

Ph.D. dissertation

**THE EFFECTS OF SELECTIVE SEROTONIN REUPTAKE  
INHIBITORS ON THE FUNCTION OF DISTINCT  
SEROTONIN RECEPTOR SUBTYPES IN THE RAT**

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**Abbreviations**

<b>5-HIAA</b>	5-hydroxyindole acetic acid
<b>5-HT</b>	5-hydroxytryptamine, serotonin
<b>8-OH-DPAT</b>	8-hydroxy-2-(di-N-propylamino)tetralin
<b>ANOVA</b>	analysis of variance
<b>BW 723C86</b>	1-[5-thienylmethoxy-1-1H-3-indoyl] propan-2-amine hydrochloride
<b>CCK</b>	cholecystokinin
<b>CNS</b>	central nervous system
<b>CRH</b>	corticotropin releasing hormone
<b>DA</b>	dopamine
<b>ECS</b>	electroconvulsive shock
<b>EEG</b>	electroencephalography
<b>EMG</b>	electromyography
<b>FH</b>	Fawn-Hooded
<b>GAD</b>	generalized anxiety disorder
<b>GABA</b>	gamma-aminobutyric acid
<b>LSD</b>	lysergic acid diethylamide
<b>LY-53857</b>	6-methyl-1-(methylethyl)-ergoline-8beta-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate
<b><i>m</i>-CPP</b>	<i>m</i> -chlorophenylpiperazine
<b>mRNA</b>	messenger ribonucleic acid
<b>NA</b>	noradrenaline
<b>NAN-190</b>	1-(2-methoxyphenyl)-4-[4-2(2-phthalimmido)butyl]piperazine
<b>NRT</b>	nucleus reticularis thalami
<b>OCD</b>	obsessive-compulsive disorder
<b><i>p</i>-CPA</b>	<i>p</i> -chlorophenylalanine
<b>PTSD</b>	post-traumatic stress disorder
<b>SB-206553</b>	N-3-pyridinyl-3,5-dihydro-5-methyl-benzo[1,2-b:4,5-b']dipyrrole-1(2H)-carboxamide
<b>SB-215505</b>	6-chloro-5-methyl-1-[5-quinolylcarbonyl]indoline
<b>SB-242084</b>	6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy) pyrid-5-yl carbonyl] indoline
<b>SD</b>	Sprague-Dawley

<b>SEM</b>	standard error of the mean
<b>SSRI</b>	selective serotonin reuptake inhibitor
<b>SWD</b>	spike-wave discharge
<b>TCA</b>	tricyclic antidepressant
<b>WAG/Rij</b>	Wistar albino Glaxo strain, bred in Rijswijk
<b>WAY-100635</b>	N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl cyclohexanecarboxamide maleate

## 1. **Introduction**

The discovery of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) has emerged as a major therapeutic advance in psychiatry and marked a milestone in psychopharmacology. The first of the SSRIs (also called serotonin-specific reuptake inhibitors), fluoxetine (Prozac) was introduced in the United States in 1988 and has launched a new era in psychotropic drug therapy [191]. SSRIs, including fluoxetine, sertraline, paroxetine, citalopram and fluvoxamine, are widely prescribed for a range of behavioral and psychiatric problems. In addition to being established treatments for depression, SSRIs have well-documented efficacy in the anxiety disorders, panic disorder and OCD. Evidence suggests that SSRIs are effective in the treatment of dysthymic disorder, social phobia, premenstrual syndrome, bipolar disorder depression and bulimia nervosa. Research is currently focusing on new indications, including post-traumatic stress disorder (PTSD) and generalized anxiety disorder (GAD) [185]. Clinical experience supported by ongoing research continues to expand on the broad array of therapeutic applications for this class of medication [176]. SSRIs appear to be effective in some patients with neurocardiogenic syncope, may reduce the frequency and severity of migraine headaches and are possibly effective in reducing the pain of diabetic neuropathy [176]. In combination with tricyclic antidepressants, SSRIs offer a potent therapy for fibromyalgia [176]. A side effect of SSRIs coincidentally provides therapy for premature ejaculation [176]. Selective serotonin reuptake inhibitors are widely used because of their safety, tolerability, and demonstrated efficacy [185]. Prior to the SSRIs, all psychotropic medications were the result of chance observation. SSRIs were developed for inhibition of the neuronal uptake pump for 5-HT, a property shared with the tricyclic antidepressants (TCAs), but without affecting the other various neuroreceptors (i.e., histamine, acetylcholine and adrenergic receptors) or fast sodium channels, effected by TCAs. Thus SSRIs have a more favourable side-effect profile than TCAs. The surfeit of biological substrates, receptors and neuroanatomical pathways for 5-HT are candidates to mediate not only the therapeutic actions of SSRIs but also their side effects. Adverse effects as well as therapeutic effects of SSRIs appear to exhibit a topography with neuroanatomic site and pathway specific actions for each physiological function and involving different

receptor subtypes [170]. With the clarification of multiple discrete projection pathways for serotonin in the central nervous system, research is attempting to map a topography of serotonin physiology by elucidating which function(s) any given pathway controls [18, 33, 142]. Knowledge about serotonergic neurotransmission has been expanding rapidly. Recent research has delineated at least 14 molecularly different serotonin receptor subtypes and multiple, discrete neuronal and nonneuronal pathways and mechanisms that mediate the many functions of serotonin. It certainly appears plausible that serotonin's involvement in mediating different physiological effects likely involves different 5-HT receptor subtypes but one receptor subtype may be involved in several physiological actions [18, 142]. Few serotonin receptor-selective drugs are available for clinical use. SSRIs remain the agents with greatest therapeutic utility, although the mechanisms underlying their delayed efficacy, which clearly result from adaptive consequences following repeated administration rather than early uptake inhibition of serotonin by itself, are incompletely understood and appear to involve changes in signal transduction and gene expression in serotonergic and other neurotransmitter systems [142]. The immediate actions of SSRIs are mostly side effects such as nausea, anxiety, nervousness and headache [170, 185]. Therapeutic effects of SSRIs usually take weeks to become apparent. The explanation for curative effects characteristic of SSRIs is likely found in delayed neurochemical adaptations. A leading hypothesis explaining the late therapeutic effects as well as the development of tolerance to side effects involves the desensitization of pre- and postsynaptic serotonin receptors [170, 184, 185]. It has also been postulated that 5-HT is a modulator, which modulates a homeostasis between dopamine, noradrenaline and GABA, which are involved in thought process, anxiety and mood. This homeostasis is plausibly disturbed in certain psychiatric disorders (e.g. depression) and serotonergic drugs may reinstate the homeostasis [185].

## **2. Background**

### **2.1. Serotonin receptors involved in the mechanism of SSRIs in anxiety: assumptions based on animal models and human studies**

As described above, SSRIs have well documented efficacy in certain anxiety disorders (panic disorder, obsessive-compulsive disorder, social phobia and generalized

anxiety disorder) [170, 185]. Although inhibition of serotonin reuptake occurs immediately after administration of an SSRI, the clinical effect is characterised by delayed onset and it is generally only the side-effects of these agents which are manifest immediately [170, 185]. In some patients, SSRIs may precipitate or exacerbate anxiety, jitteriness and agitation and that may cause a decrease in treatment acceptance. Furthermore, some studies report increase in suicide attempts and suicidal ideation in patients in the early phase of SSRI treatment [54, 96, 162] although other investigations fail to confirm such findings [62, 94, 192]. The initial adverse effects often attenuate with time or dose reduction [170, 185]. Clinical studies report that these anxiogenic effects are mimicked by the serotonin<sub>2C/2B</sub> receptor (5-HT<sub>2C/2B</sub>) agonist *m*-chlorophenylpiperazine (*m*-CPP) [5, 97, 143]. Intravenously as well as orally administered single dose of *m*-CPP was found to induce anxiety in healthy volunteers and induce panic attacks in panic disorder patients [5, 107, 111].

In animal models of anxiety, SSRI antidepressants are, in general, characterized by an anxiogenic-like profile after acute administration [75]. Anxiogenic-like effect of fluoxetine and citalopram in the rat social interaction test and in the elevated plus maze test was described in earlier studies, although this response disappeared after chronic SSRI treatment [49, 76, 179]. Corresponding with clinical studies, anxiogenic effects of *m*-CPP similar to SSRI-induced responses were observed in animal experiments as well [104, 179]. Pharmacological characterization of the effects of *m*-CPP led to the hypothesis that activation of 5-HT<sub>2C</sub> receptors may mediate anxiety in humans and rodents although pharmacological tools used in these studies were not able to differentiate between 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors [9, 11, 13, 30, 104, 143]. Furthermore, the role of other serotonin receptors, namely 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, or other mechanisms, e.g., noradrenergic and neuropeptide systems have been suggested in acute and/or chronic effects of SSRI antidepressants [30, 49, 102, 112, 179, 180], but only one study used a 5-HT<sub>2C</sub> receptor subtype-selective antagonist [49].

The serotonin<sub>1A</sub> (5-HT<sub>1A</sub>) receptor is the most extensively studied of the serotonin receptors and belongs to the large family of seven transmembrane domain G-protein coupled receptors. It is clear that 5-HT<sub>1A</sub> receptors are located both postsynaptic to 5-HT neurones (in forebrain regions), and also as autoreceptors on the 5-HT neurones themselves at the level the soma and dendrites in the mesencephalic and medullary raphe nuclei [18]. 5-HT<sub>1A</sub> autoreceptors which are localized on serotonergic cells and

dendrites in the raphe nuclei of the brain stem are believed to regulate synaptic 5-HT release through an inhibitory influence on serotonergic impulse flow [166]. Clinical and animal studies have suggested the involvement of 5-HT<sub>1A</sub> receptors in anxiety, depressive disorders, thermoregulation, feeding and sexual behaviour [18, 46, 114, 149]. The 5-HT<sub>2C</sub> and the 5-HT<sub>2B</sub> receptors are members of the 5-HT<sub>2</sub> receptor family. It is generally accepted that 5-HT<sub>2</sub> receptors are coupled to G-proteins and activate phospholipase C and release calcium via phosphatidylinositol hydrolysis in the brain and other tissues [44, 90]. 5-HT<sub>2C</sub> receptors are most likely involved in several neurobehavioural processes including anxiety, stereotype movements, and penile erection [13, 97, 104, 190]. Activation of this receptor subtype efficiently alters the release of other neurotransmitters like noradrenaline, adrenaline, dopamine, glutamate or gamma-amino butyric acid (GABA) [50, 51, 133] and the release of neuropeptides like corticotropin releasing hormone (CRH), oxytocin and vasopressin [12, 13, 37].

## **2.2. Serotonin receptors involved in grooming behaviour: possible implications in obsessive-compulsive disorder (OCD)**

Acute SSRI treatment was reported to induce self-grooming, a stereotype behaviour in rats [179, 180] and correspondingly, the 5-HT<sub>2C/2B</sub> receptor agonist *m*-CPP is also known to produce dose-dependent self-grooming [13]. Excessive grooming in animals is regarded similar to the symptoms of obsessive-compulsive disorder (OCD) and other obsessive-compulsive (OC)-spectrum disorders in humans including trichotillomania [73, 74, 147, 160]. A growing body of evidence about OCD, including the clinical treatment, brain imaging, and neuropharmacologic studies, links this classic psychiatric syndrome to 5-HT<sub>2</sub> receptors. The behavioural and neuroendocrine effects of the 5-HT<sub>2B/2C</sub> agonist *m*-CPP were studied in patients with OCD and healthy controls. Twelve patients and 20 controls were given a single dose of 0.5 mg/kg of *m*-CPP, administered orally under double-blind, placebo-controlled, random-assignment conditions. Following *m*-CPP, but not following placebo, patients with OCD experienced a transient but marked exacerbation of obsessive-compulsive symptoms [198]. Some indication that this symptom exacerbation did not simply represent a nonspecific response to the anxiogenic effects of *m*-CPP comes from studies using anxiogenic agents in OCD patients. Unlike patients with panic disorder in whom panic

attacks occurred when exposed to yohimbine, lactate or carbon dioxide, patients with OCD did not manifest significantly greater anxiety or panic attacks with these other agents, nor did they manifest any exacerbation of OCD symptoms [143]. Pretreatment with orally administered metergoline, a non-selective 5-HT<sub>2C</sub> antagonist which by itself had no effects on OCD symptoms, obliterated the increases in *m*-CPP-induced exacerbation of OCD symptoms [153].

In rats, *m*-CPP-induced self-grooming can be attenuated by mianserin, LY-53857 and metergoline which are antagonists with high affinity for the 5-HT<sub>2C</sub> and the 5-HT<sub>2B</sub> receptor site [13]. These data suggest the involvement of 5-HT<sub>2C</sub> or 5-HT<sub>2B</sub> receptors in SSRI-induced self-grooming as well. As described above, 5-HT<sub>2C</sub> receptors are reportedly involved in several neurobehavioural processes including anxiety, stereotype movements, and penile erection [13, 97, 104, 190]. However, there are little available data on the functional effects of central 5-HT<sub>2B</sub> receptor activation. Studies on the actions of the 5-HT<sub>2B</sub> receptor agonist BW 723C86 indicate a role for the 5-HT<sub>2B</sub> receptor in anxiety. BW 723C86 has been reported to have an anxiolytic-like profile in the rat social interaction test after subcutaneous administration or after direct injection into the medial amygdala, which contains detectable amounts of 5-HT<sub>2B</sub> receptor-like immunoreactivity [52, 53, 101]. In a recent experiment BW 723C86 was reported to reduce the frequency of grooming bouts of rats in observation cages [100].

### **2.3. The effect of chronic SSRI treatment on 5-HT receptor sensitivity: presumptions regarding therapeutic mechanism of action and long-term side effects**

Since it generally takes time for the numerous therapeutic actions of SSRIs to develop or for the transient side effects of SSRIs to abate, this has led to a search for delayed neurobiological actions of SSRIs which might explain their late pharmacological actions. One such plausible delayed action of SSRIs is the desensitization of presynaptic 5-HT<sub>1A</sub> and 5-HT<sub>1D</sub> receptors [170, 185]. Presynaptic serotonin receptors are located on the cell body and neighbouring dendrites, as well as on the axon terminal. Such receptors are mostly autoreceptors, which exert potent negative feedback on 5-HT release. Normally, stimulation of 5HT<sub>1A</sub> somatodendritic autoreceptors located in the midbrain raphe decreases neuronal firing rates and thus

reduces 5-HT in projection sites [170, 185]. This is what happens both under normal physiological conditions, and by the acute initiating actions of an SSRI. The chronic increase in 5-HT transmission generated by SSRIs has been suspected to desensitize somatodendritic 5-HT<sub>1A</sub> and terminal 5-HT<sub>1D</sub> autoreceptors, thus allowing a greater release of 5-HT per action potential [24, 25]. Desensitization of postsynaptic 5-HT<sub>1A</sub> receptor function, which may be a direct consequence of the increased 5-HT release, has also been proposed as a basic mechanism of the antidepressant and anxiolytic action of SSRIs. Animal studies confirm that repeated injections of fluoxetine and paroxetine produce a gradual desensitization of hypothalamic 5-HT<sub>1A</sub> receptors, whereas the specific noradrenaline uptake inhibitor desipramine does not produce this effect [117, 118]. Decrease in hypothalamic 5-HT<sub>1A</sub> receptor responsivity was also observed in humans treated with fluoxetine [112, 113, 115].

As mentioned above, 5-HT<sub>2C</sub> receptor activation is suspected to be responsible for *m*-CPP-induced acute anxiety-type effects in the rat social interaction test, as well as *m*-CPP-induced penile erection, self-grooming, and hypolocomotion [49, 134-136, 194]. The consequences of long-term increase in CNS 5-HT concentration on 5-HT<sub>2C</sub> receptor function have been investigated earlier. Chronic treatment with the SSRI's paroxetine and fluoxetine significantly attenuated the effect of *m*-CPP on locomotion and rears in rats [102] and chronic fluvoxamine treatment produced matching results [194]. Furthermore, 28 days of fluoxetine treatment significantly reduced the anxiogenic effect of *m*-CPP in the social interaction test in the rat [30]. Incidentally, chronic fluoxetine treatment also attenuates the effects of other anxiogenic compounds (i.e. CRH and CCK) and increases social affiliation [179, 180]. Following chronic paroxetine treatment, both the prolactin and hyperthermic responses to *m*-CPP were significantly attenuated in humans [159]. These long-term effects of chronic SSRI treatment are suspected to be the consequence of 5-HT<sub>2C</sub> receptor desensitisation [30, 102, 159, 194].

The enduring side-effect, erectile dysfunction has been associated with SSRI pharmacotherapy [87, 170, 185]. Systemic delivery of *m*-CPP induces penile erections in rats [13, 14]. The ability of the antidepressant trazodone to induce penile erection in patients treated for depression has been reported repeatedly and sometimes explained by the existence of *m*-CPP as one of its metabolites [163]. The use of selective 5-HT<sub>2</sub> receptor agonists and antagonists demonstrated that the proerectile effect of *m*-CPP is due to the selective activation of 5-HT<sub>2C</sub> receptors [135]. Accordingly, double-labelling

studies showed that neurons in the sacral parasympathetic nucleus and the dorsal gray commissure of the L6-S1 spinal segments - retrogradely labelled from the corpus cavernosum with pseudoviruses - exhibited 5-HT<sub>2C</sub> receptor immunoreactivity [17]. Such localization is in favour of a physiologic role of the 5-HT<sub>2C</sub> receptor in the control of penile erection. Based on these studies, we may assume that 5-HT<sub>2C</sub> receptor desensitisation could be responsible for erectile dysfunction associated with SSRI treatment.

#### **2.4. The effect of serotonin depletion on 5-HT<sub>2</sub> receptor function**

The first major theory of depression, the monoamine hypothesis, proposed that low levels of one or more of the brain monoamine neurotransmitters - serotonin, noradrenaline and dopamine - could produce depression. A refinement of this hypothesis is that depressive illness may arise, specifically, from decreased brain 5-HT function [131]. Additional lines of evidence were subsequently generated that corroborated the involvement of 5-HT in depressive illness. Studies reported that levels of the 5-HT precursor tryptophan were decreased in depressed patients [3], mood lowering effects arose following administration of a tryptophan-free diet [196] and administration of tryptophan produced an antidepressant effect [167]. These are just a few of the studies that implicated impaired 5-HT neurotransmission in the pathogenesis of depression. One of the factors probably associated with low levels of 5-HT in depression is altered sensitivity of 5-HT receptors [132].

Studies of 5-HT<sub>2</sub> binding in blood platelets found increased 5-HT<sub>2</sub> binding of depressed patients compared to normal controls. Increased 5-HT<sub>2</sub> receptor functional response as measured by phosphoinositide turnover and 5-HT-induced platelet aggregation was also observed in depressed patients [132]. Mikuni et al. [132] reported that the effects of 5-HT to increase intracellular calcium in platelets was greater in depressed patients than in controls, which is consistent with the hypothesis of 5-HT<sub>2</sub> receptor sensitisation in depression.

Previous studies report, that *p*-chlorophenylalanine (*p*-CPA) irreversibly inhibits tryptophane hydroxylase, and it causes a massive, long-lasting, over 70 and 90% decrease in the brain 5-HT concentration after 2x50 and 2x350 mg/kg, respectively [40, 108, 144]. The sensitisation of 5-HT receptors after depletion of brain 5-HT by *p*-CPA

has been described earlier [36, 80]. *p*-CPA treatment resulted in a significant upregulation of 5-HT<sub>2C</sub> receptor mediated phosphoinositide hydrolysis and an increased sensitivity to *m*-CPP and LSD discriminative stimulus in rats [36, 58]. Furthermore, the increased expression of 5-HT<sub>2C</sub> mRNA isoforms encoding receptors with higher sensitivity to serotonin was observed in serotonin-depleted mice [80].

## **2.5. The effect of SSRI treatment on epilepsy: studies in the WAG/Rij rat**

Anticonvulsant effect of the SSRI fluoxetine and its most important metabolite norfluoxetine has been recently reported in the pentylenetetrazol-induced mouse epilepsy model [99]. Furthermore, pretreatment with fluoxetine or norfluoxetine significantly increased both the rate and duration of survival, demonstrating a significant protective effect against pentylenetetrazol-induced epilepsy.

Epilepsy comprises a group of disorders that are characterized by recurrent unprovoked seizures causing a transitory impairment of brain function due to a paroxysmal excessive cortical excitability and aberrant synchronization [146]. There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and is probably involved in the enhanced seizure susceptibility observed in some genetically epilepsy prone rodents [35, 47, 48, 55, 61]. Generally, agents that elevate extracellular serotonin (5-HT) levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both limbic and generalized seizures [123, 124, 157, 195]. Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsion [34, 55, 172, 173].

Despite the fact that a large number of 5-HT receptors with different anatomical localizations and functions have been identified within the last two decades [18, 90] there are only few studies investigating the role of 5-HT receptor subtypes in the modulation of seizure activity, and the results are sometimes controversial depending on the used experimental epilepsy model.

Rats of the WAG/Rij strain exhibit spontaneously occurring spike–wave discharges (SWDs), accompanied by behavioral phenomena and pharmacological reactivity very similar to human absence epilepsy. Therefore, WAG/Rij rats are among the most frequently investigated genetic models of absence epilepsy [42, 43, 183].

Administration of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT causes marked, dose-dependent increases in the number and mean cumulative duration of spike-wave discharges in WAG/Rij rats [55, 61]. However, the antagonism of 5-HT<sub>1A</sub> receptors may increase or augment seizure severity in rats with stage 1 kindled seizures [188], and activation of 5-HT<sub>1A</sub> receptors can retard the development of amygdaloid kindling [186].

Activation of 5-HT<sub>2</sub> receptors causes, in general opposite effects as 5-HT<sub>1A</sub> receptors. For example, 5-HT<sub>2</sub> receptors play a facilitatory role in the developmental seizure process in amygdaloid kindling [186]. Extracellular and intracellular recordings revealed that 5-HT-induced inhibition of burst firing in nucleus reticularis thalami (NRT) is mediated through 5-HT<sub>2</sub> receptors with possible involvement of 5-HT<sub>2C</sub> receptors [130]. Other experiments using pharmacological probes have also implicated 5-HT<sub>2</sub> receptors in the development of amygdala-kindled limbic seizures [186] and the expression of electrically induced generalized seizures [158] in rodents. The possible involvement of 5-HT<sub>2C</sub> receptors has been suggested by the finding that mutant mice lacking this receptor subtype are extremely susceptible to audiogenic seizures and are prone to spontaneous death from seizures, suggesting that serotonergic neurotransmission mediated by 5-HT<sub>2C</sub> receptors suppresses neuronal network hyperexcitability and seizure activity [29, 178]. In addition, studies investigating the role of 5-HT<sub>2C</sub> and 5-HT<sub>2B</sub> receptors in the generation of pentylenetetrazol and electroshock-evoked seizures in rodents revealed that the observed anticonvulsant effects of *m*-CPP are likely to be mediated by activation of 5-HT<sub>2C</sub> receptors in these models [182]. In contrast, the results of a recent study obtained in rats with stage 1 kindled hippocampal seizures suggest that the antagonism of 5-HT<sub>2B/C</sub> do not lower or raise seizure threshold [188].

### 3. **Objectives**

#### 3.1. **Objective One**

A limiting factor in ongoing studies of SSRI-induced anxiety has been the absence of subtype-selective compounds [60, 90, 105]. The purpose of **Study One** was to explore the role of distinct 5-HT receptor subtypes involved in anxiety through the rat social interaction test, using novel, subtype-selective antagonists. To inspect the possible role of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors in acute SSRI-induced anxiety, WAY-100635, a subtype-selective 5-HT<sub>1A</sub> receptor antagonist [59] and SB-242084, a subtype-selective 5-HT<sub>2C</sub> receptor antagonist [105] were used as a pretreatment before administration of the SSRI antidepressant fluoxetine. In addition to social interaction another measure of anxiety, a stereotype behaviour, self-grooming was also tested.

#### 3.2. **Objective Two**

As mentioned before, a restraining aspect of previous inspections on role of the various 5-HT receptors involved in grooming behaviour has been the lack of subtype-selective agents [60, 90, 105]. The purpose of **Study Two** was to explore the role of 5-HT<sub>2C</sub> and 5-HT<sub>2B</sub> receptors in self-grooming in rats observed in single cages, using the selective 5-HT<sub>2C</sub> antagonist SB-242084 [31, 32, 105] the selective 5-HT<sub>2B</sub> antagonist SB-215505 [103] and the 5-HT<sub>2C/2B</sub> agonist *m*-CPP [90, 143].

#### 3.3. **Objective Three**

Changes in several aspects of serotonergic functions have been documented in the Fawn-Hooded (FH) rat strain [8, 78, 181]. There is evidence for a decreased storage and transporter function of 5-HT in FH rats [181]. Platelet 5-HT content was found to be markedly lower in FH rats than in other rat strains [181]. Similarly, decreased concentrations of 5-HT and 5-HIAA in the brainstem of FH rats have been reported [8]. Based on these results, as well as behavioural studies, this rat strain has been proposed to be a useful genetic model for depression and anxiety disorders [2, 8, 98, 161].

The objective of **Study Three** was to compare the effects of 7 and 16 days treatment with the SSRI fluoxetine on responses to the selective 5-HT<sub>1A</sub> agonist 8-

hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in control Sprague-Dawley (SD) and FH rats. We measured lower lip retraction as a model of 5-HT<sub>1A</sub> receptor function that is mediated by receptors located in the median raphe, and decrease in body temperature, as a widely used model of 5-HT<sub>1A</sub> receptor function in the rat [16, 20, 22, 86].

### **3.4. Objective Four**

The aim of **Study Four** was to test the effects of chronic SSRI treatment on 5-HT<sub>2C</sub> receptor sensitivity in the rat CNS. We examined the alterations in 5-HT<sub>2C</sub> receptor function through *m*-CPP-induced self-grooming and penile erection after chronic fluoxetine treatment in Sprague-Dawley (SD) rats.

### **3.5. Objective Five**

In **Study Five** we aimed to inspect changes in 5-HT<sub>2C</sub> receptor sensitivity in Sprague-Dawley (SD) rats after *p*-CPA-induced serotonin depletion. We used *m*-CPP-induced self-grooming and penile erection as an indicator of 5-HT<sub>2C</sub> receptor function.

### **3.6. Objective Six**

In order to explore the effect of the SSRIs on seizure generation, in **Study Six** we have investigated the consequences of fluoxetine administration in the genetic absence epilepsy model WAG/Rij rats with the application of the 5-HT<sub>2C</sub> receptor antagonist SB-242084 and the selective 5-HT<sub>1A</sub>-receptor antagonist WAY 100635.

#### **4. Materials and Methods**

All procedures used were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The protocols were approved by the local Ethical Committee.

##### **4.1. Study One**

Male Sprague-Dawley rats (240-330 g, Crl:CD<sup>R</sup>BR, Charles River, Hungary), were used in the studies. The animals (4 per cage) were kept under standard condition, with standard food (Charles River, Hungary) and water freely available. The temperature was  $21 \pm 1$  °C, the 12 hour light-dark cycle started at 06.00 hour.

Rats were housed in a room adjacent to the testing room at least for two weeks before the tests. The animals were randomly assigned to treatments. In the low light (5 lux), familiar arena conditions animals were individually pre-exposed to the test box and the injections three times on days preceding the test. Rats were tested for social interaction with an unknown test partner that did not differ by more than 15 g in weight. At the end of each test the box was thoroughly wiped with detergent and dried to remove odours. The animals were tested in a random order between 9.00 and 13.00 h in an adjacent room for 7.5 min. The evenly illuminated box (60 x 60 x 40 cm), was marked to 10 cm compartments by lines on the floor.

The behaviour of animals was recorded on videotape. It was scored later by a person unaware of the drug treatment. The following behaviours were included in total social interaction: sniffing partner, anogenital sniffing, peaceful following, grooming partner, under crawling, over climbing, chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing and wrestling. Rearing, crossing of lines and self-grooming were also scored.

SB-242084 (6-chloro-5-methyl-1-[(2-[2-methylpyrid-3-yloxy]pyrid-5-yl(carbamoyl)indoline) dihydrochloride, Sigma-Adrich, Budapest, Hungary) and fluoxetine hydrochloride (kindly donated by EGIS Pharmaceuticals., Ltd., Budapest, Hungary) were dissolved in 10% solution of 2-hydroxypropyl- $\beta$ -cyclodextrin (Research Biochemicals International, Natick, MA, USA). WAY-100635 maleate (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl cyclohexanecarboxamide maleate,

Research Biochemicals International, Natick, MA, USA) was dissolved in physiological saline. WAY-100635 was administered s.c., all other drugs i.p., in a volume of 1 ml/kg. SB-242084 was administered 5 min, WAY-100635 20 min before fluoxetine, which was administered 20 min before the test.

The data were analyzed using one- or two-way analysis of variance followed by Tukey-Kramer test and Kruskal-Wallis test followed by Mann-Whitney rank sum test (Super ANOVA and StatViewSE+Graph, Abacus Concepts, Berkeley, CA, USA). Each treatment group consisted of 10-14 animals that is, 5-7 pairs of rats. Each rat was tested only one time. Data on the figures and in the text are expressed as mean $\pm$ SEM.

#### 4.2. Study Two

Male Sprague-Dawley rats (280-340 g, CrI:CD<sup>R</sup>BR, Charles River, Hungary), were used in the studies. The animals (4 per cage) were kept under standard condition, as described above.

In these experiments animals were placed to single observation cages immediately after *m*-CPP or vehicle injections. Display of grooming behaviour was scored every 15 s by a trained person, beginning with the injection of the compounds [13]. Vibration, face and head washing, body grooming, scratching, paw licking, head shaking and genital grooming were included as components of grooming behaviour. Animals were scored for 30 min, thus, the maximum of the available score during the observation period was 120.

1-[3-chlorophenyl]piperazine hydrochloride (*m*-CPP, Research Biochemicals International, Natick, MA, USA) was dissolved in physiological saline. SB-242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy) pyrid-5-yl carbamoyl] indoline; kindly provided by Dr. G. Kennett, Winnersh, Wokingham, UK) was dissolved in a solution of 25 mM citric acid and 10% hydroxypropyl- $\beta$ -cyclodextrin (Research Biochemicals International, Natick, MA, USA) and neutralised by diluted NaOH. SB-215505 (6-chloro-5-methyl-1-[5-quinolylcarbamyl]indoline, kindly provided by Dr. G. Kennett, Winnersh, Wokingham, UK) was dissolved in a solution of 25 mM citric acid and 40% hydroxypropyl- $\beta$ -cyclodextrin (Research Biochemicals International, Natick, MA, USA) and neutralised by diluted NaOH. All drugs were injected intraperitoneally in a

volume of 1 ml/kg. SB-242084 and SB-215505 were administered 20 min before the agonist as a pretreatment.

The data were analyzed using Kruskal-Wallis test followed by Mann-Whitney rank sum test (StatViewSE+Graph, Berkeley, CA, USA). Groups consisted of 6-8 animals. Each rat was tested only once. Data in the figures and in the text are expressed as mean±SEM.

### 4.3. Study Three

Male Sprague-Dawley (CrI:CD<sup>R</sup>BR, Charles River, Hungary) and Fawn-Hooded (IRL/N BR, Inbr. Gen. 1988 at Jean Dodds, F33, Charles River, Germany) rats (260-330 g) were used in the studies. The animals were kept under standard conditions, as described in Study One.

All studies were performed in rats placed into perspex cylinder restraints. They were previously exposed at least 4 times to the experimental conditions. By the 4th exposure rats were clearly adapted to the situation, thus no hypermotility, struggling or other sign of stress were observed. Treatment groups in the dose-response study consisted of 6-16 animals (n=7-8 at 0 and 15 mg/kg, n=15-16 at 30 and 60 mg/kg, n=6 at 120 mg/kg). The number of animals at 30 and 60 mg/kg were doubled because of the high scattering of the masseter-eyeball syndrome. Groups in the chronic fluoxetine study consisted of 11-11 fluoxetine treated rats and 9-9 controls treated with the vehicle of fluoxetine. Body temperature, lower lip retraction and masseter-eyeball syndrome were measured or scored parallel in the same experiments. The animals were placed in plexiglass restrainers which allow partial movement. For body temperature measurements, rectal probes (Yellow Springs Instruments, Ohio, USA) were inserted to a depth of 6 cm and remained there for at least 45 min. Injections of vehicle (saline), or 8-OH-DPAT were administered 15 min after the probes were inserted.

(+)-8-Hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemicals International, Natick, MA, USA) was dissolved in physiological saline. It was administered into a tail vein (1ml/kg).

In the chronic fluoxetine study, animals were treated i.p. once a day with fluoxetine hydrochloride (10 mg/kg in a volume of 1 ml/kg, kindly donated by EGIS Pharmaceuticals, Budapest, Hungary) or vehicle. Fluoxetine was dissolved in 20%

solution of 2-hydroxypropyl- $\beta$ -cyclodextrin (Research Biochemicals International, Natick, MA, USA). The same animals were tested with 8-OH-DPAT (60 mg/kg, i.v.) repeatedly 2-3 days before and 7 and 16 days after the beginning of chronic fluoxetine or vehicle treatment. The 8-OH-DPAT injection was administered 24 hours after the last fluoxetine treatment.

For the pharmacological characterization of the masseter-eyeball syndrome WAY-100635 maleate (Research Biochemicals International, Natick, MA, USA) was used as a pretreatment in Fawn-Hooded rats ( $n=12-12$ ). It was dissolved in physiological saline, and injected s.c. in a dose of 0.2 mg/kg, 20 min before 8-OH-DPAT (30 mg/kg, i.v.).

Body temperature was recorded continuously for at least 60 min. A fall in temperature was observed within 5 min after injections of 8-OH-DPAT which persisted for at least 30 min. Changes in body temperature 30 min after 8-OH-DPAT compared to baseline were calculated and used for statistical evaluation at all doses.

For a more complete observation of lower lip retraction a mirror was placed below each rat. The animals were scored at 5, 10, 15, 20, 25, 30, 35 and 40 min after injection as follows: 0=lower incisors are not visible (not different from non-treated rats), 0.5=lower part of incisors partly visible, 1.0=major part of incisors clearly visible, 1.5=lower incisors are completely visible. Thus, a total score of 12 per rat was the maximum score achievable.

The masseter-eyeball syndrome (a more detailed description is given in the Results section) was observed and videotaped in preliminary experiments. In the present studies the animals were scored at 15 second intervals for 40 min as follows: 0=syndrome is not present, 1.0=syndrome is present. Thus, a total score of 160 per rat was the maximum score achievable. The animals were scored by two observers, both of whom were blind to the treatment.

Changes in body temperature compared to baseline (immediately before drug administration), total scores and time for behavioural data are given as means+ SEM for the groups. Statistical analysis of the data was performed with Super Anova (Brain Powers Inc., California) and StatViewSE+Graph (Abacus Concepts, Inc., Berkeley, CA) softwares. Treatment and strain effects were analysed by one- or two-way analysis of variance, analysis of variance for repeated measures and Kruskal-Wallis test. For post hoc comparisons of temperature data, social interaction and rearing time Tukey-Kramer test, for comparison of lower lip retraction, masseter-eyeball syndrome and number of

crossings Mann-Whitney rank sum test were run after the analysis of variance or the Kruskal-Wallis test indicated significant main differences.

#### 4.4. Study Four

Male Sprague-Dawley rats (280-340 g, CrI:CD<sup>R</sup>BR, Charles River, Hungary), were used. The animals were kept under standard conditions, as described previously. Animals were placed to single observation cages immediately after *m*-CPP injections. Display of grooming behaviour was scored as described in Study Two.

Penile erections were observed and quantified for 30 mins as described previously [13]. The characteristic behaviour consisted of pelvic thrusts immediately followed by an upright position presenting an emerging, engorged penis which the rat proceeds to lick, eating the ejaculate.

1-[3-chlorophenyl]piperazine hydrochloride (*m*-CPP, Research Biochemicals International, Natick, MA, USA) was dissolved in physiological saline and it was administered in a dose of 0.6 mg/kg. In the chronic fluoxetine study, animals were treated once a day with fluoxetine HCl (10 mg/kg, kindly donated by EGIS Pharmaceuticals, Budapest, Hungary) or vehicle. Fluoxetine was dissolved in 20% solution of 2-hydroxypropyl- $\beta$ -cyclodextrin (Research Biochemicals). All drugs were injected intraperitoneally in a volume of 1 ml/kg.

The data were analyzed using one - way analysis of variance (ANOVA). Newman-Keuls test was used for post-hoc comparisons (StatSoft Inc., Tulsa, OK, USA). Groups consisted of 7-12 animals, each rat was tested only once. Data in the figures and in the text are expressed as mean $\pm$ SEM.

#### 4.5. Study Five

Male Sprague-Dawley rats (280-340 g, CrI:CD<sup>R</sup>BR, Charles River, Hungary), were used in the study. The animals were kept under standard conditions, as described above. Animals were placed to single observation cages immediately after *m*-CPP injections. Grooming behaviour and penile erections were observed and scored as described above.

1-[3-chlorophenyl]piperazine hydrochloride (*m*-CPP, Research Biochemicals International, Natick, MA, USA) was dissolved in physiological saline and it was

administered in a dose of 0.3, 0.6 or 2 mg/kg . DL *p*-chlorophenylalanine methylester hydrochloride (*p*-CPA, SIGMA, Budapest, Hungary) was dissolved in physiological saline, and it was administered in a dose of 50, 100 or 350 mg/kg, two times each, 72 and 48 h before the experiment. All drugs were injected intraperitoneally in a volume of 1 ml/kg.

The data were analyzed using one - way analysis of variance (ANOVA). Newman-Keuls test was used for post-hoc comparisons (StatSoft Inc., Tulsa, OK, USA). Groups consisted of 7-12 animals, each rat was tested only once. Data in the figures and in the text are expressed as mean±SEM.

#### **4.6. Study Six**

12-15 month old, adult male Wistar Albino Glaxo rats from Rijswijk, Netherlands (WAG/Rij rats), body weights 370-420 g were kept in a 12:12 h light-dark cycle under standard conditions. Ancestors were purchased from the REPGO-institute of TNO at Rijswijk, the Netherlands. Animals were chronically equipped with EEG and EMG electrodes. Surgery was performed under anesthesia with 2% halothane in oxygen (Fluotec 3) using a Kopf stereotaxic instrument. Stainless steel screw electrodes were implanted on the dura mater over the cortex: two in the frontal region (co-ordinates with skull surface flat and bregma zero-zero: A 2.0, L 3.0) and two in the parieto-occipital region (A -6.0, L 3.0) (115). The ground electrode was placed over the cerebellum. In addition stainless steel coil spring electrodes encased in silicone rubber tubing similar to pacemaker leads (Subcutaneous Electrode Wire, Plastics One Inc., Roanoke U.S.A.) were sewn into the neck muscles for EMG recording. The leads of the electrodes were soldered to a miniature connector, which was head mounted with cranioplastic cement (Plastics One Inc., Roanoke U.S.A.) and mounting screws. An electromagnetic transducer activated by cable movements was used to record motor activity. Recovery after surgery was at least 10 days.

In order to habituate the animals to the recording conditions, the rats were connected to the recording cables, and received intraperitoneal (i.p.) injections of physiological saline for at least 3 days before the experiments. The animals were attached to a multichannel amplifier by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement for the animals. EEG, EMG

and motor activity were recorded for 24-hour periods, starting at light onset. EEG, EMG and motor activity signals were amplified (amplification factor approx. 5000 for EEG and motor activity, 20000 for EMG respectively), conditioned by analog filters (filtering: below 0.53 Hz and above 30 Hz at 6 dB/octave) and subjected to an analogue-to digital conversion with a sampling rate of 64 Hz. The digitized signals were displayed on a PC monitor and stored on computer for further analysis.

Fluoxetine (5.0 mg/kg, i.p., fluoxetine hydrochloride kindly donated by EGIS pharmaceuticals Ltd., Budapest, Hungary) or vehicle (saline or 10% solution of 2-hydroxypropyl- $\beta$ -cyclodextrin, Research Biochemicals International) was administered after pretreatment with SB-242084 (0.2 mg/kg, i.p.), WAY-100635 (0.2 mg/kg, i.p., N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-N-2-pyridinyl cyclohexanecarboxamide maleate, Research Biochemicals International) or vehicle. Combined pretreatment with SB-242084 (0.2 mg/kg, i.p.), and WAY-100635 (0.2 mg/kg, i.p.) compared to vehicle/vehicle was also applied to further characterize the effect of fluoxetine. Pretreatments preceded treatments by 5 minutes. All i.p. treatments were injected in a volume of 1 ml/kg.

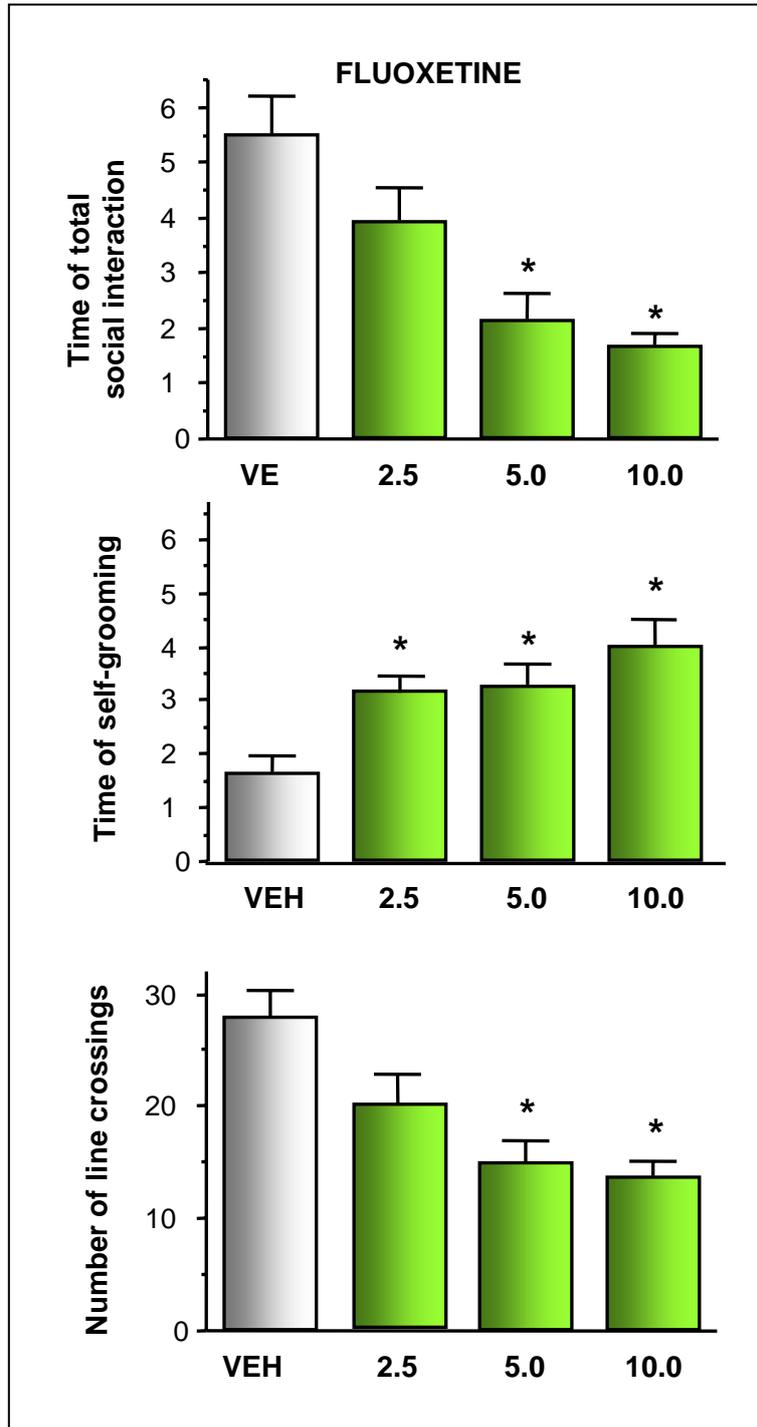
All EEG and EMG recordings were made at least 10 days after surgery. EEG signals filtered below 0.53 Hz and above 30 Hz were subjected to an analogue to digital conversion by a polygraph, designed for experimental sleep studies, with a sampling rate of 64 Hz and were stored on a PC. The animals were kept in plastic cages, connected to the polygraph by a flexible recording cable and rotating connector hanging in a moveable arm above the chamber, permitting free movement. Time of spike-wave discharges (SWDs) and number of paroxysms were summarized for each rat in 30 min intervals for 2 h after drug treatments, scored by visual inspection. Statistical analysis was performed by analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. Mean $\pm$ S.E.M. values of 6-7 animals are shown on the Figures.

## 5. Results

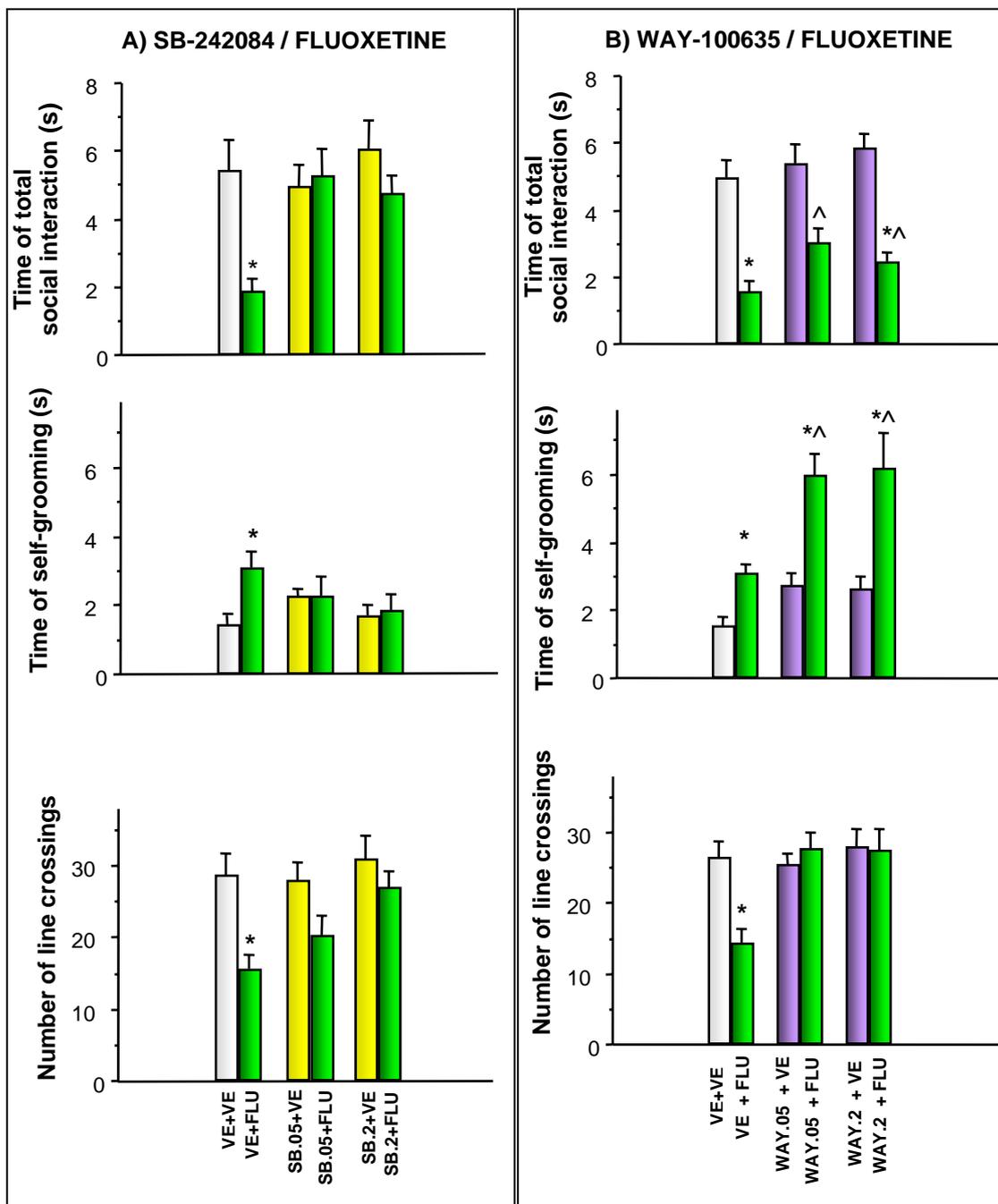
### 5.1. Study One

The selective serotonin reuptake inhibitor fluoxetine (2.5-10 mg/kg, i.p.) caused dose-dependent decrease in time of total social interaction ( $F(3,42)=7.68$ ,  $P<0.001$ ), increase in self-grooming and decrease in line crossings (**Figure 1**). Minimal effective dose for time of total social interaction and number of line crossings was 5.0 mg/kg. Pretreatment with the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 (0.05 and 0.2 mg/kg, i.p.) significantly reversed effects of fluoxetine (**Figure 2A**) on time of total social interaction ( $F(2,68)=6.14$ ,  $P<0.01$  for pretreatment x treatment interaction). SB-242084 reversed also other effects, thus, self-grooming and decrease in line crossings induced by fluoxetine but the dose response effects of SB-242084 on the various parameters were different. Anxiety-related effects, like social interaction and self-grooming responses, were completely reversed by 0.05 mg/kg of SB-242084, but a higher dose was needed for the distinct reversal of hypolocomotion.

Pretreatment with the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (**Figure 2B**; 0.05 and 0.2 mg/kg, s.c.) failed to reverse the effects of fluoxetine on time of total social interaction or self-grooming (pretreatment x treatment interactions  $F(2,68)=0.71$ ,  $P=0.49$  and  $F(2,68)=0.37$ ,  $P=0.69$  for the two parameters, respectively). Even more, WAY-100635 alone slightly increased self-grooming, and, as a pretreatment, it augmented self-grooming response caused by fluoxetine (pretreatment effect,  $F(2,68)=3.30$ ,  $P<0.05$ ). In contrast, it reversed the effect of fluoxetine on line crossings very efficiently (pretreatment x treatment interaction  $F(2,68)=5.81$ ,  $P<0.005$ ). WAY-100635 alone did not have any effect on time of total social interaction or line crossings.



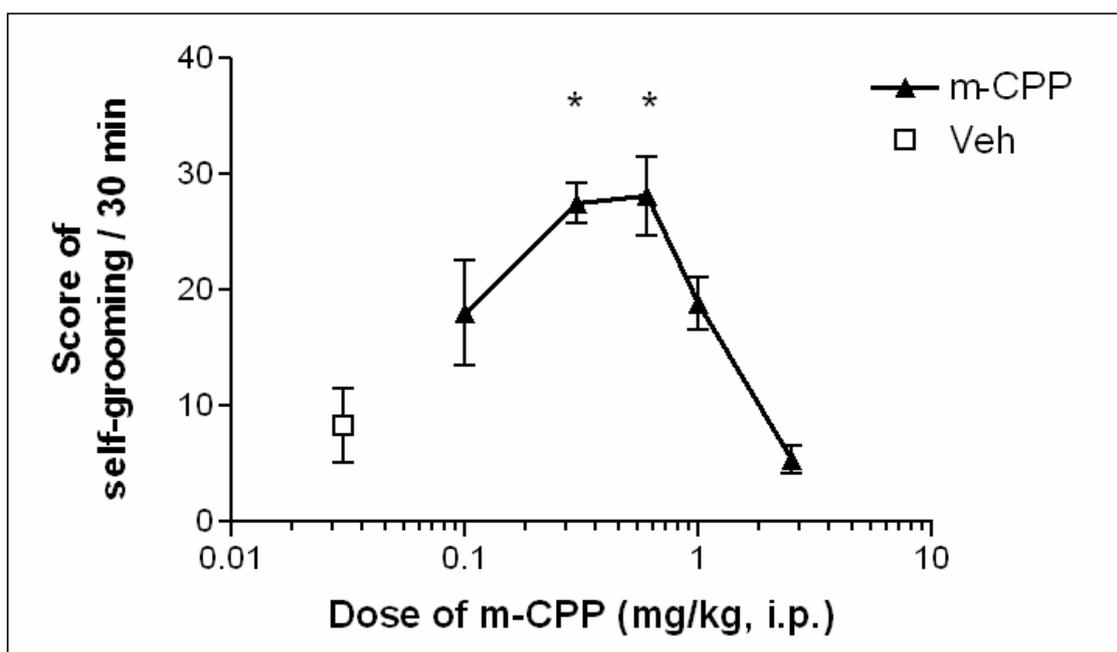
**Figure 1.** Effects of different doses of the SSRI fluoxetine (2.5-10.0 mg/kg, i.p.) in the social interaction anxiety test under low light, familiar arena conditions. Significant effects ( $P < 0.05$ ) compared to vehicle are denoted by \*.



**Figure 2.** Effects of fluoxetine (5.0 mg/kg, i.p.) after pretreatment with (A) the 5-HT<sub>2C</sub> receptor antagonist SB-242084 (0.05 and 0.2 mg/kg, i.p.), and (B) the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (0.05 and 0.2 mg/kg s.c.) or vehicle in the social interaction anxiety test under low light, familiar arena conditions. Significant effects ( $P < 0.05$ ) compared to vehicle/vehicle are denoted by \*; significant effects ( $P < 0.05$ ) of fluoxetine compared to the antagonist+vehicle are denoted by ^.

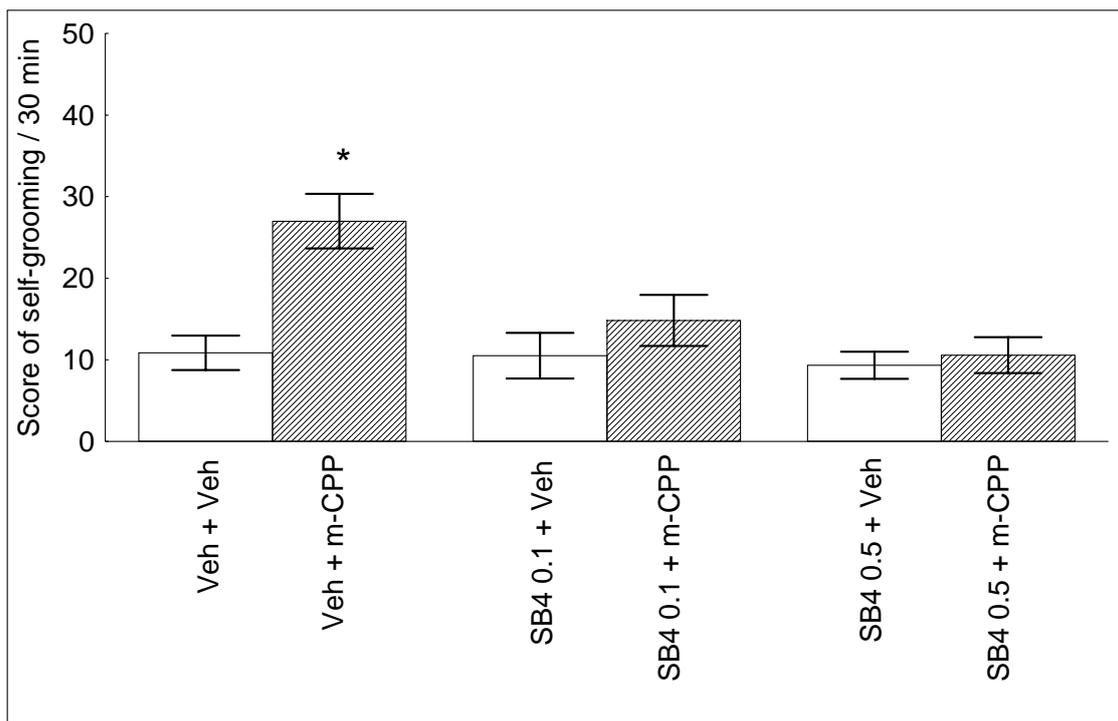
## 5.2. Study Two

Effect of different doses of the 5-HT<sub>2C/2B</sub> receptor agonist *m*-CPP (0.1-2.5 mg/kg, i.p.) on self-grooming is shown in **Figure 3**. *m*-CPP caused dose-dependent increase of self-grooming ( $H(5,41)=14.81$ ,  $P<0.02$ ). The effect showed a bell-shaped, bimodal dose-response curve. Maximal effect was observed at the dose of 0.6 mg/kg of *m*-CPP.



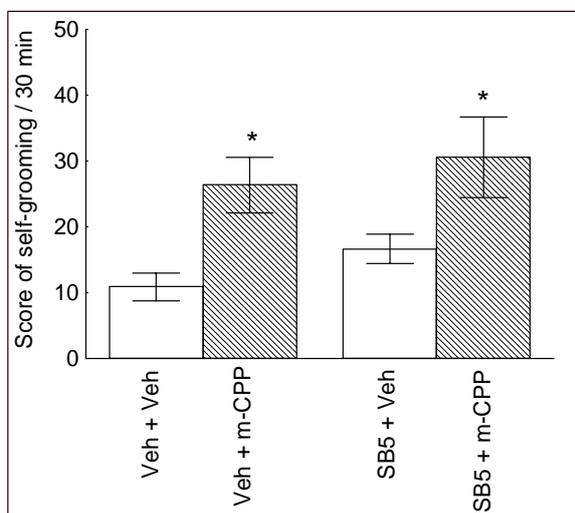
**Figure 3.** Effect of different doses of *m*-CPP compared to vehicle control (saline) on self-grooming. Each point represents the mean $\pm$ SEM of 6-8 animals. Veh= vehicle control, \*= Significant effect of *m*-CPP compared to vehicle, Mann-Whitney rank sum test,  $P<0.05$ . Results of the Kruskal-Wallis tests are given in the text.

Effect of the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 (20 min pretreatment) on *m*-CPP-induced self-grooming is shown in **Figure 4**. SB-242084 (0.1 and 0.5 mg/kg, i.p.) dose-dependently inhibited *m*-CPP-induced (0.6 mg/kg) self-grooming ( $H(5,39)=13.98$ ,  $P<0.02$ ). SB-242084 alone did not induce self-grooming.



**Figure 4.** Effect of the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 (SB4) pretreatment (0.1 and 0.5 mg/kg, i.p.) compared to vehicle (10% hydroxypropyl- $\beta$ -cyclodextrin) on *m*-CPP- (0.6 mg/kg, i.p., vehicle: saline) induced self-grooming. Each column represents the mean $\pm$ SEM of 6-8 animals. Veh= appropriate vehicle control, SB4 0.1= SB-242084 0.1 mg/kg, SB4 0.5= SB-242084 0.5 mg/kg. \*= Significant effect compared to vehicle/vehicle, Mann-Whitney rank sum test,  $P < 0.05$ . Results of the Kruskal-Wallis tests are given in the text.

Effect of the selective 5-HT<sub>2B</sub> antagonist SB-215505 (20 min pretreatment) on *m*-CPP-induced self-grooming is shown in **Figure 5**. Pretreatment with SB-215505 (1 mg/kg, i.p.) did not alter the effect of *m*-CPP (0.6 mg/kg) on self-grooming ( $H(3,27)=11.47$ ,  $P < 0.01$ ). SB-215505 alone did not induce self-grooming.



**Figure 5.** Effect of the selective 5-HT<sub>2B</sub> receptor antagonist SB-215505 (SB5) pretreatment (1 mg/kg, i.p.) compared to vehicle on *m*-CPP- (0.6 mg/kg, i.p.) induced self-grooming. Each column represents the mean $\pm$ SEM of 6-8 animals. Veh= appropriate vehicle control, SB5= SB-215505 1 mg/kg, i.p., \*= Significant effect compared to the appropriate control, Mann-Whitney rank sum test,  $P < 0.05$ . Results of the Kruskal-Wallis tests are given in the text.

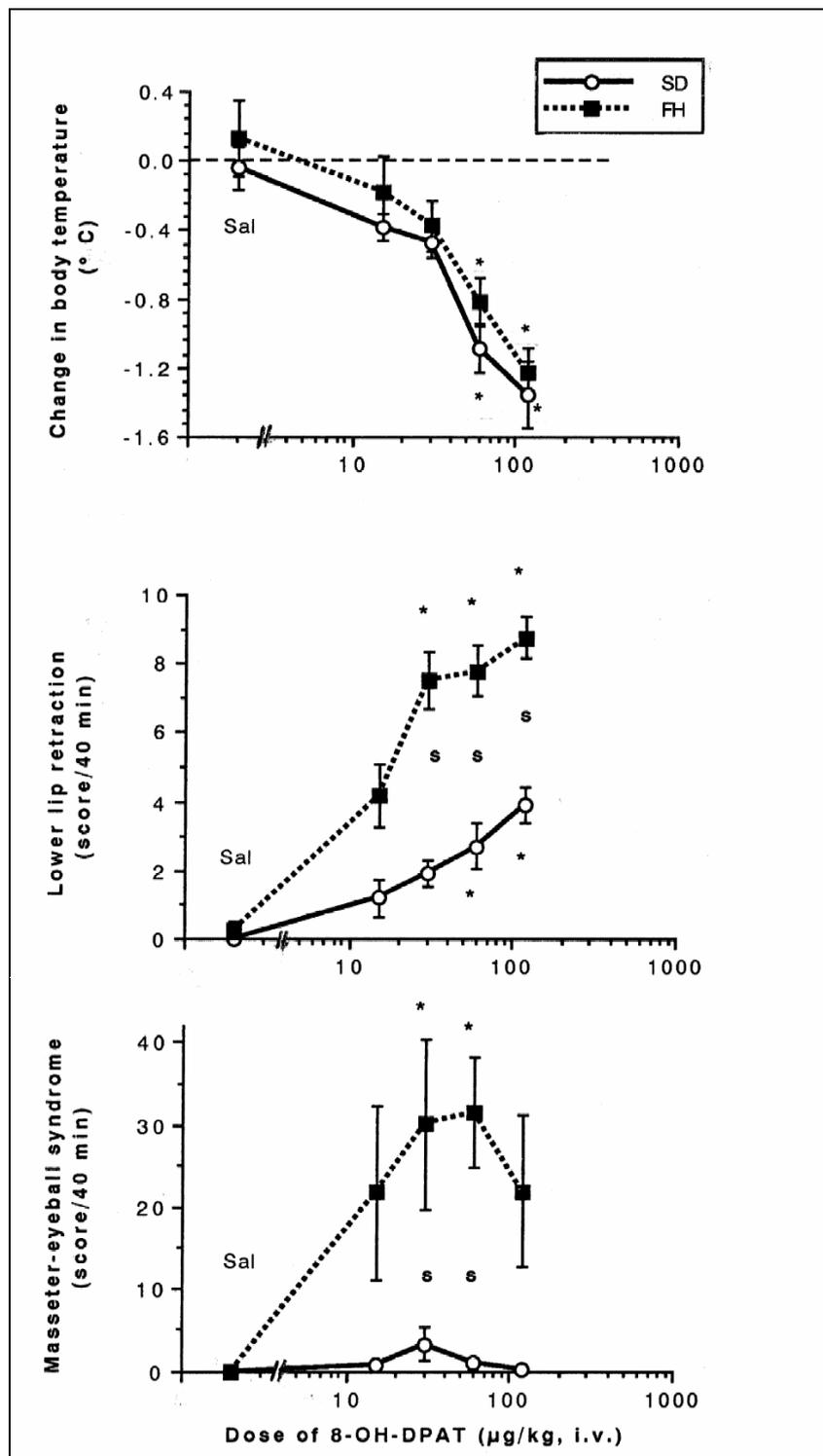
### 5.3. Study Three

Effects of different doses (15-120 mg/kg, i.v.) of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT in SD and FH rats are shown in **Figure 6**. In SD rats, 8-OH-DPAT caused a dose-dependent decrease in body temperature ( $F(4,46)=6.692$ ,  $P<0.001$ ) and an increase in lower lip retraction ( $H=17.447$ ,  $df=4$ ,  $P<0.01$ ).

In FH rats, 8-OH-DPAT dose-dependently decreased body temperature ( $F(4,41)=7.642$ ,  $P<0.001$ ). The effect on temperature reduction was slightly lower in FH compared to SD rats, but there was no significant strain effect or strain-dose interaction. When data of all animals challenged with 60 mg/kg 8-OH-DPAT were combined, including the dose response study and the chronic fluoxetine study preceding fluoxetine or chronic vehicle, the decrease in body temperature in FH rats was significantly lower ( $-1.165\pm 0.073$ ,  $n=36$ , and  $-0.894\pm 0.052$ ,  $n=35$ , in SD and FH animals, respectively,  $P<0.05$ ).

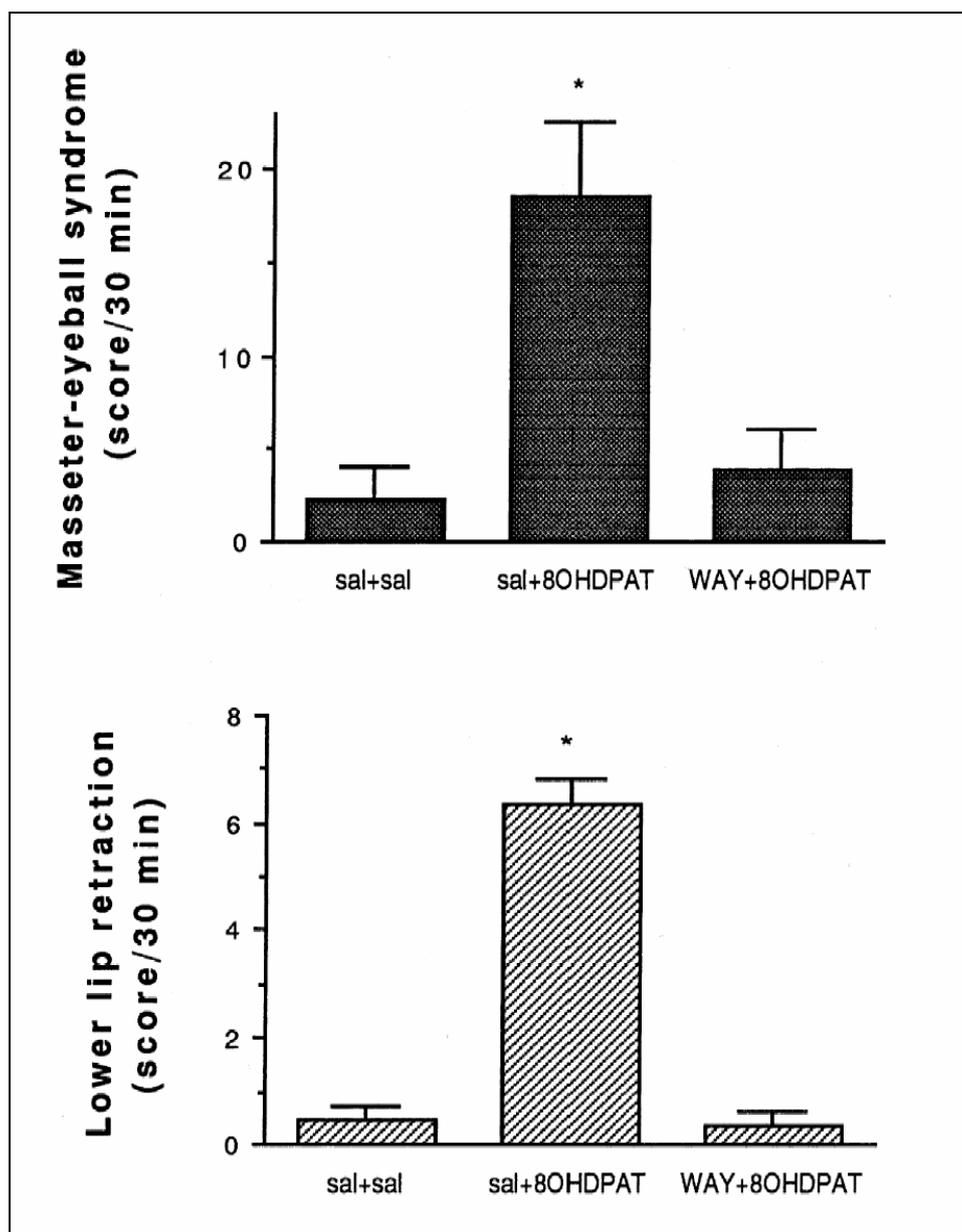
In contrast, 8-OH-DPAT caused lower lip retraction with much higher efficacy in FH rats ( $H=25.37$ ,  $df=4$ ,  $P<0.001$ ). The strain-dose interaction was also significant ( $F(4,86)=6.679$ ,  $P<0.001$ ).

We observed in most FH but only in a few SD rats, a special neurological syndrome induced by 8-OH-DPAT, a clonic movement of the masseters that extended to the ocular muscles resulting in an in-and-out movement of the eyeballs. The syndrome resembled a nystagmus-type focal symptom with high frequency, although the orientation of the eye movement was clearly in-and out, like a bobbing of the eyeballs. This syndrome was observed and videotaped in preliminary studies and scored also in these experiments. It was evident in 81% of the FH rats challenged with different doses of 8-OH-DPAT. There was a significant general drug effect ( $H=17.906$ ,  $df=4$ ,  $P<0.01$ ), although high interindividual differences were observed. The maximal effect was achieved at 30-60 mg/kg, i.v. At this dose the syndrome was present in an average 17% of the 40 min observation period in FH, and less than 4% in SD animals. Thus, there was also a highly significant strain difference ( $Z=-5.667$ ,  $P<0.001$ ). Masseter-eyeball syndrome was clearly dose-dependent up to 30 mg/kg, i.v., when previously selected SD or FH rats were used, based on a positive response to 8-OH-DPAT.



**Figure 6.** Effects of different doses of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT or saline on body temperature, lower lip retraction and masseter-eyeball syndrome in Sprague-Dawley (SD) and Fawn-Hooded (FH) rats. Group differences marked on the figure were calculated by analysis of variance followed by Tukey-Kramer test (body temperature), or Kruskal-Wallis test followed by Mann-Whitney rank sum test (lower lip retraction and masseter-eyeball syndrome).\*: Significant effects of 8-OH-DPAT compared to saline,  $P < 0.05$ . s: Significantly different effects in the FH compared to the SD strain,  $P < 0.05$ .

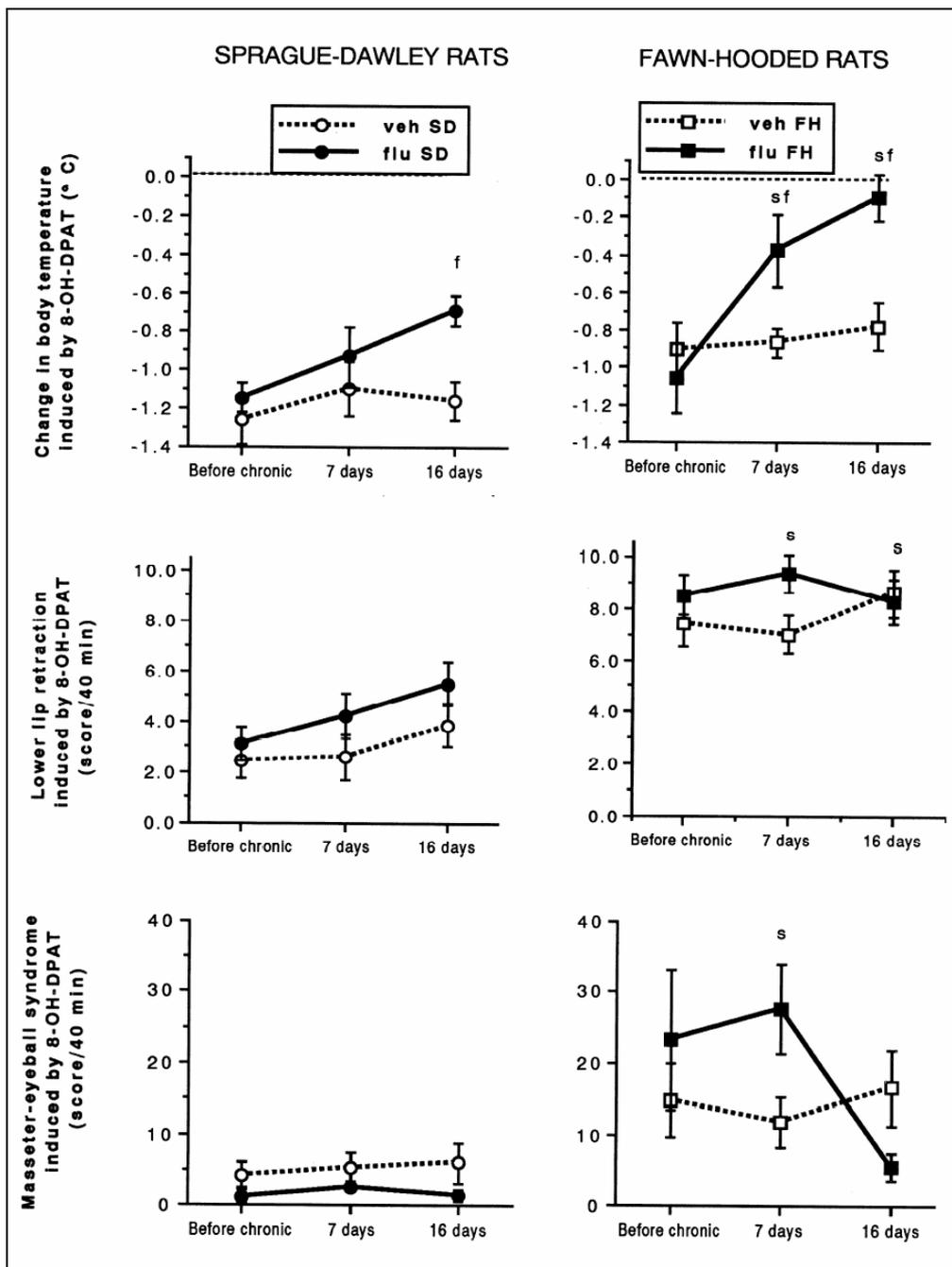
Effects of the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 pretreatment on 8-OH-DPAT-induced masseter-eyeball syndrome are shown in **Figure 7**. WAY-100635 in a dose of 0.2 mg/kg s.c., completely blocked the effect of 8-OH-DPAT (30 mg/kg, i.v.) on the masseter-eyeball syndrome and also on lower lip retraction.



**Figure 7.** Effects of the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (0.2 mg/kg, s.c.) on 8-OH-DPAT (30 µg/kg, i.v.)-induced masseter-eyeball syndrome and lower lip retraction in Fawn-Hooded rats (n=12 in all groups). \*: Significant response compared to saline/saline treated control group, P<0.05.

Effect of chronic fluoxetine treatment on the effects of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT in SD and FH rats are presented in **Figure 8**. 8-OH-DPAT(60 mg/kg, i.v.)-induced effects were studied in rats challenged 2-3 days before and 7 and 16 days after the beginning of fluoxetine (10 mg/kg/day, i.p.) or vehicle treatment. 8-OH-DPAT was administered 24 hours after the last fluoxetine or vehicle treatment. In SD rats the effect of 8-OH-DPAT on body temperature was decreased after fluoxetine ( $F(2,30)=4.362$ ,  $P<0.05$ ). This decrease became significant only 16 days after the beginning of fluoxetine treatment, and even at that time it was relatively small (-40%). In contrast, fluoxetine caused no decrease in 8-OH-DPAT-induced lower lip retraction. Chronic treatment with vehicle failed to cause any significant effect.

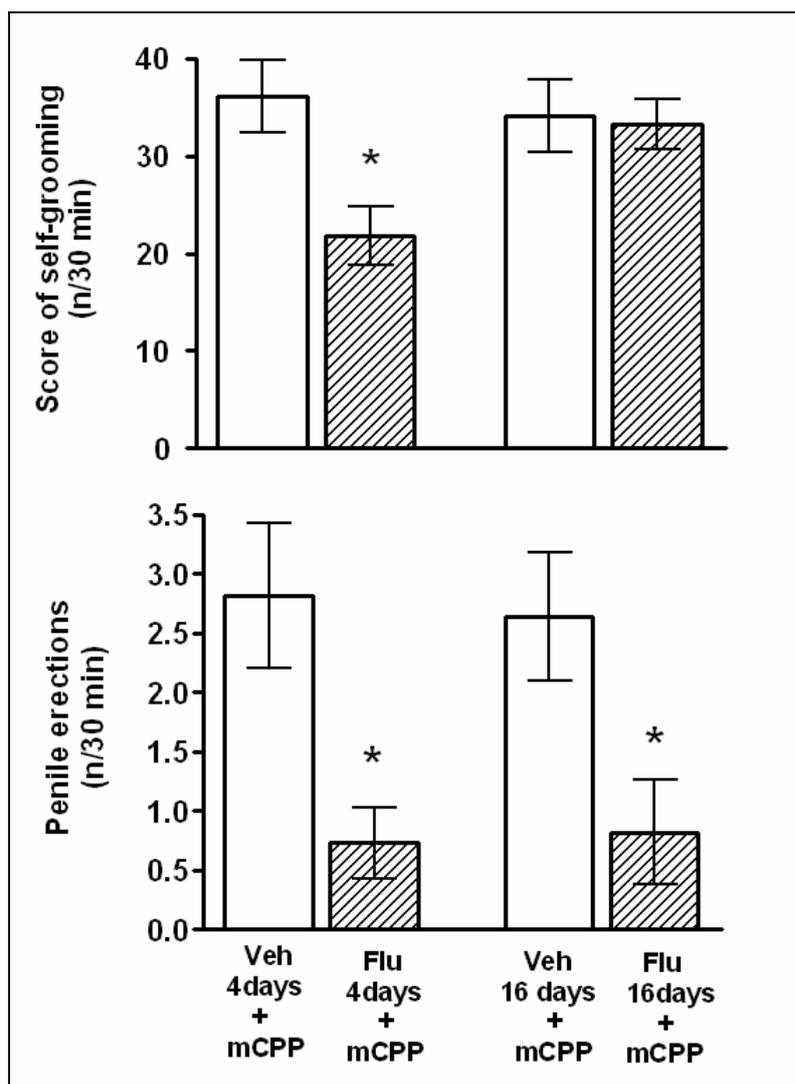
In FH rats the SSRI fluoxetine caused a much faster and more complete reduction in the 8-OH-DPAT-induced temperature effect (-65 % and -91 % after 7 and 16 days of fluoxetine, respectively). Thus, in FH rats, treatment with fluoxetine for 16 days abolished the hypothermia induced by 8-OH-DPAT. Strain ( $F(2,55)=12.778$ ,  $P<0.001$ ) and general fluoxetine effects were also significant ( $F(2,55)=13.100$ ,  $P<0.001$ ). Fluoxetine treatment failed to change lower lip retraction response in FH rats. A marked reduction was observed also in the masseter-eyeball syndrome in FH rats 16 days after fluoxetine treatment ( $H=12.089$ ,  $df=2$ ,  $P<0.01$ ). Chronic vehicle treatment failed to cause any significant effect in FH rats.



**Figure 8.** Effects of chronic fluoxetine treatment (10 mg/kg/day, i.p.) or vehicle on the effects of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (60 µg/kg, i.v.) on body temperature, lower lip retraction and masseter-eyeball syndrome in Sprague-Dawley (SD) and Fawn-Hooded (FH) rats. Group differences marked on the figure were calculated by analysis of variance followed by Tukey-Kramer test (body temperature), or Kruskal-Wallis test followed by Mann-Whitney rank sum test (lower lip retraction and masseter-eyeball syndrome). s: Significant difference between the FH and SD rats compared to the same treatment group of the other strain,  $P < 0.05$ . f: Significant fluoxetine effect compared to the vehicle control of the same strain,  $P < 0.05$ .

#### 5.4. Study Four

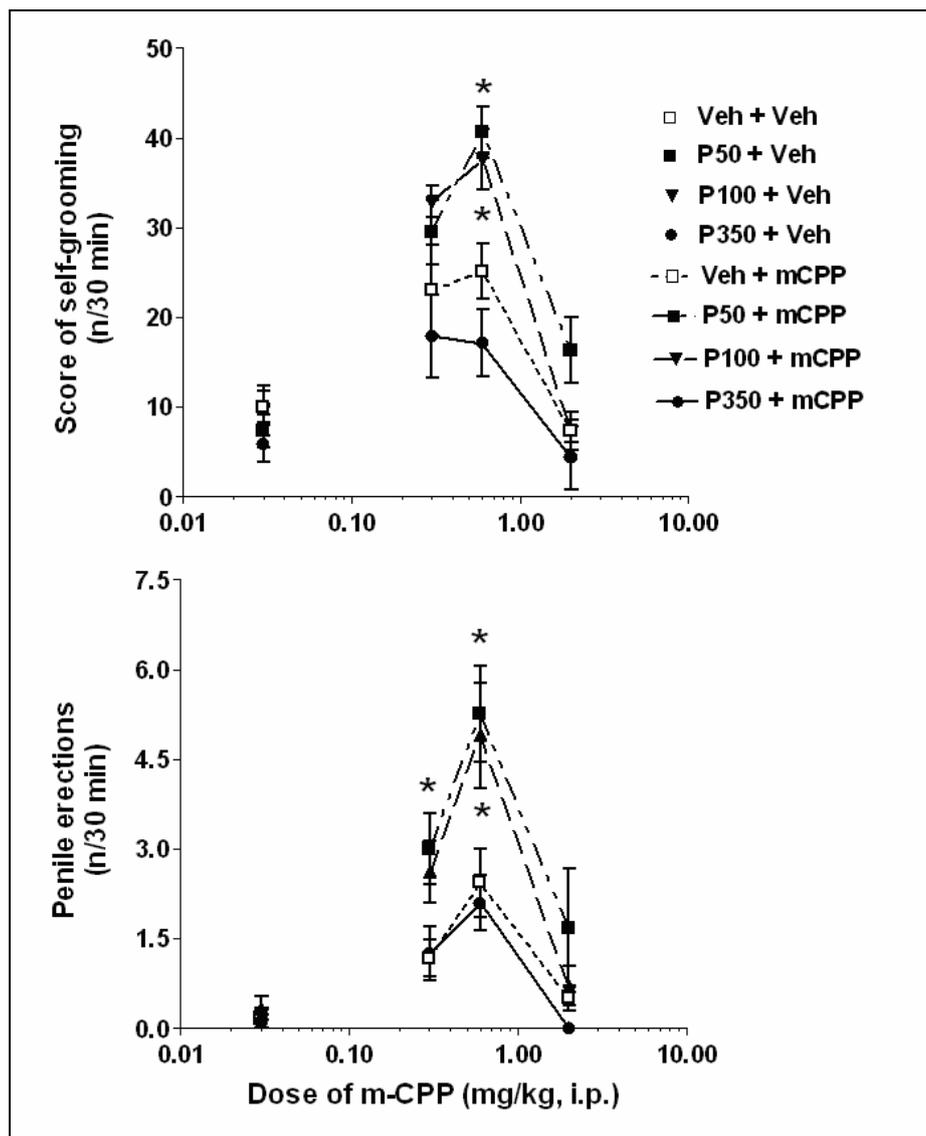
Penile erection and self-grooming induced by the 5-HT<sub>2C</sub> receptor agonist *m*-CPP were significantly reduced after 4 days of chronic fluoxetine treatment. This reduction was persistent after 16 days of fluoxetine treatment in the case of *m*-CPP-induced penile erection, but disappeared in the case of self-grooming (Figure 9).



**Figure 9.** Effect of chronic fluoxetine treatment (10 mg/kg/day, i.p., 4 and 16 days) on self-grooming and penile erections induced by *m*-CPP (0.6 mg/kg, i.p.). Each data point represents the mean  $\pm$  SEM values for 7-12 animals. \* denotes significant effect compared to vehicle/*m*-CPP,  $P < 0.05$  (Newman-Keuls post-hoc test). Flu denotes fluoxetine, Veh denotes appropriate vehicle control.

### 5.5. Study Five

2x50 and 2x100 mg/kg *p*-CPA pre-treatment significantly enhanced both *m*-CPP-induced self-grooming and penile erection. 2x350 mg/kg *p*-CPA failed to significantly alter *m*-CPP-induced responses (**Figure 10**).



**Figure 10.** Effect of *p*-CPA pre-treatment (2x50, 2x100 and 2x350 mg/kg, i.p., 72 and 48 h before the experiment) on self-grooming and penile erections induced by *m*-CPP (0.3, 0.6 and 2 mg/kg, i.p.). Each data point represents the mean  $\pm$  SEM values for 7-9 animals. \* denotes significant effect compared to vehicle/*m*-CPP,  $P < 0.05$  (Newman-Keuls post-hoc test). P50, P100 and P350 denote respective doses of *p*-CPA pre-treatment. Veh denotes appropriate vehicle control.

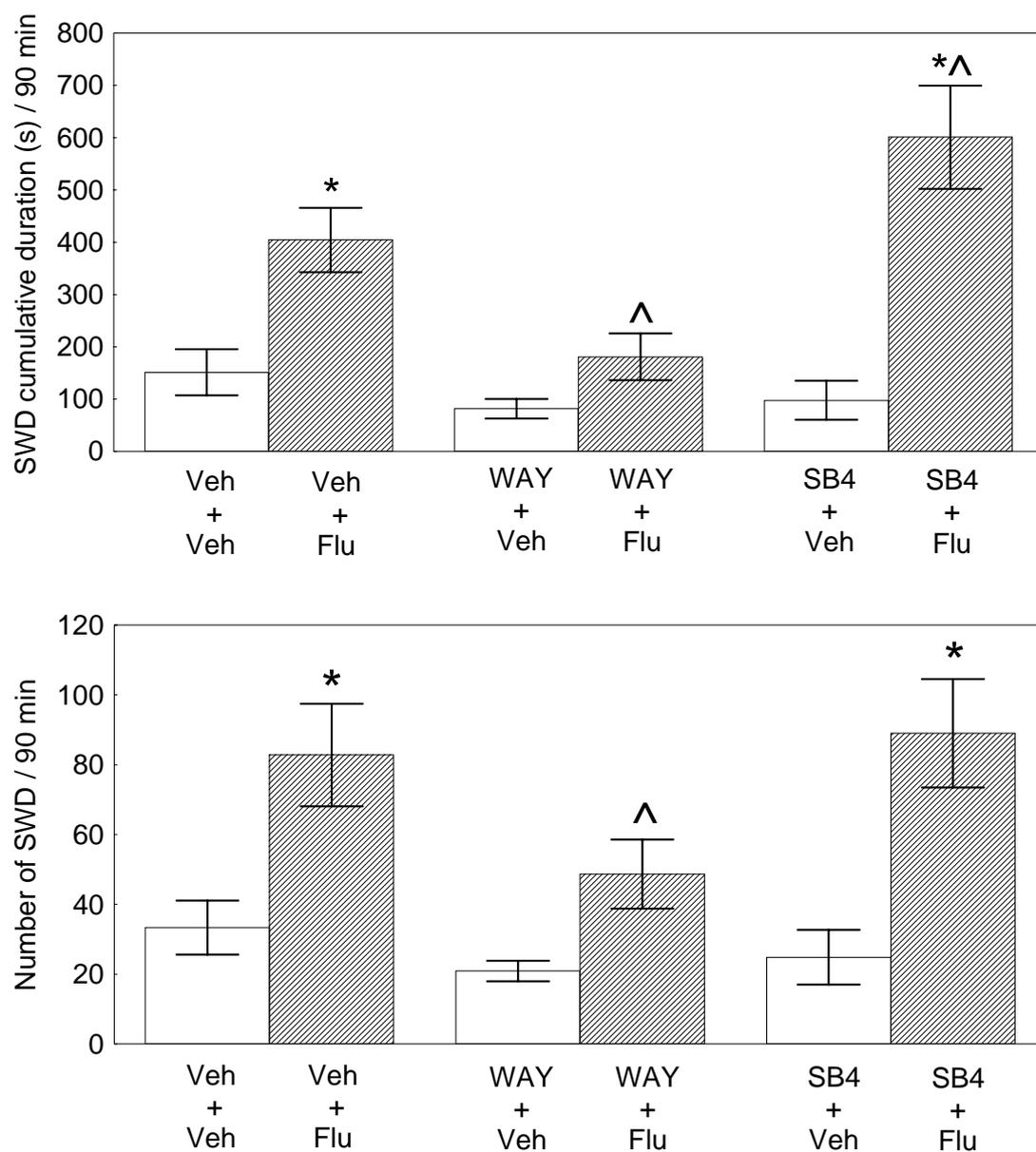
## 5.6. Study Six

The SSRI fluoxetine (5.0 mg/kg, i.p.) alone caused significant, albeit moderate increase in the cumulative duration and number of SWDs. This effect started 30 min after the drug treatment and lasted for about 1.5 hours. Pretreatment with the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (0.2 mg/kg, i.p) significantly attenuated the effect of fluoxetine. In contrast, after pretreatment with the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 (0.2 mg/kg, i.p) the effect of fluoxetine was significantly enhanced (**Figure 11**). Combined pretreatment, namely SB-242084 (0.2 mg/kg, i.p.) plus WAY-100635 (0.2 mg/kg, i.p) failed to alter the effect of fluoxetine (**Figure 12**).

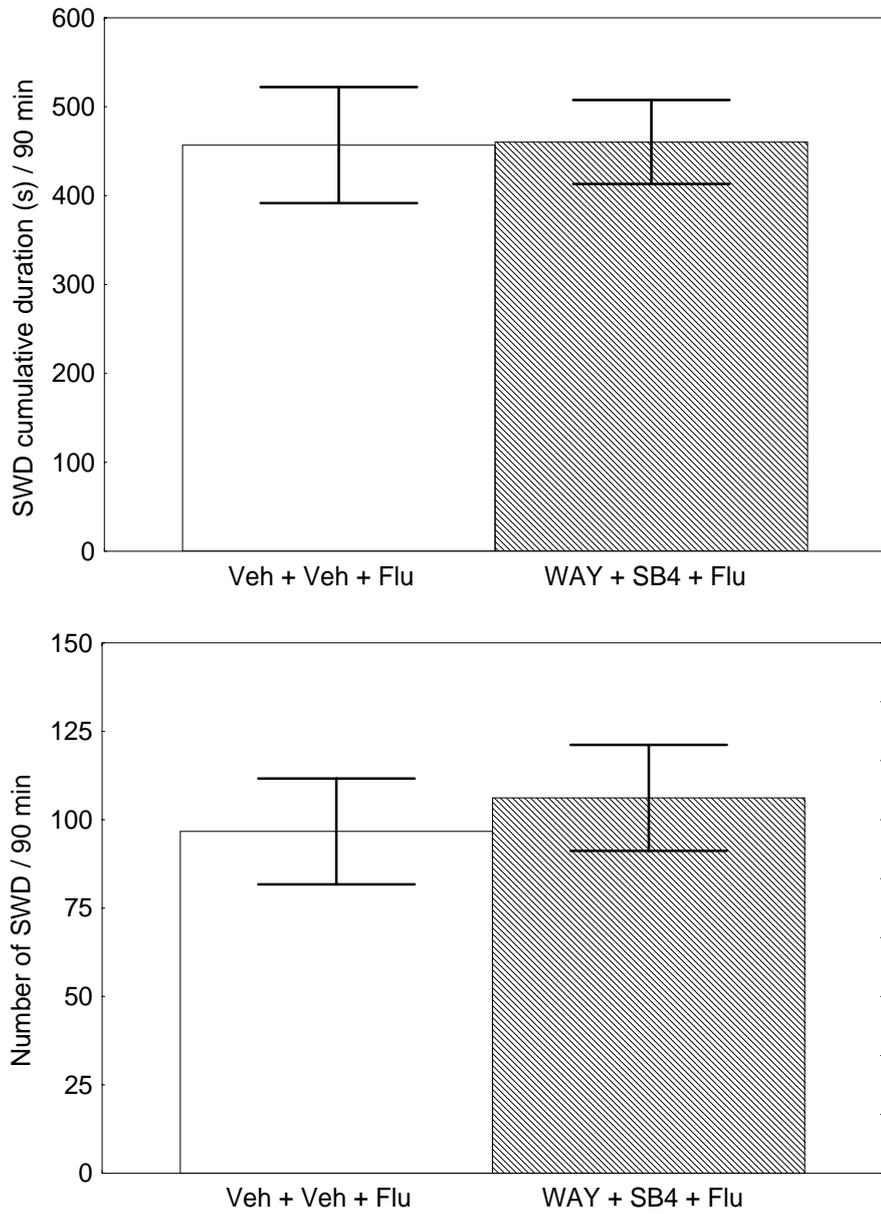
## 6. Discussion

### 6.1. Serotonin receptors mediating acute anxiogenic effects of SSRIs

Anxiogenic-like effect of fluoxetine [30, 179, 180] and citalopram [49] in the social interaction test was described in earlier studies. Acute administration of these compounds caused significant decrease in the time of total social interaction and fluoxetine caused also a decrease in the number of line crossings, and an increase in time of self-grooming [30, 49, 179, 180]. Under low light, familiar arena conditions the surrounding is comfortable for the animals, and thus, basal anxiety is low, so anxiogenic compounds, including e.g., corticotropin releasing hormone (CRH) or the 5-HT<sub>2</sub> agonist *m*-CPP efficiently alter parameters like social interaction [98, 104, 179] or self-grooming [98, 179]. The number of line crossings, a measure of locomotor activity, is decreased by some, e.g., cholecystokinin (CCK) or higher doses of *m*-CPP, but not all compounds that increase anxiety [104, 179, 180]. Anxiogenic action of SSRI antidepressants is also supported by data that fluoxetine caused significant *c-fos*-like immunoreactivity in the central nucleus of amygdala, and paraventricular nucleus and increased ACTH and corticosterone secretion [57, 63].



**Figure 11.** Effects of fluoxetine (Flu; 5.0 mg/kg, i.p.) alone, after pretreatment with WAY-100635 (WAY; 0.2 mg/kg, i.p.), SB-242084 (SB4; 0.2 mg/kg, i.p) or vehicle on the cumulative duration of spike-wave discharges (SWD, above) and number of paroxysms (below) over 90 minutes. Fluoxetine alone caused significant increase in SWDs. Pretreatment with WAY-100635 attenuated, SB-242084 enhanced the effect of fluoxetine. The effect of WAY+Flu and SB+Flu were significant ( $p < 0.05$ ) compared to Veh+Flu, denoted by  $\wedge$ . After pretreatment with SB-242084, fluoxetine caused marked, significant increase in the cumulative duration of SWDs and number of paroxysms compared to the control pretreatment. The effect of Veh+Flu was significant compared to Veh+Veh, and SB4+Flu was significant compared to SB4+Veh, (both  $p < 0.05$ ) denoted by \*.



**Figure 12.** Effects of fluoxetine (Flu; 5.0 mg/kg, i.p) after co-administration of WAY-100635 (WAY; 0.2 mg/kg, i.p.) and SB-242084 (SB4; 0.2 mg/kg, i.p) compared to vehicle/vehicle pretreated group on spike-wave discharges (SWD).

The 5-HT<sub>2</sub> receptor family consists of three subtypes, namely, the 5-HT<sub>2A</sub>, the 5-HT<sub>2B</sub> and the 5-HT<sub>2C</sub> (formerly 5-HT<sub>1C</sub>) receptor, and several antagonists, like ritanserin has similar affinity for all three subtypes [18].

Immunohistochemical analysis of the 5-HT<sub>2C</sub> receptor protein revealed that the most abundant 5-HT<sub>2C</sub>-like immunoreactive cell bodies, beside the chorioid plexus, were in the anterior olfactory nucleus, medial and interrelated amygdaloid nuclei, hippocampus layers CA1 to CA3, laterodorsal and lateral geniculate thalamic nuclei, caudate-putamen and several areas of the cortex (including piriform and frontal), consistent with this receptor being located postsynaptic to serotonergic neurons. Immunopositive neurons were also found in the dorsal raphe, suggesting that 5-HT<sub>2C</sub> receptors may be on some serotonergic neurons [41, 122, 154]. The abundance of 5-HT<sub>2C</sub> positive neurones in limbic areas, hippocampus, hypothalamus, thalamus, cortex and the striatum are concordant with the role of this receptor in anxiogenesis, feeding, neuroendocrine function, locomotor activity and seizure generation, as suggested from the effect of 5-HT<sub>2C</sub> receptor compounds [12, 14, 18, 105, 106, 186].

SB-242084, the first subtype-selective 5-HT<sub>2C</sub> receptor antagonist, has very high affinity (pK<sub>i</sub>=9.0) for the cloned human 5-HT<sub>2C</sub> receptor and has over 100- and 158-fold selectivity over the cloned human 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors [105]. Furthermore, it has at least 100-fold selectivity for this receptor over all other receptors tested [105]. SB-242084 reversed the actions of fluoxetine very efficiently in **Study One**; in the dose of 0.05 mg/kg it reversed decrease of social interaction and increase in self-grooming caused by fluoxetine. These data, together with a recently published study of Dekeyne et al. [49], where SB-242084 effectively reversed anxiogenic action of citalopram, strongly suggest that 5-HT<sub>2C</sub> receptors mediate the anxiogenic actions of SSRI antidepressants.

SB-242084 administered alone failed to alter anxiety-related behaviours under low light, familiar arena conditions, but pretreatment with this compound significantly reversed fluoxetine-induced decrease in total social interaction, increase in self-grooming and hypolocomotion. Because of the receptor binding profile of this compound, these data suggest that SSRIs and *m*-CPP mediate anxiogenic responses by activation of 5-HT<sub>2C</sub> receptors. However, other explanations might also be possible,

thus first, the effect of fluoxetine on locomotion may confound the interpretation of the data, second, the anxiolytic effect of SB-242084 per se may lead to a false conclusion. To discuss the first question, our data show that decreases in locomotion caused by fluoxetine are, in some cases parallel to its effect on total social interaction, which might suggest that the decrease in social interaction may be caused by the effects on locomotion. However, anxiogenic and hypolocomotion effects show clear dissociation in the case of *m*-CPP and citalopram, which has been described in other studies [49, 104]. Thus, *m*-CPP failed to decrease locomotion at the doses of 0.5 mg/kg, but it caused significant decrease on time of total social interaction. A similar dissociation was found in the case of citalopram [49, 104]. The efficacies of SB-242084 on SSRI-induced social interaction and locomotion also dissociated, namely, anxiety-like effects were entirely reversed by 0.05 mg/kg SB-242084, while higher doses of the compound were needed for the complete reversal of hypolocomotion (see Results). In addition, WAY-100635 fully reversed hypolocomotion caused by fluoxetine but it failed to reverse decrease in social interaction. Thus, the decrease and reversal in social interaction can not be considered as a secondary effect of locomotion. Furthermore, in addition to social interaction, we used another measure of anxiety, namely self-grooming. This stereotype behavior is increased by aversive stimuli and anxiogenic compounds like CRH or *m*-CPP, measured either in the social interaction test or in single cages [13, 98, 179]. Conversely, self-grooming is attenuated by anxiolytic compounds under high anxiety conditions. Under certain conditions, this parameter is a more sensitive and less variable measure than time of total social interaction [98, 179]. For example, WAY-100635 pretreatment enhanced self-grooming response but not social interaction caused by fluoxetine in this study, and the minimal effective dose for fluoxetine was lower for self-grooming than for social interaction (see Results). It is likely that augmentation of this stereotype behavior by WAY-100635 is caused by the enhancement of the effect of fluoxetine on extracellular 5-HT, as it was shown in microdialysis studies [125]. This enhancing mechanism is most probably explained by WAY-100635 blocking the negative feedback mediated by 5-HT<sub>1A</sub> autoreceptors on serotonergic neurons, thus augmenting synaptic 5-HT release.

The anxiogenic-like effects of fluoxetine are abolished after chronic treatment [30, 179, 180]. This finding is in parallel with the clinical and experimental data obtained with chronic SSRI treatment [121, 170, 185, 189]. There is a higher activation

of postsynaptic receptors by the excess extracellular 5-HT produced by chronic compared to acute SSRI treatment [6, 19, 93, 170]. Furthermore, disappearance of fluoxetine-induced acute anxiety is accelerated by additional subchronic administration of WAY-100635 [30]. The antagonists used for characterization of receptors that mediate the effect of fluoxetine used in the work of Bristow et al., [30] could not differentiate between 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors. By the use of the subtype-selective SB-242084, our data show that fluoxetine-induced anxiety is mediated by 5-HT<sub>2C</sub> receptors. Thus, the mechanism of action of delayed attenuation of fluoxetine-induced anxiety possibly includes desensitization of 5-HT<sub>2C</sub> receptors. This is supported also by the clinical data that *m*-CPP exacerbates symptoms of OCD before, but not after chronic treatment with clomipramine [197], and *m*-CPP-induced neuroendocrine and hyperthermic responses are attenuated after chronic citalopram treatment [159]. In rats, *m*-CPP-induced hypolocomotion was attenuated after chronic SSRI treatment [102] and the role of 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> receptors have been suggested in these responses of *m*-CPP [7, 9, 11, 13, 30]. In addition, the effectiveness of SB-242084 in self-grooming and social interaction responses provide further evidence for the involvement of 5-HT<sub>2C</sub> receptors in *m*-CPP-induced anxiety, which supports the view that chronic SSRI treatment causes desensitization of 5-HT<sub>2C</sub> receptors.

Fluoxetine has modest affinity for 5-HT<sub>2C</sub> receptors [18, 151]. In an in vivo functional model for 5-HT<sub>2C</sub> receptors, however, it failed to have any significant 5-HT<sub>2C</sub> receptor antagonist action [95]. This might be explained by its 5-HT-mimetic, reuptake inhibiting effect [95]. Indeed, its affinity for the 5-HT uptake site is much higher than its affinity for the 5-HT<sub>2C</sub> receptor [151]. The dose of fluoxetine used in our study was based on the dose-response curves (**Figure 1**).

An increase in serotonergic function produced by SSRI antidepressants underlies their clinical effectiveness in anxiety disorders and major depression [6, 170, 185]. Interestingly, acute increase in 5-HT neurotransmission usually increases fear and anxiety-like behavior [75, 179, 180], although it may have also an opposite effect depending on the type of the test [85]. Graeff et al. suggested that at least three serotonergic pathways are involved in the regulation of anxiety, thus, separate pathways facilitate conditioned fear, inhibit inborn fight/flight reactions and promote resistance to chronic, unavoidable stress [70]. SSRIs and *m*-CPP increase anxiety in the social

interaction test or in the elevated plus maze in rats [75, 98, 104, 180]. Anxiogenic effects of *m*-CPP in clinical studies are also well known [5, 97, 143], and anxiety related transient effects of SSRI antidepressants, like agitation or jitteriness have been described [170, 185, 189]. Stress hormones, like ACTH and cortisol or corticosterone are also released after acute administration of these compounds in both humans and rodents [12, 143]. However, ritanserin, a non-subtype-selective 5-HT<sub>2</sub> receptor antagonist that reverses some effects of *m*-CPP, may also increase or decrease anxiety [10, 97]. Furthermore, activation of the 5-HT<sub>2C</sub> receptor efficiently alters release of other neurotransmitters like noradrenaline, adrenaline, dopamine or glutamate [12, 50, 133], and neuropeptides like CRH, oxytocin and vasopressin [12, 13, 37]. Some of these neurotransmitters systems may mediate anxiolytic and antidepressant activity of SSRIs. The role of CCK in SSRI-induced anxiety-like effects is supported by the data that citalopram-induced decrease in exploratory behavior is reversed by a CCK<sub>B</sub> receptor antagonist [126]. A progressive attenuation of the firing activity of locus coeruleus noradrenergic neurons by sustained SSRI treatment has been shown in electrophysiological studies [177]. Furthermore, CCK or CRH-induced anxiety is also decreased after chronic SSRI treatment [179, 180]. Thus, different anatomical pathways and other neurotransmitter/neuropeptide systems and receptors may mediate or modulate 5-HT<sub>2C</sub> receptor-induced changes.

In conclusion, our studies provide evidence that acute anxiogenic effects of SSRI antidepressants are reversed by pretreatment with low doses of the subtype-selective 5-HT<sub>2C</sub> receptor antagonist SB-242084, and thus, they suggest that anxiety related side effects of acute SSRI treatment, like agitation or jitteriness, may be mediated by 5-HT<sub>2C</sub> receptors.

## **6.2. Serotonin receptors involved in excessive self-grooming**

As described in earlier studies, acute SSRI treatment induces self-grooming, a stereotype behaviour in rats [179, 180] and correspondingly, the 5-HT<sub>2C/2B</sub> receptor agonist *m*-CPP is also known to produce dose-dependent self-grooming [13]. The affinities of the 5-HT<sub>2</sub> receptor agonist *m*-CPP for receptor subtypes are 5-HT<sub>2B</sub> > 5-HT<sub>2C</sub> > 5-HT<sub>2A</sub> [18, 143], but functional characterization on recombinant human

receptors showed that, compared to 5-HT, *m*-CPP has 65%, 24% and 22% relative efficacies on 5-HT<sub>2C</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> receptors, respectively [155].

Pharmacological studies with different antagonists revealed that most effects of *m*-CPP on the central nervous system are mediated by 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors rather than by 5-HT<sub>2A</sub> receptors [13, 15, 104]. This is true also for anxiety-type effects and self-grooming, behaviours that are evident at low, 0.3-0.6 mg/kg doses of *m*-CPP [13, 98, 104].

Bimodal dose-response curves of *m*-CPP on self-grooming were described earlier [13]. Pharmacological studies suggested the role of 5-HT<sub>2C</sub> receptors in these responses [13]. Although compounds used in those studies could not clearly differentiate between 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors, data about low concentration and expression of 5-HT<sub>2B</sub> receptors in the central nervous system and the opposite for 5-HT<sub>2C</sub> receptors supported this conclusion [1, 89, 91, 110, 138]. However, additional studies confirmed the expression of 5-HT<sub>2B</sub> receptors in the rat brain [52] and activation of the 5-HT<sub>2B</sub> receptor was reported to inhibit grooming behaviour [100].

In **Study Two** we demonstrate that pretreatment with low doses of the 5-HT<sub>2C</sub> receptor antagonist SB-242084 effectively reverses actions of *m*-CPP, and pretreatment with SB-215505, a 5-HT<sub>2B</sub> receptor antagonist does not alter *m*-CPP induced grooming. These results confirm that 5-HT<sub>2C</sub> and not 5-HT<sub>2B</sub> receptor activation is responsible for *m*-CPP-induced self-grooming. Our results are in line with recent studies, which reported that the 5-HT<sub>2B</sub> agonist BW 723C86 did not produce any of the behavioural effects associated with administration of *m*-CPP [100]. Furthermore, the selective 5-HT<sub>2B</sub> antagonists, LY 202146 and LY 266097, failed to block *m*-CPP-induced hypolocomotion in mice [65, 100]. Thus, there is compelling evidence that predominantly 5-HT<sub>2C</sub> and not 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> receptors mediate the in vivo effects of *m*-CPP in the CNS. Therefore we can conclude that *m*-CPP is a useful tool for testing 5-HT<sub>2C</sub> receptor function in vivo.

We can assume that 5-HT<sub>2C</sub> receptors also play a role in obsessive-compulsive disorder (OCD) in humans. *m*-CPP was reported to exacerbate symptoms of OCD [198] and pretreatment with metergoline, an antagonist with very high affinity to 5-HT<sub>2C</sub> receptors obliterated the increases in *m*-CPP-induced exacerbation of OCD symptoms [153]. Recent studies have found further intriguing similarities between excessive self-

grooming in animals and OCD in humans [73, 74, 147, 160]. Previous studies report, that *m*-CPP-induced self-grooming is in whole, or in part mediated via the hypothalamic paraventricular nucleus [11, 14]. The role of the paraventricular nucleus has been postulated also in OCD [165]. Furthermore, the localization of 5-HT<sub>2C</sub> receptors show a remarkable association with the so-called “OCD-circuit”. Studies on the immunohistochemical distribution of 5-HT<sub>2C</sub> receptors in the rat brain [41] show that 5-HT<sub>2C</sub> receptors are highly represented in the orbitofrontal cortex, the anterior cingulate cortex, and the caudate nucleus. These structures are referred to as the “OCD-circuit”, because they show abnormal metabolic activity in OCD patients [72]. Therefore, we can truly expect that our findings on self-grooming may also be relevant to clarifying serotonergic mechanisms in OC-spectrum disorders.

### **6.3. The effect of chronic fluoxetine treatment on 5-HT<sub>1A</sub> receptor function in two different rat strains**

Fawn-Hooded (FH) rats spend significantly less time in social interaction compared to SD rats [98], exhibit high immobility in the forced swim test and high voluntary ethanol intake [8, 150, 161]. Thus, this rat strain has been proposed as an animal model for depression, alcohol abuse, and anxiety [8, 150, 161].

8-OH-DPAT-induced decrease in body temperature is mediated selectively by stimulation of 5-HT<sub>1A</sub> receptors. The response is antagonized by selective antagonists, even stereoselectivity of pindolol has been shown [21, 67, 86].

It is generally admitted that in mice 5-HT control of body temperature is attributed to somatodendritic 5-HT<sub>1A</sub> autoreceptors [67]. Although a presynaptic mechanism has been suggested also in the rat [69, 86], there is also evidence that decrease in body temperature is mediated by postsynaptic 5-HT<sub>1A</sub> receptors [16, 137, 193]. Despite the extensive literature, data about the site and location of 5-HT<sub>1A</sub> receptors mediating temperature reduction are contradictory.

In **Study Three** we found only moderate differences in 8-OH-DPAT-induced hypothermia between FH and the control SD rats. In earlier studies, markedly attenuated temperature reduction to 8-OH-DPAT and ipsapirone were found [8, 78]. These differences may be attributed to the variations in the route of administration of the drug and experimental conditions.

In SD rats we found a significant decrease in hypothermic effect of 8-OH-DPAT after chronic fluoxetine treatment. Similar results, namely reduction in temperature, behavioural and hormone responses to 5-HT<sub>1A</sub> receptor agonists after chronic SSRI treatment were described earlier [66, 68, 117, 118]. Although there is evidence for a decrease of 5-HT<sub>1A</sub> receptor-mediated hypothermic response after chronic SSRI treatment and also after electroconvulsive shocks (ECS), repeated ECS increases the responsiveness of 5-HT<sub>1A</sub> receptors in the hippocampus and upregulates 5-HT<sub>1A</sub> binding sites in the cortex [39, 148]. Furthermore, the sensitivity of the 5-HT<sub>1A</sub> somatodendritic autoreceptor in the dorsal raphe remains normosensitive after repeated ECS [23, 79]. Sustained administration of the 5-HT<sub>1A</sub> receptor agonist flesinoxan failed to modify the responsiveness of hippocampal CA3 pyramidal neurones [81]. In contrast, long-term treatment with SSRI antidepressants attenuated functions of hypothalamic 5-HT<sub>1A</sub> receptors [117, 118]. Interestingly, somewhat similar antianxiety effects were described by chronic treatments with SSRI antidepressants or by ipsapirone, a 5-HT<sub>1A</sub> receptor antagonist [180].

In the FH rats platelet 5-HT content is significantly lower than in SD animals and there is evidence for a decreased storage and transporter function of 5-HT in the FH rats [181]. Similarly, there is evidence for decreased concentrations of 5-HT and 5-HIAA in the brainstem of FH rats [8]. The role of impaired transporter function in the alterations of 5-HT<sub>1A</sub> receptor function in FH rats is strongly supported by the findings that the hypothermic response to 8-OH-DPAT was completely absent in transgenic mice lacking the 5-HT transporter [120].

Interestingly, studies that provided evidence for the decreased function of 5-HT<sub>1A</sub> receptors after chronic SSRI treatment failed to show any decrease in 5-HT<sub>1A</sub> receptor number or the concentration of its mRNA after chronic SSRI treatment [119, 169]. Thus, post-receptor mechanisms, rather than changes at the receptor level might be involved in SSRI-induced desensitization of 5-HT<sub>1A</sub> receptors [119, 145]. The fast and more complete reduction in the hypothermic response in FH rats suggests that a faster and more complete desensitization in depression- and anxiety disorders may occur. A recent clinical study that found significant reduction in temperature and hormone responses without any significant effect on mood and other psychological variables to the 5-HT<sub>1A</sub> agonist ipsapirone after chronic fluoxetine treatment in normal subjects also suggests this hypothesis [112]. Several studies have shown that the

hypothermic response to 5-HT<sub>1A</sub> receptor agonists is decreased also in untreated depressed patients [45, 116]. Thus, studies on 5-HT<sub>1A</sub> receptor function in animal models of depression and anxiety may help us understand the role of this receptor subtype in mood disorders.

8-OH-DPAT-induced lower lip retraction is mediated by receptors located in the median raphe nucleus [20]. This response was prevented by pretreatment with the subtype-selective 5-HT<sub>1A</sub> receptor silent antagonist WAY-100635. This finding is consistent with the view that this behaviour is selectively mediated by activation of 5-HT<sub>1A</sub> receptors [22, 141]. This effect is most likely mediated by somatodendritic autoreceptors in the median raphe nucleus, and this receptor population is located far from those that mediate decrease in body temperature [16, 20]. We found a higher efficacy of 8-OH-DPAT-induced lower lip retraction in FH rats. Interestingly, a decrease in the number of 8-OH-DPAT binding sites was observed in the brainstem of FH rats earlier [92]. These data suggest that postreceptorial 5-HT<sub>1A</sub> receptor function in the median raphe nucleus may be increased in FH rats.

The lack of any decrease in lower lip retraction after chronic fluoxetine treatment in either SD or FH rats is an intriguing finding. There is a general consensus that the acute administration of SSRIs leads to a blockade of 5-HT reuptake, local increase in extracellular 5-HT, activation of 5-HT autoreceptors and a reduction of 5-HT neuronal activity [6, 25]. Furthermore, several studies found a desensitization of somatodendritic 5-HT<sub>1A</sub> autoreceptors after chronic SSRI treatment [93, 109], although others failed to show a similar effect [27, 88].

In electrophysiological studies two day treatment with different SSRIs markedly attenuated firing activity of 5-HT-containing neurones, but these neurones regained their normal firing activity during a two-week treatment [25]. Furthermore, acute administration of an SSRI produced a small and transient increase in extracellular 5-HT concentrations in the rat frontal cortex, whereas continuous infusion for 14 days resulted in a robust, 6-fold increase in extracellular 5-HT concentrations in in vivo microdialysis studies [6]. In general, these data were interpreted as a result of somatodendritic autoreceptor desensitization after chronic SSRI treatment [25]. However, recent evidence from microdialysis studies may provide another explanation. Local application of 8-OH-DPAT in the medial prefrontal cortex decreased extracellular 5-HT, thus, postsynaptic 5-HT<sub>1A</sub> receptors in this region also participate in the control of 5-HT

release [38]. Consequently, adaptive changes of such receptors must be taken into account when trying to understand the mechanism for the changes of terminal 5-HT release and clinical effects of these drugs. Another possible explanation for the difference might be that the vast majority of studies dealing with the function of somatodendritic autoreceptors concentrated on the dorsal raphe nucleus. Acute effects of 5-HT<sub>1A</sub> receptor activation using electrophysiological techniques were lower in some [26, 168] but not in other studies [82] in the median compared to the dorsal raphe nucleus. In our studies, we did not find any desensitization in the function of 5-HT<sub>1A</sub> receptors that are located in the median raphe nucleus. These data, together with previous results, suggest that there may be differences in neuronal functions related to the 5-HT<sub>1A</sub> receptor desensitization process in median and dorsal raphe nuclei. Significantly lower basal firing rate [82] or a much lower ratio of repetitive firing of median raphe 5-HT neurones [83] may explain differences in adaptive changes in 5-HT<sub>1A</sub> receptor function of dorsal versus median raphe nuclei after chronic SSRI treatment.

The masseter-eyeball syndrome caused by 8-OH-DPAT was frequently observed in FH rats, but it was a rare event in SD rats. WAY-100635, is a selective, silent 5-HT<sub>1A</sub> receptor antagonist [77]. Pretreatment with this compound completely blocked the effect of 8-OH-DPAT, thus, this response is mediated by 5-HT<sub>1A</sub> receptors. Muscles of the head and face of the rats involved in this response are located close to those that mediate lower lip retraction, that suggest at least some similarity in the two responses. In addition, both responses are elicited more efficiently in FH rats. The difference in the effect of fluoxetine, however, suggests that other 5-HT receptor subtypes may also modulate this effect, and change in the function of these other modulatory receptors may interfere with changes in 5-HT<sub>1A</sub> receptor functions caused by chronic SSRI treatment. Further studies, including lesion of 5-HT neurons, are needed to characterize the exact mechanism that mediates the masseter-eyeball syndrome.

In conclusion, our data reveal a clear strain difference in the regulation of 5-HT<sub>1A</sub> receptor systems. FH rats evidently demonstrate altered 5-HT<sub>1A</sub> receptor function as well as faster and more enhanced desensitization of 5-HT<sub>1A</sub> receptors. In addition, they have serotonergic defects and behavioural alterations similar to those observed in mood and anxiety disorders. Hence, the observed differential effects of fluoxetine on 5-

HT<sub>1A</sub> receptor function might help us to understand the regulation of 5-HT<sub>1A</sub> receptor systems in depressed patients.

#### 6.4. The effect of chronic fluoxetine treatment on 5-HT<sub>2C</sub> receptor function

As discussed above, 5-HT<sub>2C</sub> receptor activation is responsible for SSRI- as well as *m*-CPP-induced self-grooming, hypolocomotion, and acute anxiety-type effects in the rat social interaction test. Recent studies with selective 5-HT<sub>2C</sub> receptor agonists and antagonists revealed that 5-HT<sub>2C</sub> receptor activation (also induced by *m*-CPP or SSRIs) causes penile erection in the rat [135, 136]. The consequences of long-term increase in CNS 5-HT concentration on 5-HT<sub>2C</sub> receptor function have also been investigated earlier. Chronic treatment with the SSRI's paroxetine and fluoxetine significantly attenuated the effect of *m*-CPP on locomotion and rears in rats [102] and chronic fluvoxamine produced matching results [194]. Furthermore, long-term fluoxetine administration significantly reduced the anxiety-like effects of *m*-CPP and other anxiogenic compounds in the social interaction test in the rat [30, 179, 180]. Following chronic paroxetine treatment, both the prolactin and hyperthermic responses to *m*-CPP were significantly attenuated in humans [159]. These effects of chronic SSRI treatment are suspected to be the consequence of 5-HT<sub>2C</sub> receptor desensitisation [30, 102, 159, 194]. The results of **Study Four** largely correspond with these previous reports. *m*-CPP-induced penile erection decreased significantly after both 4 and 16 days of chronic fluoxetine treatment. This effect is most probably the result of the desensitisation of 5-HT<sub>2C</sub> receptors located on neurons in the sacral parasympathetic nucleus and the dorsal gray commissure of the L6-S1 spinal segments [17].

*m*-CPP-induced self-grooming was significantly decreased after 4 days of fluoxetine administration. However, after 16 days of fluoxetine treatment the self-grooming-inducing effect of *m*-CPP returned. The inconsistency between the effects of chronic fluoxetine treatment on *m*-CPP-induced penile erection and self-grooming could be explained by the different anatomical localizations involved in these responses. The hypothalamic paraventricular nucleus was reported to mediate *m*-CPP-induced self-grooming [14] whereas 5-HT<sub>2C</sub> receptor-immunopositive spinal neurons are suspected to be mainly responsible for *m*-CPP-induced penile erection [17]. Studies report that the effects of chronic SSRI treatment on 5-HT<sub>2C</sub> receptors are diverse in different regions of

the CNS: increase or no change of 5-HT<sub>2C</sub> receptor density have been reported [184]. Moreover, the effect of chronic SSRI treatment on extracellular 5-HT concentration is also brain-region dependent [184]. Thus, the difference in the anatomical localizations mediating *m*-CPP-induced penile erection and self-grooming may play a role in the diverging effects of fluoxetine treatment seen after 16 days.

### **6.5. The consequences of serotonin depletion on 5-HT<sub>2C</sub> receptor responsivity**

*p*-chlorophenylalanine (*p*-CPA) irreversibly inhibits tryptophane hydroxylase, and it causes a long-lasting, over 70 and 90% decrease in brain 5-HT concentration after 2x50 and 2x350 mg/kg, respectively [40, 108, 144]. The sensitisation of 5-HT receptors after depletion of brain 5-HT by *p*-CPA has been reported in other animal studies [36, 80]. *p*-CPA-induced 5-HT depletion increases 5-HT<sub>2C</sub> receptor mediated phosphoinositide hydrolysis and an increased sensitivity to *m*-CPP and LSD discriminative stimulus [36, 58]. Furthermore, the increased expression of 5-HT<sub>2C</sub> mRNA isoforms encoding receptors with higher sensitivity to serotonin was observed in serotonin-depleted mice [80].

As observed in **Study Five**, both *m*-CPP-induced self-grooming and penile erection were significantly enhanced after 2x50 and 2x100 mg/kg *p*-CPA treatment, which effect is most likely caused by sensitisation of 5-HT<sub>2C</sub> receptors. Although 2x350 mg/kg *p*-CPA failed to enhance *m*-CPP-induced responses, previous studies report that *p*-CPA decreases not only 5-HT but also noradrenaline (NA) and dopamine (DA) concentrations in the rat brain at the dose of 2x350 mg/kg. In contrast, 2x50 and 2x100 mg/kg *p*-CPA has no effect on DA and NA concentrations [144]. As previously reported, DA plays an important role in regulating grooming behaviour [164, 175] and both DA and NA are implicated in the control of penile erection [4, 64, 171]. Therefore, the decrease in brain DA and NA concentrations after 2x350 mg/kg *p*-CPA treatment could modify the effects of *m*-CPP on penile erection and self-grooming.

The monoamine hypothesis of depression proposes that low levels of one or more of the brain monoamine neurotransmitters - serotonin, noradrenaline and dopamine - could produce depression. A refinement of this hypothesis is that depressive illness may arise, specifically, from decreased brain 5-HT function [131]. One of the factors associated with low levels of 5-HT in depression is a possible change in 5-HT

receptor sensitivity [132]. Increased 5-HT<sub>2</sub> receptor binding in blood platelets and an elevated 5-HT<sub>2</sub> receptor functional response was observed in depressed patients compared to healthy controls [132]. These studies are consistent with the hypothesis of 5-HT<sub>2</sub> receptor sensitisation in depression.

In **Study Five** we demonstrate that 5-HT<sub>2C</sub> receptor sensitization does indeed occur after experimentally-induced, long-term reduction in brain 5-HT concentration. Our results may be helpful in understanding possible alterations of 5-HT receptor function in psychiatric disorders (e.g. depression) associated with decreased CNS serotonin levels.

### **6.6. The effect of fluoxetine on epileptic activity in WAG/Rij rats: involvement of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors**

As described earlier, the SSRI fluoxetine and its metabolite norfluoxetine was reported to have anticonvulsant properties in mice with pentylenetetrazol-induced seizures [99]. Hence, in **Study Six** we investigated the effects of fluoxetine on epileptic activity in the WAG/Rij rat strain, an animal model of absence epilepsy.

Absence epilepsy is characterized by the spontaneous occurrence of bilateral synchronous spike-wave discharges (SWDs) that involve the entire cortical mantle [42, 156, 183]. Thalamus, as a pacemaker structure for the rhythmic cortical oscillations very likely represents the primary neuronal dysfunction underlying the generation of spike-wave discharges [42, 127, 156, 174]. Within the thalamus, sleep spindles are generated as a recurrent interaction between thalamocortical and thalamic reticular cells [174]. It has been suggested based on the resemblance in the EEG and the similar circadian pattern that spike-wave discharges are modified sleep spindles [127, 174, 183]. Sleep spindles and other rhythmic oscillations (e.g. delta waves) are produced by thalamic neurons by their ability to spontaneously generate rhythmic bursts of action potentials due to the interaction of the Ca<sup>2+</sup> current I<sub>T</sub> and the inward “pacemaker” current I<sub>h</sub>. The amplitude, or voltage sensitivity of I<sub>h</sub> adjusts the rate at which the thalamic cells oscillate and this sensitivity is adjusted by the release of modulatory neurotransmitters [128].

Previous studies [55, 56, 61] demonstrated that the 5-HT<sub>1A</sub> agonist 8-OH-DPAT increases the number and cumulative duration of SWDs in WAG/Rij rats. The actions

of 8-OH-DPAT on SWDs could be attenuated by pretreatment with NAN-190, a 5-HT<sub>1A</sub> receptor antagonist [61]. Activation of 5-HT<sub>1A</sub> receptors was reported to cause a direct hyperpolarization of thalamocortical neurons *in vitro* in the ferret dorsal thalamus through an increase in potassium conductance [139, 140]. Hyperpolarization due to the activation of a potassium current results in an enhancement of burst firing in thalamocortical neurons, which enables the generation of intrinsic oscillations, e.g. sleep spindles and generalized spike-wave seizures [129, 139]. Therefore, 8-OH-DPAT possibly increases SWDs through 5-HT<sub>1A</sub> receptor-mediated hyperpolarization and a consequent increase in burst firing in thalamocortical neurons.

We found that fluoxetine caused a moderate but significant increase in the number and cumulative duration of spike-wave discharges (SWDs). Thus, in the Wag/Rij rat fluoxetine has pro-epileptic effects. As mentioned above, the 5-HT<sub>1A</sub> agonist 8-OH-DPAT also increases epileptic activity in WAG/Rij rats [55, 56, 61], therefore, it could be assumed that increased synaptic 5-HT concentration and subsequent 5-HT<sub>1A</sub> receptor activation mediates the pro-epileptic effect of fluoxetine.

To find out more about 5-HT receptors mediating the effect of fluoxetine in this animal model of absence epilepsy, the combination of fluoxetine, the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635, and the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 were administered.

As expected, WAY-100635 pretreatment significantly diminished the effect of fluoxetine on epileptic activity in WAG/Rij rats in our study. In contrast, pre-treatment with the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 significantly enhanced the effect of fluoxetine on the cumulative duration of SWDs. This result suggests that fluoxetine-induced increase in endogenous 5-HT produces a dual effect on SWDs: the activation of 5-HT<sub>1A</sub> receptors enhances, and stimulation of 5-HT<sub>2C</sub> receptors inhibits epileptic activity in WAG/Rij rats.

Our findings are consistent with experiments demonstrating that the 5-HT<sub>2C</sub> agonist *m*-CPP weakly elevated seizure threshold in the mouse maximal electroshock seizure threshold test and also provided appreciable protection against pentylenetetrazol-induced myoclonic and tonic seizures in mice and forelimb tonic seizures in rats [182].

The administration of the 5-HT<sub>2C</sub> receptor subtype-selective antagonist SB-242084 alone was not associated with significant changes in the epileptiform activity in our study. This finding is in agreement with results that antagonism of 5-HT<sub>2B/2C</sub> receptor

subtype with SB-206553 alone did not lower the threshold to myoclonus, forelimb and/or hindlimb tonus in mice or rats [182], and that the highly selective 5-HT<sub>2C</sub> antagonist SB-242084 did not produce proconvulsant activity in the rat maximal electroshock seizure threshold test even after administration at very high acute dose (30 mg/kg, p.o.) [105]. Thus, under normal physiological conditions, at basic serotonergic tone, 5-HT<sub>2C</sub> receptor activation does not seem to inhibit epileptic activity.

The inability of 5-HT<sub>2C</sub> receptor antagonists to reduce seizure threshold in adult rodents contrast with the observed characteristics of mutant mice lacking the 5-HT<sub>2C</sub> receptor [178]. The mutant mice undergo spontaneous tonic-clonic convulsions and by 2-3 months of age exhibit enhanced susceptibility to pentylenetetrazol and audiogenic-induced seizures [29, 84, 178]. Although other epilepsy models in addition to WAG/Rij rats have to be tested, our results together with the above mentioned studies suggest that pharmacological blockade of the receptor and “knock-out” of the receptor gene may result different effects. This might be explained by strain differences, developmental or neuroadaptive changes in the brain.

To further verify our findings we tested the effect of fluoxetine after pretreatment with both the selective 5-HT<sub>1A</sub>-receptor antagonist WAY-100636 and the selective 5-HT<sub>2C</sub> receptor antagonist SB-242084. After the co-administration of the two antagonists, the effect of fluoxetine on SWDs remained unchanged. Thus, WAY-100636 and SB-242084 compensated each others effects, and it can be assumed that not only 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub>, but also other 5-HT receptor subtypes are involved in the effect of fluoxetine on epileptic activity. One such candidate is the 5-HT<sub>7</sub> receptor, which has also been found to be involved in the serotonergic mechanisms regulating SWDs [71].

Briefly, we can state, that fluoxetine moderately, but significantly promotes the generation of spike-wave discharges in the WAG/Rij rat model of absence epilepsy. In our model fluoxetine did not have antiepileptic actions, as reported in the pentylenetetrazol-induced mouse epilepsy model [99]. However, fluoxetine and its metabolite norfluoxetine were proven to efficiently block neuronal Ca(2+) channels [99, 187]. Therefore, it can be assumed, that the Ca(2+) channel-blocking effect of fluoxetine and norfluoxetine may explain their anticonvulsant effects on pentylenetetrazol-induced seizures in mice [99].

Our studies provide evidence that the effect of fluoxetine in the WAG/Rij rat is exerted through inhibitory and excitatory actions of 5-HT at different 5-HT receptor subtypes. Stimulation of 5-HT<sub>2C</sub> receptors by increase in endogenous 5-HT concentration inhibits SWDs, although this inhibitory effect is not significant at basal 5-HT tone. In contrast, activation of 5-HT<sub>1A</sub> receptors by increased 5-HT concentration (or 5-HT<sub>1A</sub> agonists) promotes SWDs but the role of other 5-HT receptors (e.g. the 5-HT<sub>7</sub> receptor) must be considered as well.

## **7. Conclusions**

Selective serotonin reuptake inhibitors are extensively used for the treatment of a wide range of behavioral and psychiatric disorders [28, 152, 170, 176, 185]. Therapeutic effects of SSRIs usually take weeks to become apparent, the immediate actions of these agents are mostly side effects [170, 185]. The mechanisms underlying the delayed efficacy are incompletely understood and appear to involve adaptive changes in signal transduction and gene expression in serotonergic and other neurotransmitter systems [142].

In some patients treated with SSRIs [170, 185] and in animal models acute SSRI administration can induce anxiety [49, 76, 179]. Our studies provide evidence that anxiogenic effects of SSRIs in the rat social interaction test are mediated by activation of 5-HT<sub>2C</sub> receptors. Furthermore, stimulation of the 5-HT<sub>2C</sub> receptor can also induce excessive self-grooming, a stereotype behaviour which is associated with increased anxiety and is characteristic for animal models of obsessive-compulsive disorder. The involvement of 5-HT<sub>2C</sub> receptors in OCD has been previously suggested also by clinical studies [153, 198] and our results further support this hypothesis.

A leading theory explaining the late therapeutic effects as well as the development of tolerance to side effects involves the desensitization of pre- and postsynaptic serotonin receptors [170, 184, 185]. 8-OH-DPAT-induced hypothermia was attenuated in rats after chronic fluoxetine treatment in our experiments, which supports the hypothesis of 5-HT<sub>1A</sub> receptor desensitisation as a consequence of long-term SSRI therapy. Interestingly, this effect was faster and more potent in the Fawn-Hooded rat strain, which is considered as an animal model of depression and anxiety [150, 161]. We found that 5-HT<sub>2C</sub> receptor-mediated responses, *m*-CPP-induced self-grooming and

penile erection also diminished following chronic fluoxetine treatment, which reinforces the theory of post-synaptic 5-HT receptor desensitisation associated with therapeutic effects of serotonergic antidepressants. Our results can also provide clues for the clarification of the mechanisms underlying the frequent and enduring adverse effect of SSRIs, erectile dysfunction [170].

Decreased brain 5-HT function and increased sensitivity of certain 5-HT receptors have been proposed as mechanisms associated with depressive disorders [131, 132]. This assumption is supported by our experiments, which demonstrated that chemically induced, lasting depletion of brain 5-HT resulted in enhanced responses to *m*-CPP, which indicates an increase in 5-HT<sub>2C</sub> receptor sensitivity.

Previous studies in the WAG/Rij rat strain suggested a modulatory role of 5-HT on epileptic activity in this animal model of human absence epilepsy. In our experiments, fluoxetine-induced increase in brain 5-HT neurotransmission produced an upsurge in epileptic activity, however simultaneous inhibitory and excitatory effects were detected, which are exerted through activation of different 5-HT receptor subtypes. Stimulation of 5-HT<sub>2C</sub> receptors appear to inhibit spike-wave discharges (SWDs), although this inhibitory effect is not significant at basal 5-HT tone. In addition, our studies further confirm that activation of 5-HT<sub>1A</sub> receptors promotes the generation of SWDs.

In conclusion we can state that acute and chronic effects of SSRIs are mediated by a variety of 5-HT receptor subtypes. Our studies, focusing primarily on the role of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors provide evidence for their role in modulating spike-wave discharges, anxiety, stereotype behaviour, thermoregulation and penile erection. The results presented in our thesis may facilitate the understanding of serotonergic mechanisms underlying certain psychiatric as well as neurological conditions in humans (e.g. depression, anxiety disorders and epilepsy), and help us clarify the complex and diverse effects of SSRI pharmacotherapy.

## **8. Clinical relevance**

In our present work we demonstrated that activation of 5-HT<sub>2C</sub> receptors is responsible for the induction of excessive self-grooming and the acute anxiogenic effects of SSRIs in animal experiments. Our research helps to understand serotonergic mechanisms involved in OCD [198] and processes underlying SSRI-induced anxiety, an adverse effect observed in some patients treated with selective serotonin reuptake

inhibitors [185]. The future development of selective 5-HT<sub>2C</sub> antagonistic compounds could not only offer treatment for some side-effects of SSRIs but may also represent an entirely new class of anti-OCD and anxiolytic drugs.

The presented results provide evidence for the desensitisation of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors as a consequence of chronic SSRI treatment. These findings can help to clarify the mode of action of SSRIs producing the late therapeutic effects [170] as well as the long-term side-effects (e.g. sexual dysfunction) of SSRI pharmacotherapy [87]. Our studies reinforce the hypothesis on the key role of 5-HT<sub>1A</sub> receptor desensitisation associated with SSRI treatment [170], which may progress the discovery of improved, faster-acting antidepressants. Furthermore, based on our research we can also assume that the 5-HT<sub>2C</sub> receptor may present a potential target for the treatment of erectile dysfunction [17].

The chemically-induced depletion of brain 5-HT produced an increased sensitivity of 5-HT<sub>2C</sub> receptors in the animals tested. Reduced brain 5-HT function and increased sensitivity of some 5-HT receptors have been proposed as mechanisms associated with depressive disorders [131, 132]. Our experiments support these assumptions and may possibly elucidate neurotransmitter and receptor alterations lying beneath the pathogenesis of depression.

Studies conducted in the WAG/Rij rat strain offer a great potential to understand human absence epilepsy [42, 183]. Our investigations on the effects of SSRIs in WAG/Rij rats present additional evidence for the central modulatory role of the serotonergic system in absence epilepsy. Based on recent literature, this role appears to be increasingly relevant considering the close correlation between the circadian pattern of 5-HT neurotransmission and vigilance states. The growing number of prescribed medications affecting a wide range of 5-HT receptors renders our studies even more pertinent. Furthermore, our results may prove to be valuable for the development of novel antiepileptic medications.

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**12. Other related publications by the author**

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## 14. Summary

### **The effects of selective serotonin reuptake inhibitors on the function of distinct serotonin receptor subtypes in the rat**

Selective serotonin reuptake inhibitors (SSRIs) have well-documented efficacy in depression, dysthymic disorder, panic disorder, obsessive-compulsive disorder, social phobia, bulimia nervosa and several other psychiatric and neurological conditions.

Although inhibition of serotonin reuptake occurs immediately after administration of an SSRI, the clinical effect is characterized by delayed onset and it is generally only the side effects of these agents, which are manifest immediately. Anxiety is one of the most common early adverse effects of SSRI treatment, and SSRIs were reported to have an anxiogenic-like profile after acute administration in animal experiments. In our present work, we provide evidence that acute fluoxetine treatment dose-dependently increases anxiety in rats in the social interaction test. Furthermore, using subtype selective receptor antagonists, we prove that this anxiogenic effect is mediated by activation of 5-HT<sub>2C</sub> receptors. Our studies demonstrate that 5-HT<sub>2C</sub> receptor activation also causes self-grooming, a stereotype behaviour which is associated with increased anxiety and is characteristic for animal models of obsessive-compulsive disorder.

Desensitisation of 5-HT receptors associated with chronic SSRI treatment is a leading theory explaining the late therapeutic effects and the development of tolerance to side effects. In our experiments, physiological and behavioural responses mediated by 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors were attenuated after chronic fluoxetine treatment, which supports the hypothesis of 5-HT receptor desensitisation.

Our studies proved that chemically induced, lasting depletion of brain 5-HT resulted in enhanced responses to *m*-CPP, which indicates an increase in 5-HT<sub>2C</sub> receptor sensitivity. This result supports the assumption that decreased brain 5-HT transmission is associated with increased sensitivity of 5-HT<sub>2</sub> receptors, a mechanism believed to play a role in depressive disorders.

Previous studies in the WAG/Rij rat strain suggested a modulatory role of 5-HT on epileptic activity in this an animal model of human absence epilepsy. In our

experiments, fluoxetine-induced increase in brain 5-HT neurotransmission produced an upsurge in epileptic activity, however simultaneous inhibitory and excitatory effects were detected, which are exerted through activation of different 5-HT receptor subtypes. Stimulation of 5-HT<sub>2C</sub> receptors appear to inhibit spike-wave discharges (SWDs), although this inhibitory effect is not significant at basal 5-HT tone. In addition, our studies further confirm that activation of 5-HT<sub>1A</sub> receptors promotes the generation of SWDs.

In conclusion we can state that acute and chronic effects of SSRIs are mediated by a variety of 5-HT receptor subtypes. Our studies, focusing primarily on the role of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors provide evidence for their role in modulating anxiety, stereotype behaviour, thermoregulation, penile erection and spike-wave discharges. The results presented in the thesis may facilitate the understanding of serotonergic mechanisms underlying certain psychiatric as well as neurological conditions in humans (e.g. depression, anxiety disorders and epilepsy) and help to clarify the complex and diverse effects of SSRI pharmacotherapy. Furthermore, our studies may promote the development of future treatments targeting the serotonergic system in a wide range of psychiatric and neurological disorders.

## 15. Összefoglalás

### **A szelektív szerotonin felvétel gátló antidepresszánsok hatása egyes szerotonin receptor altípusok működésére patkányban**

A szelektív szerotonin felvétel gátló antidepresszánsok (SSRI-k) hatásossága számos pszichiátriai kórképben igazolt. Ezek közé tartozik a depresszió, a disztímia, a pánikzavar, a kényszerbetegség, a szociális fóbia és a bulímia.

Annak ellenére, hogy a szerotonin felvétel gátlása röviddel az SSRI gyógyszerek bevitelét követően már megtörténik, a klinikai hatás késleltetett, és a kezelés kezdeti szakaszában legfeljebb csak a mellékhatások jelentkeznek. Az SSRI-k egyik ilyen lehetséges és viszonylag gyakori mellékhatása a szorongás. Akut alkalmazás mellett a szelektív szerotonin felvétel gátló gyógyszerek állatkísérletekben is szorongáskeltő hatást váltanak ki. A társas magatartás tesztben, patkányokon végzett vizsgálataink is a fluoxetin akut, dózisfüggő szorongáskeltő hatását bizonyították. Altípus-szelektív szerotonin receptor antagonistá vegyületek alkalmazásával kimutattuk, hogy a fluoxetin szorongást fokozó hatását az 5-HT<sub>2C</sub> receptorok aktiválása közvetíti. Vizsgálataink során bebizonyosodott, hogy az 5-HT<sub>2C</sub> receptorok aktivációja fokozza patkányokban a sztereotip mosakodást (self-grooming) amely a szorongással összefüggő magatartás, és ennek fokozott formája állatokban több szempontból is a humán kényszerbetegségre jellemző kompulzív viselkedés megfelelője.

Az egyik vezető elmélet szerint az SSRI-k terápiás hatásában és a mellékhatások iránti tolerancia kialakulásában egyes szerotonin receptorok deszenzitizációja fontos szerepet játszik. Vizsgálatainkban krónikus fluoxetin kezelést követően a kísérleti állatokban az 5-HT<sub>1A</sub> és 5-HT<sub>2C</sub> receptorok által közvetített egyes élettani és viselkedési válaszok mérséklődtek, ami alátámasztja az SSRI terápia következtében kialakuló szerotonin receptor deszenzitizáció hipotézisét.

A központi idegrendszer szerotonin koncentrációjának kémiai úton történő tartós csökkentése kísérleteinkben fokozta az 5-HT<sub>2C</sub> receptor agonista *m*-CPP hatását (fokozódott a self-grooming és az erekciós válasz), ami arra utal, hogy alacsony agyi 5-HT szint mellett az 5-HT<sub>2C</sub> receptorok érzékenysége fokozódik. Ez az eredmény megerősíti azokat a korábbi klinikai vizsgálatokat, melyek csökkent szerotonin

transzmisszót és az 5-HT<sub>2</sub> receptorok szenzitizációját mutatták ki depressziós betegekben.

Az absence epilepszia genetikai állatmodelljén, a WAG/Rij patkánytörzsen végzett korábbi kísérletek a szerotonin, mint szabályozó neurotranszmitter szerepét bizonyították. Vizsgálatunkban fluoxetin adását követően emelkedett a kísérleti állatokban a tüske-hullám kisülések gyakorisága és időtartama. A fluoxetin és egy 5-HT<sub>2C</sub> receptor antagonistá vegyület együttes alkalmazásával ez a hatás szignifikánsan fokozódott, míg az 5-HT<sub>1A</sub> antagonistá csökkentette az SSRI epileptiform aktivitást növelő hatását. Ezek az eredmények arra utalnak, hogy a szerotonin több, különböző 5-HT receptoron keresztül modulálja a tüske-hullám kisüléseket, és e receptorok aktivációja egymással ellentétes hatást eredményezhet.

Összegzőképpen megállapíthatjuk, hogy az SSRI-k akut és krónikus hatásait számos 5-HT receptor altípus közvetíti. Vizsgálataink elsősorban az 5-HT<sub>1A</sub> és az 5-HT<sub>2C</sub> receptorokra irányultak, és bizonyították azok meghatározó szerepét a szorongás, a sztereotip mosakodás, az erekció, a testhőmérséklet és a tüske-hullám aktivitás szabályozásában. Továbbá kimutattuk e receptorok szenzitivitásának megváltozását a központi idegrendszer tartós 5-HT koncentráció változásának függvényében. Eredményeink elősegítik az egyes pszichiátriai és neurológiai kórképeket nagymértékben befolyásoló szerotonerg rendszer megértését, az SSRI antidepresszánsok hatásmechanizmusának tisztázását, és hozzájárulhatnak új, szerotonin receptorokra ható gyógyszerek kifejlesztéséhez.