

Effects of chronic escitalopram treatment on vigilance and quantitative EEG following REM-deprivation and acute dosing of a 5-HT_{2C} receptor antagonist

Ph.D. thesis

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1 Introduction

1.1 Sleep-wake cycle and theta oscillations

Electroencephalography (EEG) measures the difference between electrode potentials which originates from the summarized electrical activity of numerous, synchronously firing neurons located perpendicularly to the cortex. Electric signals of the rodent brain can be detected by electrodes implanted epidurally into the skull. Sleep can be divided into REM (rapid eye movement) and non-REM (NREM) sleep. REM phase is characterized by the complete atonia of skeletal muscles; hippocampal theta oscillations dominate on cortical EEG recordings in rodents. Theta waves are also frequent during wakefulness related to different mnemonic functions in humans, and during exploratory locomotor activity in rodents. Sleep-wake regulation is a very complex process, in which several neuronal systems are involved. These serotonergic neurons fire most actively during wakefulness, decrease their activity during NREMS and fall silent during REMS. The relationship between wake- and sleep-promoting neurons appears to be mutually inhibitory, if either side obtains a small advantage over the other; it turns the neurons off on the other side. Transitions occur rapidly but not instantly, due to the large number of neurons; the time taken varies with species and complexity of the brain. The balance between mutual inhibitions is influenced mainly by circadian and homeostatic factors. One of the most important properties of sleep-wake regulation is to ensure the stable state of sleep and wake.

1.2 Sleep and depression

Sleep disturbances are typical symptoms of depression. Sleep fragmentation and insomnia occur frequently, and the latter can be not only a symptom of, but also a risk factor for depression. So, this indicates a strong bi-directional relationship between the pathomechanism of insomnia and depression. Furthermore, enhanced REM sleep pressure, namely increased time spent in REM and reduced REM sleep latency are also characteristic symptoms of depression. Most antidepressant drugs decrease REM sleep, and REM sleep deprivation may alleviate the symptoms of depression. In order to maintain the

antidepressant effect of REM sleep deprivation, the therapy has to be applied for several days or weeks, which latency is the same as the one during antidepressant drug therapy; the adaptation of 5-HT_{1A} receptors is supposed to be in the background of the therapeutic potential. Several serotonergic antidepressants and the 5-HT_{1A} agonist pindolol enhanced the therapeutic effect of sleep deprivation and attenuated the occurrence of relapses following rebound sleep. Unfortunately, the mood improvement is only transient; relapse occurring with the first episode of sleep is associated with high REM sleep pressure. In conclusion, REM sleep dysregulation may have a crucial role in the pathomechanism of depression, however, its precise mechanism of action is still unknown. According to the reciprocal interaction hypothesis, depression is caused by the enhanced cholinergic sensitivity, by the cholinergic-aminergic imbalance, which may explain the REM sleep pressure. Additionally, it might be a rationale for the mood improvement after REM deprivation, since the elongation of wakefulness may prevent the cholinergic dominance during the night, and increase the monoaminergic activity in the morning. In spite of the positive therapeutic effect, REM deprivation is not an adequate method in clinics, as it is difficult to carry on and its effect is not certain and only transient. Sleep suppression disturbs the homeostasis, produces typically bad mood, anxiety and cognitive decline in healthy individuals; it can play a role in the pathomechanism of anxiety.

1.3 REM sleep deprivation in rodents, the “flower pot” method

Sleep deprivation is difficult to investigate in humans, so methods applied in rodents are especially important to understand better the mechanisms of sleep. Sleep disturbances which are typical of depression can be often observed in rodent models of depression. In one of our experiments, we used the “flower pot” procedure to deprive REM sleep. During this method, when the animal reaches REM, it falls in the water due to the muscle atony, and awakes. Rebound sleep after REM deprivation is characterized by increased REM-pressure, which manifests in enhanced time spent in REM sleep and reduced REM latency. Besides REM deprivation, the „flower pot” method definitely causes a very stressful pathological condition due to isolation,

immobilization, falling into the water, soaking etc.; rats are under uncontrollable, inescapable, sub-chronic stress exposure. REM-deprivation is not a well-established model of depression yet, although it influences several systems related to stress. The “flower pot” protocol has limitations, but it meets some of the criteria for a valid animal model, and has good face and construct validity.

1.4 SSRI antidepressants

The selective serotonin reuptake inhibitors (SSRIs: escitalopram, citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline) are the most widely used antidepressants nowadays. According to the monoaminergic hypothesis of depression, the extracellular concentration of monoamines, such as serotonin (5-HT), may be deficient. Furthermore, the neurotransmitter receptor hypothesis proposes that monoaminergic receptors may be up-regulated (increase in the number and/or the sensitivity of receptors) which may lead to a depressed state. SSRIs enhance the extracellular concentration of 5-HT, since they interfere with the reuptake of it from the synaptic cleft. 5-HT accumulates mainly in the somatodendritic area of neurons, where binding to the 5-HT_{1A} receptors decreases the neuronal activity. So, SSRIs acutely attenuate the serotonergic activity and cause anxiety. Due to the evaluation of their therapeutic effect and to the disappearing of the acute side effects, such as anxiety, several weeks of treatment is necessary. Decreases in the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptor functions may be especially important for the development of their therapeutic effectiveness. SSRIs reduce REM sleep and increase REM latency as well as wake time after acute dosing. The interruption of antidepressant drug therapy causes REM-rebound, for which the enhanced cholinergic activity is supposed to be responsible. Escitalopram is the most selective and most effective SSRI, but not all patient responses for the treatment adequately.

1.5 The 5-HT_{2C} receptor antagonists

The 5-HT_{2C} receptor antagonists seem to be promising ligands for the augmentation of antidepressant activity and for the acute anxiolysis. Decrease in 5-HT_{2C} receptor responses after chronic SSRI treatment has

been found to disinhibit the mesolimbic dopamine system, which may play a crucial role in the development of the antidepressant action. In addition, several antidepressants (e.g. fluoxetine, ritanserin, agomelatine, trazodone) and most atypical antipsychotics have 5-HT_{2C} receptor antagonist property. Due to the earlier lack of subtype selective ligands with high affinity, profound investigation of 5-HT_{2C} receptors has begun only recently. These receptors are widely distributed in the central nervous system and involved in the regulation of anxiety, sleep, learning, locomotor activity, hormone secretion and appetite. Some highly selective 5-HT_{2C} receptor antagonists, such as SB-242084 had anxiolytic effect and elevated the power of theta waves (5-9 Hz) according to the quantitative sleep analysis (Q-EEG) in rodents. Hippocampal theta rhythm is generated by the GABA (gamma-aminobutyric acid)-ergic and cholinergic neurons of the medial septum. These neurons get strong serotonergic innervation from the raphe nuclei. In these brain areas, the 5-HT_{2C} receptors are located postsynaptically on serotonergic axon terminals and as somatodendritic heteroreceptors on GABAergic interneurons. Activation of these receptors exerts tonic inhibition on the theta waves via GABAergic interneurons. The 5-HT_{2C} receptor antagonists elevate theta oscillations in rodents, which waves are strongly related to learning and memory processes.

2 Aims

The focus of recent research was on the sleep effects of chronic escitalopram treatment in rats.

- Rebound sleep following the “flower pot” method has been extensively investigated earlier, but Markov chain analysis of the sleep architecture has not been studied yet. Furthermore, effects of chronic SSRI treatment on the rebound sleep are still lacking. In the first experiment (Escitalopram-Rebound), we examined (1) the macro- (2) and the micro-architecture (Markov chains) of sleep in the first 2 hours of the rebound period, following the 72-hour-long “flower pot” procedure; (3) Q-EEG spectra were also analysed. In addition, we

explored (4) how the chronic escitalopram administration (10 mg/kg for 24 days, osmotic minipump) influences the sleep pattern changes.

- Changes in several 5-HT_{2C} receptor functions after chronic antidepressant treatment have already been studied, but there is no information regarding theta oscillations. In the second experiment (Escitalopram-5-HT_{2C}receptor), we examined (5) how chronic escitalopram (10 mg/kg for 21 days, osmotic minipump) affects the theta-increasing activity of the acute (1 mg/kg, i.p.) 5-HT_{2C} receptor antagonist SB-242084 in the first 3 hours of the passive phase in freely-moving rats, under undisturbed conditions.

3 Materials and Methods

3.1 Study design

Both experiments were carried out in male *Wistar* rats, which were equipped with fronto-parietal EEG and electromyographic (EMG) electrodes in the neck muscle. Motor activity was detected by the movement of the cable transferring the EEG and EMG signals. For the chronic escitalopram treatment an osmotic minipump (2ML4, ALZET, 2.5 µl/hour, 28 days, DURECT Corporation, USA) was implanted subcutaneously under the skin of the back of each animal. Minipumps were filled with escitalopram-oxalate solution (10 mg/kg/day, dissolved in solution of 0.3 N HCl in distilled water) or its solvent. Rats were habituated to the recording conditions before the experiments.

Escitalopram-Rebound

We explored how influences the chronic escitalopram treatment (for 24 days) the first 2 hours of rebound sleep following 3-day-long REM sleep deprivation. On the 21th day of escitalopram administration, at the beginning of the passive phase, each RD animal was placed on a platform situated in the middle of a round water tank. Food was available ad libitum during the whole procedure. At the end of the 72-hour-long REM deprivation, rats were removed from the platforms and they were placed into the recording cages, similarly to HC rats. The first

2 hours of rebound sleep were recorded. Rats were randomly divided into 4 groups on the basis of the chronic treatment [escitalopram (SSRI) or vehicle (VEH)] and REM sleep deprivation [(RD) or keeping in home cage (HC)]:

- HC-VEH; n=6
- HC-SSRI; n=7
- RD-VEH; n=7
- RD-SSRI; n=7

Escitalopram-5-HT_{2C} receptor

In this experiment we investigated, how influences the chronic escitalopram pretreatment (21 days) the effects of the acute 5-HT_{2C} receptor antagonist SB-242084 (SB, 6-chloro-5-methyl-1-[[2-(2-methyl-3-pyridyl)oxy]-5-pyridyl carbamoyl], 1 mg/kg, dissolved in 10 % (2-hydroxypropyl)- β -cyclodextrin) treatment on the Q-EEG spectra. The acute intraperitoneal (i.p.) treatment was administered immediately at the beginning of the passive phase and then the 3-hour-long EEG recording started. Rats were randomly divided into 4 groups on the basis of the chronic pretreatment (SSRI or VEH) and the acute treatment (SB-242084 or vehicle, veh):

- VEH+veh; n=6
- VEH+SB; n=6
- SSRI+veh; n=7
- SSRI+SB; n=6

3.2 Sleep analysis and statistical evaluation

The EEG recordings were visually scored by the *SleepSign for Animal* sleep analysis software (Kissei Comtec America, Inc., USA) for 4 s periods (epoch) as follows:

- **AW (active wakefulness):** the EEG is characterized by low amplitude activity at alpha (8-13 Hz), beta (14-30 Hz), gamma (30-60 Hz) and theta (5-9 Hz) frequencies accompanied by high EMG and motor activity.

- **PW (passive wakefulness):** the EEG is characterized by low amplitude activity at alpha (8-13 Hz), beta (14-30 Hz), gamma (30-60 Hz) and theta (5-9 Hz) frequencies accompanied by high EMG activity, and low motility.
- **SWS-1 (light slow wave sleep):** high voltage delta waves (0.5-4 Hz) interrupted by low voltage fast EEG activity (spindles 6-15 Hz) accompanied by reduced EMG and motor activity.
- **SWS-2 (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):** unusual association of theta waves (5-9 Hz) and high-amplitude spindles (mean 12.5 Hz), appearing in brief periods just prior to or after REM sleep.
- **REM (rapid eye movement) sleep:** regular theta waves (5-9 Hz) accompanied by silent EMG and motor activity with occasional twitching.

Quantitative spectrum (Q-EEG) analyses of sleep were evaluated in both experiments. Q-EEG calculates the electric power in μV^2 at each frequency by fast Fourier transformation. Epochs with artefacts were discarded from the analysis by visual inspection. Q-EEG was analysed in the frequency range of 1.25-60 Hz, and we particularly focused on the theta band (5-9 Hz).

Escitalopram-Rebound

Hypnograms were evaluated by the following methods:

- Descriptive statistical analysis,
- Markov chain model,
- Q-EEG analysis.

Descriptive statistical analysis

Sleep parameters evaluated in the summarized first 2 hours of rebound sleep are the followings:

- Total time spent in stages;

- The number and average duration of vigilance stage episodes [(a stage episode was defined as a period of stage lasting for ≥ 4 s (1 epoch))];

In the abovementioned parameters, sleep stages were classified as follows: Wake (AW and PW), NREM sleep (SWS-1, SWS-2 and IS), REM sleep and total sleep (NREM and REM sleep).

- Sleep fragmentation: the number of awakenings that disconnected any sleep periods;
- REM sleep latency: the time elapsed from the start of sleep until the occurrence of the first REM sleep episode;
- Sleep latency: the time elapsed from the beginning of the passive phase until the first SWS-1 epoch;
- Length of the first REM item: the length of the first uninterrupted REM sleep period (IS epochs are permitted after REM);

Data were evaluated by two-way analysis of variance (ANOVA) statistics [factors: treatment (VEH or SSRI) and REM deprivation (HC or RD)], followed by Tukey's honest significant difference test. $p < 0.05$ was defined as statistically significant. For statistical analysis and graphs, Prism 6 (GraphPad Software, Inc., USA) software was used.

Analysing hypnograms as Markov chains

We modelled hypnograms as time-continuous Markov chains. The Markov chain model is a nonlinear regression modelling technique based on numerical optimization. Our primary interests were not stage transition rates themselves but rather how different they are compared to the control group. Therefore we normalized our data, so the reported rates are relative rates compared to the HC-VEH group (normalized transition rate, NTR). Instead of formal statistical tests, we used the confidence intervals to declare significance. If the confidence interval did not cover 1, we declared that the treatment effect is statistically significant at $p < 0.05$ level compared to control. Also, if the confidence intervals of two groups are not overlapping, the groups significantly

differ at $p < 0.05$ level. If a $NTR > 1$, it indicates that the treatment accelerates the transitions from one state to another compared to the control group. If a $NTR < 1$, the given treatment decelerates the transitions. There is another set of parameter of interest called sojourn time. Sojourn time is the expected average time span of an event between two jumps. Sojourn times are absolute values which are provided with the corresponding 95% confidence intervals. We declared that two sojourn times are statistically different if the confidence intervals were not overlapping. To achieve convergence, we faced limitations of the applied software. First, it had a constraint that the transition rates are constant and independent from time. This limitation could have been overcome by visually segmenting the data into two stationary periods, that is, when the observed stage frequencies were relatively constant: onset phase [the first 30 minutes of rebound sleep (0–2000 s)] and a subsequent steady phase [lasted about 90 minutes (2001–7200 s)] The second limitation was that we could achieve successful convergence only with two or three stage models, namely wake and sleep states in the onset phase, and wake, REM and NREM sleep in the steady phase. We used the freely available R program with the help of an R package called msn for the Markovian analysis.

Q-EEG analysis

Three-way repeated measures ANOVA was used with three main factors [treatment (non-repeated; VEH or SSRI) and rebound (HC or RD); theta (repeated; 5-9 Hz) and alpha (repeated; 10-13 Hz) frequency bins in SWS-1, and theta (repeated; 5-9 Hz) bins in SWS-2], followed by Tukey's honest significant difference test. $p < 0.05$ was defined as statistically significant. For statistical analysis and graphs, Prism 6 (GraphPadSoftware, Inc., USA) software was used.

Escitalopram-5-HT_{2C}receptor

Q-EEG analysis

Theta frequencies (5-9 Hz) were analysed in each hours of AW (1st, 2nd and 3rd hours) and REM sleep (solely 2nd and 3rd hours). The area under the curve (AUC) values of theta frequencies (5-9 Hz) were evaluated by two-way ANOVA statistics [factors: pretreatment (VEH

or SSRI) and treatment (veh or SB)] followed by Tukey's honest significant difference test. In addition, to investigate the interrelation between group effects and theta frequencies, theta power values were submitted to repeated-measures ANOVA with two main factors: [groups (non-repeated; VEH+veh, VEH+SB, SSRI+veh or SSRI+SB) and theta frequency bins (repeated; 5-9 Hz), followed by Tukey's honest significant difference test. $p < 0.05$ was defined as statistically significant. For statistical analysis and graphs, Statistica 7.0 (Statsoft Inc., Tulsa, OK, USA) software was used.

4 Results

4.1 Escitalopram-Rebound

Descriptive statistical analysis of sleep parameters

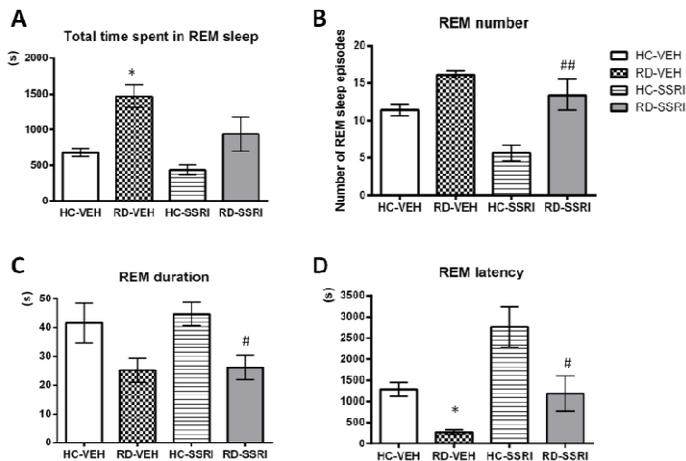


Figure 1 REM sleep parameters in the summarized first 2 hours after 3 day-long REM sleep deprivation. Total time spent in REM sleep (A) REM sleep episode number (B) and duration (C), as well as REM latency (D). Data are presented as mean \pm SEM of 6–7 animals per group. *: significant ($p < 0.05$) post hoc effect of RD-VEH group compared to the HC-VEH group; # and ##: significant ($p < 0.05$ and $p < 0.01$) post hoc effects of RD-SSRI group compared to HC-SSRI group.

REM sleep deprivation caused REM rebound compared to the control, as it elevated the total time spent in REM sleep and reduced the REM latency. The chronic SSRI treatment by itself did not affect REM parameters significantly. In addition, chronic escitalopram treatment only moderately attenuated the REM rebound (Figure 1).

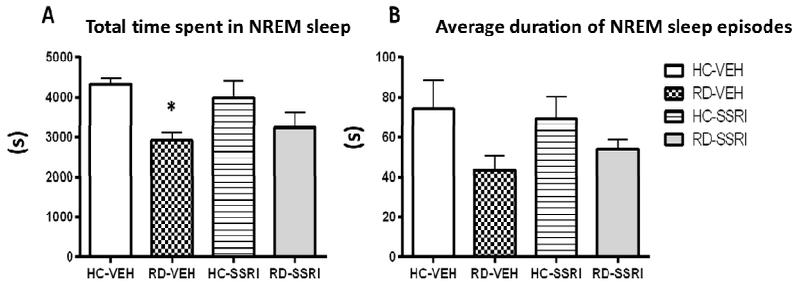


Figure 2 NREM sleep parameters in the summarized first 2 hours after 3 day-long REM sleep deprivation. Total time spent in NREM sleep (A) and NREM sleep episode duration (B). Data are presented as mean \pm SEM of 6–7 animals per group. * $p < 0.05$ means significant post hoc effects of RD-VEH group compared to the HC-VEH group;

REM sleep deprivation decreased the total time spent in NREM sleep compared to the control by the reduction of the average duration of NREM sleep episodes. Similarly to REM sleep parameters, escitalopram did not influence the effects of REM sleep deprivation on NREM parameters (Figure 2).

Analysing hypnograms as Markov chains

Onset period (first 30 minutes of rebound sleep; 0-2000 s; Figure 3 and 5)

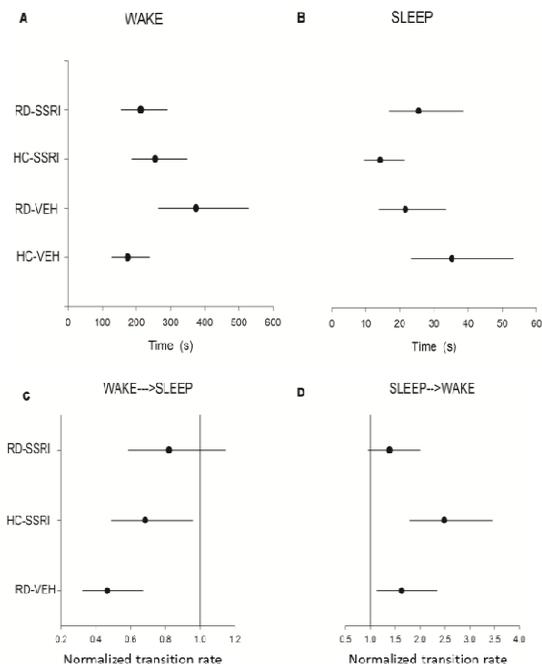


Figure 3 Sojourn times and normalized transition rates (NTR) in the onset phase (0-2000) of rebound sleep. The estimated mean of the average time spent between two jumps (sojourn time) in WAKE (A) and SLEEP(B), and the normalized transition rates (NTR, relative rates compared to the transition rate of HC-VEH group, which is 1) from WAKE to SLEEP (C) and from SLEEP to WAKE (D) states. Each point (●) represents the mean values and the line segments show the 95% confidence interval surrounding them.

Separately, both chronic escitalopram treatment and REM deprivation increased sleep fragmentation namely sleep sojourn decreased in HC-SSRI, wake sojourn enhanced in RD-VEH; however, when the two interventions were applied together, these effects did not add but rather antagonized each other (Figure 3).

Stable period (ca. 30-120 min of rebound sleep, 2001-7200 s; Figure 4 and 5)

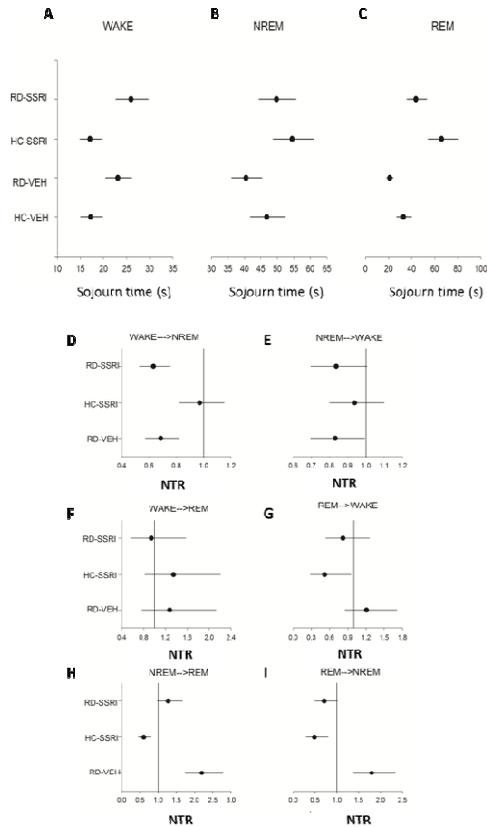


Figure 4 Sojourn times and normalized transition rates (NTR) in the steady phase of rebound sleep (ca. 30–120 min; 2001–7200 sec). The estimated mean of the average time spent between two jumps (sojourn time) in WAKE(A), NREM (B) and REM sleep(C), and the normalized transition rates (relative rates compared to the transition rate of HC-VEH group, which is 1) from WAKE to NREM(D), from NREM to WAKE(E), from WAKE to REM(F), from REM to WAKE(G), from NREM to REM (H) and from REM to NREM (I) states. Each point (●) represents the mean values and the line segments show the 95% confidence interval surrounding them.

REM sleep deprivation caused REM fragmentation (decreased REM sojourn, doubled transition rates between NREM and REM stages). In

the contrary, chronic escitalopram elongated the REM episodes (increased REM sojourn, decelerated jumps between NREM and REM stages), and prevented the changes occurring after REM deprivation (Figure 4).

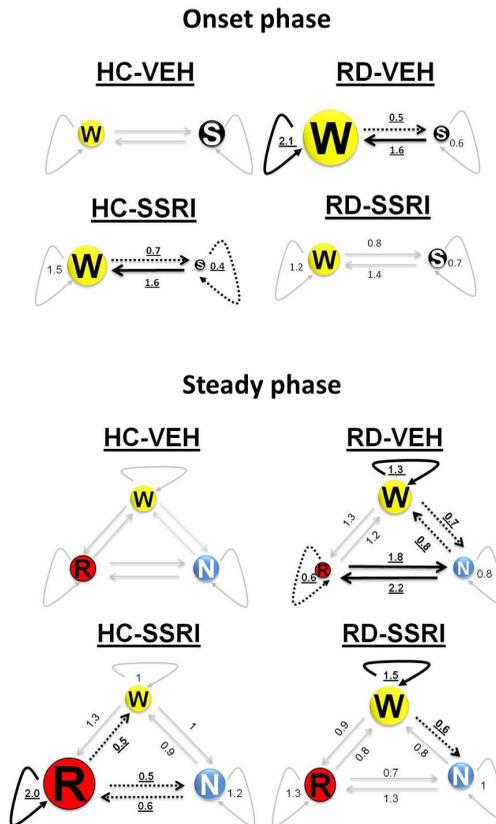


Figure 5 Illustrations of Markov chains during the onset (0-2000 s) and steady(2001-7200 s) phase. Numeric data represent the relative mean values of sojourn times (curved lines) and normalized transition rates (straigt lines) in the treatment groups compared to the mean values of the HC-VEH group (sojourn times and transition rates of the HC-VEH group are 1). The size of circles aligns the changes in sojourn times compared to HC-VEH group. Thick, uninterrupted arrows represent significant increase, dashedarrows show significant decrease and slight arrows sign no significant changes in sojourn and NTRvalues compared to the HC-VEH group. Significant alterations are highlighted in bold, underlined characters.

Q-EEG

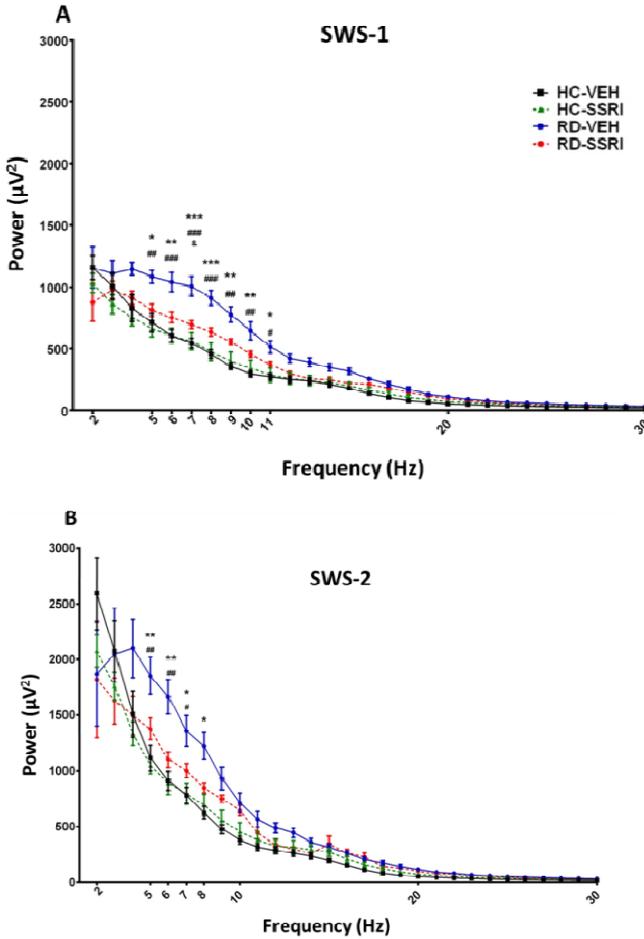


Figure 6 EEG spectra (Q-EEG) in the summarized first 2 hours of rebound sleep during slow-wave sleep stages. The theta (5–9 Hz) and alpha (10–13 Hz) frequencies during light slow-wave sleep (SWS-1) (A) and deep slow-wave sleep (SWS-2)(B).

Each point represents mean \pm SEM values of 6–7 animals per group. *, ** and ***: significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) Tukey's post hoc effects of the RD-VEH group compared to the HC-VEH group; #, ## and ###: significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) Tukey's post hoc effects of RD-VEH group compared to HC-SSRI group; &: significant ($p < 0.05$) Tukey's post hoc effect of RD-SSRI group compared to RD-VEH group;

REM sleep deprivation markedly enhanced the power of theta waves(5-9 Hz) in SWS-1 and SWS-2 stages compared to the HC groups. Furthermore, REM deprivation elevated also the power of alpha frequencies (10-13 Hz) in SWS-1. The chronic SSRI treatment prevented the abovementioned effects of REM deprivation (Figure 6).

4.2 Escitalopram-5-HT_{2C}receptor

Q-EEG

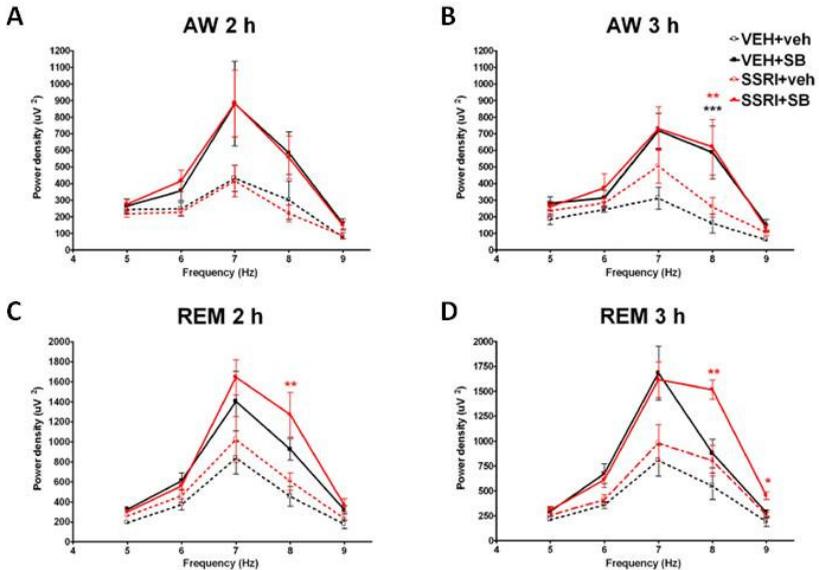


Figure 7 The power spectra of theta frequencies (5-9 Hz) during active wake (AW; A and B) and REM sleep (C and D) in the 2nd and 3rd hours after SB-242084 treatment. Each point represents mean ± SEM values of 6–7 animals per group. * and **: significant ($p < 0.05$ and $p < 0.01$) Tukey's post hoc differences between SSRI+SB and VEH+veh groups; *** significant ($p < 0.001$) Tukey's post hoc difference between VEH+SB and VEH+veh groups;

The chronic escitalopram pretreatment did not affect the theta power-increasing activity of SB-242084 in the 2nd and 3rd hours of AW and REM sleep, since there was any significant interaction between the effects of pretreatment and treatment. The most prominent increase was

observed at 8 Hz in the 3rd hour of AW, caused by VEH+SB and SSRI+SB groups. In the case of REM sleep, we found the greatest increase at 8 Hz (2nd and 3rd hours) and at 9 Hz (3rd hour) in the SSRI+SB group.

5 Conclusions

- Based on our results, we found REM rebound as well as sleep and REM fragmentation in the first 2 hours after 72-day-long „flowerpot” method. Since enhanced REM sleep pressure and REM fragmentation are characteristic features in most depressed patients and in several animal models of depression, we can conclude that the sub-chronic „flowerpot” paradigm may be capable to mimic anxiety or depression, at least regarding sleep.
- According to the results of Markov chain and Q-EEG analyses, the 24-day-long escitalopram treatment avoided most changes after REM deprivation, so we suppose a good predictive validity of the sub-chronic „flower pot” protocol, as a model of depression. Normalization in sleep transitions potentially can be a characteristic feature of SSRIs and also other antidepressants which draws attention to the applicability of Markov chain model in sleep research. The applied time-continuous Markov-model is a powerful statistical tool which might allow analysing hypnograms in finer granularity and with higher statistical power than the standard sleep metrics; however, currently the method is not without any limitations.
- Since the 21-day-long escitalopram treatment did not influence the theta activity-increasing effect of SB-242084 during active wakefulness and REM sleep, we can assume that 5-HT_{2C} receptors, which play a role in the regulation of the theta waves, are not involved in the adaptive mechanisms caused by the SSRI. Taking into account that theta oscillations are strongly associated with learning and memory processes, these results can hypothesize that the supposed memory-

enhancing property of 5-HT_{2C} receptor antagonists might remain during chronic antidepressant treatment.

6 List of publications

6.1 Publications used for the thesis

1. Kostyalik D, Katai Z, Vas S, Pap D, Petschner P, Molnar E, Gyertyan I, Kalmar L, Tothfalusi L, Bagdy G (2014a) Chronic escitalopram treatment caused dissociative adaptation in serotonin (5-HT) 2C receptor antagonist-induced effects in REM sleep, wake and theta wave activity. *Experimental brain research* 232:935-946.

2. Kostyalik D, Vas S, Katai Z, Kitka T, Gyertyan I, Bagdy G, Tothfalusi L (2014b) Chronic escitalopram treatment attenuated the accelerated rapid eye movement sleep transitions after selective rapid eye movement sleep deprivation: a model-based analysis using Markov chains. *BMC Neurosci* 15:120.

6.2 Other publications

1. Bergman P, Adori C, Vas S, Kai-Larsen Y, Sarkanen T, Cederlund A, Agerberth B, Julkunen I, Horvath B, Kostyalik D, Kalmar L, Bagdy G, Huuttoniemi A, Partinen M, Hokfelt T (2014) Narcolepsy patients have antibodies that stain distinct cell populations in rat brain and influence sleep patterns. *Proc Natl Acad Sci U S A* 111:E3735-3744.

2. Katai Z, Adori C, Kitka T, Vas S, Kalmar L, Kostyalik D, Tothfalusi L, Palkovits M, Bagdy G (2013) Acute escitalopram treatment inhibits REM sleep rebound and activation of MCH-expressing neurons in the lateral hypothalamus after long term selective REM sleep deprivation. *Psychopharmacology (Berl)* 228:439-449.

3. Vas S, Katai Z, Kostyalik D, Pap D, Molnar E, Petschner P, Kalmar L, Bagdy G (2013) Differential adaptation of REM sleep latency, intermediate stage and theta power effects of escitalopram after chronic treatment. *J Neural Transm* 120:169-176.

4. Volk B, Nagy BJ, Vas S, Kostyalik D, Simig G, Bagdy G (2010) Medicinal chemistry of 5-HT_{5A} receptor ligands: a receptor subtype with unique therapeutical potential. *Curr Top Med Chem* 10:554-578.