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THE EFFECT OF ESCITALOPRAM AND A 5-HT_{2C} RECEPTOR ANTAGONIST ON THE EEG GAMMA OSCILLATIONS

PhD thesis

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List of Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5-HT_{1A}R	serotonin 1A receptor
5-HT_{2A}R	serotonin 2A receptor
5-HT_{2C}R	serotonin 2C receptor
ANOVA	analysis of variance
AW	active wake
CNS	central nervous system
EEG	electroencephalography / electroencephalographic
EMG	electromyography / electromyographic
ESC	chronic escitalopram treatment
GABA	γ-aminobutyric acid
ip.	intraperitoneal / intraperitoneally
IS	intermediate stage of sleep
non-REMS	non rapid eye movement sleep
PV	parvalbumin
PW	passive wake
qEEG	quantitative electroencephalography
REMS	rapid eye movement sleep
SB	acute SB-242084 treatment
SEM	standard error of mean
SERT	serotonin transporter
SSRI	selective serotonin reuptake inhibitor
SWS	slow-wave sleep
SWS-1	light slow-wave sleep
SWS-2	deep slow-wave sleep
veh	acute vehicle treatment
VEH	chronic vehicle treatment
W	wakefulness

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1. Introduction

Electroencephalography (EEG) records the electrical activity of the brain, thus provides objective, real-time information about the actual (physiological or pathological) state of the brain. This method enables us, for example, to distinguish sleep-wake stages, to identify epileptic seizures, moreover, to investigate neuronal oscillations, event-related potentials, brain network connectivity, or even, indirectly, cognitive functions.

The use of EEG in the diagnosis of various neurological diseases (epilepsy, sleep disorders such as sleep apnea and narcolepsy, etc.) is common, and EEG is a valuable tool for experimental studies in neuroscience and neuropsychopharmacology (pharmaco-EEG) as well. A classical approach is to study the sleep-wake architecture. On the other hand, quantitative EEG (qEEG) studies alterations of neuronal oscillations.

1.1. Rhythms of the brain

EEG oscillatory pattern, recorded as field potentials on scalp in humans or on cortical surface in rodents, stems from the synchronous electrical activity of a great number of cortical neurons, and reflects alterations in network functions of the brain. These oscillations are highly conserved in mammals, regardless of species and brain size (1), hence they provide translatable results across rodent and human studies (2). In this section, I shortly present the various oscillations, their typical presence in the EEG during different sleep-wake (vigilance) stages, and their disturbances in depression, focusing on gamma oscillations.

1.1.1. Frequency bands of the EEG

EEG signals (waves), localized in time and space, are characterized by amplitude (voltage) and frequency. While amplitude refers to the number of synchronous firing cells in the area detected by the electrode in that moment, oscillations within different frequency bands are associated with different brain functions. Thus, frequency provides a reasonable basis for classification.

Brain oscillations cover >4 orders of magnitude in frequency, from the infraslow (<0.01 Hz) to ultrafast rhythms, and include at least 10 oscillation classes (1). The most commonly used, well-established ones are the followings (the exact limits may slightly

vary between studies): delta (1-4 Hz), theta (5-9 Hz), alpha (10-13 Hz), beta (14-29 Hz), and gamma (≥30 Hz) oscillations. In our studies (3, 4) and this thesis we define gamma frequency band between 30-60 Hz, that is also known as low-gamma band. **Table 1** presents a short synopsis.

Table 1 - Basic EEG rhythms and their characteristics (3, 5)

EEG rhythm	Frequency band (Hz)	EEG pattern	Typical vigilance stage in rats
Delta	0.5-4		Slow-wave sleep
Theta	5-9		REMS, wakefulness
Alpha	9-13		Light slow-wave sleep
Beta	14-29		Wakefulness
Gamma	30-60		Wakefulness

Gamma band oscillations are relatively high-frequency components of the EEG spectrum. In the generation of these oscillations, parvalbumin-positive (PV+) and somatostatin-positive GABAergic interneurons have been demonstrated to play an important role (6, 7). Gamma oscillations are thought to reflect neuronal population dynamics, and have been associated with several cognitive and sensory functions, neural plasticity and memory processes in both animals and humans (8, 9). Conversely, an abnormal gamma EEG activity has been described in several psychiatric conditions (9), detailed in section 1.1.4.

1.1.2. Sleep and wakefulness

Sleep is a fundamental phenomenon, its good quality and quantity are vital for physical and mental health. The occurrence and timing of the three main vigilance stages, wakefulness, rapid eye movement sleep (REMS), and non-REMS is regulated in the brain by multiple neuroanatomic circuits and related neurochemical systems. The EEG that visualizes alterations in the global pattern of neuronal activity and the electromyogram (EMG) that monitors changes in muscle tone are excellent biomarkers of sleep-wake stages in both humans and animals (10). Each stage is accompanied by a characteristic EEG pattern, allowing the differentiation and separated examination.

Wakefulness is characterized by low-voltage, high-frequency (fast) EEG activity and high muscle tone. These fast oscillations stem from synchronized neuronal activity in small, functionally interrelated areas. In contrast, theta rhythm that is also typical in this stage, is generated over more widespread areas and synchronizes faster, locally generated rhythms (beta and gamma) that presumably provide a temporal framework for higher-order CNS functions such as conscious awareness, attention, memory formation (5). In contrast, non-REMS is defined by high-voltage, low-frequency (slow, therefore this stage is also called slow-wave sleep, SWS) EEG and reduced muscle tone. The predominant frequency band during non-REMS is delta (5). Delta EEG power is commonly interpreted as marker of sleep intensity. During REMS, the behavioral components of sleep and the electrophysiological profiles of wakefulness are combined, as low-voltage fast EEG activity are coupled with muscle atonia (complete loss of muscle tone). Hence, this stage is also known as paradoxical sleep. Rapid eye movements are peculiar REMS phenomena in contrast to the slow rolling eye movements occurring during non-REMS. The marked, synchronous theta activity results in a characteristic EEG pattern (5).

These main stages of the sleep-wake architecture can be further divided for a more detailed analysis. In our rat experiments, we differentiated two stages of wakefulness (active and passive, primarily based on locomotor activity), and four stages of non-REMS (light and deep slow-wave sleep, and intermediate stage of sleep, based on EEG patterns), in addition to REMS (3, 4, 11).

1.1.3. Relevance of EEG in neuropsychiatric diseases

Neuronal oscillations and sleep-wake architecture are key mechanisms that support synaptic plasticity and cognitive functions, disruption of which has been described in numerous neuropsychiatric disorders including depression (12, 13). Cognitive dysfunctions in depression might include learning, memory processes, sustained attention, as well as executive function (14).

Impaired sleep-wake architecture is common in depressed patients and also in animal models of depression, namely, disinhibition of REMS (demonstrated as increased total REMS time and reduced REMS latency), reduction of non-REMS, and impaired sleep continuity are characteristic symptoms (13, 15, 16). As both REMS and non-REMS have a role in memory formation, these alterations of the sleep-wake architecture are suggested

to affect memory processes (17). In addition, most antidepressants affect the sleep-wake cycle, in particular, generally suppress REMS, i.e. they induce effects in the opposite direction to those observed in depression (18).

Abnormal EEG oscillatory activity in depressed patients has also been widely described, affecting mostly theta, alpha and beta frequencies (19-21). However, changes in the gamma band are less investigated, despite the abovementioned role of these oscillations in cognitive and sensory functions (22, 23). Depressed patients have been found to display increased gamma activity in response to emotional stimuli (negative words), that also suggests gamma-band EEG as a marker of semantic information processing (24). Another study has demonstrated a correlation between attention deficit and low-gamma (and beta) power in patients with depression (25). The alteration of late gamma-band responses to positive information has been linked to depression recurrence risk, in humans (26). Importantly, a temporal association between beta as well as gamma oscillations and secretory pulses of cortisol has been demonstrated in humans (27). Considering that hyperactivity of the hypothalamic-pituitary-adrenal axis, that is among the most consistent physiological findings in depression, disrupts cortisol secretion in depressed patients (28), the relevance of high-frequency oscillations in this disorder is further supported. Nevertheless, evidence from animal experiments have also confirmed the relationship of gamma activity and depression. In mice, the involvement of gamma oscillation (namely, the restoration of gamma activity at the network level) in the remission of depression-like behavior (induced by chronic restraint stress) has been described (29). In rats subjected to another model of depression, chronic early life stress, has been shown to disrupt maturation of gamma oscillations in the hippocampus (30).

Besides, further supporting the key role of gamma rhythm in normal brain functioning, disturbed gamma band oscillations have been observed in several other neuropsychiatric diseases, such as attention deficit hyperactivity disorder (31). In schizophrenia, negative symptoms have been correlated with decreased gamma activity, while an increase has been found during positive symptoms, e.g. hallucinations (32). Additionally, increased gamma activity has been reported in epileptic patients, whereas reduced gamma activity can be observed in Alzheimer's disease (33). Notwithstanding, gamma oscillations have been reported to weaken with age, even in healthy elderly (34).

Gamma power represents a physiological correlate of enhanced excitatory to inhibitory (E/I) balance, whereas reduced E/I balance has been suggested in major depression (35). Aberrant network function of gamma-generating GABAergic interneurons is considered to be involved in the development of cognitive and mood impairments in mental disorders such as bipolar depression (36, 37). In line with this, postmortem studies have demonstrated decreased numbers of PV+ interneurons in the prefrontal cortex and hippocampus in bipolar patients. As a consequence, it has been hypothesized that such alterations of these specific GABAergic neurons might reflect vulnerability toward mental disorders (38, 39). As PV+ interneurons are essential for proper brain function but are highly susceptible to environmental disturbances (leading to their role in several neuropsychiatric disorders), novel therapeutic strategies targeting them selectively have been proposed recently (40). The clinical potential of restoring gamma signaling via transcranial brain stimulation has also been suggested in several neuropsychiatric disorders (41, 42).

1.1.4. Gamma oscillations as biomarker of depression

To identify reliable and sensitive biomarkers of major depression, that would support implementing precision medicine approaches in psychiatry, is challenging yet crucial for improving diagnosis as well as treatment of this increasingly common and debilitating disorder (43). Several parameters of the EEG during both wakefulness and sleep have been proposed as possible biomarkers of depression and predictors of treatment response, for example, prefrontal qEEG cordance (13). In recent years, as a growing body of evidence suggests an important role of gamma activity in the pathomechanism and course of depression, alterations of these oscillations also have been implicated as novel biomarker or endophenotype of the disease (43). Key findings on the role of gamma oscillations in depression suggest the followings. Under certain circumstances it might distinguish depressed patients from healthy subjects, moreover, bipolar from unipolar depression. Importantly, abnormal gamma activity is also present in animal models of depression, as pointed out previously. On the other hand, numerous antidepressant treatments (pharmacological agents as well as other therapies) have been reported to affect gamma activity too. However, it is still unknown whether these alterations are

indicators of disease status, causative mediators of therapeutic effects, or signs of side effects (43, 44).

1.2. Serotonin in the brain

Within the central nervous system (CNS), the monoamine neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) participates in numerous regulatory functions such as sleep-wake behavior, mood, cognition, sexual function, thermoregulation, and appetite. It has been established that 5-HT dysfunction is involved in the pathophysiology of depression. Accordingly, most antidepressant drugs act on the serotonergic system, namely, cause an increase in synaptic levels of 5-HT. Serotonergic neurons fire most actively in wakefulness, less intensively in non-REMS, and fall silent in REMS (45). So, it is not surprising that serotonergic antidepressants can influence the sleep-wake cycle too, namely, increased wakefulness (i.e. insomnia) and REMS suppression are commonly observed side effects of these drugs (18, 45). The serotonergic system has been considered an important modulator of sleep-wake architecture (45) as well as brain oscillations (46-48), although the involved mechanisms and receptors are complex and poorly understood.

The effects of 5-HT are mediated through 14 distinct 5-HT receptor subtypes (49), among which we focus in this thesis on serotonin 2C receptors (5-HT_{2C}Rs). However, the most important target of currently used serotonergic antidepressants is the serotonin transporter (SERT or 5-HTT), the protein that carries 5-HT from the synaptic cleft back to the presynaptic neuron (reuptake).

1.2.1. Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed, first-line antidepressants. Primarily targeting SERT, these drugs increase extracellular 5-HT levels via inhibiting its reuptake. Escitalopram, the more effective *S*-enantiomer of *RS*-citalopram (the racemate) with high selectivity for SERT, is a leading member of the class, due to its relatively good efficacy and tolerability profiles (50, 51).

SSRIs including citalopram and escitalopram have been reported to affect the sleep-wake cycle in humans (52, 53), mice (54, 55), and rats (56-58). For instance, acute treatment with escitalopram has been reported to inhibit REMS generation and deep non-

REMS, while following chronic treatment with the drug, all these changes diminished except some increase in REMS latency (59). Effects on EEG rhythms have also been described, namely, a reduction of low-frequency oscillations in humans (60) and rats (57). A decrease in theta power has been reported during active wakefulness and REMS following acute but not chronic escitalopram administration in rats (59). In patients with moderate major depression, an increase in combined delta-theta (2.5-8 Hz) power and a decrease in alpha power have been found 1 week after escitalopram treatment (61).

Although the EEG spectral effects of escitalopram on lower frequencies have been intensively investigated, our knowledge regarding gamma oscillations is limited. The available studies in humans (62) and in rats (63) failed to find an effect of escitalopram on gamma oscillations. However, several evidence suggests the influence of escitalopram on connectivity (64-66), attention (67), memory (68, 69), and synaptic plasticity (70, 71) – CNS functions those disturbances in depression have been inevitably demonstrated.

Considering the role of gamma rhythm in depression, it is essential to elucidate how these oscillations are affected by antidepressants at the beginning of treatment (acute effects) as well as during its maintenance (chronic effects). Importantly, the onset of therapeutic action of SSRIs including escitalopram is delayed (presumably due to receptor desensitization), indicating that acute and chronic effects often differ (as seen above too). Moreover, regarding the relationship between cognitive functions and gamma oscillations, research on gamma effects of escitalopram might provide new insights to cognitive aspects of antidepressant therapy (3).

1.2.2. 5-HT_{2C}R antagonists

5-HT_{2C}Rs are widely distributed in the CNS, and have an important role in the 5-HTergic regulation of numerous functions including sleep, anxiety, memory processes, as well as hormonal secretion, feeding behavior, and locomotor activity (72, 73). 5-HT_{2C}R dysfunction has been implicated in, among other pathological conditions, depression and anxiety (73, 74). Furthermore, a great number of antidepressant, anxiolytic, and antipsychotic drugs possess affinities to 5-HT_{2C}Rs as well, that antagonism might be involved in the efficacy of these drugs (73-75). Therefore, 5-HT_{2C}R has been proposed as a promising target in developing new therapies for several neuropsychiatric disorders including depression (73, 74).

Whereas the stimulation of 5-HT_{2C}Rs in certain brain regions has been found to be anxiogenic, subtype-selective 5-HT_{2C}R antagonists such as SB-242084 produced marked anxiolytic effects (75-77). Moreover, the selective 5-HT_{2C}R antagonists SB-242084 and RS-102221 have been reported to exert fast-onset (5 days versus 14 days in case of citalopram) antidepressant-like effects in mouse models of depression (78). Other work has shown that 5-HT_{2C}R blockade potentiated the antidepressant and anxiolytic, while reduced motor side effects of the SSRI fluoxetine in mice, highlighting the potential advantages of this combination (79). Thus, a growing body of evidence supports the idea that selective 5-HT_{2C}R antagonists may be beneficial in the therapy of depression and/or anxiety, although no clinical studies have been conducted yet.

A few articles have been published on qualitative or quantitative EEG effects of selective 5-HT_{2C}R antagonists, reporting reduced time spent in REMS and enhanced theta power during active wakefulness and REMS (77, 80). However, until our work, none of them have investigated effects on gamma oscillations. Considering these agents as putative antidepressants (alone or in combination with an SSRI) and taking into account the relationship between gamma rhythm and depression, it is relevant to fill this gap in the literature (4).

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2. Objectives

Based on the above mentioned aspects, our aims were the followings:

1. We intended to investigate how gamma activity varies during each sleep-wake stage, namely, active and passive wakefulness, light and deep slow-wave sleep, intermediate stage of sleep, and rapid eye movement sleep (AW, PW, SWS-1, SWS-2, IS, and REMS, respectively).
2. Does the SSRI escitalopram influence gamma power during any of the sleep-wake stages, when acutely administered?
3. Does escitalopram influence gamma power during any of the sleep-wake stages, when chronically administered?
4. Is there any similarity or difference between the acute and chronic effects of escitalopram on gamma power?
5. Does the 5-HT_{2C}R antagonist SB-242084 influence gamma power during any of the sleep-wake stages, when acutely administered?
6. Is there any similarity or difference between the chronic effects of escitalopram and the acute effects of SB-242084 on gamma power?
7. Does the combination of chronic escitalopram and acute SB-242084 influence gamma power during any of the sleep-wake stages?

To examine these questions, we conducted two series of EEG experiments, using rats equipped with EEG/EMG electrodes. The first, hereafter referred to as *"Escitalopram experiment"* was aimed to find answers to the previous 1-4. questions, whereas the second *("SB-242084 experiment")* to questions 5-7.

We gained EEG data from frontoparietal derivation during the first 3 h of passive phase, immediately after the injections. For further details, see Methods sections of the two publications, on which this thesis is based (3, 4).

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3. Results

3.1. Escitalopram experiment

3.1.1. Gamma power showed prominent variance with sleep-wake stages

To examine the potential alterations of gamma activity during different sleep-wake stages, we summarized EEG power values in the gamma frequency band (30-60 Hz) in each stage, and pooled data of control rats, i.e. those treated with acute or chronic vehicle. As shown in **Figure 1**, total gamma band power was clearly sleep-wake stage-dependent (significant one-way ANOVA for repeated measures, $F_{5.0,60.0} = 129.4$, $p < 0.0001$).

The highest gamma activity was observed in AW and REMS. According to Bonferroni *post hoc* results, no significant difference was found between these two stages. In contrast, all the other stages showed significantly lower gamma band power compared to AW. While gamma power in PW was 75% of that in AW, in non-REMS stages it was notably less, namely, 23% in SWS-1, 12% in SWS-2, and 16% in IS, respectively.

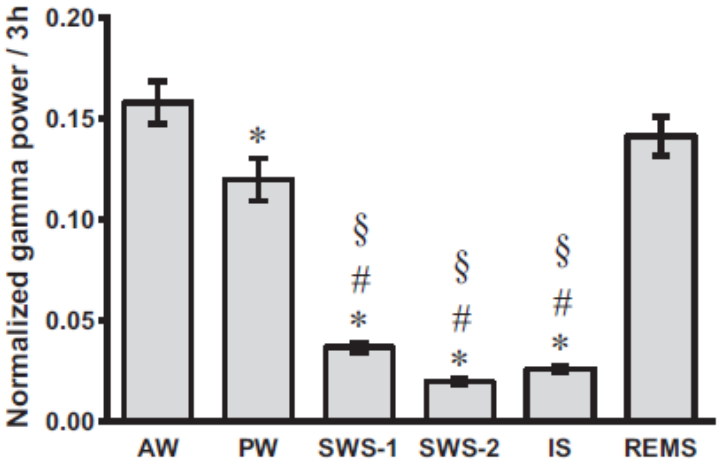

Figure 1 - Differences of gamma band (30-60 Hz) total power between sleep-wake stages in the control groups. The columns represent mean ± SEM values of $n = 13$ animals in the summarized first 3 h of passive (light) phase during active and passive wakefulness (AW, PW), light and deep slow-wave sleep (SWS-1, SWS-2), intermediate stage of sleep (IS), and rapid eye movement sleep (REMS), respectively. Significant *post hoc* results, compared to AW (* $p < 0.01$), PW (# $p < 0.01$) and REMS (§ $p < 0.01$) are marked.

Based on these results, we present EEG power spectra in the six sleep-wake stages grouped as follows:

- Stages associated with relatively high gamma activity: AW, PW, and REMS (**Figure 3** for *Escitalopram experiment*, **Figure 5** for *SB-242084 experiment*),
- Stages associated with relatively low gamma activity: SWS-1, SWS-2, and IS (**Figure 4** for *Escitalopram experiment*, **Figure 6** for *SB-242084 experiment*).

When all sleep-wake stages are shown (**Figure 1**, **Figure 2**) and when describing the results, we present them in the order in which they usually follow each other during the sleep-wake cycle.

3.1.2. Effects of acute escitalopram on gamma power

According to results of two-way ANOVA for repeated measures, acute escitalopram did not affect gamma power significantly during wake stages (AW, PW, **Figure 3 A, B**) or during slow-wave sleep stages (SWS-1, SWS-2, **Figure 4 A, B**). During IS, acute escitalopram treatment caused a reduction in gamma power (treatment effect: $F_{1,12}$ = 4.925, p = 0.0465, **Figure 4 C**). Finally, acute escitalopram reduced gamma power markedly during REMS (treatment effect: $F_{1,11}$ = 5.613, p = 0.0372, frequency × treatment interaction: $F_{30,330}$ = 3.246, p < 0.0001, **Figure 3 C**). REMS data had been calculated from only n = 6 rats, due to the REMS suppressing effect of acute escitalopram.

3.1.3. Effects of chronic escitalopram on gamma power

Similarly to acute treatment, neither chronic escitalopram did affect gamma power significantly during wake stages (AW, PW, **Figure 3 A, B**). However, in contrast to acute treatment, chronic administration of escitalopram caused a marked increase in gamma power during SWS-1 (treatment effect: $F_{1,11}$ = 12.10, p = 0.0052, frequency × treatment interaction: $F_{30,330}$ = 5.660, p < 0.0001, **Figure 4 A**) and during SWS-2 (treatment effect: $F_{1,11}$ = 9.667, p = 0.0099, frequency × treatment interaction: $F_{30,330}$ = 6.508, p < 0.0001, **Figure 4 B**). During IS, chronic escitalopram caused an elevation in gamma power (frequency x treatment interaction: $F_{30,330}$ = 5.114, p < 0.0001, **Figure 4 C**). Lastly, during REMS, no significant effect was seen after chronic escitalopram treatment (**Figure 3 C**).

3.1.4. Acute and chronic escitalopram induced different changes in gamma power

As a short summary, our results demonstrated that escitalopram administered acutely or chronically altered EEG power in the gamma band differently. The effects showed prominent sleep-wake stage-dependency. **Figure 2** presents these alterations for the summarized gamma frequency band (30–60 Hz), while **Figure 3** and **Figure 4** provide a deeper insight showing power spectra with individual frequencies of the gamma range.

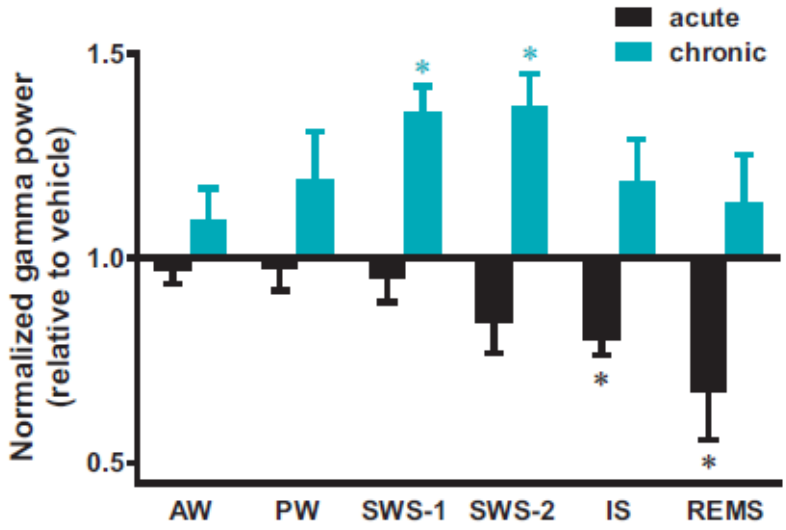

Figure 2 - Changes of gamma band (30-60 Hz) total power after acute and chronic escitalopram treatment during active and passive wakefulness (AW, PW), light and deep slow-wave sleep (SWS-1, SWS-2), intermediate stage of sleep (IS), and rapid eye movement sleep (REMS). Each column represents mean ± SEM values of n = 6-7 animals per group, relative to the appropriate vehicle control, in the summarized first 3 h of passive (light) phase. * significant ANOVA results ($p < 0.05$)

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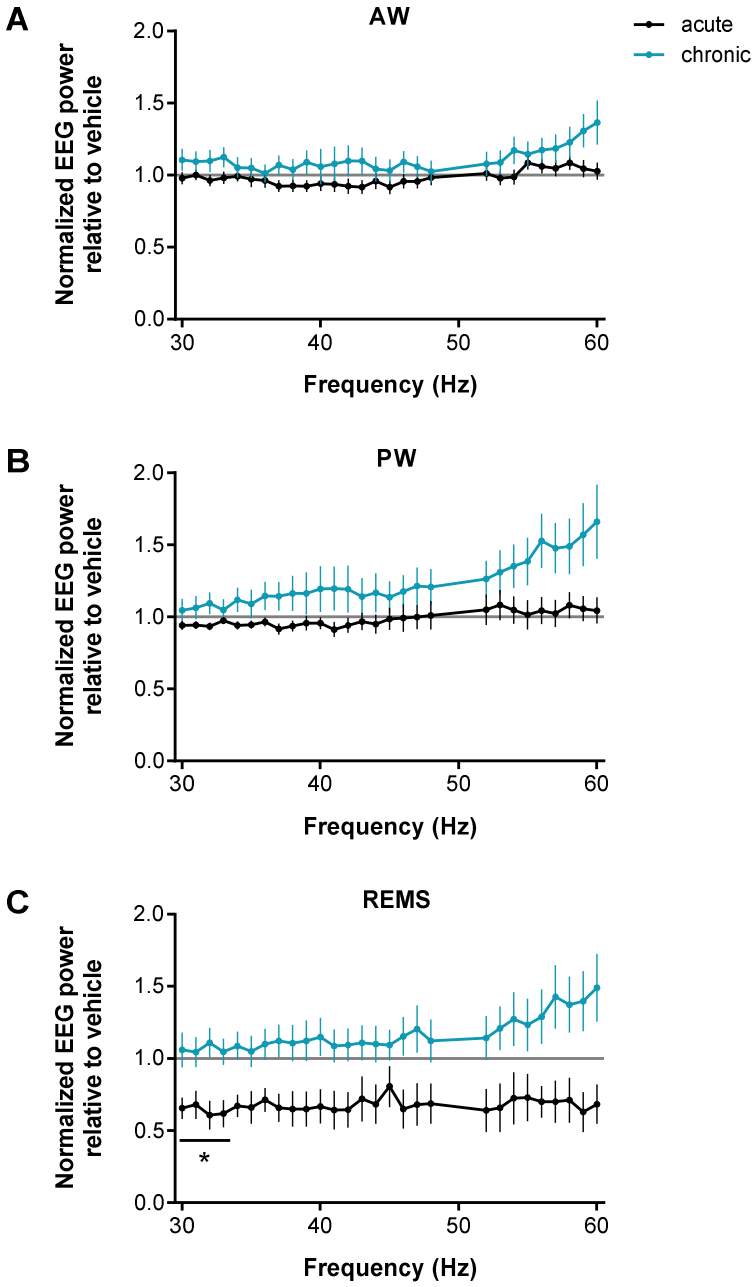

Figure 3 - Changes of the EEG spectral power in the gamma frequency band after acute and chronic escitalopram treatment during sleep-wake stages associated with higher gamma activity: (A) active wakefulness (AW), (B) passive wakefulness (PW), and (C) rapid eye movement sleep (REMS). Each point represents mean ± SEM values of n = 6-7 animals per group, relative to the appropriate vehicle control, in the summarized first 3 h of passive (light) phase. * significant *post hoc* results ($p < 0.05$)

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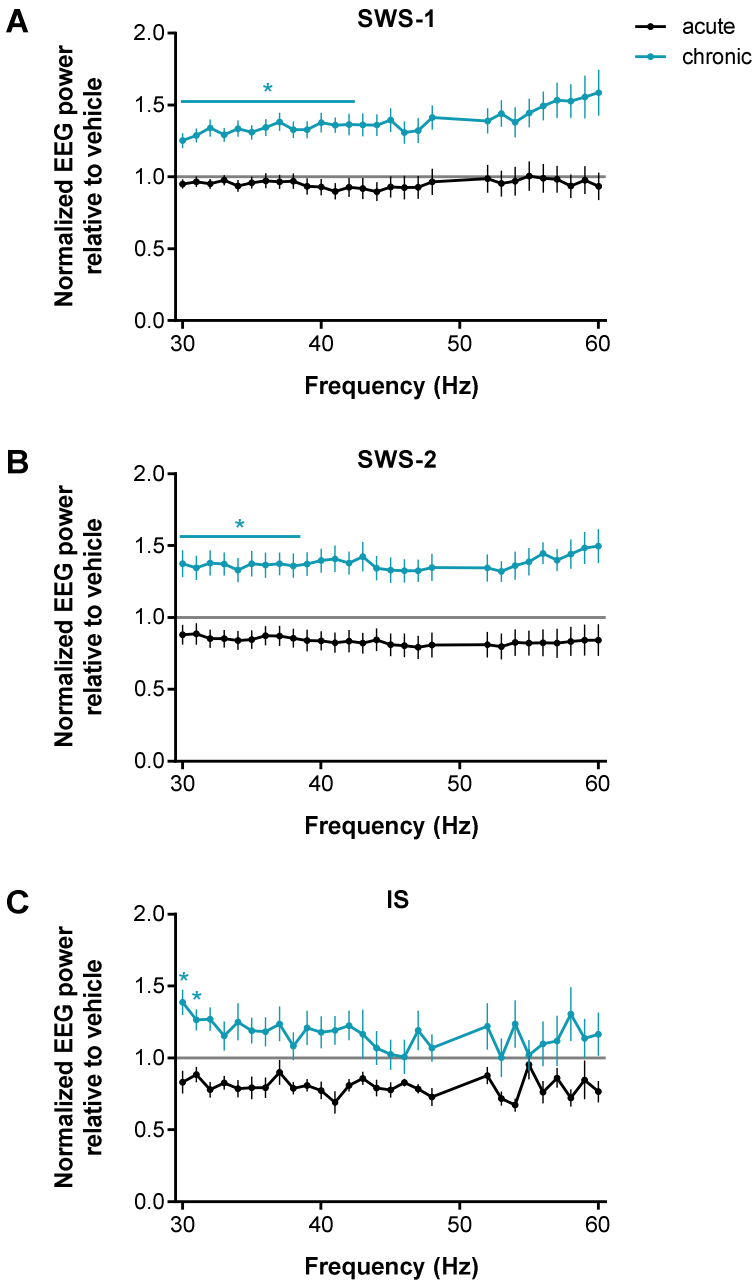

Figure 4 - Changes of the EEG spectral power in the gamma frequency band after acute and chronic escitalopram treatment during stages associated with lower gamma activity: (A) light slow-wave sleep (SWS-1), (B) deep slow-wave sleep (SWS-2), and (C) intermediate stage of sleep (IS). Each point represents mean ± SEM values of n = 6-7 animals per group, relative to the appropriate vehicle control, in the summarized first 3 h of passive (light) phase. * significant *post hoc* results ($p < 0.05$)

3.2. SB-242084 experiment

In the second experiment, we investigated and compared the effects of chronic escitalopram (ESC+veh), acute SB-242084 (VEH+SB), their combination (ESC+SB), and a control group (VEH+veh) on EEG gamma power during the foregoing six sleep-wake stages.

3.2.1. Effects of acute SB-242084 on gamma power

During AW, no significant alterations were seen (two-way ANOVA for repeated measures, treatment effect: $F_{3,20} = 1.917$, $p = 0.1594$; treatment x frequency interaction: $F_{81,540} = 1.218$, $p = 0.1081$; **Figure 5 A**). Meanwhile during PW, acute treatment with SB-242084 caused a moderate elevation in gamma power (treatment effect: $F_{3,20} = 2.217$, $p = 0.1176$; treatment x frequency interaction: $F_{81,540} = 2.519$, $p < 0.001$; **Figure 5 B**). The most prominent effects were found in slow-wave sleep stages. Not only chronic escitalopram, but also acute SB-242084, as well as their combination caused an elevation in gamma power. This observation was supported by significant results of ANOVA in SWS-1 (treatment effect: $F_{3,20} = 3.692$, $p = 0.0290$; treatment x frequency interaction: $F_{81,540} = 2.693$, $p < 0.001$; **Figure 6 A**) and in SWS-2 (treatment effect: $F_{3,20} = 4.787$, $p = 0.0113$; treatment x frequency interaction: $F_{81,540} = 1.911$, $p < 0.001$; **Figure 6 B**). In IS, the increase in gamma power was not significant (treatment effect: $F_{3,20} = 2.349$, $p = 0.1031$, treatment x frequency interaction: $F_{81,540} = 1.241$, $p = 0.0878$; **Figure 6 C**). Finally, during REMS, the acute SB-242084 treatment did not affect gamma power (treatment effect: $F_{3,19} = 2.515$, $p = 0.0891$; treatment x frequency interaction: $F_{81,513} = 1.226$, $p = 0.1014$; **Figure 5 C**).

3.2.2. Acute SB-242084 and chronic escitalopram induced similar changes in gamma power

Bonferroni *post hoc* results revealed no significant differences during any of the sleep-wake stages between VEH+SB and ESC+veh groups, suggesting similar effects of acute SB-242084 and chronic escitalopram on EEG gamma power.

3.2.3. Effects of the combination of acute SB-242084 and chronic escitalopram on gamma power

As seen in **Figure 5** and **Figure 6**, the combined treatment caused apparently similar effects on gamma power to acute SB-242084 (and chronic escitalopram) alone. No significant *post hoc* differences were found when comparing either VEH+SB vs. ESC+SB or ESC+veh vs. ESC+SB groups, confirming the similarity of the effects and suggesting that the gamma power effects of the two treatments did not interfere. (In the ESC+SB group, we could calculate from data of $n = 5$ rats only, as one rat failed to enter REMS in this time period.)

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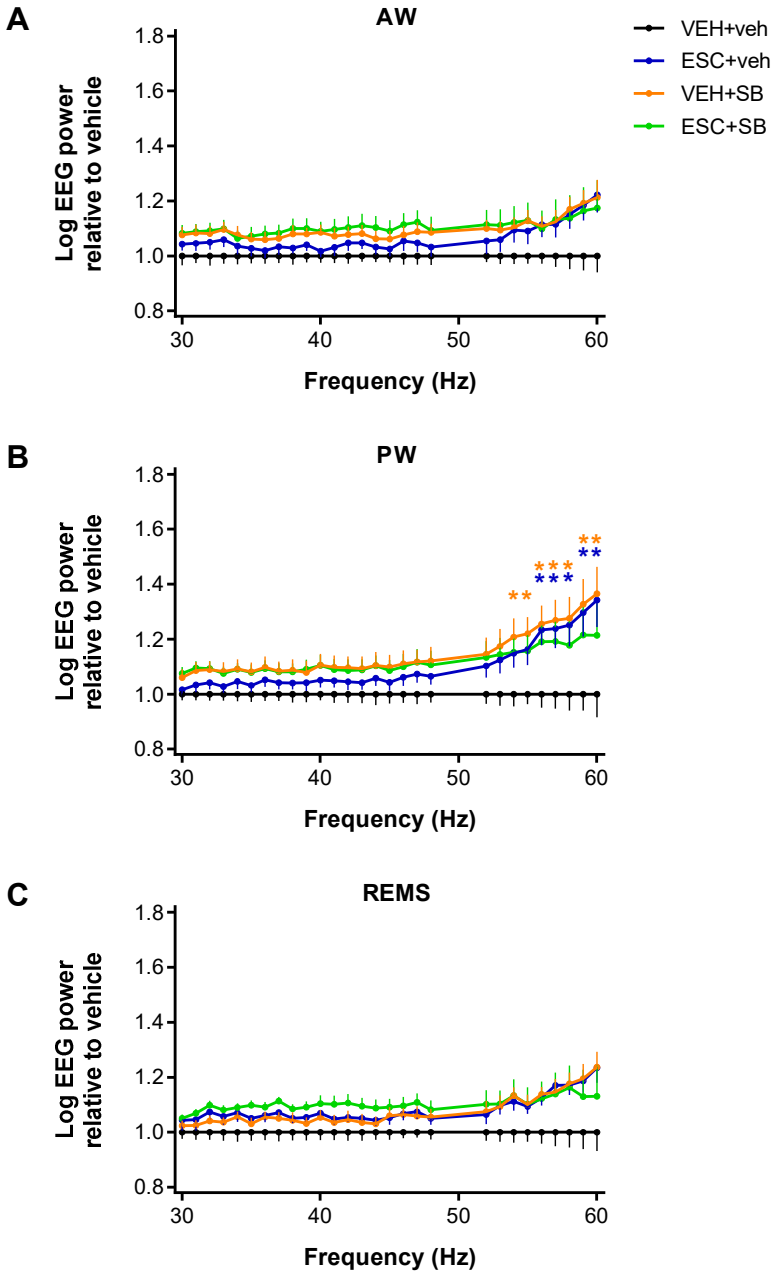

Figure 5 - Changes of the EEG spectral power in the gamma frequency band after the administration of chronic escitalopram (ESC+veh), acute SB-242084 (VEH+SB), and their combination (ESC+SB) during stages associated with higher gamma activity: (A) active wakefulness (AW), (B) passive wakefulness (PW), and (C) rapid eye movement sleep (REMS). Each point represents mean ± SEM values of $n = 6$ animals per group, in the summarized first 3 h of passive (light) phase. * significant *post hoc* results ($p < 0.05$) compared to VEH+veh

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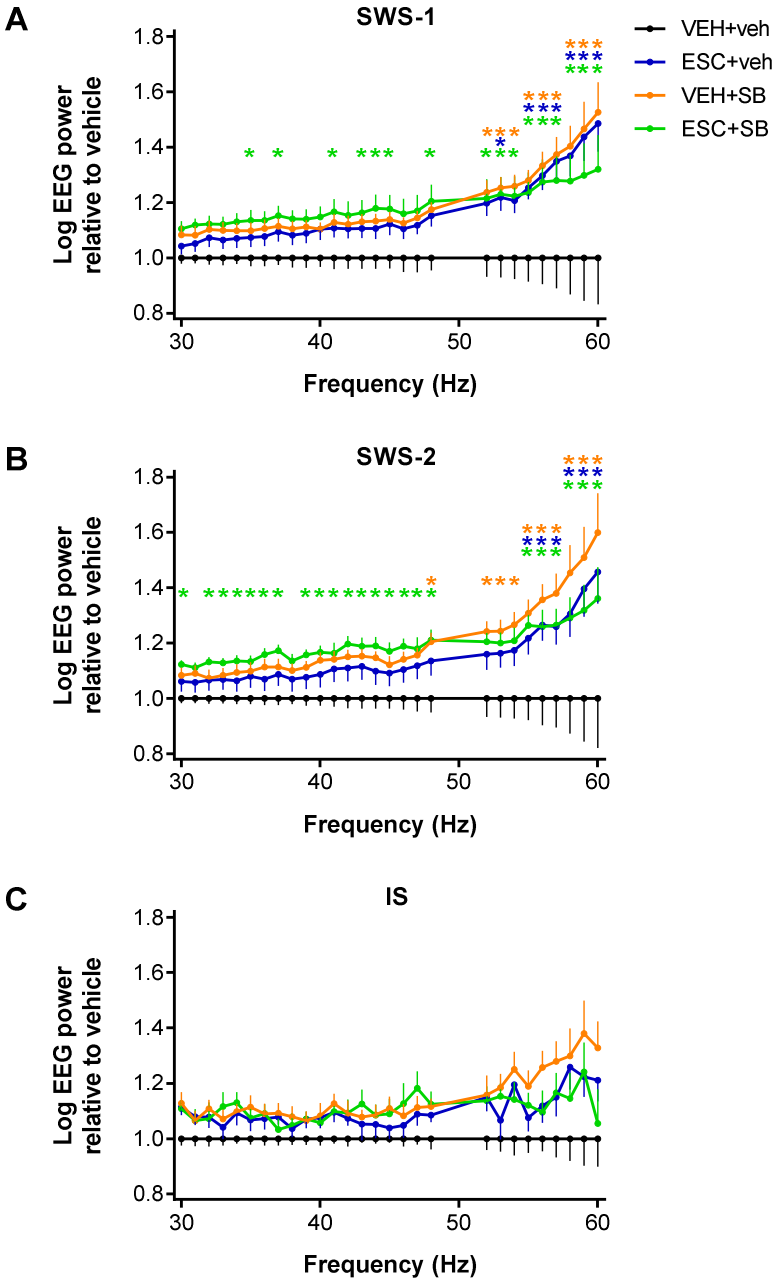

Figure 6 - Changes of the EEG spectral power in the gamma frequency band after the administration of chronic escitalopram (ESC+veh), acute SB-242084 (VEH+SB), and their combination (ESC+SB) during stages associated with lower gamma activity: (A) light slow-wave sleep (SWS-1), (B) deep slow-wave sleep (SWS-2), and (C) intermediate stage of sleep (IS). Each point represents mean ± SEM values of $n = 6$ animals per group, in the summarized first 3 h of passive (light) phase. * significant *post hoc* results ($p < 0.05$) compared to VEH+veh

The explanation of the different data transformation methods used in the two studies is the following. In case of *Escitalopram experiment*, we normalized data, i.e. divided power values at each frequency bin by the total power of the whole EEG spectrum (1-60 Hz) in the given stage, in order to reduce inter-individual variability in the absolute power of the animals. The advantage of normalization in case of EEG power data is well demonstrated by the fact that it reduces SEM values of the group more than applying logarithmic or other transformation. This method was not applicable in case of *SB-242084 experiment*, due to the marked theta power elevation during AW and REMS induced by SB-242084 treatment (80). In these data sets, as the denominator (total power including theta) would be higher, the value of the fraction (normalized power per Hz) would be lower in the whole spectrum (including gamma band). We must note that these decreases result from treatment effects on lower frequencies, not on gamma band, thus, these normalized data would be biased. For this reason, we chose logarithmization instead of normalization in case of *SB-242084 experiment*. In contrast, in *Escitalopram experiment*, the theta power decrease caused by acute escitalopram was so moderate that it did not affect our main results (3, 59). According to two-way ANOVA results of logarithmized absolute EEG power data:

- AW: neither acute ($F_{1,12} = 2.175$, $p = 0.1660$), nor chronic escitalopram ($F_{1,11} = 3.247$, $p = 0.0990$) had an effect on gamma power;
- PW: neither acute ($F_{1,12} = 1.208$, $p = 0.2933$), nor chronic escitalopram ($F_{1,11} = 4.110$, $p = 0.0676$) had an effect on gamma power;
- SWS-1: acute escitalopram did not affect gamma power ($F_{1,12} = 1.785$, $p = 0.2063$), while chronic escitalopram had an effect ($F_{1,11} = 5.742$, $p = 0.0355$) on gamma power;
- SWS-2: acute escitalopram did not affect gamma power ($F_{1,12} = 1.536$, $p = 0.2389$), while chronic escitalopram had a trend for effect ($F_{1,11} = 4.437$, $p = 0.0589$) on gamma power;
- IS: acute escitalopram did not affect gamma power ($F_{1,12} = 1.821$, $p = 0.2021$), while chronic escitalopram had an effect ($F_{1,11} = 5.872$, $p = 0.0338$) on gamma power;
- REMS: both acute escitalopram ($F_{1,11} = 5.600$, $p = 0.0374$), and chronic escitalopram had an effect ($F_{1,11} = 5.562$, $p = 0.0379$) on gamma power

basically in accordance with results of normalized data.

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4. Discussion

This work is aimed to present and summarize our findings about the effects of the SSRI antidepressant escitalopram (both acutely and chronically) and the 5-HT_{2C}R antagonist SB-242084 (with or without chronic escitalopram pretreatment) on the EEG gamma power in different sleep-wake stages.

To understand the possible background of our results, it is essential to take a closer look on the origin of the investigated brain rhythm. Gamma oscillations have been described in most cortical and subcortical areas of the brain, although in the activity registered by the EEG on cortical surfaces, the contribution of specific structures, mostly cortex, hippocampus, and thalamus is predominant. As mentioned in section 1.1, their generation is linked to PV+ GABAergic interneurons (6, 81), and to a lesser extent, to somatostatin-expressing interneurons (7). Assemblies of these interneurons generate high-frequency (>30 Hz) network oscillations, via exerting strong, rhythmic inhibition onto pyramidal neurons as well as other fast-spiking interneurons (8).

At first, we presented that the total power of gamma oscillations varied with sleep-wake stages. Our results showing the highest gamma activity in AW and REMS are not surprising considering that these vigilance states are characterized by the highest levels of cortical activation. During PW and non-REMS stages, a significantly lower gamma power was observed, in line with the descending cortical activation levels from wakefulness to sleep. In accordance with our findings, a similar pattern has been reported in rats (82), whereas extra- and intracranial recordings (of epileptic patients) demonstrated higher gamma activity in wakefulness and lower in REMS and non-REMS (83). During both wakefulness and REMS, the desynchronized EEG activity resulting in high gamma power has been associated with an elevated cholinergic activity (84). However, the intra- and interhemispheric coherence of gamma rhythm has been reported significantly lower during REMS in comparison to that during wakefulness (82).

During wakefulness, an elevated gamma oscillatory activity has been related to focused attention (85), cognition (86), and sensory processing (87) in both humans and animals (8, 9). REMS has a crucial role in memory consolidation, specifically, promoting synaptic potentiation, via enhanced high-frequency oscillations and increased expression of genes related to plasticity (17, 88-90). Finally, the presence of gamma oscillations has

also been established during non-REMS in humans (91-93), monkeys (92), cats (94), and rats (95), also with an important role in memory consolidation (96). Whereas the role of gamma oscillations during wakefulness is extensively investigated, their functions and importance during sleep are still not fully understood.

4.1. Effects of escitalopram

Our results from the first experiment showed that the widely used SSRI antidepressant escitalopram differently modified the EEG gamma power in rats when administered acutely (10 mg/kg, ip.) and chronically (10 mg/kg/day for 21 days, osmotic minipumps). Moreover, the effect of the drug had a clear sleep-wake stage-dependency. Whereas acute treatment with escitalopram decreased gamma power in REMS and IS exclusively, chronic treatment with the same dose of the drug markedly increased gamma power in SWS-1 and SWS-2. Nonetheless, gamma power in AW and PW did not change as a result of either acute or chronic escitalopram administration.

In this experiment, we chose 10 mg/kg escitalopram dose, based on literature data demonstrating the effect of this treatment on sleep and EEG parameters (59, 80) as well as depressive-like behavior (97, 98) and extracellular 5-HT levels (99, 100) in rats. We analyzed effects in the first 3 h after the ip. injections (from lights on, i.e. in the passive phase), with respect to the 1.5 h half-life time of escitalopram reported in rats (101).

In wakefulness, our results revealed that neither acute nor chronic escitalopram treatment affected gamma activity. Accordingly, no alteration has been detected in frontal cortical EEG gamma power (30-50 Hz) in rats during AW, although in that study, escitalopram was applied in a smaller dose, 2 mg/kg (102). In (healthy) humans, no alteration has been found in gamma peak frequency when taking 10 mg/day escitalopram for 7-10 days (62), although another study has reported an increase in gamma power (103). Aside from these miscellaneous results (of studies using scarcely comparable methods), considering the crucial role of gamma oscillations in cognition, no negative effect of escitalopram on psychomotor function has been found in clinical studies with healthy participants (104) and depressed patients (105). Hence, we presumed that escitalopram, applied acutely or chronically, did not affect cortical network functions occurring in wakefulness reflected by EEG gamma oscillations recorded on cortical surfaces (3).

In contrast to wakefulness, during REMS (where a relatively high gamma activity was seen, similarly to wakefulness) and also during IS (the transition stage of REMS), a significant decrease in gamma power was found after acute escitalopram treatment. Interestingly, following chronic treatment, these changes disappeared. Several lines of evidence support the central role of REMS in the pathophysiology of depression. Characteristic alterations in REMS architecture, such as shortened REMS latency and increased REMS density, are commonly observed in depressed patients (13) and animal models of depression (106). Furthermore, most antidepressants inhibit REMS (13), while selective REMS deprivation induces antidepressant effect in humans (107). On the other hand, the active contribution of REMS has also been proposed in negative memory consolidation (108). For example, in patients suffering from depression, the amount of REMS or the awakenings from REMS have been reported to correlate with lower self-appraisal, higher suicidality scores, and more frequent negative emotions in REMS dreams in comparison to non-REMS dreams (109, 110). Therefore, it has been hypothesized that pathophysiological alterations in REMS might enhance the vulnerability to depression, including the recurrence of the disease (108). Considering the role of REMS in both depression and memory processes (111), the gamma power decreasing effect of acute escitalopram treatment during REMS and IS raises the question: is this alteration a critical early step in the development of the antidepressant effect or a side effect of the drug (3)? Clinical findings about the influence of antidepressants on memory are not consistent. For instance, while Wadsworth et al. have linked impaired episodic (but not semantic or working) memory with SSRI treatment, Gorenstein et al. have found the clinical relevance of disturbances in memory and psychomotor performances by SSRIs uncertain (105, 112). Furthermore, the SSRI-induced pharmacological REMS suppression (by acute fluvoxamine) has been reported to improve skill memory consolidation in healthy humans (113). Hence, disturbed consolidation of procedural memory, that is commonly observed in depressed patients, is unlikely to be a result of REMS suppression caused by antidepressants (114). In addition, chronic treatment with escitalopram has been demonstrated to improve both immediate and delayed verbal and nonverbal memory as well as work productivity in depressed patients (115). Importantly, both REMS and non-REMS stages are of high importance in memory processes. Whereas REMS favors procedural and emotional (i.e.

non-declarative) memory consolidation, non-REMS supports formation of declarative memories (17). However, in healthy humans, the suppression of REMS and SWS, declarative memory and motor consolidation have been correlated (116). Our results showed that chronic treatment with escitalopram increased the EEG gamma power in non-REMS stages (SWS-1 and SWS-2), while acute treatment with the drug failed to produce this effect. Le Van Quyen et al. have described that population activity of cortical neurons in non-REMS is presumably orchestrated by high-frequency (beta and gamma) oscillations, and through this mechanism these rhythms might be involved in the reactivation of information during sleep (92). Considering the role of non-REMS in memory formation and the function of high-frequency (including gamma) oscillations in non-REMS, we hypothesized that elevation in gamma power caused by chronic escitalopram might affect local network communication in non-REMS, thus, it might serve as a marker of its antidepressant effect (3). This hypothesis is supported by findings demonstrating that chronic administration of different SSRIs improved declarative memory (both emotional and neutral) in depressed patients, by improving anterior cingulate function (117). However, further studies are needed to prove this concept.

As described earlier, in the generation of gamma oscillations, a crucial role of PV+ fast-spiking interneurons exerting feed-back and feed-forward inhibition of pyramidal neurons has been demonstrated (118). Alterations in the synchrony of gamma oscillations (manifested as EEG power) can be induced either by directly modulating pyramidal neurons or by modulating other cells of the network thus indirectly influencing pyramidal neurons. Regarding the effect of escitalopram on gamma oscillations (and other brain rhythms as well), the elevation of extracellular 5-HT levels is crucial. However, PET studies in humans have provided evidence that at the beginning of treatment, 5-HT levels rather reduce in serotonergic projection areas (119). The marked difference between acute and chronic effects of escitalopram may serve as an explanation of these findings. In acute treatment, escitalopram activates somatodendritic 5-HT_{1A} autoreceptors on neurons of the dorsal raphe nucleus, resulting in decreased firing rate of serotonergic neurons. However, in long-term treatment, the desensitization of 5-HT_{1A}Rs allows the recovery of the firing rate of these neurons (120, 121), that explains the delayed onset of clinical effect. Of all cortical regions in the brain, prefrontal cortex is the area most enriched in 5-HT receptors: the co-expression of 5-HT_{1A}Rs and 5-HT_{2A}Rs has been described in ~80% of neurons

including excitatory pyramidal neurons (122) and PV+ fast-spiking interneurons (123). Activation of 5-HT_{1A}Rs induce hyperpolarization of pyramidal neurons, whereas activation of 5-HT_{2A}Rs leads to depolarization, reduced after-hyperpolarization, and increased firing rate as well as excitatory postsynaptic potentials. In cortical areas, 5-HT administration has been reported to cause a biphasic effect, namely, hyperpolarization followed by depolarization (124), that might be attributed to the aforementioned frequent co-expression of 5-HT_{1A}Rs and 5-HT_{2A}Rs (125, 126). Puig et al. have shown that 5-HT potently controls (primarily downregulates) prefrontal gamma oscillations (30-80 Hz), by finely tuning the amplitude through 5-HT_{1A}R (and to a lesser extent through 5-HT_{2A}R) expressing fast-spiking interneurons, inducing an overall reduction in gamma power (123). In line with this, 5-HT_{1A}R activation has been shown to suppress ketamine-induced hippocampal gamma rhythm, as a result of reduced hyperpolarization and afterpolarization frequency of CA3 pyramidal neurons, but not PV+ interneurons (127). I have to note that, similarly to 5-HT_{1A}R desensitization, chronic SSRI treatment has been reported to induce adaptive changes in the function of 5-HT_{2A}Rs too, although with miscellaneous results (128-131). Taken together, the differential gamma activity alteration caused by acute and chronic escitalopram treatment our results revealed, might be the consequence of a biphasic effect of both 5-HT_{1A}Rs and 5-HT_{2A}Rs (3).

4.2. Effects of SB-242084

Results of the second experiment showed that the acute administration of the 5-HT_{2C}R antagonist SB-242084 (1 mg/kg, ip.) enhanced the EEG gamma power in SWS-1, SWS-2, and PW, in rats. Interestingly, these effects seemed very similar to the effects of chronic escitalopram treatment in most sleep-wake stages, in other words, the selective antagonism on 5-HT_{2C}R resulted in similar effects on gamma oscillations as the chronic inhibition of 5-HTT and additional effects escitalopram induces (4).

In this experiment, we chose 1 mg/kg SB-242084 dose, based on former literature data demonstrating the effect of this treatment on sleep architecture and EEG parameters (80, 132-134) as well as behavior (78, 135, 136) in rodents. Notably, Kantor et al. have reported that although both 0.3 and 1.0 mg/kg doses of SB-242084 produced anxiolysis, prominent effects on the sleep-wake cycle (wake promotion and SWS-2 suppression) were observed only in case of the 1.0 mg/kg dose, in Sprague-Dawley rats (77).

A limited amount of literature data is available about the qEEG effects of SB-242084 or other 5-HT_{2C}R antagonists, and until our study, none of them investigated gamma oscillations. However, Schulz et al. analyzed the acetylcholine and physostigmine induced gamma power altering effects of antipsychotic drugs in rat hippocampal slices (in the CA3 region), and found that whereas 5-HT_3Rs enhanced and dopamine D_3 receptors inhibited gamma oscillations, 5-HT_{2C}Rs did not affect them (137). Accordingly, in the abovementioned study, Puig et al. have reported that although 5-HT *per se* affected gamma activity, this effect was not mediated through 5-HT_{2C}Rs, but through 5-HT_{1A}Rs and 5-HT_{2A}Rs, in anesthetized rats (123). Besides, gamma activity is modulated by other monoamine neurotransmitters as well. A growing body of evidence supports the role of the dopaminergic system in synchronizing fast-spiking interneurons and modulating gamma oscillations (138, 139).

5-HT_{2C}Rs have been found on GABAergic interneurons in numerous areas of the brain, and are also expressed by dopaminergic neurons, but not by serotonergic and noradrenergic neurons. Several preclinical data suggest the role of these receptors in the modulation of monoaminergic (serotonergic, noradrenergic, and also dopaminergic) systems (74, 140), that we must take into consideration to explain our findings. Constitutive activity of 5-HT_{2C}Rs has been described to inhibit the activity of dopaminergic neurons, that could be reversed by 5-HT_{2C}R antagonists. Both SB-242084 and SB-206553 increased basal dopamine release in the rat nucleus accumbens and striatum. Yet, the SB-242084 induced dopamine release was modest and limited, in comparison to SB-206553 (141).

Meanwhile, SSRIs have also been suggested in the regulation of dopaminergic signaling. Whereas acute treatment with escitalopram has been found to increase the firing rate of dopaminergic neurons in the ventral tegmental area (142), others have observed a decrease in firing rate of these neurons following a 2-week-long escitalopram treatment, in anesthetized rats (143). The possible implication of both 5-HT_{2C}R antagonists and SSRIs in dopaminergic signaling might serve as an explanation for the similar effects on gamma oscillations, however, more studies are needed to reinforce this idea. Nevertheless, this similarity might be an indirect evidence of the antidepressant and/or anxiolytic effect of SB-242084.

4.3. Combination of SSRIs and 5-HT$_{2c}$R antagonists

In the second experiment, effects of SB-242084 on the EEG gamma power were investigated not only alone but also after chronic escitalopram pretreatment. According to our results, similar alterations were induced by chronic escitalopram, acute SB-242084, and their combination in most vigilance stages, namely, all of the treatments caused an enhancement of gamma power in SWS-1 and SWS-2. These findings suggest that the two treatments did not interfere (neither potentiation nor inhibition was apparent), i.e. the 5-HT$_{2C}$R antagonist did not modify any further the effects of the SSRI on gamma activity, or vice versa (4).

Our study was the first to investigate gamma power effects of this combination, although Kostyalik et al. have studied effects on lower frequencies and on sleep-wake architecture, also in rats. They have reported that SB-242084 increased PW and suppressed REMS, which latter effect was attenuated by chronic escitalopram pretreatment. QEEG analysis revealed increased theta power in AW and REMS by SB-242084, which effect however was not altered by chronic escitalopram (80). The SSRI-related attenuation in particular EEG parameters induced by the 5-HT$_{2C}$R antagonist has been associated with the presumed dissociative adaptation of 5-HT$_{2C}$Rs (80), that might be the explanation of our results as well. On the other hand, non-EEG studies have demonstrated the interaction of the two treatments when applied in combination. In a rat study using microdialysis, acute SB-242084 or RS-102221 (another 5-HT$_{2C}$R antagonist) have been found to augment citalopram-induced 5-HT increases in the ventral hippocampus and prefrontal cortex (144). Conversely, SB-242084 pretreatment has been reported to reverse the citalopram-induced increased fear expression, in rats (145). Moreover, after a chronic (21-day-long) fluoxetine pretreatment, acute SB-242084 treatment has been shown to reduce depression- and anxiety-like behaviors, and to ameliorate certain SSRI-induced side effects, in mice (79).

Considering our hypothesis that the enhanced gamma activity in non-REMS, affecting local network communication, might serve as a marker of an antidepressant effect, and the promising results of behavioral studies, applying SB-242084 alone or at the beginning of a chronic treatment with escitalopram might be beneficial.

4.4. Relationship of gamma with other frequency bands

As a result of cross-frequency coupling (an effective mechanism to functionally link active cortical circuits), slower rhythms, theta in particular, modulate the power of gamma oscillations (8, 146). Gamma oscillations often occur synchronously with theta during active wakefulness and REMS, moreover, gamma and theta waves occur in the same brain regions (5, 147). Hippocampal theta oscillations have been extensively investigated for decades. These waves play a crucial role in memory processes and several other CNS functions, in both humans and animals (148-151), and are under ascending serotonergic control by the brainstem raphe nuclei (152). Thus not surprisingly, escitalopram has been demonstrated to affect these oscillations, according to literature data. While acute escitalopram has been reported to decrease theta power in the most relevant stages, namely, AW and REMS, chronic treatment failed to produce such an effect, in rats (3, 59). As an explanation, the acute SSRI-induced increase in 5-HT levels leads to somatodendritic 5-HT_{1A}R activation, resulting in reduced theta activity (153), whereas the desensitization of 5-HT_{1A}Rs is likely to cause the lack of theta-reducing effects following chronic escitalopram treatment (3, 121). Although our results showed no effect on gamma oscillations after escitalopram administration during AW, the acute treatment caused a power reduction during REMS, that may be related to the theta decrease in this stage. However, more accurate studies are needed to investigate this possible relationship. Meanwhile, in case of acute SB-242084 treatment, an opposite effect, namely, a theta power increase has been reported during AW and REMS, in rats (77, 80). Accordingly, 5-HT_{2C}Rs have been demonstrated to play a key role in the regulation of hippocampal theta activity (123, 154). As SB-242084 did not affect gamma activity during AW and REMS, these effects seem to be governed by independent mechanisms.

The less studied beta band oscillations are relatively high-frequency waves, adjacent to gamma band, and these two types of oscillations have some common features. Beta waves have been implicated in sensorimotor and motor processes (155, 156), and also in depression, namely, the dominance of these oscillations in relation to other bands during resting-state condition has been described as a unique qEEG alteration in depressed patients (19, 157). Escitalopram has also been shown to alter beta oscillations, most prominently during SWS-1 and SWS-2. Whereas a reduced beta power has been observed after acute escitalopram treatment (that did not affect gamma band), interestingly, a

parallel increase with the elevated gamma band power has been found as a result of chronic escitalopram treatment (3). The relevance of these findings is further supported by the work of Le Van Quyen et al. underlining the involvement of both beta and gamma oscillations during SWS in memory consolidation, specifically, the reactivation of information (92). Regarding SB-242084, previous data suggest no effect on beta oscillations in any of the sleep-wake stages (77).

4.5. Gamma oscillations as potential biomarker of antidepressant response

In terms of using the EEG as a measure of antidepressant effects, several lines of evidence support that a variety of early changes in a number of EEG parameters correlate with antidepressant response, moreover, that in certain circumstances the EEG can identify patients likely to respond to treatment (158). Fitzgerald and Watson have reviewed literature data supporting the role of gamma oscillations as biomarker of depression, and have hypothesized that optimal mood may be associated with an "optimal amount" of gamma activity or a proper balance across certain involved brain regions, such as prefrontal cortex or hippocampus (43, 159). Although in this thesis, gamma signaling was not investigated region-specifically, the examination of sleep-wake stage-dependency is also of high importance and a less frequently applied aspect. The presented results further support the hypothesis about the relationship between EEG gamma alterations and antidepressant effects.

4.6. Limitations

The findings of this thesis should be interpreted in the context of some limitations. First, we used small sample sizes. Second, we did not investigate whether the effects of the drugs on the EEG gamma oscillations are dose-dependent. Third, we recorded EEG using one derivation (from the left frontoparietal cortical surface), resulting in limited spatial resolution, as written above. Fourth, the upper frequency limit of our analysis was 60 Hz due to the nominal sampling rate. Further studies are required to involve these aspects and to extend the analysis to higher frequencies.

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5. Conclusions

The EEG gamma oscillations during wakefulness were not affected by escitalopram treatment either acutely or chronically, suggesting that the SSRI did not interfere with brain functions linked to the synchrony of gamma rhythm in this stage. Considering the role of REMS in both depression and memory functions, the gamma power decreasing effect of acute escitalopram treatment indicates that administration of the drug might affect memory processes at the beginning of therapy. However, this effect may also be an early step in the development of antidepressant effect. The enhancement of gamma power during non-REMS caused by chronic escitalopram treatment may reflect an improvement in local network communication, that is likely to serve as a marker of therapeutic effect. While acute and chronic escitalopram altered gamma activity differently, acute SB-242084 and chronic escitalopram induced similar effects in this frequency band of the EEG, that supports preclinical findings demonstrating that short-term 5-HT_{2C}R antagonist treatment may have antidepressant effects. The combination of the two treatments did not cause any further alteration of gamma power, suggesting that the increased serotonergic tone by the chronic SSRI pretreatment did not affect 5-HT_{2C}R function.

Still, further studies are needed to clarify the molecular background underlying gamma rhythms. Also, the relationship between gamma signaling, aberrant cognitive network functions and behavior should be further investigated in depression or its animal models, and particularly, how conventional and putative antidepressants influence these. Solving this question might enable us to identify reliable EEG biomarkers to predict or test the efficacy of antidepressant therapies in an accurate, non-invasive, yet cost-effective way.

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6. Summary

Identifying reliable biomarkers that objectively reflect the underlying neurobiological processes is of high importance for improving both the diagnosis and the treatment of depression. A growing body of evidence supports the role of gamma oscillations, measured by electroencephalography (EEG), in the development and course of depression, and also in testing antidepressant efficacy. Escitalopram, a selective serotonin reuptake inhibitor (SSRI), is one of the most commonly prescribed antidepressant drugs. With regard to its different clinical effects in acute and chronic treatments, in this work, we examined the effect of escitalopram on gamma power after both acute and chronic treatments. As gamma power varies across the sleep-wake cycle, we analyzed it in six vigilance stages. As part of the antidepressant effect of chronic escitalopram treatment, the adaptation of serotonin 2C receptors (5-HT_{2C}R) has been suggested. Therefore, in a subsequent experiment, we investigated how SB-242084, an antagonist of 5-HT_{2C}R, affects gamma activity (the role of 5-HT_{2C}R), and whether pretreatment with chronic escitalopram modifies these effects (the influence of increased serotonergic tone on 5-HT_{2C}R function). Our main results are the followings: (1) escitalopram acutely decreased gamma power during rapid eye movement sleep, (2) in contrast, chronically it increased gamma power during light and deep slow-wave sleep. (3) The acute SB-242084 treatment, similarly to chronic escitalopram, increased gamma power during slow-wave sleep stages. (4) This latter effect was not affected by the pretreatment with chronic escitalopram.

Taken together, the difference between the acute and chronic effects of escitalopram on gamma power is in line with previous data reporting markedly different therapeutic and side effects with short- and long-term application of the drug. Our findings also suggest that 5-HT_{2C}R antagonists could be potential new compounds of antidepressant therapies in monotherapy or in combination to enhance the effect of SSRI antidepressants. Our results confirm the presumed relationship of gamma oscillations and the clinical effect of antidepressant treatments, further supporting the idea that gamma oscillations could serve as a potential quantitative EEG biomarker in tracking the pathophysiological process of depression and in testing the efficacy of antidepressant therapies.

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7. Összefoglalás – Summary in Hungarian

A depresszió és az antidepresszáns gyógyszerek kutatása során felmerült az igény olyan biomarkerek azonosítására, melyek megbízhatóan és objektíven tükrözik a háttérben zajló neurobiológiai folyamatokat, ezáltal fejlesztve a diagnosztikus és terápiás lehetőségeket. Egyre több tudományos bizonyíték támasztja alá az elektroenkefalográfiával (EEG) mérhető gamma oszcillációk szerepét a depresszió kialakulásában és lefolyásában, a kezelés hatékonyságában. Az escitalopram, a szelektív szerotoninvisszavétel-gátlók (SSRI) képviselője, az egyik leggyakrabban felírt antidepresszáns gyógyszer. Figyelembe véve a szer akut és krónikus adagolás során eltérő klinikai hatásait, az escitalopram gamma teljesítményre kifejtett hatásait mind akut, mind pedig krónikus kezelést követően vizsgáltuk. Mivel a gamma teljesítmény az alvás-ébrenlét ciklus során jellemző módon változik, az elemzés hat vigilancia stádiumban történt. A krónikus escitalopram kezelés antidepresszáns hatásához hozzájárulhat a szerotonin 2C receptorok (5-HT_{2C}R) adaptációja. Egy következő kísérletben ezért azt vizsgáltuk, hogy hogyan hat a 5-HT_{2C}R antagonista SB-242084 a gamma aktivitásra (5-HT_{2C}R szerepe), illetve hogy a krónikus escitaloprammal való előkezelés hogyan módosítja e hatásokat (a fokozott szerotonerg tónus befolyásolja-e a 5-HT_{2C}R funkcióját). Fő eredményeink: az escitalopram akutan adagolva csökkentette a gamma teljesítményt REM alvás alatt, krónikusan ezzel szemben növelte a gamma teljesítményt felszínes és mély lassú hullámú alvás során. Az akut SB-242084 kezelés, a krónikus escitalopramhoz hasonlóan, növelte a gamma teljesítményt a lassú hullámú alvásstádiumok alatt. Ez utóbbi hatást a krónikus escitaloprammal történő előkezelés nem befolyásolta.

Az escitalopram akut és krónikus hatásai közt megfigyelt különbség összhangban áll a rövid és hosszú távú alkalmazás során eltérő terápiás, illetve mellékhatásokkal. A kapott eredmények megerősítik a 5-HT_{2C}R antagonisták potenciális antidepresszánsként való alkalmazhatóságát monoterápiában vagy SSRI-vel való kombinációban. Eredményeink alátámasztják továbbá a gamma oszcillációk és az antidepresszáns terápiák klinikai hatékonyságának feltételezett kapcsolatát, valamint a hipotézist, miszerint a gamma oszcillációk kvantitatív EEG biomarkerként szolgálhatnak a depresszió patofiziológiai folyamatai, valamint az antidepresszáns hatás nyomon követésében.

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9. Bibliography of the candidate's publications

9.1. Publications related to the PhD thesis

1. **Papp N**, Koncz S, Kostyalik D, Kitka T, Petschner P, Vas S, Bagdy G. (2019) Acute 5-HT2C Receptor Antagonist SB-242084 Treatment Affects EEG Gamma Band Activity Similarly to Chronic Escitalopram. Front Pharmacol, 10: 1636.
   IF: 4.225 (2019)

2. **Papp N**, Vas S, Bogathy E, Katai Z, Kostyalik D, Bagdy G. (2018) Acute and chronic escitalopram alter EEG gamma oscillations differently: relevance to therapeutic effects. Eur J Pharm Sci, 121: 347-355.
   IF: 3.532 (2018)

9.2. Publications not related to the PhD thesis

1. Bogathy E, **Papp N**, Vas S, Bagdy G, Tothfalusi L. (2019) AM-251, A Cannabinoid Antagonist, Modifies the Dynamics of Sleep-Wake Cycles in Rats. Front Pharmacol, 10: 831.
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2. Bogathy E, **Papp N**, Tothfalusi L, Vas S, Bagdy G. (2019) Additive effect of 5-HT2C and CB1 receptor blockade on the regulation of sleep-wake cycle. BMC Neurosci, 20: 14.
   IF: 2.811 (2019)

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