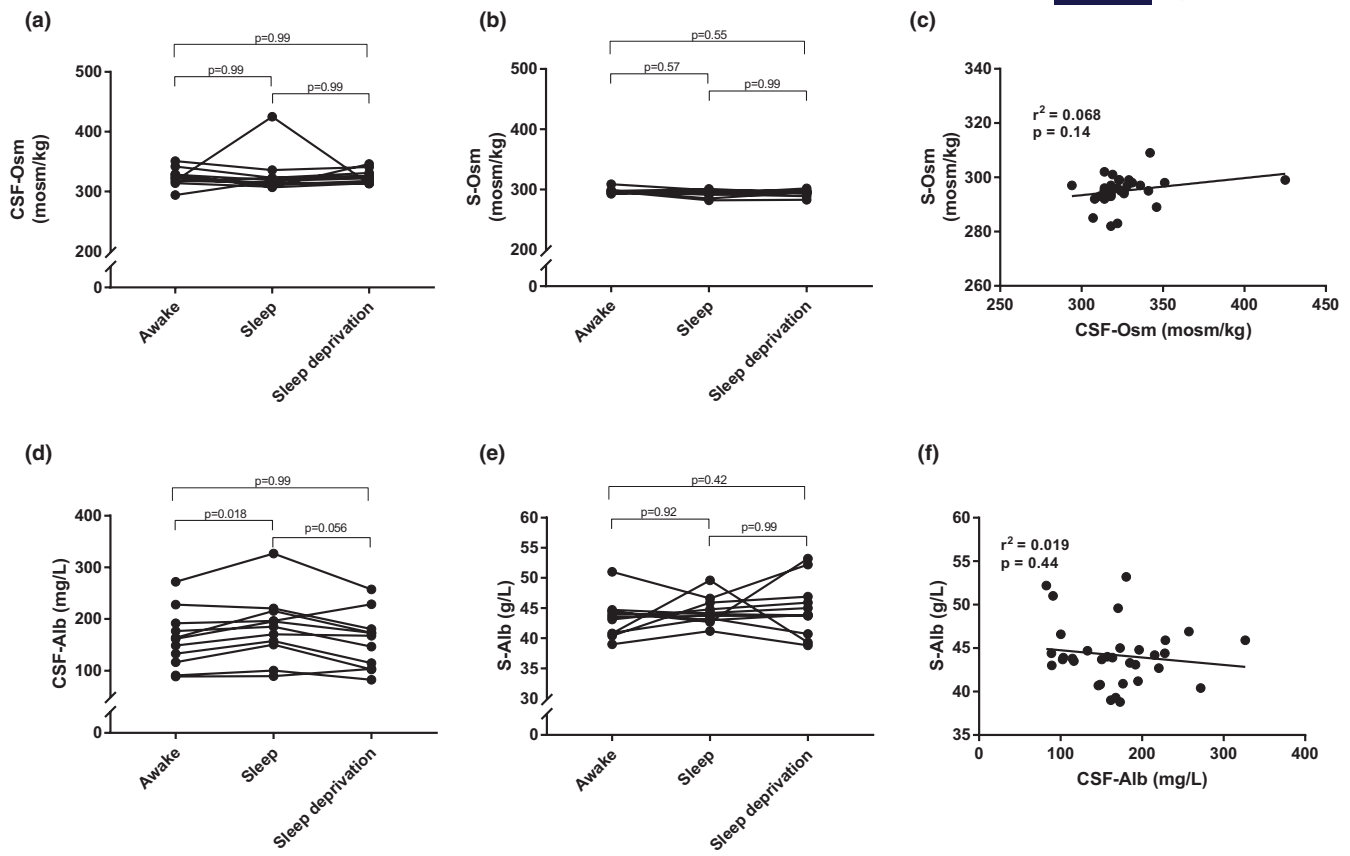

FIGURE 2 Osmolality and albumin concentration in cerebrospinal fluid (CSF) and serum during awake, following sleep and following sleep deprivation. (a) CSF and (b) serum osmolality during awake, following sleep and following sleep deprivation. (c) Correlation between CSF and serum osmolality during awake. (d) CSF and (e) serum albumin concentration during awake, following sleep and following sleep deprivation. (f) Correlation between CSF and serum albumin concentration during awake ($n = 11$)

in mice. In our study of human CSF, we reproduced the result with respect to $[K^+]_E$, but not with respect to $[Ca^{2+}]_E$ and $[Mg^{2+}]_E$. The discrepancy may be explained by the site of sampling, as lumbar CSF may not fully reflect brain ISF, and this may explain the lack of change in $[Ca^{2+}]_E$ and $[Mg^{2+}]_E$, and the smaller change in $[K^+]_E$. We detected a change in $[K^+]_E$ of about 0.1 mM in the CSF, which is probably an underestimation of the brain ISF. Reasons for an underestimation of the circadian shift may include a concentration gradient from the brain to the lumbar region, a quicker than expected shift in lumbar CSF (the participants were awake a couple of minutes before the sampling of sleep-CSF) or increase in neuromodulators, such as noradrenaline, before awaking. Nevertheless, a change of 0.1 mM would correspond to a change in the membrane potential of about 1 mV (according to the Goldman equation). Moreover, if changes in $[K^+]_E$ affect excitatory more than inhibitory neurons (Rasmussen et al., 2020), even small changes in $[K^+]_E$ could significantly contribute to the modulation of network excitability.

Although our data do not allow us to establish whether the change in $[K^+]_E$ is mediating or occurring as a consequence of circadian rhythmicity in network excitability, previous studies favour a mediating role. Studies in mice have demonstrated that imposed changes in $[K^+]_E$ can shift brain states, as determined by changes in EEG patterns and behaviour (Ding et al., 2016; Rasmussen et al., 2019). This is also supported by a study that used a mathematical neuronal model to show sleep- and awake-like firing patterns facilitated by changes in extracellular potassium concentration (Rasmussen, Jensen, & Heltberg, 2017). In conclusion, our results support the notion of a circadian regulation of $[K^+]_E$ in human CSF.

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AUTHOR CONTRIBUTIONS

All authors participated in designing the experiments. M. F. and M. O. conducted the experiments. M. F. designed and performed the analyses. M. F. and E. H. wrote the paper. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors present no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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