Effects of selective REM deprivation and stress on the architecture of subsequent sleep rebound and some hypothalamic neuropeptides

Ph.D. theses

Tamás Kitka

Semmelweis University

Szentágothai János School of Ph.D. studies

Supervisor: Prof. György Bagdy D.Sc.

Official reviewers: Dr. Csaba Fekete D.Sc.

Dr. András Boros Ph.D.

Head of the Final Examination Committee:

Prof. Attila Fonyó D.Sc.

Members of the Final Examination Committee:

Dr. Róbert Bódizs Ph.D.

Dr. Lucia Wittner Ph.D.

Budapest

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1 Introduction

1.1 Stages of sleep

The stages of sleep are basically, but not exclusively identified based on their electroencephalographic (EEG) characteristics. This method gives information about the synchronicity of neuronal activation at the observed region; hence the amplitude of the EEG sign is high if the neurons show synchronous activation. Generally, the EEG waves are classified based on their frequency. The borders of EEG bands are not clearly defined, thus the exact values used by different workgroups may vary. The bands studied are usually the following: slow oscillation (0.5-1 Hz), delta (1-4 Hz), theta (5-7 or 5-9 Hz), alpha (8-13 or 10-20 Hz), beta (14-30 or 20-30 Hz) and gamma (30-60 Hz).

The sleep consists of REM (rapid eye movement) sleep and SWS (slow wave sleep). Synonyms for these names also exist: PS (paradoxical sleep) for REM and NREM (non-REM) for SWS. In animal studies, two substages of SWS are defined (SWS1 and SWS2).

1.2 The melanin-concentrating hormone

The MCH (melanin-concentrating hormone) is a 19-amino acid cyclic neuropeptide that has been first described in salmon pituitary as a hormone responsible for skin paling. It has been shortly described that this peptide is also expressed in the mammalian brain, but it doesn’t affect skin color there. In mammals, MCH-containing neurons are situated in the tuberal hypothalamus and zona!subzona incerta

MCH-ergic neurons send projections to several brain regions involved in the regulation of sleep/waking and food intake. Several peptides were found to be colocalized with MCH. For example. most of MCH-ergic neurons express nesfatin and GABA (gamma-aminobutyric acid), and a portion of them also contain CART (cocaine- and amphetamine-regulated transcript).

The role of MCH-ergic system in the regulation of sleep and waking has been studied by several workgroups. Their results equivocally prove that MCH-ergic neurons reach their maximal activity during REM sleep, and pharmacological, immunological or genetical modulation of this system affects time spent is REM sleep. Contrary, the effect of MCH-ergic activity on the architecture of REM sleep is unknown. There are results in

the literature supporting the role of MCH in the modulation of either duration and frequency of REM sleep episodes, but other findings also exist that confute both roles of this system.

MCH-ergic neurons can be divided into two subpopulations based on their CART-immunoreactivity (CART-IR): there are CART-containing and non-CART-IR MCH-ergic neurons. These subpopulations also differ regarding their projection areas: CART-IR neurons send fibers to the cerebral cortex, the medial septal complex (a region having a known role in the generation of hippocampal theta), the hippocampus, but also have descending fibers reaching the tectum, dorsolateral periaqueductal grey and dorsal raphe. In the brainstem, the densest innervation from this subpopulation is received by the dorsal paragigantocellular nucleus. In contrast, the non-CART-IR subpopulation of MCH-ergic neurons innervates some regions in the lower brainstem and spinal cord.

Both subgroups of MCH-ergic cells are activated during the sleep rebound following REM-deprivation using a small platform (for the description of this method see chapter **Hiba! A hivatkozási forrás nem található.**). However, it has to be noted that this protocol is very stressful for the animals and the MCH-ergic system is known to play a role in behavioural and thermoregulatory effects of stress. Furthermore, several stress-induced changes can be blocked by using MCHR1- (MCH receptor 1) antagonists. Thus it can be presumed that the stress caused by this method can play a role in the aforementioned activation of the MCH-ergic neurons during sleep rebound.

1.1 Orexins

Orexin A and B (also known as hypocretin 1 and 2) have been first described in 1998. The first identified role of these peptides is the modulation of food intake. These two orexins are spliced from the same precursor, prepro-orexin. The localization of orexin-containing neurons is reminiscent of the one of MCH-ergic cells: the somae are situated at the dorsal LH (lateral hypothalamus), PFA (perifornical area) and PH (posterior hypothalamus). These neurons send fibers to almost the entire central nervous system, but the majority of their terminals can be found at brain regions related to the regulation of food intake, autonomic control and sleep and waking.

Orexinergic neurons cease firing during sleep, with the exception of limb twitches during REM sleep. Accordingly, activation of orexinergic system has been shown to reduce the time spent in sleep, and also the time spent in REM sleep. Its latter effect is exerted through the decreased frequency of REM sleep episodes.

Orexinergic and MCH-ergic neurons have reciprocal monosynaptic connections. MCH-ergic cells express orexin 1 receptor, and correspondingly orexin A and B have been shown to excite the MCH-containing neurons. The excitability of orexinergic neurons by orexin-A can be attenuated using MCH, and this effect of MCH is diminished in MCHR1 knockout mice.

1.2 The "flower-pot" REM-deprivation technique

In this method, the animal is placed on a small, round platform surrounded by water. The size of this platform is sufficient for the animal to stand on it, but it is not large enough to lie down even when curled. Thus as the animal reaches REM sleep, it falls in the water because of the muscle atony and awakes. Usually two platforms of different sizes are used: a smaller one described above and a larger one as control. This latter one is big enough for the animal to sleep curled, so it is possible to reach REM sleep, but the two platforms are similar in terms of other circumstances.

It is known that sleep deprivation using both small and large platforms decreases time spent in SWS, and also causes severe stress. However, it is proven that the basic difference between the sleep pattern of animals on a small or large platform is present in the time spent in REM sleep, the two platforms are similar in terms of time spent in SWS. Furthermore, they are also similar regarding the stress caused. Based on these data, we can presume that it is possible to study the effects of selective REM deprivation by comparing the effects of these two platforms, although neither of them is capable to evoke selective REM deprivation. Hence the comparison of the effects of these two platforms will be referred hereafter as effects of selective REM deprivation.

2 Aims

The effects of "flower pot" method on sleep pattern, as well as the effects of repeated sleep deprivation and rebound have been studied by several workgroups. However, the comparison of the sleep patterns throughout 24 hours after sleep deprivation by a small and a large (stress-control) platform has not been described yet. So the effects of selective REM deprivation were not separated from the effects of stress caused by this method, and the time course of the sleep rebound was also unknown. The aim of our experiment on the architecture of REM rebound was to perform this comparison, and thus to describe the time course of the sleep architecture during the sleep rebound caused by the selective REM deprivation. Thereafter, we studied the connection between the architecture of sleep rebound caused by the selective REM deprivation and the endogenous activation of hypothalamic MCH-ergic and orexinergic neurons. Furthermore, we also aimed to clarify whether the activation of CART-IR and non-CART-IR subpopulations of MCH-ergic neurons is associated with the architecture of REM sleep in a similar, or different manner.

Summarizing the aims of the experiments:

- The aim of the first experiment was to describe (1) the architecture of REM rebound, and (2) the time course of sleep rebound following selective REM deprivation (Kitka et al, 2009).
- In the second experiment, we intended to clarify that (3) which subpopulation (CART-IR, non-CART-IR) of MCH-ergic neurons has neurophysiological connection with the REM rebound following selective REM deprivation (Kitka et al, 2011).
- Besides, we studied (4) whether the activation of MCH- and orexin-containing neurons can be correlated with the changes in sleep pattern caused by selective REM deprivation. In other words, we aimed to test the possibility that these neuronal populations play an important role in the regulation of sleep architecture following selective REM deprivation.

3 Materials and methods

3.1 Study design

Male Wistar rats were equipped with frontoparietal EEG electrodes and EMG electrodes in the neck muscles in both experiments. Motor activity was recorded by the registration of the movements of the cable for EEG and EMG signs. After the recovery from the surgery, animals were habituated to the experimental conditions. The following animal groups were used:

- HC (home cage) group: animals were kept in their home cages during the time of sleep deprivation
- SP (small platform) group (only at the second experiment): animals spent 72 hours on a small platform
- LP (large platform) group (only at the second experiment): animals spent 72 hours on a large platform
- SPR (small platform, sleep rebound) group: animals spent 72 hours on a small platform, and after that a polysomnographic recording was performed during the sleep rebound
- LPR (large platform, sleep rebound) group: animals spent 72 hours on a large platform, and after that a polysomnographic recording was performed during the sleep rebound

The diameter of the small and the large platform was 6.5 cm and 13 cm, respectively. After the sleep deprivation, a 23-hour-long or a 3-hour-long sleep rebound was performed in the case of the first or the second experiment, respectively. In the second experiment, animals were transcardially perfused immediately after the sleep deprivation (HC, SP and LP groups) or immediately after the sleep rebound (SPR, LPR). The sleep deprivation and rebound was started at lights on at all cases. Water and food was available *ad libitum* for all animals during both experiments.

3.2 Evaluation of the recordings

The vigilance states were classified by SleepSign for Animal sleep analysissoftware (Kissei Comtec America, Inc., USA) for 4 s periods over 23 h as follows:

- AW (active wakefulness): the EEG is characterized by low amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied by high EMG and motor activity
- PW (passive wakefulness): the EEG is characterized by low amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied by high EMG activity
- SWS1 (light slow wave sleep): high voltage slow cortical waves (0.5–4 Hz) interrupted by low voltage fast EEG activity (spindles 6–15 Hz) accompanied by reduced EMG and motor activity
- SWS2 (deep slow wave sleep): continuous high amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity
- IS (intermediate stage of sleep): a brief stage just prior to REMS and sometimes just after it, characterized by unusual association of high-amplitude spindles (mean 12.5 Hz) and low-frequency (mean 5.4 Hz) theta rhythm
- REM (rapid eye movement) sleep: low amplitude and high frequency EEG activity with regular theta waves (5–9 Hz) accompanied by silent EMG and motor activity with occasional twitching

After the automatic scoring, recordings were visually verified. In the case of the study on the architecture of REM-rebound, the following parameters were calculated for each animal, with hourly resolution:

- Time spent in each sleep stage
- TSWS (total slow wave sleep): SWS1 + SWS2
- SWS2%: time spent in SWS2 in the percent of TSWS (indicated the average deepness of SWS)
- TW (total wakefulness): AW + PW
- Average length of REM sleep episodes
- Number or REM sleep episodes

In the case of the study on the neurobiological background of REM sleep architecture during the rebound, only the pattern of REM sleep was evaluated (time spent in REM, average length of REM episodes, number of REM episodes). The so-called short REM attempts (sRa-s) were not taken into the calculation of the number and average REM

sleep episodes. Thus a REM episode was defined to be at least 16 s long and not interrupted by 16 s or longer period of other vigilance stage.

3.3 Immunohistochemistry

Immunohistochemistry was performed only at the study on the neurobiological background of REM sleep architecture during the rebound. Animals were deeply anesthetized, transcardially perfused, their brains were removed and 4 series of 50 μm thick sections were made from their hypothalami.

All primary antibodies were produced in rabbit, except from the MCH/CART/cFos triple immunohistochemistry, where we used goat anti-MCH. Microwave treatment was used in order to avoid antibody cross-reaction and to block the horse radish peroxidase. In case of double immunohistochemistry, we visualized the cFos-containing and MCH- or orexin-containing neuronal elements with nickel-diaminobenzidine and diaminobenzidine, respectively. In case of triple immunohistochemistry, the visualization was performed using FITC-Tyramide (cFos), Alexa 594 (CART) and Streptavidin-Pacific Blue (MCH).

We quantified the MCH-immunoreactive (MCH-IR) cells at the zona incerta (ZI), lateral hypothalamus (LH), paraventricular area and perifornical area (PFA); as well as the orexin-IR cells at the LH, PFA, dorsomedial and posterior hypothalamus for the double immunohistochemistry using a Reichert visopan microscope. For the triple immunohistochemistry, labelled neuronal elements were counted at the ZI, PFA and LH using a Nikon Eclipse E 8000 confocal microscope.

3.4 Statistical evaluation

We used repeated measures ANOVA (analysis of variance) on the data of predefined time intervals at the study on the architecture of REM rebound. The factors were “group” (non-repeated) and “time” (repeated). Studied intervals were: 2-24 hours, 2-6 hours, 7-12 hours, 2-12 hours, 13-24 hours. Besides, the data of each hour were evaluated using one-way ANOVA (factor: “group”). In case of significant effect on “group” ($p<0.05$), we performed a Tukey’s post hoc test using the factor “group”. A difference between groups was considered to be significant in case of significance ($p<0.05$) in the post hoc test.

Daily rhythms were evaluated using cosinor analysis (fitting of a sinusoid curve) with a period time of 24 hours. We considered a difference between groups to be significant if the 95% confidence intervals did not overlap. The studied parameters of the curve were acrophase (the place of the maximal value), mesor (average value) and amplitude.

The data of different groups coming from the double immunohistochemistry and the evaluation of REM sleep pattern were compared using one-way ANOVA (factor: "group"). We used factorial ANOVA for the statistical evaluation of triple immunohistochemistry (factors: "group" and "CART-immunoreactivity"). We performed a Tukey's post hoc test in case of significant ANOVA effect of "group".

Interindividual correlations were studied using linear regression. As the distribution of the percentage of cFos-IR neuronal elements within orexin-IR cells was exponential-like in most comparisons, we used the logarithm with base 10 for the regression analysis of this rate.

STATISTICA 7.0 (Statsoft, Inc, Tulsa, OK, USA) and Time Series Analysis Seriel Cosinor 6.0 Lab View (Expert Soft Technologie) software were used for the statistical analysis. Data in all figures are expressed as mean ± SEM.

4 Results

4.1 Study on the 24-hour architecture of the REM-rebound

The architecture of sleep rebound following selective REM-deprivation, and its time curve were studied in this experiment (Kitka et al, 2009).

4.1.1 Sleep parameters

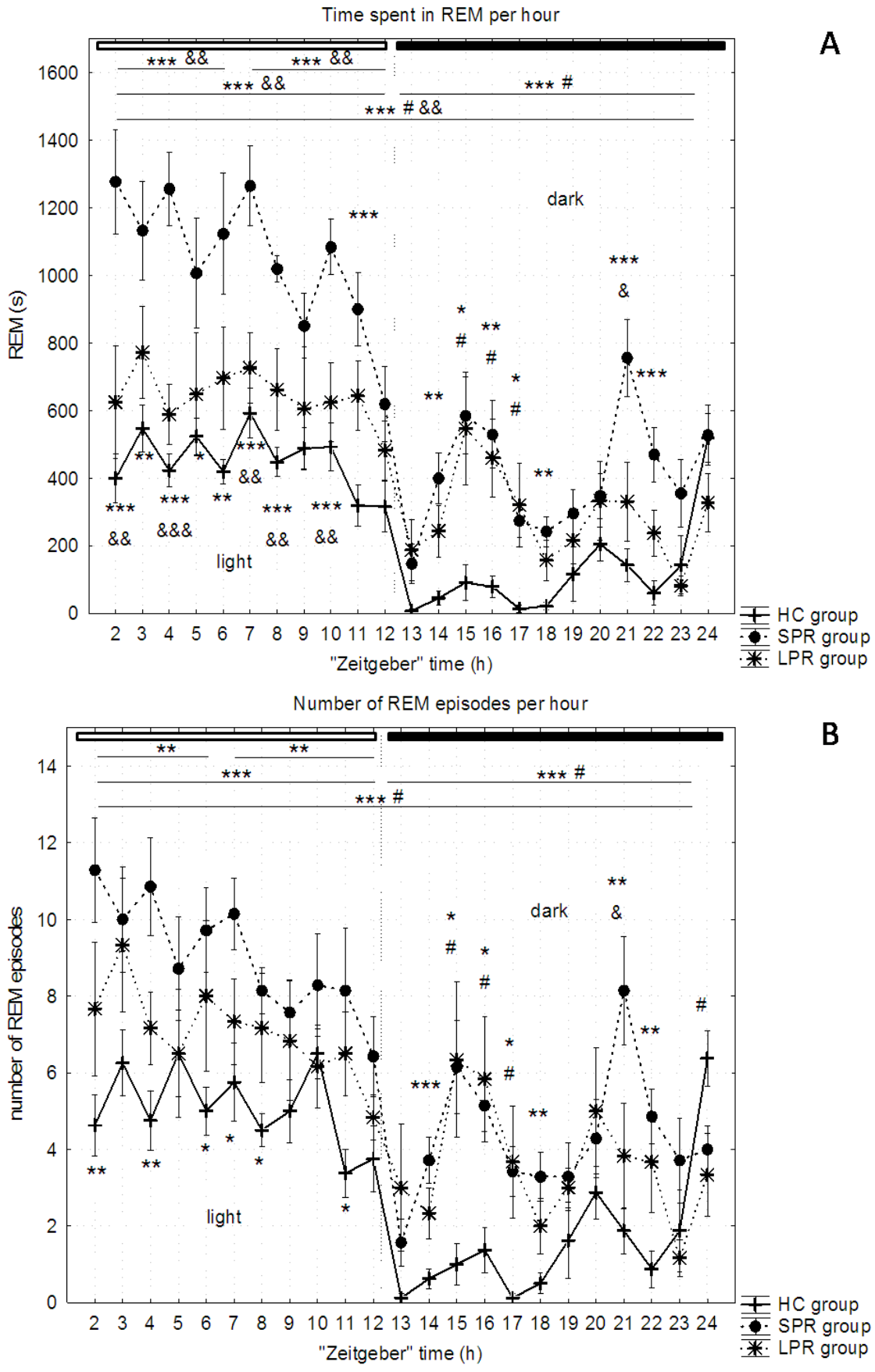
Several differences were seen between the sleep pattern of the three groups throughout the whole recorded day. In general, the data of SPR group were different from the data of HC group during the whole day. The data of LPR group showed differences from the SPR and HC group mostly during the passive and active phase, respectively. For the sake of clarity, the results of this experiment (significant differences) are demonstrated in a table (Table 1). The results regarding the architecture of REM sleep are shown in more details (Figure 1).

Table 1 (next page). Differences between the sleep parameters of HC (home cage), SPR (small platform, sleep rebound) and LPR (large platform, sleep rebound) groups. AW: active wakefulness, PW: passive wakefulness, SWS1: light slow wave sleep, SWS2: deep slow wave sleep, IS: intermediate stage of sleep, REM: rapid eye movement sleep, sRa: short REM attempt, TSWS: total slow wave sleep, TW: total wakefulness, SWS2%: SWS2 in the percent of TSWS. The light (passive) phase started at the beginning of the measurement and lasted for 12 hours. One sign: $p<0.05$, two signs: $p<0.01$, three signs: $p<0.001$. ▲: increased,▼: decreased

	SPR group compared to LPR group				
	2-24 h	2-6 h	7-12 h	2-12 h	13-24 h
AW					
PW					
SWS1		▼			
SWS2					
IS					
REM	▲▲	▲▲	▲▲	▲▲	
REM no.					
REM length	▲▲	▲	▲	▲▲	
REM no. incl. sRa-s					
REM length incl. sRa-s	▲	▲	▲	▲▲	
TSWS		▼			
TW	▼				
SWS2%					

	SPR group compared to HC group				
	2-24 h	2-6 h	7-12 h	2-12 h	13-24 h
AW	▼				▼▼
PW					
SWS1					▲▲
SWS2					
IS					
REM	▲▲▲	▲▲▲	▲▲▲	▲▲▲	▲▲▲
REM no.	▲▲▲	▲▲	▲▲	▲▲▲	▲▲▲
REM length	▲▲	▲	▲▲	▲▲	
REM no. incl. sRa-s	▲▲▲	▲▲	▲▲	▲▲	▲▲
REM length incl. sRa-s	▲▲▲	▲▲	▲▲	▲▲	▲
TSWS		▼▼▼		▼	▲▲
TW	▼▼▼		▼▼		▼▼▼
SWS2%					

	LPR group compared to HC group				
	2-24 h	2-6 h	7-12 h	2-12 h	13-24 h
AW					▼
PW					
SWS1	▲				▲▲
SWS2					▼
IS					
REM	▲				▲
REM no.	▲				▲
REM length					
REM no. incl. sRa-s	▲				▲
REM length incl. sRa-s					
TSWS					
TW	▼▼▼				▼▼
SWS2%					▼


Time spent in REM per hour
A
REM (s)
"Zeitgeber" time (h)
light
dark
HC group
SPR group
LPR group
Number of REM episodes per hour
B
number of REM episodes
"Zeitgeber" time (h)
light
dark
HC group
SPR group
LPR group

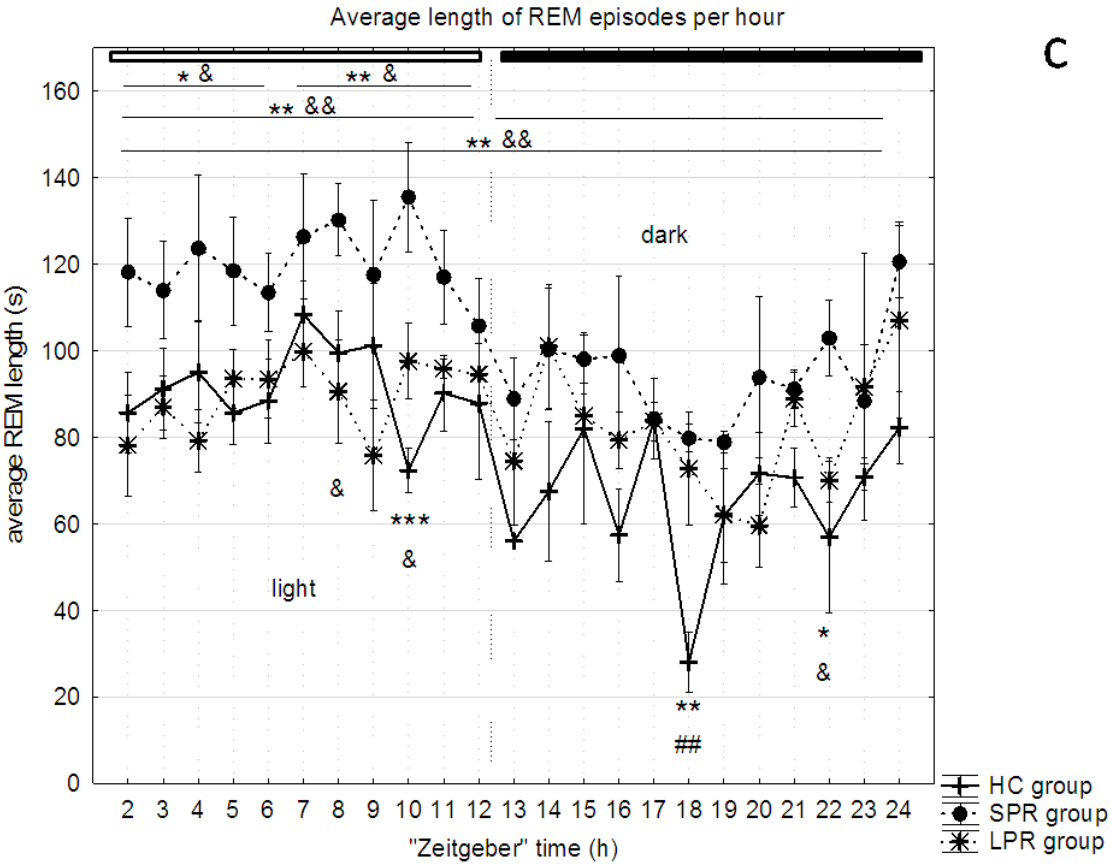


Figure 1 Time spent in REM sleep (A), number (B) and average length (C) of REM sleep episodes throughout 24 hours following the 72-hour-long sleep deprivation period. HC: home, SPR: small platform sleep rebound, LPR: large platform, sleep rebound. *: difference between HC and SPR, #: difference between HC and LPR, &: difference between SPR and LPR groups. One sign: p<0.05, two signs: p<0.01, three signs: p<0.001

4.1.2 Evaluation of daily rhythms

We did not find any alterations between the acrophases of any groups or studied parameters, meaning that we did not find any phase-shift. Thus only the amplitude and mesor values will be demonstrated (Table 2).

Table 2 The results of the cosinor analysis. HC: home cage, SPR: small platform sleep rebound, LPR: large platform sleep rebound group; AW: active wakefulness, PW: passive wakefulness, SWS1: light slow wave sleep, SWS2: deep slow wave sleep, IS: intermediate stage of sleep, REM: rapid eye movement sleep, sRa: short REM attempt, TSWS: total slow wave sleep, TW: total wakefulness, SWS2%: SWS2 in the percent of TSWS. *: difference between HC and SPR, #: difference between HC and LPR, &: difference between SPR and LPR groups (p<0,05)

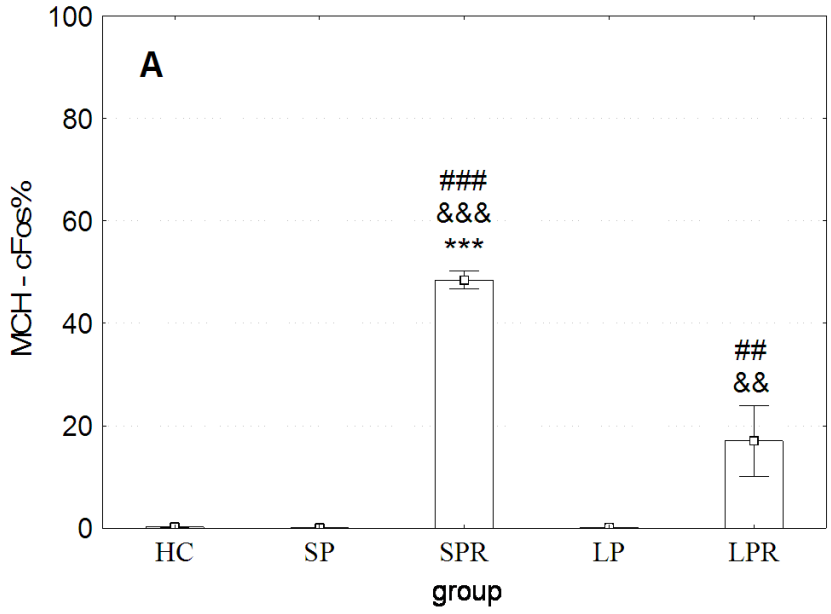
	group	p(H0)	amplitude			mesor		
			mean	CI min	CI max	mean	CI min	CI max
AW (s)	HC	0	995	860	1130	1130	1030	1230
	SPR	0	595 *	475	716	820 *	733	907
	LPR	0	640 #	496	784	861 #	757	964
PW (s)	HC	0,0127	118	40,3	197	730	674	785
	SPR	0	192	137	247	540*&	500	579
	LPR	0,0007	210	104	317	696	620	773
SWS1 (s)	HC	0	329	253	405	646	591	701
	SPR	0,4346	50,6 *	-26,6	128	775 *&	722	829
	LPR	0,0038	200	84,1	316	1020 #	935	1100
SWS2 (s)	HC	0	555	456	653	699	629	768
	SPR	0	300 *	220	318	582 &	525	639
	LPR	0	347 #	243	451	362 #	288	436
IS (s)	HC	0	96,3	79,5	113	112	99,9	124
	SPR	0	92,6	71,3	114	161 *&	145	176
	LPR	0	131	91,1	170	205 #	176	234
REM (s)	HC	0	250	215	256	283	257	308
	SPR	0	442 *&	373	511	723 *&	673	772
	LPR	0	231	163	299	457 #	408	507
REM no.	HC	0	2,67	2,22	3,12	3,33	3	3,65
	SPR	0	2,52	1,79	3,25	5,49 *	4,97	6,02
	LPR	0	2,19	1,39	2,99	5,62 #	4,69	5,84
REM no. (incl. sRa-s)	HC	0	3,32	2,78	3,87	4,32	3,93	4,7
	SPR	0	3,5	2,68	4,32	7,88 *&	7,29	8,47
	LPR	0	2,64	1,76	3,53	6,45 #	5,81	7,08
TSWS (s)	HC	0	770	659	881	1340	1270	1420
	SPR	0	260 *&	172	348	1360	1290	1420
	LPR	0	496 #	391	600	1380	1310	1460
TW (s)	HC	0	1110	972	1250	1860	1760	1960
	SPR	0	788 *	653	923	1360 *	1260	1460
	LPR	0	844	692	996	1560 #	1450	1670
SWS2%	HC	0	18,4	13	23,7	45,8	42	49,5
	SPR	0	13,8	8,79	18,8	40,5 &	36,9	44
	LPR	0	13,7	7,47	20	22,9 #	18,5	27,3

4.2 Study on the neurobiological background of the architecture of REM-rebound

Slimilarly to the previous experiment, we have seen that the SPR animals spent more time in REM sleep than the LPS animals during the rebound following the 72-hour-long sleep deprivation (p=0.011). In addition, this difference was accompanied by an increase in the average length of REM episodes (p=0.017), while the number of REM episodes was unchanged.

4.2.1 Morphological data

The ratio of orexin-cFos and MCH-cFos double labelled neurons is demonstrated by Figure 2, while the ratio of cFos-IR neurons within the CART-IR and non-CART-IR subpopulations of MCH-containing neurons is shown at Figure 3.



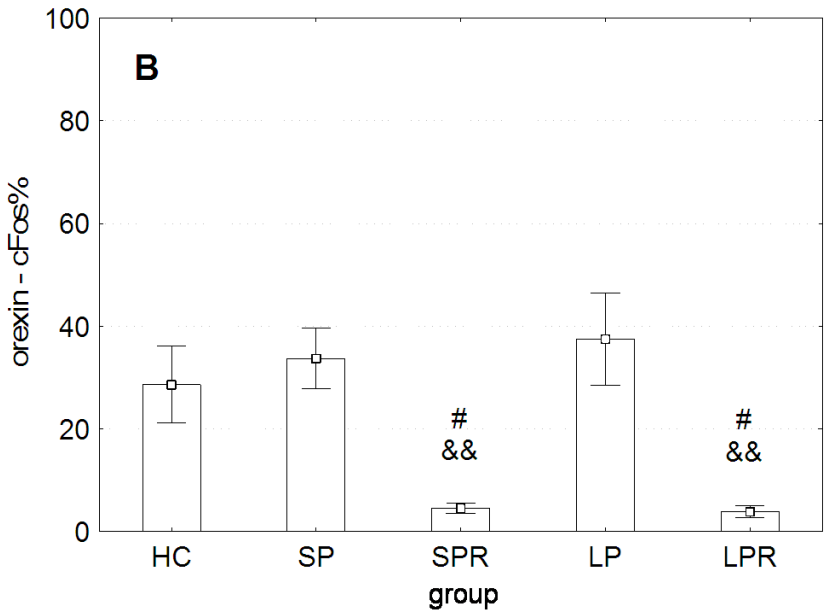
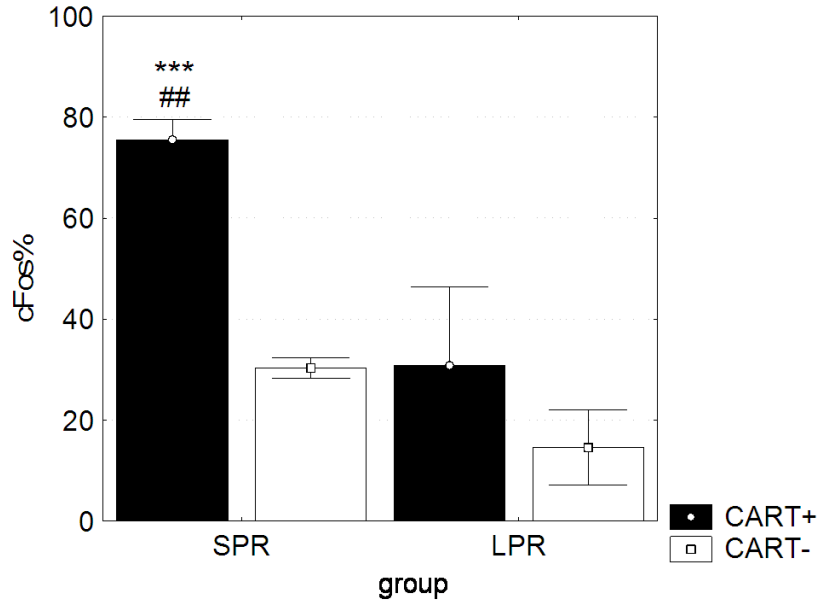


Figure 2 Percent of cFos-staining cells within MCH- (melanin-concentrating hormone) immunoreactive (A) and orexin-immunoreactive (B) neurons at all studied brain regions after the 3-hour-long rebound following 72-hour-long sleep deprivation. HC: home cage, SP: small platform sleep deprived, SPR: small platform sleep rebound, LP: large platform sleep deprived, and LPR: large platform sleep rebound; #: difference compared to HC group, &: difference compared to respective non-sleep rebound group, *: difference between SPR and LPR groups; one sign: p<0.05, two signs: p<0.01, three signs: p<0.001

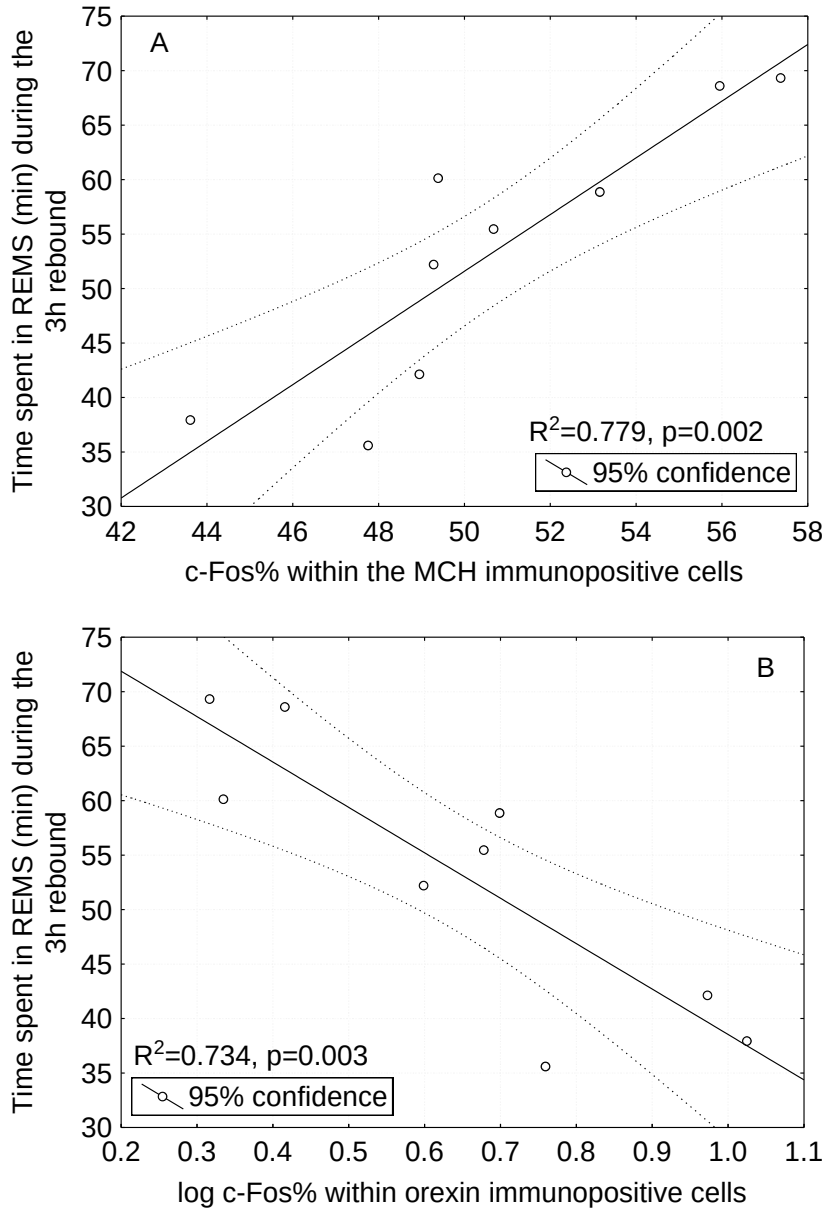


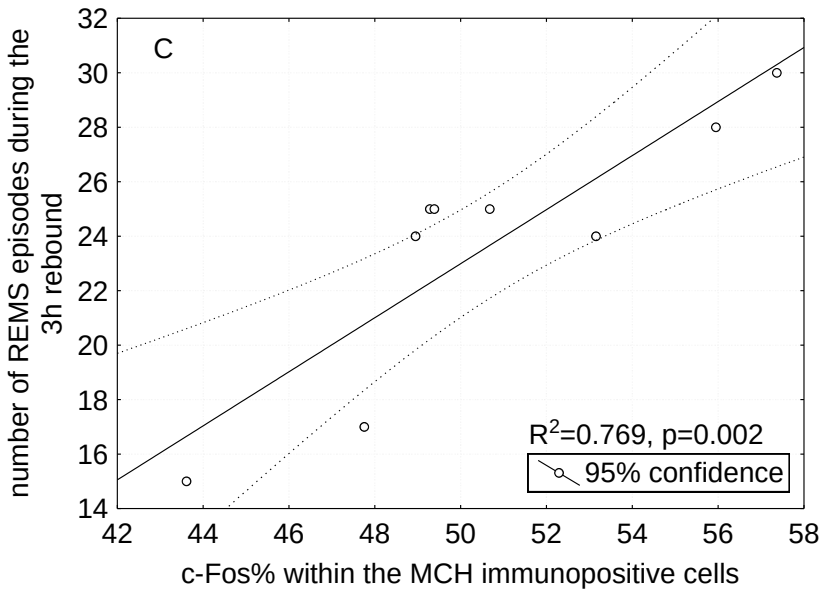
3. ábra. Percent of cFos immunoreactive cells within the CART-IR and non-CART-IR subpopulations of melanin-concentrating hormone-containing neurons after the 3-hour-long rebound following 72-hour-long sleep deprivation. SPR: small platform sleep rebound, LPR: large

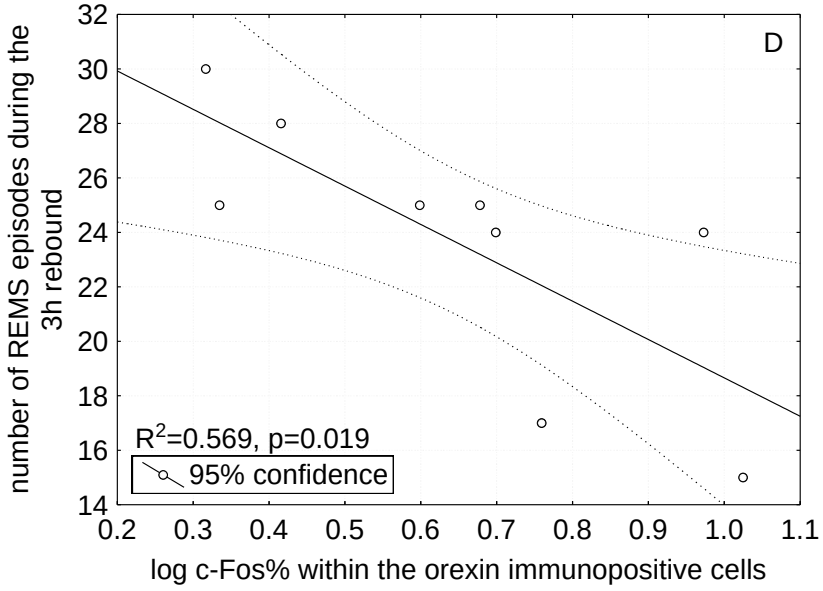
platform sleep rebound, CART-IR: cocaine- and amphetamine regulated transcript-immunoreactive; ***: p<0.01 compared to SPR non-CART-IR cell group, ##: p<0.01 compared to LPR CART-IR cell group

4.2.2 Interindividual correlations

In order to elucidate the relation between the architecture of REM sleep and the activation of MCH- and orexin-containing neurons, we performed a linear regression on the data of SPR group. The correlations found are demonstrated by Figure 4. None of the studied immunohistochemical parameters have shown a correlation with the average length of REM episodes.




C
number of REMS episodes during the 3h rebound
c-Fos% within the MCH immunopositive cells
R2=0.769, p=0.002
95% confidence


D
number of REMS episodes during the 3h rebound
log c-Fos% within the orexin immunopositive cells
R2=0.569, p=0.019
95% confidence

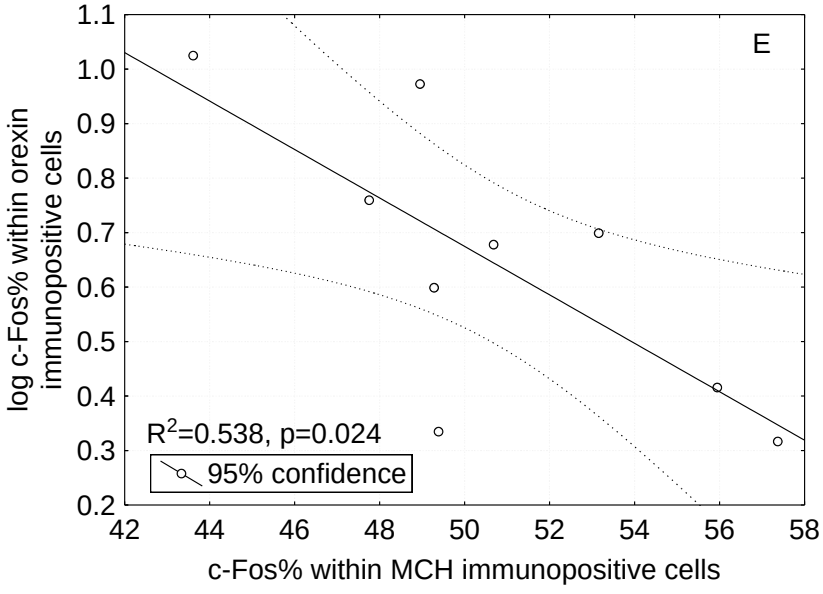

E
log c-Fos% within orexin immunopositive cells
c-Fos% within MCH immunopositive cells
R2=0.538, p=0.024
95% confidence

Figure 4 (previous page) Correlations between individual parameters in the small platform sleep rebound group. (A) Time spent in REM during the 3 h rebound and the percentage of cFos double-stained neurons within the MCH-IR (melanin-concentrating hormone-immunoreactive) cells, (B) time spent in REM during the 3 h rebound and the percentage of cFos double-stained neurons within the orexin-IR cells, (C) number of REM episodes during the 3 h rebound and the percentage of cFos double-stained neurons within the MCH-IR cells, (D) number of REM episodes during the 3 h rebound and the percentage of cFos double-stained neurons within the orexin-IR cells, (E) percentage of cFos double-stained neurons within the MCH-IR cells and the percentage of cFos double-stained neurons within the orexin-IR cells

5 Conclusions

1. Although the rebound following sleep deprivation using both a small or a large platform resulted in an elevation of the number of REM sleep episodes, we did not find significant alteration between this parameter of the sleep rebound groups. In contrast, the average length of REM episodes was increased during the rebound following a small platform sleep deprivation compared to the large platform sleep rebound group. Based on this result, we propose that the elongation of REM episodes is characteristic for the REM rebound caused by the selective REM deprivation; while the increased number of REM episodes is a consequence of the stress factors (new environment, humidity, immobilisation), or the general sleep deficit caused by the "flower pot" method.
2. The increased time spent in REM sleep caused by the selective REM deprivation is characteristic for the subsequent passive phase. In contrast, the increased time spent in slow wave sleep can be observed during the forthcoming active phase, when the slow wave sleep becomes superficial in the case of the LPR (stress-control) group
3. Based on our results, the non-CART-IR subpopulation of MCH-ergic neurons show similar activation during the rebound following sleep deprivation by a small or a large platform. Contrarily, the activation of the CART-containing subpopulation is higher during the rebound caused by the sleep deprivation using a small platform, than using a large one. This result suggests that the activation of this neuronal population may be related to the REM-rebound caused by a selective REM deprivation.

4. Despite the findings that the selective REM deprivation results in the elongation of REM episodes during the subsequent sleep rebound, and the activation of MCH-containing neurons is apparent parallelly, we did not find an interindividual correlation between these parameters. In contrast, there was a significant correlation between the number of REM episodes and the activation of MCH- and orexin immunoreactive neurons. In accordance with their known effects on REM sleep, this correlation was positive in the case of MCH-ergic and negative in the case of orexinergic neurons. Therefore we can presume that the pattern of REM rebound caused by selective REM deprivation (i.e. the increased average length of REM episodes) is not regulated by the MCH-ergic neurons. Thus we may conclude that the neurobiological background of this phenomenon is still unknown, and elucidating its regulation requires further experiments.

6 List of own publications

6.1 Publications used for the dissertation

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