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Research report

The murine serotonin syndrome – Evaluation of responses to 5-HT-enhancing drugs in NMRI mice

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HIGHLIGHTS

- Different 5-HT-enhancing drugs induce variable responses in NMRI mice.
- Five SS signs common for the different drugs were identified in NMRI mice.
- Combination of serotonin (5-HT)-enhancing drugs has a potentiation effect.

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ABSTRACT

In humans, the ingestion of the combination of two or more serotonin (5-HT)-enhancing drugs but also of a single drug in overdose can induce serious adverse effects, which are characteristics of the serotonin syndrome (SS). In mice, acute administration of direct and indirect 5-HT agonists also leads to behavioral and autonomic responses, but in literature different responses are thought to be essential. In order to detect common behavioral SS responses induced by 5-HT-enhancing drugs with different mechanisms of action, we investigated the effects of the 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP), the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX), and the monoaminooxidase (MAO) inhibitor tranylcypromine (TCP) in male NMRI mice. The drugs were administered alone or in combination to investigate additive effects or drug potentiation. Moreover, we compared the 5-HT responses to the effects induced by the dopamine, noradrenaline, and cholinergic agonists, apomorphine (APO), atomoxetine (ATO), and oxotremorine (OXO). Our results show that the studied 5-HT-enhancing drugs induced a different number of concomitant responses. The following five responses consistently and dose-dependently occurred in NMRI mice: flat body posture, hindlimb abduction, piloerection, tremor, and decreased rearings. Like in humans, the combination of 5-HT-enhancing drugs leads to a potentiation of drug effects. With the exception of flat body posture the responses are not specific for serotonergic hyperactivity. The findings demonstrate that the SS in NMRI mice is a suitable animal model for preclinical research, if it is taken into account that the spectrum of typical responses to 5-HT enhancing drugs may differ depending on drug and mouse strain and that some responses might be evoked by activation of other transmission systems, too.

1. Introduction

In humans, the serotonin syndrome (SS) is a potentially life threatening disorder, induced by drugs raising the activity of the serotonergic transmission system. An SS usually develops when two drugs, which increase extracellular concentration of serotonin (5-HT) or act as an agonist at 5-HT receptors, are ingested together. However, it can also be induced by single drug use in overdose. The symptomatology of the SS in man includes a triad of motor symptoms (e.g. myoclonus, tremor), autonomic responses (e.g. hyperthermia, tachycardia), and altered mental state (e.g. agitation, confusion; see [1,2]).

Abbreviations: 5-HT, serotonin; 5-HTP, 5-hydroxy-L-tryptophan; APO, apomorphine; ATO, atomoxetine; FLX, fluoxetine; MAO, monoamine oxidase; OXO, oxotremorine; SS, serotonin syndrome; SRI, serotonin reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCP, tranylcypromine.

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In laboratory rodents, a syndrome of serotonergic hyperactivity can be evoked by the same drugs and drug combinations as in man [see 3,4]. A prerequisite for the translational application of the murine SS as a preclinical screening test for unwanted drug effects is to evaluate the predictive validity of the assessed responses and their specificity.

In mice, several responses of motor and autonomic systems have been reported following the administration of 5-HT-enhancing drugs or direct 5-HT agonists [see 3,4]. However, it is still a matter of debate which responses are essential to determine the SS in mice [4–7]. Different authors consider different responses as relevant and do not make use of the same spectrum of responses [e.g. 3,5,7,8,9–11]. In order to examine which signs can be reliably provoked in a typical outbred mouse strain, we systematically investigated the effects of three 5-HT-enhancing drugs with different mechanisms of action, i.e. the 5-HT precursor 5-hydroxtryptophan (5-HTP), the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX), and the unspecific monoamine oxidase (MAO) inhibitor tranylcypromine (TCP), given alone and in combination, in NMRI mice. The spectrum of the examined behavioral signs included fourteen responses that were frequently reported in literature and can concomitantly be assessed [3,5,7,12].

The second aim of this study was to characterize the interaction of 5-HT-enhancing drugs, i.e. to clarify if the effect of two 5-HT-enhancing drugs concurrently administered is an additive one or a drug potentiation effect. It is known from clinical studies that combined ingestion of two 5-HT-enhancing drugs with different mechanisms of action in therapeutic doses are more likely to elicit an 5-HT toxidrome, suggesting a potentiation of unwanted drug effects [13,14]. In animal studies the "5-HTP potentiated behavioral syndrome" [15] and the behavioral syndrome after "tryptophan loading" [16] are examples for presumed potentiation effects in rats and mice. However, in these studies, 5-HTP or tryptophan were given in sub-effective doses. In our study combinations of 5-HT-enhancing drugs in mild effective doses were used enabling us to analyze drug interactions.

In laboratory rodents, dopamine and noradrenaline agonists as well as parasympathomimetics also induce motor and autonomic effects, which are known to resemble some of the SS responses. The third aim of our study in NMRI mice was hence to clarify which distinct responses to 5-HT-enhancing drugs are specific and which coincide with responses to agonists at dopamine, noradrenaline, and acetylcholine receptors. Therefore, we studied the effects induced by the dopamine receptor agonist apomorphine (APO), the noradrenaline reuptake inhibitor atomoxetine (ATO), and the unspecific muscarinic receptor agonist oxotremorine (OXO).

2. Materials and methods

2.1. Animals

Male NMRI mice (HsdWin:NMRI) at an age of 10 weeks were used in all experiments. The mice (n = 12–14 per group) were obtained from Harlan–Winkelmann (Horst, Netherlands) and were habituated in the animal facility for at least two weeks. The mice were pair-housed under standardized conditions (23 ± 2 °C room temperature, 50 ± 5% humidity) with an artificial 12/12 h light/dark cycle (lights on 06.00–18.00 h, illumination strength ca. 250 lx). Food (Altromin 1326, Lage, Germany) and tap water were available *ad libitum*. Experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the Berlin State Authority ("Landesamt für Gesundheit und Soziales").

2.2. Drug treatment

Fluoxetine (FLX; European Directorate for the Quality of Medicines & HealthCare (EDQM)), tranylcypromine (TCP; (±)-*trans*-2-phenylcyclopropan-1-amine hydrochloride; Sigma–Aldrich), apomorphine (APO; (R)-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo[de,g]quinolone-10,11-diol hydrochloride; Tocris Bioscience), oxotremorine (OXO; 1-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidinone sesquifumarate; Sigma–Aldrich), and atomoxetine (ATO; (R)-N-methyl-γ-(2-methylphenoxy)-benzenepropanamine hydrochloride; Tocris Bioscience) were freshly dissolved in 0.9% saline. 5-hydroxy-L-tryptophan (5-HTP; Sigma–Aldrich) was freshly prepared as a suspension in 0.9% saline with 1% Cremophor EL. All drugs and vehicle were injected intraperitoneally (i.p.) at a volume of 10 ml/kg body weight.

2.2.1. Experiment 1

In order to investigate SS responses after single drug administration the following dose ranges were used based on previous studies [7,10]: for 5-HTP 80, 160, and 320 mg/kg, for FLX 10, 20, and 40 mg/kg, and for TCP 1, 2, and 4 mg/kg. For ethical reasons, the doses of these drugs were below the lethal one. The maximal doses we have chosen for 5-HTP, FLX, and TCP were about 1/4–1/3 of the respective LD50 administered i.p. in mice [17–19]. Since no significant differences were observed, the vehicle groups for 5-HTP, FLX and TCP (n = 5, 5, and 4, respectively) were pooled to one control group.

2.2.2. Experiment 2

For the investigation of drug interactions we concomitantly administered 5-HTP with FLX, FLX with TCP, and 5-HTP with TCP in their minimal effective dose obtained by *Experiment 1*, i.e. 80 mg/kg 5-HTP, 10 mg/kg FLX, and 2 mg/kg TCP respectively. All drug combinations were administered as one injection.

2.2.3. Experiment 3

The doses of APO (4 mg/kg), OXO (0.5 mg/kg), and ATO (2 mg/kg) were chosen according to Lau et al. [20] and Maehara et al. [21] and to our own experience.

2.3. Behavioral test

The mice were transferred to the experimental room 12 h prior to the beginning of the experiment. On the following day between 08.00 and 12.00 h, the experiments were carried out in a sound attenuated chamber. For each 90 min trial four mice were tested at the same time. Immediately after drug or vehicle injection, each mouse was placed in the center of a black painted Makrolon type II cage (260 mm × 200 mm × 140 mm) and its behavior was evaluated by an observer blind to drug treatment. Between each trail, the cages were cleaned with a solution of water and detergent.

The assessment method for the SS responses was adapted from previous studies [7,9,10]. Beginning 5 min post-injection, the occurrence of the following SS responses was assessed every 5 min for 1 min by instantaneous sampling and was rated as present (=1) or absent (=0):

- Flat body posture (the greater ventral part of the body is in contact with the cage floor and the animal moves similar to a reptile. To our experience flat body posture is often accompanied by hindlimb abduction)
- Head weaving (repetitive movement of the head from side to side, like watching a tennis match)
- Hindlimb abduction (both hindlimbs are splaying out to the side. Toes of the distal limb are also often splayed out.)

- Tremor (shivering encompassing the whole body at rest)
- Straub tail (a rigid dorsiflexed tail in a sharper angle at the base of the tail)
- Backward walking (the animal continuously moves backwards for some cm)
- Hunched back (dorsal arching of the spine relative to a line between the neck and the rump with distinctive flexion of the back muscles)
- Piloerection (erected body hair, most prominent in the region of the neck)
- Head shakes (short and firm movement of the head in any direction comparable to the Pinna reflex reaction)
- Forepaw treading (the animal is at rest and moves the forepaws alternating and repetitively)
- Head twitches (jerky movements of the head only toward the neck).

Additionally, as a measure for vertical activity the number of rearings (i.e. both forepaws lifted off the cage floor) was counted every 5 min for 1 min. At the end of the experiment, the animals were removed from the cage and the occurrence of salivation, rated as present (=1) or absent (=0), was determined by closely examining the muzzle of the mice for patches of damp fur. Moreover, the number of defecation boli in the experimental cage was counted. The occurrence of any other effect mentioned in literature, e.g. myoclonic seizures [22], was also registered by the observer.

Horizontal motor activity was not determined because of the restricted area of the observation cages. Drug effects on rectally recorded body temperature were not included in this study as it would have interfered with the behavioral observation.

2.4. Statistical analysis

Since no significant behavioral changes were detected in the last 30 min (60–90 min), we only analyzed the data of the first 60 min (0–60 min), resulting in 12 assessment points. The scores measured by instantaneous sampling were summed up for each a mouse with a possible maximum value of 12 for each response. The number of rearings was summed up over the 12 assessment points. For all treatment groups the means ± SEM for each response were calculated, except for salivation. Sign of salivation were only observed in the OXO group and appeared there in all animals. Hence, for salivation descriptive statistics were used.

In order to detect dose response effects (*Experiment 1*) or treatment effects (*Experiments 2 and 3*), the data were analyzed using one-way ANOVAs followed by post hoc Dunnett's tests in comparison to vehicle.

In order to investigate the synergistic effect of the drug combinations, we calculated the respective sums of the single drug effects for each SS response and analyzed the data by Mann–Whitney-*U*-tests. For all tests a probability value of 0.05 was considered to be statistically significant. In Tables 1–3, only those SS responses are shown where significant differences were detected.

3. Results

3.1. Experiment 1

5-HTP. Out of all three drugs, 5-HTP induced the broadest spectrum of SS responses. Post hoc analysis revealed that, in comparison to vehicle, the higher doses of 5-HTP (i.e. 160 mg/kg and 320 mg/kg) significantly increased the occurrence of the following seven responses: flat body posture, hindlimb abduction, piloerection, tremor, forepaw treading, head twitches, and hunched back (see Table 1). Additionally, both doses significantly decreased the

Table 1
Effects of 5-HTP, FLX, and TCP on SS responses in NMRI mice.

Response	Treatment vehicle	5-HTP			*p* value	FLX			*p* value	TCP			*p* value
		80 mg/kg	160 mg/kg	320 mg/kg		10 mg/kg	20 mg/kg	40 mg/kg		1 mg/kg	2 mg/kg	4 mg/kg	
Sum scores of													
Flat body posture	0.14 ± 0.10	0.54 ± 0.18	**1.77 ± 0.47**	**2.77 ± 0.51**	<0.001	0.00 ± 0.00	**3.00 ± 1.01**	**8.23 ± 1.03**	<0.001	0.38 ± 0.14	0.77 ± 0.34	**2.93 ± 0.85**	<0.001
Hindlimb abduction	0.29 ± 0.13	2.08 ± 0.69	**3.46 ± 0.76**	**4.23 ± 0.71**	<0.001	0.42 ± 0.42	**3.42 ± 1.14**	1.54 ± 0.64	0.006	2.54 ± 0.84	**6.85 ± 1.10**	**5.57 ± 0.82**	<0.001
Piloerection	2.86 ± 0.84	**11.08 ± 0.61**	**11.31 ± 0.38**	**12.00 ± 0.00**	<0.001	4.50 ± 1.41	**10.83 ± 0.99**	**11.85 ± 0.15**	<0.001	5.54 ± 1.32	**8.92 ± 0.92**	**11.86 ± 0.15**	<0.001
Tremor	0.00 ± 0.00	1.54 ± 0.42	**5.46 ± 1.05**	**6.08 ± 0.92**	<0.001	0.33 ± 0.26	**4.00 ± 0.79**	**3.62 ± 0.90**	<0.001	0.23 ± 0.12	1.00 ± 0.41	**2.79 ± 0.79**	<0.001
Forepaw treading	0.00 ± 0.00	0.69 ± 0.33	**1.54 ± 0.51**	**4.62 ± 0.60**	<0.001	1.00 ± 0.92	1.00 ± 0.92	0.08 ± 0.08	ns	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	ns
Head twitches	0.07 ± 0.07	**5.00 ± 0.84**	**7.85 ± 0.76**	**9.23 ± 0.47**	<0.001	0.92 ± 0.92	0.92 ± 0.92	0.00 ± 0.00	ns	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	ns
Hunched back	0.00 ± 0.00	0.92 ± 0.37	**2.00 ± 0.57**	**1.92 ± 0.49**	0.002	**2.08 ± 1.06**	**10.25 ± 0.66**	**12.00 ± 0.00**	<0.001	0.00 ± 0.00	0.08 ± 0.08	0.14 ± 0.10	ns
Rearing [*n*]	62.79 ± 3.05	**26.92 ± 6.59**	**14.15 ± 3.87**	**6.15 ± 2.77**	<0.001	61.92 ± 4.33	**15.67 ± 4.25**	**0.54 ± 0.24**	<0.001	55.23 ± 5.28	49.62 ± 3.87	**29.64 ± 4.05**	<0.001
Defecation boli [*n*]	13.43 ± 1.88	**19.92 ± 1.45**	16.69 ± 1.37	10.08 ± 1.26	<0.001	15.58 ± 1.36	**6.00 ± 1.23**	**1.77 ± 0.60**	<0.001	14.92 ± 1.75	11.00 ± 1.64	12.79 ± 1.00	ns

Data are presented as means ± SEM. *P* values for treatment effects were calculated by one-way ANOVAs for each drug and response. Dose response effects were analyzed by Dunnett's tests versus vehicle, indicated as bold numbers. ns = not significant; 5-HTP = 5-hydroxy-L-tryptophan; FLX = fluoxetine; TCP=tranylcypromine.

Table 2
Effects of 5-HTP, FLX, and TCP in combination on SS responses in NMRI mice.

Response	Treatment vehicle	Drug combination			*p* value	Addition of sum scores/numbers of the single drugs		
		5-HTP + FLX 80 + 10 mg/kg	FLX + TCP 10 + 2 mg/kg	5-HTP + TCP 80 + 2 mg/kg		5-HTP/FLX 80/10 mg/kg	FLX/TCP 10/2 mg/kg	5-HTP/TCP 80/2 mg/kg
Sum scores of								
Flat body posture	0.38 ± 0.38	9.54 ± 0.40*	3.15 ± 1.02	7.33 ± 1.08*	<0.001	0.58 ± 0.19	0.77 ± 0.34	1.31 ± 0.40
Hindlimb abduction	1.31 ± 0.84	9.38 ± 0.60*	8.38 ± 0.99	11.83 ± 0.17	<0.001	2.67 ± 0.73	7.23 ± 1.22	8.92 ± 1.39
Piloerection	2.00 ± 0.91	11.62 ± 0.21*	11.00 ± 0.52	12.00 ± 0.00*	<0.001	15.67 ± 1.73	13.08 ± 1.61	20.00 ± 1.26
Tremor	0.69 ± 0.69	4.54 ± 0.84*	3.85 ± 0.85*	8.42 ± 0.63*	<0.001	1.83 ± 0.52	1.31 ± 0.56	2.54 ± 0.40
Forepaw treading	0.46 ± 0.46	4.38 ± 0.49*	0.23 ± 0.12	2.17 ± 0.67	<0.001	1.75 ± 0.99	0.92 ± 0.92	0.69 ± 0.33
Head twitches	0.62 ± 0.62	4.38 ± 0.72	0.69 ± 0.26	6.92 ± 0.87	<0.001	5.83 ± 0.92	0.85 ± 0.85	5.00 ± 0.84
Hunched back	0.23 ± 0.23	0.69 ± 0.24	0.31 ± 0.13	4.17 ± 0.98*	<0.001	2.83 ± 0.98	2.00 ± 1.01	1.00 ± 0.36
Rearing [*n*]	65.23 ± 6.71	3.69 ± 1.10*	37.77 ± 7.71*	13.50 ± 2.45*	<0.001	89.33 ± 8.53	106.85 ± 76.62	76.62 ± 7.88
Defecation boli [*n*]	12.23 ± 1.31	5.77 ± 0.99*	10.31 ± 1.37*	17.17 ± 1.94*	<0.001	35.75 ± 2.29	25.38 ± 1.67	30.92 ± 1.83

Data are presented as means ± SEM. *P* values for treatment effects were calculated by one-way ANOVAs for each drug combination and response. Treatment effects of the drug combinations were analyzed by Dunnett's tests versus vehicle, indicated as bold numbers. * $p < 0.05$ analyzed by Mann–Whitney-*U*-tests versus the addition of the sums scores/numbers of single drugs. 5-HTP = 5-hydroxy-L-tryptophan; FLX = fluoxetine; TCP = tranylcypromine.

numbers of rearings (Table 1). At the lowest dose (80 mg/kg), 5-HTP increased the occurrence of piloerection and head twitches and also the number of defecation boli, whereas the number of rearings was significantly decreased (Table 1).

FLX. In comparison to 5-HTP, less responses were evoked by FLX treatment. Compared to vehicle, the administration of 20 mg/kg and 40 mg/kg FLX induced significant increases only in the following five SS responses, flat body posture, hindlimb abduction (although only significant when treated with 20 mg/kg FLX), piloerection, tremor, and hunched back (Table 1). In addition, significant decreases were revealed for number of rearings and defecation boli (Table 1). At the lowest dose (10 mg/kg), FLX only induced an increase in the occurrence of hunched back (Table 1).

TCP. Of the three drugs, TCP elicited the fewest number of SS responses. At the highest dose (4 mg/kg), TCP only induced significant increases in the occurrence of flat body posture, hindlimb abduction, piloerection, and tremor, and additionally it decreased the number of rearings (Table 1). Treatment with 2 mg/kg TCP significantly enhanced the occurrence of hindlimb abduction ($p < 0.001$) and piloerection ($p < 0.001$), whereas 1 mg/kg TCP had no significant effects on the assessed responses (Table 1).

Comparing all three single drug treatments, the following five responses were consistently observed: flat body posture, hindlimb abduction, piloerection, tremor, and decreased rearings. Backward walking, head shakes, head weaving, salivation, and Straub tail did not occur after treatment with 5-HTP, FLX and TCP in NMRI mice in any of the used doses (data not shown).

3.2. Experiment 2

The minimal effective dose of each drug was used for investigating drug interaction, i.e. the doses, derived by *Experiment 1*, which induced at least one SS response. 5-HTP at 80 mg/kg evoked four responses, 10 mg/kg FLX induced one and 2 mg/kg TCP two responses (see Table 1). All three drug combinations increased the number of SS responses and at least the five aforementioned responses (i.e. flat body posture, hindlimb abduction, piloerection, tremor, and a decrease in rearings) were observed (see Table 2). *5-HTP + FLX.* The combination of 80 mg/kg 5-HTP and 10 mg/kg FLX additionally induced an increase in forepaw treading and head twitches and a decrease of defecation boli (Table 2).

FLX + TCP. The combination of 10 mg/kg FLX with 2 mg/kg TCP induced no further SS responses than the five aforementioned SS responses (see Table 2).

5-HTP + TCP. After administration of 5-HTP (80 mg/kg) and TCP (2 mg/kg) the broadest spectrum of responses was observed. In addition to the five aforementioned SS responses forepaw treading, head twitches, hunched back, and an increased number of defecation boli occurred (see Table 2).

Likewise to the single treatments, all three combinations had no significant effects on backward walking, head shakes and weaving, salivation and Straub tail (data not shown).

In order to characterize the synergism of the drug combinations, the effects were compared to the respective addition of sum scores/numbers of the single drugs (see Table 2). Arithmetically, the sum scores/numbers for seven SS responses of the *5-HTP + FLX* combination were significantly higher than the respective sums of the single drug effects. Also, sum scores/numbers of the *5-HTP + TCP* combination exceeded the sums of the single drug effects in case of six SS responses. The over-additive effect of the *FLX + TCP* combinations was not as pronounced: only the sum scores/numbers of three SS responses differed from the sums of the single drug effects (see Table 2).

3.3. Experiment 3

Following the treatment with the noradrenaline agonist ATO only two of the five common SS responses in NMRI mice, i.e. hindlimb abduction and piloerection, were observed (Table 3). The administration of the dopamine agonist APO also increased

Table 3
Effects of ATO, APO, and OXO on the five common SS responses and the Straub tail response in NMRI mice.

Response	Treatment vehicle	ATO 2 mg/kg	APO 4 mg/kg	OXO 0.5 mg/kg	*p* value
Sum scores of					
Flat body posture	0.13 ± 0.13	0.15 ± 0.10	0.07 ± 0.07	0.00 ± 0.00	ns
Hindlimb abduction	0.13 ± 0.13	**3.46 ± 0.56**	**3.21 ± 0.62**	**11.38 ± 0.40**	<0.001
Piloerection	0.56 ± 0.24	**10.23 ± 0.67**	**7.86 ± 1.08**	**7.23 ± 0.86**	<0.001
Tremor	0.00 ± 0.00	1.23 ± 0.65	0.00 ± 0.00	**10.00 ± 0.85**	<0.001
Rearing [*n*]	55.31 ± 5.54	44.31 ± 6.82	**19.29 ± 2.93**	**0.77 ± 0.61**	<0.001

Data are presented as means ± SEM. *P* values for treatment effects were calculated by one-way ANOVAs for each drug and response. Treatment effects were analyzed by Dunnett's tests versus vehicle indicated as bold numbers. ns = not significant; ATO = atomoxetine; APO = apomorphine; OXO = oxotremorine.

hindlimb abduction and piloerection. Additionally, APO decreased the number of rearings (see Table 3). The most responses in this experiment were induced by the acetylcholine agonist OXO. The administration of OXO increased the occurrence of hindlimb abduction, piloerection, and tremor, and decreased the number of rearings (Table 3). For the first time salivation was observed, which was displayed in all 13 animals treated with OXO. Moreover, the administration of APO and OXO induced in some animals the Straub tail response (sum scores for APO: 1.43 ± 0.64, for OXO: 2.00 ± 0.62; $p = 0.002$ vs vehicle: 0.00 ± 0.00).

Of the five common SS responses induced by 5-HT-enhancing drugs in NMRI mice, flat body posture was not evoked by ATO, APO, or OXO. Also, backward walking, changes in defecation, forepaw treading, hunched back, head twitches, head weaving and head shakes were not observed (data not shown).

4. Discussion

In literature, flat body posture, hindlimb abduction, tremor, head weaving, backward walking, Straub tail, and hyperactivity are the most frequently reported signs for describing the SS in mice [see 3]. Head twitches and decrease in body temperature are also often referred to as signs of serotonergic hyperactivity [3]. In order to prove, whether this spectrum of signs is reliably induced by different 5-HT-enhancing drugs we investigated the effects of 5-HTP, FLX, and TPH on the behavioral SS responses in male NMRI mice. The selected drugs increase the extracellular 5-HT concentration by different mechanisms of action and have been frequently used to evoke SS responses in rodents [7,10,23–25].

In our study, we observed in NMRI mice five common signs that were induced by all three 5-HT-enhancing drugs, i.e. flat body posture, hindlimb abduction, tremor, piloerection, and decrease in rearing. Likewise to humans, where the symptoms of the SS are thought to be a continuous spectrum from side-effects to toxicity [26], the severity and number of responses increased with ascending dosage. Although, the spectrum of signs is confirmed by other murine SS studies [5,7–10,15,27], it only partially overlaps with the most frequently reported responses. Most likely, strain differences contribute to the diverging spectrum of signs as it was previously shown that the intensity of head weaving and hindlimb abduction induced by the 5-HT precursor tryptamine can differ between mouse strains due to different tryptamine concentrations in the brain [28]. Also the pharmacodynamics of the serotonergic drugs can influence the range of evoked behavioral SS responses. In this study, the used drugs increase extracellular 5-HT concentration and therefore activate all 5-HT receptors. Hence, it is conceivable that distinct behavioral responses might occur more pronounced if only specific 5-HT receptor subtypes are stimulated. In the same mouse strain, for example, we observed a rigid tail (also known as Straub tail or spontaneous tail flick response) when the full 5-HT_{1A}-receptor agonist 8-OH-DPAT (1.25 and 2.5 mg/kg) was administered [8]. Although we used rather high doses [17–19], this response did not occur after the administration of the three 5-HT-enhancing drugs.

Moreover, our results revealed that 5-HTP and FLX unequally evoked additional signs to the five common responses. Most responses were observed after treatment with the higher doses (160 and 320 mg/kg) of 5-HTP (8 out of 14), whereas TCP (4 mg/kg) and FLX (20 mg/kg) induced 5 and 7 responses out of 14, respectively. The fact that 5-HTP magnifies 5-HT synthesis could account for the efficacy of this drug. Brain microdialysis experiments in rats demonstrated that extracellular 5-HT increased to about 300% following already a relatively low dose (75 mg/kg) of 5-HTP [29]. A further increase of 5-HTP doses raised the 5-HT release, measured by an *in vivo* voltammetry study, in a proportional manner [30]. The tremendous elevation of central 5-HT by 5-HTP was explained by conversion of 5-HTP to 5-HT, not only in serotonergic but also in dopaminergic neurons [30]. Hence, the broader spectrum of responses induced by 5-HTP in comparison to TCP and FLX might be related to a stronger stimulation of specific 5-HT receptor subtypes. Head twitches, for example, are regarded to be mediated by 5-HT_{2A} receptor activation [22,31–34], forepaw treading is induced by stimulation of 5-HT_{1A} receptors [11], and 5-HTP induced defecation is a well-known effect elicited by peripheral action of 5-HT, especially at 5-HT_4 receptors [35]. Moreover, the hunched back has been shown to be due to stimulation of 5-HT_{2C} receptors [36]. 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 an unselective inhibitor of MAO A and B, thus inhibiting also the degradation of dopamine and noradrenaline. FLX is known to increase noradrenaline and dopamine extracellular levels in brain [37]. These secondary pharmacological properties might have interfered with some of the SS responses.

In our study backward walking, head waving, head shakes, and salivation could not be evoked by all three 5-HT-enhancing drugs. In accordance to our results, backward walking, head shaking and salivation also failed to appear in other mice studies after treatment with 5-HTP [10,27]. Surprisingly, we did not observe the Straub tail response in all treatment groups, although this response is reported in corresponding studies [7,10,27]. As we have stated above, we assume that strain differences and procedural variables may account for the variations in study results.

In summary, flat body posture, hindlimb abduction and tremor together with piloerection and decreased rearings turned out to be the common SS responses following 5-HT-enhancing drugs in NMRI mice. When establishing the model of SS in the lab, 5-HTP may serve as the most appropriate positive control. However, it has to be considered that other 5-HT-enhancing drugs that are used in pharmacological research may induce a diverse spectrum of behavioral SS responses as shown by FLX and TCP.

The second aim of our study was to investigate, whether the interaction of 5-HT-enhancing drugs can be described as an over-additive effect. In laboratory rodents, a potentiation effect by combination of 5-HT-enhancing drugs has been assumed and it has been termed as the “5-HTP potentiated behavioral syndrome” [15,38] and the behavioral syndrome “after tryptophan loading” [16]. Drug potentiation (or over-additive synergism) can be pharmacologically defined as the joint effect of two drugs that is greater than the algebraic sum of their individual effects [39,40]. In order to characterize the interaction pharmacologically, we identified first the minimal effective doses of 5-HTP (80 mg/kg), FLX (10 mg/kg), and TCP (2 mg/kg), i.e. the dose which evoked at least one of the SS responses in our experiments. Our results revealed that all combinations of the three 5-HT-enhancing drugs led to an increased number of SS responses compared to single drug administration. We additionally observed that the sum scores for each response obtained by the drug combinations exceed markedly the addition of the sums scores of the single drug effects. The only exception is piloerection that reached almost the maximum sum already following low drug doses, indicating a ceiling effect. Taken together, our findings of the drug combination experiments argue for an over-additive synergistic effect. This is supported by brain microdialysis studies in rats, which have shown that the elevation of 5-HT levels by concomitantly given drugs is enlarged when compared to the administration of each single drug. FLX caused a 2–4-fold increase in extracellular 5-HT concentration in the neostriatum and hypothalamus. When 5-HTP, in a dose which alone had only a small effect was added to FLX, the 5-HT release was 10–16-fold increased [41,42]. Also clinical studies suggest a potentiation effect, since the SS in man is most frequently reported after the ingestion of MAO

inhibitors together with SRI or 5-HT releaser even in therapeutic doses [26,43]. Hence, the SS in NMRI mice induced by the drug combinations seems to mirror the increased risk by concomitant ingestion of two serotonin agonists.

In order to characterize the specificity of the SS model and to examine a further aspect of predictive validity, the five common responses induced by the serotonergic drugs used in this study were compared to the effects of rather high doses of agonists at the dopamine receptor (APO), the acetylcholine receptor (OXO), and the noradrenaline receptor (ATO). Of the five common responses induced by the three 5-HT-enhancing drugs, hindlimb abduction, piloerection, tremor, and decrease in rearing were variably demonstrated after the administration of APO, OXO, and ATO. We observed marked differences between the types of tremor: 5-HT-induced tremor was mild, whereas the OXO-induced tremor was severe and vigorous. Tremor induced by ATO was similar to 5-HT-induced tremor but was displayed only temporary in few mice. The only response which was solely induced by the three 5-HT-enhancing drugs, but not by APO, OXO, and ATO, was flat body posture. This observation is in line with the general assumption that flat body posture is a 5-HT_{1A} receptor mediated drug effect, especially by postsynaptic 5-HT_{1A} receptors [see 44]. Interestingly, in some animals treated with OXO and APO the Straub tail response was induced. The Straub tail has also been reported by others in APO or nicotine treated mice [45,46]. Taken together, the comparison of the effects of the four different neurotransmitter agonists suggests that similar responses can also be evoked by drugs primarily affecting other transmission systems. Therefore, when a drug screening test is performed, we suggest to perform corresponding receptor antagonist studies.

Summarizing, we identified a spectrum of SS responses in NMRI mice that reliably and robustly manifested following the administration of 5-HTP, FLX, and TCP: flat body posture, hindlimb abduction, piloerection, tremor, and decrease in rearing. Subsequently, we demonstrated that the combinations of 5-HTP, FLX, and TCP have a drug potentiation effect. Our results strengthen the predictive validity of the murine SS, arguing for its application as a translational tool in safety drug screening and basic research. However, it has to be considered that the observed responses can partly be induced by activation of other transmission systems. The five identified common responses belong to the frequently reported signs and should be at least examined when studying the SS in NMRI mice. However, we recommend to broaden the spectrum of responses, when other mouse strains are investigated.

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