

A validated animal model for the Serotonin Syndrome

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Declaration

I hereby declare that the work presented in this thesis has been conducted independently and without any inappropriate support, and that all sources of content, experimental or intellectual, are suitably referenced and acknowledged.

I further declare that this thesis has not been submitted before, either in the same or a different form, to this or any other university for a degree.

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Berlin, den 01.09.2014

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Abbreviations

5-HT	serotonin, 5-Hydroxytryptamine
5-HT _{1A} receptor	subtype 1A of the 5-HT receptor
5-HT _{2A} receptor	subtype 2A of the 5-HT receptor
5-HTP	5-hydroxy-L-tryptophan, 5-HT amino acid precursor
5-HTT	5-HT transporter
5-MeO-DMT	5-methoxy-dimethyltryptamine, unspecific 5-HT receptor agonist
5-MeO-T	5-methoxy-tryptamine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin, specific 5-HT _{1A} receptor agonist
ATO	atomoxetine, norepinephrine uptake inhibitor
bupirone	N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride
DOI	1-(2,5-dimethoxy-4-iodophenyl)-propan-2-amine
FLX	fluoxetine, selective 5-HT uptake inhibitor
LSD	lysergic acid diethylamide, (6aR,9R)-N,N-diethyl-7-methyl-4,6,6a,7,8,9-hexahydroindolo-[4,3-fg] quinoline-9-carboxamide, 5-HT _{2A} receptor agonist
MAO	monoamine oxidase
MAOI-I	monoamine oxidase inhibitor
MAOA-I	monoamine oxidase type A inhibitor
MAOB-I	monoamine oxidase type B inhibitor
MAOA/B-I	monoamine oxidase type A/B inhibitor
OXO	oxotremorine, unspecific muscarinic receptor agonist
pCA	para-chloroamphetamine, 5-HT releaser
SNRI	5-HT/norepinephrine uptake inhibitor
SS	5-HT syndrome
SRI	5-HT (re)uptake inhibitor
SSRI	selective 5-HT (re)uptake inhibitor
TCA	tricyclic antidepressant
TCB-2	(4-bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine hydrobromide, specific 5-HT _{2A} receptor agonist
TCP	tranylcypromine, monoamine oxidase type A/B inhibitor

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1 GENERAL INTRODUCTION

The serotonergic transmission system is a finely regulated system, which is involved in numerous physiologic functions such as cardiovascular, pulmonary and gastrointestinal function (Berger, Gray et al. 2009). It is in particular known for its involvement in the regulation of emotional processes, e.g. stress, fear and hormonal secretion. Additionally, the neurotransmitter serotonin (5-HT) has an important role for pathophysiologic conditions such as depression.

Currently, depression is one of the most widespread diseases globally (Stafford, MacDonald et al. 2001). It is assumed that 10-20% of the population goes through at least one depressive episode during their life (Hirschfeld 2012). Despite this estimation, large numbers of depressive patients are either not diagnosed or not adequately treated ("Recommendations for therapy by the drug commission of the German medical association", 2006). Nevertheless, awareness of this fact is rising lately. In recent years, the volume of antidepressant prescriptions, which are primarily 5-HT enhancing drugs, has been increasing due to the rising number of prescription, introduction of novel serotonergic drugs and extension of the medical indications (Hemels, Koren et al. 2002, Moore, Yuen et al. 2009).

The Serotonin Syndrome (SS) is an adverse, toxic drug reaction of 5-HT enhancing drugs (Boyer and Shannon 2005a). In the majority of these cases, only moderate symptoms are found (Sun-Edelstein, Tepper et al. 2008). Nevertheless, life-threatening cases of the SS were reported (Birmes, Coppin et al. 2003, Ener, Meglathery et al. 2003a).

Helpful is that criteria for diagnosis of a SS in human have been revised and specified over the years (Sternbach 1991b, Hegerl, Bottlender et al. 1998, Dunkley, Isbister et al. 2003, Boyer and Shannon 2005a). However, special cases of an SS such as atopic SS are often either not diagnosed or misdiagnosed, e.g. as a malignant neuroleptic syndrome (Boyer and Shannon 2005a). The symptoms are over large parts similar and not specific for either of them. The SS itself is characterized by the triad of

autonomic dysfunction (i.e. tachycardia, diarrhea, hyperthermia), neuromuscular excitations (i.e. hyperreflexia, tremor, myoclonus) and altered mental states (i.e. fear, agitation, confusion) (Boyer and Shannon 2005a).

The SS results from an excess of 5-HT typically following the ingestion of two 5-HT enhancing drugs. Causative drugs increase 5-HT levels in the synaptic cleft by different mechanisms of action such as an increase of 5-HT synthesis or release, prevention of 5-HT uptake or its degradation. The risk of precipitating the SS is increased if serotonergic drugs with different mechanisms of action are ingested (Bijl 2004). The broad spectrum of drugs implicated in the SS includes antidepressants, mood stabilizers, opioid analgesics, illicit drugs, and possibly drugs used in the treatment of migraine (Boyer and Shannon 2005a, Sun-Edelstein, Tepper et al. 2008, Gillman 2010).

Additional attention has focused recently on the 5-HT_{1A} receptor as a target for antidepressant action and potential triggering point for the SS when vilazodone as a dually active drug (SSRI and 5-HT_{1A} partial agonist) has expanded the available tools for the treatment of depression (de Paulis 2007). Another drug exclusively targeting the 5-HT_{1A} receptor is the 5-HT_{1A} partial agonist buspirone. Buspirone is recommended to be given in support of antidepressant medication as it is postulated to shorten the onset or even potentiate the effect of antidepressant therapy (augmentation procedure).

However, there is evidence from the clinic that buspirone in combination with the selective 5-HT uptake inhibitors (SSRI) fluoxetine or paroxetine can trigger an SS (Manos 2000, Jagstedt and von Bahr 2004). As novel compounds are developed for antidepressant therapy that are full agonists at the 5-HT_{1A} receptor, more cases of the SS are expected (Maurel, Autin et al. 2007, Si, Gardner et al. 2007, Zajdel, Subra et al. 2007).

The demand for a comprehensive animal model of the SS does therefore not only result out of necessity for assessment of toxic effects but also in its application as a tool in experimental research, e.g. for the elucidation of the serotonergic system by drugs with defined mechanism of action. Other applications are the characterization of novel drugs and as a translational tool between clinical and experimental research.

So far there have been several attempts to establish experimental animal models for the SS in rats and mice. Variations to experimentally produce an SS include combinations of SSRI, tricyclic antidepressant (TCA) or selective noradrenalin reuptake inhibitor (SNRI) with monoamine oxidase inhibitors (MAOI) (Shioda, Nisijima et al. 2004, Izumi, Iwamoto et al. 2006, Speiser, Fine et al. 2008). Administrations of 5-HT, 5-hydroxy-L-tryptophan (5-HTP) or L-tryptophan (L-Trp) alone or in combination with a MAOI are also documented in the literature (Shimomura, Mori et al. 1981, Shioda, Nisijima et al. 2004, Zajdel, Subra et al. 2007). Finally, an SS produced by 5-HT_{1A} agonists is reported as well as spontaneous appearance of a SS in 5-HT transporter knockout mice (Fox, Jensen et al. 2007, Kalueff, Fox et al. 2007).

The symptom or response complex of the SS of laboratory rodents is not clearly defined as changes or occurrences of behavioral and autonomic SS-like responses. Frequently described SS-like responses in rats and mice include: hindlimb abduction, rigor, forepaw treading, head weaving, Straub tail, tremor, flat body posture, backward walking, salivation and piloerection (Darmani and Ahmad 1999, Kalueff, LaPorte et al. 2008). Problematic is principally the focus on different responses, which are assessed in the investigations. The various authors consider different SS-like responses as relevant and never describe the whole spectrum of SS-like responses. Instead, they focus primarily on two to three parameters in their experiments that are often not comparable with each other. Further, the responses are frequently not precisely defined. Systematic studies where the SS was produced by different means under the same conditions are missing as well.

Animal models are a useful tool of research to gain insights into human conditions otherwise inaccessible. Based on careful studies of the literature, statements on face and predictive validity have been possible (Kalueff, LaPorte et al. 2008). However, predictive validity may not be applicable to ensure that drugs are antidepressants but instead that drugs could cause a SS or trigger relevant effects on certain 5-HT receptors.

The aim of this dissertation is the identification of core responses of the SS, which can serve as target parameters in a murine model of the SS. To that end, I first provide a comprehensive assessment of variants of

animal models of the SS in literature and report the outcome of a systematic analysis of the literature on experimental research of the SS in rats and mice. Second, I present the results of an experimental study of the SS using indirect 5-HT receptor agonists. The specific aims of this study were the identification of core SS-like responses in mice, the characterization of the synergism of combinations of 5-HT enhancing drugs and the specificity of the effects of 5-HT enhancing drugs. Third, I describe the results of an investigation with agonists at the 5-HT_{1A} receptor or the 5-HT_{2A} receptor. Here, the aim was to clarify the impact of these two receptors on the SS in the mice.

“Animal models of the Serotonin Syndrome (SS): A Systematic Review”

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„The Murine Serotonin Syndrome – Evaluation of Responses to 5-HT-enhancing Drugs in NMRI Mice“

p 23-29

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“The Murine Serotonin Syndrome and the 5-HT1A Receptor -- Behavioral Effects and Hypothermia”

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“Role of 5- Role of 5-HT1A and 5-HT2A Receptors in the Murine Model of the Serotonin Syndrome”

p 57-61

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6 GENERAL DISCUSSION

The SS in humans is a potentially life-threatening toxic reaction that can occur in response to treatment with drugs enhancing the serotonergic tone. The SS typically develops when two drugs are ingested, but cases of SS have been reported after one drug in overdose.

Animal models of the SS can be used to evaluate the risk of serotonergic drugs to elicit the syndrome in humans or to study the mechanisms that are involved in the SS. In rats and mice, administrations of serotonergic drugs in overdose produce various motor and vegetative signs. However, it is still a matter of debate, which signs should be the focus of the assessments. Additionally, the utility of an animal model of the SS for translational research is dependent on its predictive validity and the specificity of symptoms and neither predictive nor the specificity have been evaluated for murine models of the SS.

The aims of my dissertation were threefold: First, the identification of core responses of the SS, which can serve as reliable and robust target parameters in a murine model of the SS. To that end, an analysis was conducted on the literature of experiments in rats and mice treated with serotonergic substances and consecutive recording of the behavioral and vegetative signs. Based on the results of the analysis, the effects of treatment with representative 5-HT enhancing drugs administered to mice were studied, in order to determine whether there are reliable signs in mice. Secondly, to assess the validity of this murine model of the SS and its specificity, the response of non-serotonergic drugs known to elicit a spectrum of signs similar to the SS was studied. Third, to determine the involvement of 5-HT receptors in the triggering of the SS signs, the response in mice to treatment with specific agonist at the 5-HT_{1A} and the 5-HT_{2A} receptor was examined.

The serotonin syndrome in laboratory animals

The administration of direct and indirect 5-HT receptor agonists induces characteristic motor and vegetative signs in laboratory animals especially

rats and mice. Reported since the 1950s (Udenfriend, Weissbach et al. 1957, Page 1958), these responses in laboratory rodents result from increased serotonergic tone and some of these responses resemble the SS in man (Jacobs 1976, Kalueff, LaPorte et al. 2008).

Since the first report on “the serotonin-mediated behavioral syndrome” in rats was published in 1974 (1974), researchers have mainly evaluated the response to serotonergic drugs in rats and the majority of the publications thus reports behavioral data from this species. Over the last decades various responses have been reported and researchers proposed several response combinations, which are essential to measure the SS.

In mice, there have been attempts to establish models of the SS. However, the SS in the mouse is not clearly defined; the essential components of the SS have not been identified. The different authors consider different responses as relevant and the composition of the assessed responses varies. For mice there has not been an investigation aimed to determine “core” components of the murine SS. Additionally, there is considerable heterogeneity in animal models of the SS reported across publications. Different assessment methods, different sets of responses, and different scales when assessing the effects of serotonergic drugs make quantitative comparisons of results across laboratories problematic.

Literature analysis of the Serotonin Syndrome in laboratory rodents

To identify core responses of the SS, I conducted a systematic analysis on the literature of experimental models of the SS using laboratory animals (see chapter 2 Animal Models of the Serotonin Syndrome (SS): A Systematic Review). Data from 109 publications was analyzed, which reported for rats and mice the effects of 5-HT-enhancing drugs or 5-HT receptor agonists on behavioral and autonomic SS-like responses. These publications comprise investigations of behavioral and/or autonomic manifestations of the SS in rats (85), in mice (20), or in both species (4). Other species were excluded from the literature analysis, since publications on the toxic effects of serotonergic drugs in species other than rats or mice are rare.

The included publications were also examined for data on strain and sex of the animals and administered drugs or drug combinations. Although some authors reported differences on the spectrum of the responses due to strain and sex, these parameters were given only in few publications and thus conclusion from analysis of these parameters are problematic and thus excluded. Data from transgenic mice was also excluded as genetic animal models of the SS were the subject of a recent review (Kalueff, LaPorte et al. 2008).

The SS was typically produced by administration of combinations of two serotonergic drugs with differing mechanism of action. Serotonergic drugs, that induced serotonin-like responses, include 5-HT-enhancing drugs and direct agonists at 5-HT receptors. 5-HT enhancing drugs either increase 5-HT synthesis or release, or inhibit 5-HT uptake or degradation. 5-HT synthesis increasing drugs are 5-HT amino acid precursors such as 5-HTP. Administration of 5-HT precursors leads to increased availability of 5-HT. 5-HT releasing drugs such as fenfluramine cause release of 5-HT from synaptic vesicles, which raises 5-HT levels in the synaptic cleft. Levels of 5-HT in the synaptic cleft are decreased by the 5-HT uptake transporter (5-HTT) or by the metabolizing enzyme MAO. SRI or SSRI such as fluoxetine (FLX) block the 5-HTT and thus keep 5-HT levels high in the synaptic cleft. Degradation by MAO is inhibited by selective inhibitors of MAOA-I, e.g. clorgyline, MAOB-I e.g. deprenyl or nonselective MAOA/B-I e.g. tranylcypromine (TCP). Direct stimulation of 5-HT receptors also results in distinct responses. 5-HT receptor agonists used in studies of serotonergic toxicity include the agonist 5-MeO-DMT (Green and Youdim 1975, Green 1978, Sloviter, Drust et al. 1978), full agonists at 5-HT_{1A} receptors such as 8-OH-DPAT (Smith and Peroutka 1986, Goodwin, De Souza et al. 1987) or partial 5-HT_{1A} receptor agonists e.g. buspirone (Koenig, Meltzer et al. 1988, Blanchard, Shepherd et al. 1993, Blanchard, Griebel et al. 1997), the 5-HT_{2A/2C} receptor agonist DOI (Arnt and Hyttel 1989, Berendsen and Broekkamp 1990) and the 5-HT_{2A/2C/3} receptor agonist quipazine (Green, Youdim et al. 1976).

Analysis of the effects of drug administrations revealed that several responses were regularly observed in rats and mice. In rats, following treatment with various drugs and drug combinations in different laboratories, the most frequent serotonin-like behaviors were forepaw treading, head weaving, hind limb abduction, low body posture, Straub tail and tremor, whereas backward walking can be neglected. These set of responses is considered the “traditional SS-like behaviors”.

Hyperactivity and wet dog shakes are supportive behavioral signs for an SS in rats while temperature dysregulation, either up- or down, can be a supportive vegetative sign in rats.

In mice, the “traditional behavioral responses” best characterize the SS, with the exception of fore paw treading. Investigations should thus focus on backward walking, head weaving, hind limb abduction, low body posture, Straub tail, tremor and hyperactivity in studies on the SS. Only body temperature down-regulation is supportive of a SS in mice. Moreover, the head twitch response, which is induced by 5-HT_{2A} receptor activation, can be regarded as a reliable response of the murine SS.

Experimental determination of core symptoms of the murine Serotonin syndrome in NMRI mice

In the literature analysis, backward walking, flat body posture, hindlimb abduction, head weaving, Straub tail and tremor were identified as the most frequently reported signs for an SS in mice. In order to check whether these signs are reliably produced in mice independently of the mechanism of action, the response to treatment with different 5-HT enhancing drugs was evaluated in male NMRI mice (see chapter 5 The Murine Serotonin Syndrome – Evaluation of Responses to 5-HT-enhancing Drugs in NMRI Mice). Three 5-HT enhancing drugs well-known to elicit the SS were chosen as representative drugs: The 5-HT precursor 5-HTP, the SSRI fluoxetine and the MAOA/B-I tranylcypromine (Ener, Meglathery et al. 2003a, Boyer and Shannon 2005a), which were each studied at three different doses. The assessments consisted of all behavioral and vegetative SS-like responses included in the earlier literature analysis and were conducted for a longer

period than the typically reported 25 min in the literature and set at 60 min according to the different half-lives of the tested drugs.

The three drugs hold in common the following five signs: flat body posture, hindlimb abduction, piloerection, tremor and decrease in rearings. With ascending dosage, the number of responses and the frequency of their occurrence increased, which is similar to the continuous spectrum in humans in which the SS starts with side-effects continuing to toxicity.

This spectrum of signs only partially overlaps with the most frequently reported signs identified earlier in the literature analysis (see Table 1). While other studies in mice confirmed the observed spectrum of signs (Hwang and Van Woert 1979, Blanchard, Griebel et al. 1997, Bert, Fink et al. 2006, Fox, Jensen et al. 2007, Fox, Jensen et al. 2008, Kreilgaard, Smith et al. 2008, Diaz and Maroteaux 2011), it is highly probable that strain differences contribute to the different spectra. In an earlier study, it was shown that the severity of tryptamine-induced head weaving and hindlimb abduction differs between mouse strains, which were attributed to varying levels of tryptamine in the brain (Yamada, Sugimoto et al. 1987).

Table 1. Comparison of the most frequent responses

Responses	Literature analysis	Experimental study			
	5-HT enhancing drugs	common signs of all three drugs	5-HTP	FLX	TCP
Behavioral					
Backward walking	■				
Flat body posture	■	■	■	■	■
Forepaw treading	■		■		
Head twitches	■		■		
Head shaking					
Head weaving	■				
Hindlimb abduction	■	■	■	■	■
Hunched back			■	■	
Piloerection		■	■	■	■
Rearing, decreased		■	■	■	■
Straub tail	■				
Tremor	■	■	■	■	■
Vegetative					
Defecation boli			■	■	
Salivation					

Fields in grey or black indicate presence of response after drug administration

FLX = fluoxetine; TCP = tranylcypromine

Besides the five signs that all three drugs hold in common, the study revealed that treatment with 5-HTP or fluoxetine produced additional signs, some of which were only observed after 5-HTP or fluoxetine. Most responses were observed after treatment with the higher doses (160 and 320 mg/kg) of 5-HTP (8 out of 14 recorded responses), whereas TCP (4 mg/kg) and FLX (20 mg/kg) induced 5 and 7 responses out of 14, respectively. The efficacy of 5-HTP to increase 5-HT synthesis was demonstrated in 5-HTP treated rats, in which brain 5-HT level rose to about 300% already after low dose (75 mg/kg) of 5-HTP (Nakatani, Sato-Suzuki et al. 2008). Further increases of 5-HTP doses lead to an tremendous elevation of extracellular 5-HT levels, which was explained by the additional conversion of 5-HTP to 5-HT in dopaminergic neurons (Stamford, Kruk et al. 1990). The broader response spectrum might thus be caused by stronger

augmentation of serotonergic tone in the 5-HTP-treated mice. Besides the explanation that 5-HTP leads to a higher 5-HT release than FLX and TCP, it has to be taken into account, that both drugs possess secondary pharmacological properties including actions at other transmission systems. TCP is a nonselective MAO type A and B inhibitor, which also affects the degradation of dopamine and noradrenaline. FLX is known to increase noradrenaline and dopamine extracellular levels in brain (Bymaster, Zhang et al. 2002). These secondary pharmacological properties are a potential cause for additional effects, which contribute false-positively to the SS.

Not evoked by any of the three drugs were backward walking, head weaving and shakes, and salivation. This has also been observed in other mouse studies that evaluated the response to 5-HTP (Fox, Jensen et al. 2007, Fox, Jensen et al. 2008). The surprising absence of the Straub tail after all treatments may be accounted for by strain differences and procedural variables.

Summarizing the results, if in NMRI mice the effects of serotonergic drugs are evaluated, at least flat body posture, hindlimb abduction, piloerection and tremor as well as decreased rearings should be assessed. These parameters can serve as reliable and robust target parameters in a murine model of the SS, although it has to be taken into account that various 5-HT enhancing drugs can induce a diverse spectrum of responses. 5-HTP should be included as a standard when this murine model of the SS is established in the laboratory.

Potentiation of effects after treatment with combinations of 5-HT enhancing drugs

In laboratory rodents, drug potentiation effects have been assumed following combinations of 5-HT-enhancing drugs and it has been termed as the “5-HTP potentiated behavioral syndrome” (Ortmann, Waldmeier et al. 1980, Kreilgaard, Smith et al. 2008) and the behavioral syndrome “after tryptophan loading” (Grahame-Smith 1971). However, in mice it has never been examined whether there is a potentiating effect after administration of two serotonergic drugs.

To characterize the interaction pharmacologically, the minimal effective doses of 5-HTP, FLX or TCP were first determined. The minimal effective dose was defined as the dose evoking at least one of the SS-like responses in the previous experiments with the three serotonergic drugs each given alone. These doses were administered in combinations of two drugs.

All three combinations (5-HTP+FLX; FLX+TCP, 5-HTP+TCP) elicited at least the five common responses. Additionally, the number of responses increased and the severity of each response was markedly higher for the drug combinations than the single drugs. Piloerection is the only exception, as it reached already at lower doses the maximal possible frequency, thus indicating a ceiling effect.

These findings argue for an over-additive effect on the responses, following the administration of combinations of 5-HT enhancing drugs. Results from brain dialysis studies in rats are in support of this notion, as it was shown that 5-HT levels in the brain were dramatically elevated after two 5-HT drugs compared to single drug. FLX alone caused a 2-4fold increase in extracellular 5-HT levels, which was increased by additional treatment with 5-HTP to 10-16fold (Perry and Fuller 1993, Li, Perry et al. 1996). Moreover, results from clinical studies suggest a potentiating effect, since cases of the SS are most frequently reported after the ingestion of MAOA/B-I together with an SRI or 5-HT releaser in therapeutic doses (Gillman 1998a, Bijl 2004). The SS in mice thus seems to reflect the increased risk by concomitant ingestion of two 5-HT enhancing drugs.

The specificity of the murine model of the SS

SS-like signs have been reported after drugs targeting non-serotonergic transmission systems (2005) (2008). To assess the specificity of the murine model of the SS, I studied the response of high doses of agonists at the dopamine receptor, apomorphine (APO), the acetylcholine receptor, oxotremorine (OXO) and the noradrenaline receptor, atomoxetine (ATO). These three drugs each induce behavioral and vegetative effects which resemble some SS signs.

APO, ATO and OXO induced some of the five signs, which were identified as common signs of the SS: Hindlimb abduction, piloerection, tremor or decreased rearings were variably observed after treatment with APO, ATO or OXO (see Table 2.). Marked differences between the types of tremor were found for 5-HT enhancing drugs and OXO. While the former was mild, the latter tremor was severe and vigorous. While the ATO-induced tremor was similar to the 5-HT tremor, only few mice temporarily displayed it. Only flat body posture was solely produced by 5-HT enhancing drug treatment, which is in agreement with the assumption that this response is 5-HT_{1A} receptor mediated (see Hoyer, Hannon et al. 2002). Interestingly, I observed in some OXO or APO-treated mice the Straub tail, although other researches have also reported the Straub tail after APO or nicotine (1988, Fonck, Nashmi et al. 2003). The results from these comparisons thus suggest that drugs affecting other, non-serotonergic transmission systems can evoke similar responses. It is therefore recommended for drug screening test using the murine SS that receptor antagonist targeting the dopaminergic, the noradrenergic or the cholinergic transmission systems are included in the studies.

Table 2. Comparison of the effects of serotonergic and non-serotonergic drugs

Responses	5-HT enhancing drugs			
	5-HT enhancing drugs	ATO	APO	OXO
Flat body posture	■			
Hindlimb abduction	■	■	■	■
Piloerection	■	■	■	■
Tremor	■			■
Rearing, decreased	■		■	■

Fields in grey indicate presence of response after drug administration

ATO = atomoxetine; APO = apomorphine; OXO = oxotremorine

Involvement of 5-HT_{1A} and 5-HT_{2A} receptors in the murine Serotonin Syndrome

Only few 5-HT receptor subtypes have been implicated in the mediation of the murine SS. Predominantly implicated are pre- and postsynaptic 5-HT_{1A} receptors and the 5-HT_{2A} receptor. Several authors reported the behavioral effects of administrations of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Lucki, Nobler et al. 1984, Tricklebank, Forler et al. 1984, Yamada, Sugimoto et al. 1989, Blanchard, Griebel et al. 1997, Bert, Fink et al. 2006) and comparison of the results of the behavioral spectra induced by 8-OH-DPAT or 5-HTP reveals that there is an overlap. So far, there is no systematic study comparing the effects of drugs known to induce the SS in humans with agonists at the 5-HT_{1A} receptor. Analogous systematic studies with 5-HT_{2A} receptor agonists are also not conducted.

To determine the involvement of 5-HT-receptors in the triggering of the SS signs, the effects of treatment with the full and the partial 5-HT_{1A} agonists 8-OH-DPAT and buspirone and the 5-HT_{2A} receptor agonists TCB-2 were determined in NMRI mice.

My results show that the 5-HT_{1A}-receptor has a major role for the syndrome and that the two 5-HT_{1A} agonists with differing intrinsic activity induced a different spectrum of signs. After 8-OH-DPAT, low body posture, hindlimb abduction, Straub tail, tremor and decreased rearings were consistently observed, which coincides with results from other studies (Yamada, Sugimoto et al. 1988, Blanchard, Griebel et al. 1997, Bert, Fink et al. 2006, Fox, Jensen et al. 2007). Contrary to these studies, forepaw treading, backward walking and head weaves have not been observed. In addition was registered piloerection, which was reliably induced by 8-OH-DPAT. This is a rarely reported SS-like response in the literature (Hwang and Van Woert 1979, Diaz and Maroteaux 2011) that has so far not been reported after 5-HT_{1A} receptor agonists. The effects of the partial 5-HT_{1A} agonist buspirone on the murine SS-like symptoms have not been studied. In my study, the partial agonist buspirone was equally effective as the full agonist 8-OH-DPAT; treatment with buspirone produced most of the responses of the murine SS. The only difference in the responses of both

drugs was that solely 8-OH-DPAT treated mice showed the Straub tail. In summary, the results confirm the major role of the 5-HT_{1A} for the murine SS.

The partial agonist buspirone has a markedly lower efficacy regarding postsynaptically mediated 5-HT_{1A} receptor effects than the full agonist 8-OH-DPAT (Middlemiss and Tricklebank 1992, Millan, Rivet et al. 1992) (see Celada, Bortolozzi et al. 2013). In rats, only 5-HT_{1A} agonists with high efficacy and also 5-HT releasers but not the partial agonist buspirone triggered the Straub tail (Millan, Bervoets et al. 1991). The 8-OH-DPAT-induced Straub tail is supposed to be induced by postsynaptically located serotonergic receptors at the spinal level (Bervoets, Rivet et al. 1993, Bagdy and To 1997). In conclusion, analogous to rats, the results of these studies and my study shows that mice manifest the Straub tail only in response to treatment with high efficacy 5-HT_{1A} receptor agonists. Additionally, the Straub tail is highly selective for this 5-HT receptor subtype.

The results from the TCB-2 study suggest that the 5-HT_{2A} receptor is involved in more SS-like responses than previously assumed. In an earlier study it was reported that the 5-HT_{2A} receptor agonist TCB-2 evokes in mice the head twitch response and hypothermia (Fox *et al.*, 2010). Head twitches were also produced by TCB-2 in my experiment. It is known since the '80s that the 5-HT_{2A} receptor activation produces head twitches (Hoyer, Hannon et al. 2002, Van Oekelen, Megens et al. 2002), which has been used as parameter for the assessment of drug effects at this receptor subtype. I showed earlier that head twitches are also induced by 5-HTP administrations (see chapter 5). Both findings together argue that the head twitch is a part of the SS in the mice. Additionally, it was clearly demonstrated that TCB-2 induces also flat body posture, hindlimb abduction, piloerection and decreased rearings. Evidence for a 5-HT_{2A} receptor mediation of SS-like responses has so far only been reported in rats (Backus *et al.*, 1990; Cowen *et al.*, 1982; Green *et al.*, 1981; Green *et al.*, 1983; Green *et al.*, 1976; Nimgaonkar *et al.*, 1983; Pranzatelli and Pluchino, 1991). My findings demonstrate in mice that the 5-HT_{2A} receptor not only induces head twitches but is also involved in the induction of SS-like responses.

Summarizing, 8-OH-DPAT, buspirone and TCB-2 produce flat body posture, hindlimb abduction, piloerection and decreases of rearings (see Table 3). These responses seem not specific for either 5-HT_{1A} or 5-HT_{2A} receptor activation. Solely after 5-HTP, forepaw treading and hunched back were observed indicating that the 5-HT_{1A} or 5-HT_{2A} receptor are not involved but other 5-HT receptor subtypes. Although the results implicate that tremor is a response induced by 5-HT_{1A} receptors, it was reported in another study that paw tremor was observed after DOI, a 5-HT_{2A/2C} receptor agonist. Thus, a 5-HT_{2A/2C} receptor involvement in the induction of tremor cannot be excluded. Neither 8-OH-DPAT and buspirone nor TCB-2 treatment produced backward walking, head shaking and head weaving or salivation in mice, confirming my earlier results with 5-HT enhancing drugs. After 5-HTP, FLX or TCP, none of these four signs were consistently and reliably observed which suggest that these signs are not reliable indicator for the SS in mice.

Table 3. Overview of the SS-like responses induced by 5-HTP, 8-OH-DPAT, buspirone and TCB-2

Responses	5-HTP	8-OH-DPAT	buspirone	TCB-2
Flat body posture	■	■	■	■
Hindlimb abduction	■	■	■	■
Piloerection	■	■	■	■
Tremor	■	■	■	
Decrease in rearing	■	■	■	■
Fore paw treading	■			
Head twitches	■			■
Hunched back	■			
Straub tail				
Changes in defecation	■			■

Fields in grey indicate presence of response after drug administration

Validity of the Serotonin Syndrome in mice for the human syndrome

The murine model for the SS is suitable to study serotonergic hyperactivity for basic research purposes and to screen drugs or drug combinations for their potential risk to induce the SS in man. Since research in humans on the toxicity of serotonergic drugs is prevented by ethical considerations, the murine model of the SS provides a way to gain further insights into the condition. To be of use in preclinical research, the potential animal model of the SS must fulfil certain prerequisites. Of major importance is validity (Willner 1986, van der Staay 2006, van der Staay, Arndt et al. 2009).

Within the context of animal models, validity concerns the relationship between a model and the modeled condition (Willner 1986). It is defined as “(...) the agreement between a test score or measure and the quality it is believed to measure” (Kaplan 1997). There are several forms of validity. Particularly important for behavioral research are face, predictive and construct validity (Willner 1986, Ellenbroek and Cools 1990, van der Staay 2006, van der Staay, Arndt et al. 2009). For the evaluations of these three forms of validity, criteria have been proposed to establish whether an animal model has validity (Willner 1986, van der Staay 2006).

Although face, predictive and construct validity were originally considered equally important with regard to animal models (Willner 1986), subsequently they were put in hierarchical order. Whereas there is no consensus in the literature whether face validity or predictive validity is considered as least important (Ellenbroek and Cools 1990, van der Staay 2006), the majority of the authors give construct validity the highest priority (Ellenbroek and Cools 1990, Kalueff and Tuohimaa 2004, van der Staay 2006, van der Staay, Arndt et al. 2009).

Construct validity concerns the theoretical rationale of the test, i.e. the performance in the test agrees to the theory of how the construct should behave (Willner 1986, van der Staay 2006). In behavioral research, construct validity of an animal model is generally thought to be present if causes and processes involved in the etiology of a condition are homologous in the model species and humans (van der Staay 2006), i.e. drugs that precipitate an SS in humans should induce a similar condition in laboratory animals.

In the SS modeling of the SS in mice, construct invalidity is already assumed as the diagnosis of SS in humans includes the ingestion of substances and the same drugs produce in mice also a syndrome. Additionally, 5-HT receptors subtypes have relatively similar functions in mouse and man.

In humans, it is assumed that the 5-HT_{1A} and the 5-HT_{2A} receptor play a role in the SS (Ener, Meglathery et al. 2003a, Isbister and Buckley 2005a, Gillman 2010). Two clinical cases of the SS after ingestion of the 5-HT_{1A} receptor agonist buspirone are documented (Goldberg and Huk 1992, Manos 2000). After buspirone was added to the treatment with FLX, symptoms of the SS including myoclonus, diaphoresis and diarrhea developed and the SS was diagnosed. The relevance of the 5-HT_{2A} receptor for the SS is based on evidence that antagonism at this receptor ameliorates the SS (Kolecki 1997a, Kolecki 1997b, Graudins, Stearman et al. 1998). In several clinical cases of the SS due to ingestion of drugs such as SSRIs and MAO-Is, the treatment with the 5-HT_{2A} receptor antagonist cyproheptadine led to rapid improvement. Currently, the management of the SS includes treatment with 5-HT_{2A} receptor antagonists (Boyer and Shannon 2005a, Gillman 2005).

Similar, a major role for the 5-HT_{1A} and 5-HT_{2A} receptor is supported by the results in NMRI mice with 5-HT_{1A} and 5-HT_{2A} receptor agonists (see chapter 5). Following single administration of the 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone, the 5-HT_{1A} receptor agonists produced the core responses of the murine SS. This is corroborated by the results of several other studies (Yamada, Sugimoto et al. 1988, Yamada, Sugimoto et al. 1989, Bill, Knight et al. 1991, Blanchard, Griebel et al. 1997, Bert, Fink et al. 2006, Fox, Jensen et al. 2007, Fox, Jensen et al. 2008). It also suggests that the 5-HT_{2A} receptor has a much larger role in the murine SS than previously assumed since treatment of mice with the 5-HT_{2A} receptor agonist TCB-2 not only induced head twitches, which is well-known to be mediated by the 5-HT_{2A} receptor. Additionally, TCB-2 induced SS-like responses such as flat body posture, hind limb abduction, piloerection and decreased rearings. Results from studies in mice (Fox, Jensen et al. 2007),

rats (Green, Youdim et al. 1976, Green, Hall et al. 1981, Cowen, Grahame-Smith et al. 1982, Green, O'Shaughnessy et al. 1983, Nimgaonkar, Green et al. 1983, Backus, Sharp et al. 1990, Pranzatelli and Pluchino 1991) and pigs (Loscher, Witte et al. 1990) support the assumption that the 5-HT_{2A} receptor has a major role in the SS. Furthermore, the rationale for a role of the 5-HT_{1A} and the 5-HT_{2A} receptor in humans is in part based on results obtained from studies in animals (Nisijima, Yoshino et al. 2001, Isbister and Buckley 2005a, Shioda, Nisijima et al. 2010), in which initially evidence for an impact of these two receptor was found (Green, Heal et al. 1983, Green, O'Shaughnessy et al. 1983, Tricklebank, Forler et al. 1985, Eison and Wright 1992).

Therefore, it can be concluded that there is general agreement between human and mouse in relation to the mediation of the SS, which involves in both species primarily the 5-HT_{1A} and the 5-HT_{2A} receptor.

The pathology of the SS in humans has not been systematically and extensively studied. This is mainly due to the absence of methods allowing the direct examination of the 5-HT system during a SS in humans such as the direct measurement of 5-HT in the brain of rats or mice. Currently, assessment of 5-HT levels in humans are based on parameters such as 5-HT metabolites in the cerebrospinal fluid (McKie, Del-Ben et al. 2005) or urine (Ener, Meglathery et al. 2003a).

However, assessments of 5-HT metabolites are not able to determine heightened levels of 5-HT during a SS (Ener, Meglathery et al. 2003a). Direct examinations of the 5-HT system have been conducted in laboratory rodents. Accordingly, the effects of 5-HT enhancing drugs on brain 5-HT is well-documented in the literature on the SS. Numerous rodent studies using methods such as microdialysis reported increased 5-HT levels in the brain after 5-HT enhancing drugs (Sharp, Gartside et al. 1997, Felton, Kang et al. 2003, Nisijima, Shioda et al. 2004, Shioda, Nisijima et al. 2004, Nisijima, Shioda et al. 2007, Nakatani, Sato-Suzuki et al. 2008, Zhang, Krishnamoorthy et al. 2009, Shioda, Nisijima et al. 2010). One study investigating the effects of 5-HT enhancing drugs in mice reported that drastically increased brain 5-HT levels coincides with the manifestations of

SS-like responses (Fox, Jensen et al. 2008). The drug-induced increases of 5-HT levels in laboratory rodents may occur analogously in humans. Results from mice injected with single administrations of three SSRIs show that in mice SSRI plasma steady state levels and resulting SSRI occupancy at the uptake transporter corresponded to humans (Meyer, Wilson et al. 2001, Meyer, Wilson et al. 2004, Kreilgaard, Smith et al. 2008). Serotonergic drugs elicit the syndrome in both species and at the same time dramatically increased 5-HT levels in the rodent brain are measured. This suggests that in clinical cases of the SS 5-HT levels are also increased in the human brain.

For the murine model of the SS construct validity is thus given since the triggering drugs are the same in man and mice and the 5-HT receptor subtypes 1A and 2A have similar functions in both species.

A model has face or phenomenological validity, if it resembles the condition being modeled (Willner 1986). Specifically, the symptoms in animals resemble symptoms in humans. The focus of studies in humans was on causative drugs and on the symptomatology of serotonergic hyperactivity (e.g. Sternbach 1991a, Sporer 1995, Lane and Baldwin 1997, Gillman 1998a, Ener, Meglathery et al. 2003b, Bijl 2004, Boyer and Shannon 2005b, Gillman 2005, Isbister and Buckley 2005b, Gillman 2006, Rusyniak and Sprague 2006, Nisijima, Shioda et al. 2007, Silins, Copeland et al. 2007, Odagaki 2009, Gillman 2010, Rastogi, Swarm et al. 2011, Grubb, Kennedy et al. 2012, Iqbal, Basil et al. 2012, Perry and Wilborn 2012). Consequently, the focuses for the assessment of face validity of the murine SS as a model of the human SS are similarity of symptomatology.

The criteria for face validity apply as follows to the murine SS: Humans and mice share the same 5-HT receptors and the 5-HT system has the same relevance for body functions such as body temperature, circadian rhythm, appetite and sexual function (Berger, Gray et al. 2009). In both species, 5-HT enhancing drugs can produce a syndrome with a broad spectrum of characteristic symptoms (Boyer and Shannon 2005a, Kalueff, LaPorte et al. 2008)(see chapter 2 Animal models of the Serotonin Syndrome (SS): A Systematic Review). In humans, the SS symptoms are categorized in the triad of neuromuscular excitations, autonomous

dysregulations and mental state alterations (Boyer and Shannon 2005a). Most symptoms are neuromuscular excitations such as several forms of cloni, hypertonicity and tremor (Dunkley, Isbister et al. 2003). In mice, the symptoms can be categorized into neuromuscular and autonomous responses (Kalueff, LaPorte et al. 2008). Likewise to men, the majority of these symptoms are neuromuscular responses. The traditional SS-like responses are all neuromuscular signs. Additionally, out of the five SS-like responses that were determined as core responses of the murine SS, four are neuromuscular responses (hindlimb abduction, low body posture, tremor, decreases of rearings) (see chapter 3). The autonomic response body temperature dysregulation is also an important feature of the SS in both men and mice. Mental state alterations in humans such as agitation, confusion and hypomania relate to hyperactivity in mice (Kalueff, LaPorte et al. 2008). It can be concluded that similar responses are produced by 5-HT enhancing drugs. The specific expressions may not be identical in men and mice, and some signs are also species-specific such as the Straub tail in mice. This is explained by the differing morphology including brain structures. However, similar signs observed in humans and NMRI mice. Thus, face validity has been demonstrated by the corresponding responses of motor and autonomic system.

Predictive validity is the extent to which a model can produce results that predict future events such as behaviors or effects (Willner 1986, van der Staay 2006). In pharmacology, this means the ability of a test to predict the effects of drugs in humans, i.e. testing of drugs in a mice model correctly identifies 5-HT drugs capable of precipitating the SS in humans (van der Staay 2006). Consequently, the evaluation of the predictive validity of a murine model of the SS used in preclinical pharmacology is based on the efficacy of a range of drugs. A mice model of the SS with predictive validity has utility as a tool in drug screenings of potential antidepressants.

Evaluation of predictive validity of the SS in mice is based on an analysis of drugs known to cause the SS in humans. MAO-Is and SRI/SSRIs such as TCP and FLX, respectively, are well-known to precipitate the SS in humans (Gillman 1998b, Sun-Edelstein, Tepper et al. 2008). The same

drugs and the 5-HT precursor 5-HTP, successfully elicit the SS in mice, as confirmed by the results with 5-HTP, TCP or FLX in mice (chapter 3). Although the spectrum of registered responses differed between the three drugs, five core responses were observed after treatment with the all three drugs. Further, the concomitant ingestion of two or more 5-HT enhancing drugs is thought to increase the risk for the precipitation of the SS in humans by dramatically increasing 5-HT levels. Especially the combination of a MAO-I with another serotonergic drug is associated with many cases of the SS. In mice, similar effects are observed using TCP together with FLX and 5-HTP. The concomitant administration induced in NMRI mice all five core responses at doses that were sub-effective when given in single administration. The predictive validity has been confirmed since the triggering drugs are the same in man and mouse and the over-additive effects of drug combinations in the murine model correspond to drug interactions reported in patients. Thus, the model can identify novel serotonergic drugs if the drug administration produces the five core responses together.

To summarize my results on the evaluations of validity: The murine SS has a high level of face validity since corresponding signs are observed in men and mice. Following administration of 5-HT enhancing drugs, in both species similar neuromuscular and vegetative signs are triggered although species-specific signs are observed. Predictive validity is also confirmed for the murine model. The same drugs induce a syndrome in both species and responses are more severe when more than one 5-HT enhancing drug is administered concurrently.

Utility of the murine model of the SS in preclinical and translational research

The murine SS has utility as a tool in drug discovery, examinations on the mechanism of action of serotonergic drugs or the pathology of the SS and risk assessment. In the search for drugs that are effective in the therapy of depression and other mood disorders, the murine model can identify

compounds targeting the 5-HT system. Compounds that elicit the core symptoms of the murine SS are drugs with serotonergic action.

Another application of the model is in investigations on the mechanism of action of serotonergic drugs. For instance, the results of my investigations in mice using the SSRI FLX indicate an effect on 5-HT receptors (Chapter 4). FLX induced markedly the hunched back, which is a 5-HT_{2C} receptors mediated response (Van Oekelen, Megens et al. 2002). Evidence for an antagonist action of FLX at the 5-HT_{2C} receptors was demonstrated in previous studies using mouse and rat brain tissue (Chen, Peng et al. 1995, Palvimaki, Roth et al. 1996) and it was reported that the 5-HT_{2C} receptor has an impact on anxiety-like behavior in mice (Berendsen and Broekkamp 1994). Recently, a critical role of the 5-HT_{2C} receptor in the expression of anxiety-like behavior of mice was confirmed in 5-HT_{2C} receptor knockout mice (Heisler, Zhou et al. 2007). Although this receptor has currently little clinical relevance for the treatment of anxiety, the 5-HT_{2C} antagonist and melatonergic agonist agomelatine is successfully used in the treatment of depression (Loo, Hale et al. 2002).

It is important to consider that examinations of the SS in mice might provide insights into the pathology of the human SS. As mentioned above, methods that measure the concentration of 5-HT levels such as microdialysis are not possible in humans, primarily for ethical reasons. Investigations using microdialysis in laboratory rodents have confirmed the increased risk of serotonergic drug combinations, which have a potentiating effect on the serotonergic neurotransmission system. As long as methods allowing direct analysis of processes in the brain are not available for humans, animal models of SS provide a means to elucidate the mechanisms underlying the SS in humans.

Finally, using the murine SS in the risk assessment of serotonergic drugs seems to be the most relevant application of this model. The use of the model in drug safety screenings concerns not only novel compounds but also clinically used drugs. In humans, the SS has become an increasing clinical problem due to the introduction of novel serotonergic drugs, and the rising numbers of prescriptions for serotonergic drugs (Graudins, Stearman

et al. 1998, Isbister and Buckley 2005a, Thanacoody 2012). However, the potential of a serotonergic drug to elicit the SS is frequently not known. In view of this, a major application for the murine model of the SS is its use in the evaluation of a drug side effects profile and potential risk to produce the SS, especially in the preclinical phase. The incorporation of the murine model of the SS into the preclinical evaluation phase will be essential for risk assessment in the future. Since ingestion of two or more serotonergic drugs is associated with an increased risk of inducing the SS in humans as well as in laboratory animals, the evaluation of combinations is recommended. For this purpose, it is recommended that novel compounds are evaluated together with drugs, which are known to have a particularly high potential to precipitate the SS such as MAO-I or SRI.

Renewed interest in the 5-HT_{1A} receptor as a potential target for the therapy of several disorders underlines the importance of such drug safety screenings. Besides the use of 5-HT_{1A} receptor agonists in the treatment of anxiety and depression (Blier and Ward 2003), new lines of evidence suggest a role for such agonists in the therapy of schizophrenia and Parkinson disease (see Ohno 2011). Treatment with 5-HT_{1A} agonists attenuated side effects of antipsychotic medication such as D₂ receptor antagonist-induced extrapyramidal symptoms (Prinssen, Colpaert et al. 2002) and alleviated affective symptoms (Akimova, Lanzenberger et al. 2009). Similarly, core symptoms of Parkinson disease and side effects of anti-Parkinson medication were improved by 5-HT_{1A} agonists (Tomiya, Kimura et al. 2005, Dupre, Eskow et al. 2008). Accordingly, the idea of broadening spectrum the indications of such agonists has led to the ongoing development of drugs that target the 5-HT_{1A} receptor. When these are therapeutically used, they add to the rising number of prescriptions of serotonergic drugs and consecutively the risk of more cases of the SS.

Of major importance for the results in future preclinical drug research as well as in basic research is the standardization of the assessment procedure. Especially in respect to the selection of responses, it is recommended that at least the core SS-like responses flat body posture, hindlimb abduction, piloerection, tremor and the number of rearings are

assessed. This facilitates comparisons across drugs, studies and laboratories and improves the significance of results for research. Also, investigations of novel serotonergic drugs in the murine SS should include 5-HTP as a standard for comparison. Since this drug is known to induce the broadest spectrum of responses of the SS in humans as well as in mice, comparisons with the novel drug can provide an estimate of the efficacy of the novel drug. Additionally, it is of importance that strain differences are taken into account.

In summary, on the basis of my examination of the SS, the findings suggest that the murine SS has core SS-like responses that consistently indicate increased serotonergic tone and that it is a valid model for the humans SS. I determined core responses of the SS in NMRI mice that form together a cluster of responses, which consistently and reliably indicate increased tone of the serotonergic transmission system. Additionally, I discussed the face, predictive and construct validity of the model. Future investigations using the murine SS should study the effects of 5-HT enhancing drugs in NMRI mice, record at least the core SS-like responses and include 5-HTP as control, for a better comparability across drugs, studies and laboratories. This will increase the significance of the results in the behavioral research on the SS and may lead to the development of new serotonergic drugs, potentially with an improved side effect profile and recommendations regarding drug combinations to prevent side effects.

7 SUMMARY

The serotonin syndrome (SS) is a potentially life-threatening disorder in humans which is typically induced by ingestion of an overdose or by combination of two or more serotonin (5-HT)-enhancing drugs. In mice, acute administration of direct and indirect 5-HT agonists also leads to behavioral and autonomic effects, but in literature the murine SS is not clearly defined as different responses are thought to be essential.

The first aim of this dissertation is thus the identification of core responses of the SS, which can serve as target parameters in a murine model of the SS. It is the second aim to evaluate the specificity of the responses of murine SS and to determine the validity of these responses. The third aim is to define the impact of the 5-HT_{1A} and the 5-HT_{2A} receptor for the different SS responses.

First an overview is provided in a review of the existing versions of the animal model of the SS. With a focus on studies in rats and mice, data was extracted on the behavioral and autonomic responses following administration of serotonergic drugs administered alone or in combination. Based on the analyses of the data, a distinct set of responses was identified that are consistently observed following administration of serotonergic drugs.

In order to determine in NMRI mice common SS responses induced by 5-HT-enhancing drugs independent of the mechanisms of action, the effects of the three serotonergic drugs with differing mechanism of action were studied. The following five responses consistently and dose-dependently occurred: flat body posture, hindlimb abduction, piloerection, tremor, and decreased rearings. Additionally, combinations of drugs lead to a drug potentiation effect. The specificity of these five responses was evaluated by comparing the effects of the three serotonergic drugs with drugs targeting other transmission systems.

Finally, the effect of agonists at the 5-HT_{1A} and the 5-HT_{2A} receptor on the murine SS was examined in NMRI mice. On the basis of the findings, the major relevance of the 5-HT_{1A} receptor was confirmed. Additionally, it

was revealed that the 5-HT_{2A} receptor has more impact on the SS than previously suggested.

My findings demonstrate that the SS in NMRI mice is a suitable animal model for the SS in humans. It is a valuable tool to study serotonin-induced hyperactivity for both basic and preclinical research in order to identify drugs or drug combinations with potential risk to induce a serotonin syndrome in man. Further improvement is achieved by standardizing assessments of SS responses in rodents, which will increase the utility of animal models of the SS in translational studies. This research will be of importance in creating new effective therapeutic compounds, possibly with fewer side effects.

8 ZUSAMMENFASSUNG

Das Serotonin Syndrom (SS) ist eine potentiell lebensbedrohliche Arzneimittelwirkung im Menschen, welche in der Regel nach Einnahme von serotonergen Substanzen in Überdosis oder in Kombinationen von zwei oder mehreren auftritt. In Mäusen führt die akute Administration von direkten und indirekten Serotoninagonisten ebenfalls zu Verhaltens- und vegetativen Effekten. In der Literatur zur Maus sind diese Effekte jedoch nicht eindeutig definiert, da verschiedene Zeichen als relevant angesehen werden.

Das erste Ziel dieser Dissertation ist daher die Identifizierung von Kernsymptomen des SS, die Zielparameter für ein murines Model des SS sein können. Zweites Ziel ist die Evaluation der Spezifität der Zeichen des murinen SS und die Bestimmung der Validität der Zeichen. Das dritte Ziel ist die Relevanz der 5-HT_{1A} und 5-HT_{2A} Rezeptoren für die verschiedenen Zeichen zu bestimmen.

In einer Übersichtsarbeit wird zunächst ein Überblick über in der Literatur beschriebene Tiermodelle des SS gegeben. Der Fokus liegt auf Studien in Ratten und Mäusen, aus denen Daten extrahiert wurde zu Verhaltens- und vegetativen Zeichen, die nach Einzelgabe von serotonergen Substanzen oder in Kombination auftraten. Basierend auf der Analyse der Daten konnten Zeichen identifiziert werden, die konsistent nach der Gabe von serotonergen Substanzen beobachtet wurde.

Um in NMRI Mäusen gemeinsame Zeichen des SS zu bestimmen, die nach Gabe von serotonergen Substanzen aber unabhängig vom Wirkmechanismus auftreten, wurden die Effekte von drei serotonergen Substanzen mit unterschiedlichem Wirkmechanismus untersucht. Die folgenden Zeichen traten konsistent und Dosis-unabhängig auf: flat body posture, hindlimb abduction, piloerection, tremor, and verminderte Aufrichtebewegungen. Zusätzlich hatten Kombinationsgaben potentierende Substanzeffekte. Die Spezifität der fünf Zeichen wurde bestimmt durch einen Vergleich der Wirkungen der drei serotonergen Substanzen mit drei nicht-serotonergen.

Anschließend wurde die Validität des murinen Modells des SS überprüft auf Grundlage der aktuellen Literatur, der Übersichtsarbeit und meinen experimentellen Studien in Mäusen. Der Fokus lag dabei auf der Beteiligung der verschiedenen 5-HT-Rezeptoren und insbesondere auf dem 5-HT_{1A}-Rezeptor.

Zum Schluss werden die Ergebnisse einer Studie präsentiert, in der die Effekte von 5-HT-Rezeptoragonisten auf Zeichen des murinen SS untersucht wurden. Die Ergebnisse bestätigen die Relevanz des 5-HT_{1A} Rezeptors für das murine SS. Zusätzlich ist aufgezeigt worden, dass der 5-HT_{2A}-Rezeptor stärker am SS beteiligt ist als bisher angenommen wurde.

Meine Ergebnisse belegen, dass das SS in NMRI-Mäusen ein geeignetes Model für das SS im Menschen ist. Es ist ein wertvolles Instrument für Untersuchungen von Serotonin-induzierter Überaktivität und dies sowohl für die Grundlagen- als auch für die angewandte Forschung um potentiell gefährliche Substanz oder Substanzkombinationen zu identifizieren, die das SS im Menschen auslösen können. Weitere Verbesserungen können durch eine Standardisierung der Experimente erreicht werden, was den Nutzen für die präklinische und translationale Forschung erhöhen wird. Dies ist wichtig bei der Suche nach neuen therapeutischen Wirkstoffen mit eventuell weniger Nebenwirkungen.

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10 List of publications and contribution

This is a cumulative dissertation based on the below listed publications.

The contribution by the authors involved in the publications is listed according to the following criteria:

1. Idea, planning
2. Performing the experiment/search
3. Data analysis
4. Writing the manuscript

1. Haberzettl R¹, Bert B¹, Fink H¹, Fox M A²:

Animal Models of the Serotonin Syndrome: A Systematic Review.

***Behav. Brain Res.* 256C, 328-345.**

DOI: 10.1016/j.bbr.2013.08.045

1. Idea, planning: Haberzettl, Fink
2. Performing the search: Haberzettl
3. Data analysis: Haberzettl, Fink
4. Writing the manuscript: Haberzettl, Bert, Fink, Fox

2. Haberzettl R¹, Fink H¹, Bert B¹:

The Murine Serotonin Syndrome – Evaluation of Responses to 5-HT-enhancing Drugs in NMRI Mice.

***Behav. Brain Res.* 2014 Apr 26. pii: S0166-4328(14)00251-4.**

doi: 10.1016/j.bbr.2014.04.033 (epub ahead of print)

1. Idea, planning: Haberzettl, Bert, Fink
2. Performing the experiment: Haberzettl
3. Data analysis: Haberzettl, Bert, Fink
4. Writing the manuscript: Haberzettl, Bert, Fink

3. Haberzettl R¹, Fink H¹, Dietze S¹, Bert B¹: The Murine Serotonin Syndrome and the 5-HT_{1A} Receptor -- Behavioral Effects and Hypothermia

(Accepted: **Neuromethods**)

1. Idea, planning: Haberzettl, Bert, Dietze, Fink
2. Performing the experiment: Haberzettl
3. Data analysis: Haberzettl, Bert, Dietze, Fink
4. Writing the manuscript: Haberzettl, Bert, Dietze, Fink

4. Haberzettl R¹, Fink H¹, Bert B¹: Role of 5-HT_{1A} - and 5-HT_{2A} Receptors for the Murine Model of the Serotonin Syndrome
J Pharmacol Toxicol Methods 70(2): 129-133.

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1. Idea, planning: Haberzettl, Bert, Fink
2. Performing the experiment: Haberzettl
3. Data analysis: Haberzettl, Bert, Fink
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