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Effects of the 5-HT_{1A} Antagonist (+)-WAY-100135 on Murine Social and Agonistic Behavior

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BELL, R., P. J. MITCHELL AND H. HOBSON. *Effects of the 5-HT_{1A} antagonist (+)-WAY-100135 on murine social and agonistic behavior.* PHARMACOL BIOCHEM BEHAV **54**(1) 159–167, 1996.—Compounds previously identified as 5-HT_{1A} antagonists have subsequently been demonstrated to possess partial agonistic properties in models assessing somatodendritic autoreceptor function. This study examined the influences of (+)-WAY-100135, claimed to be the first selective 5-HT_{1A} antagonist, on offensive behaviour in male mice. Employing a resident-intruder paradigm, administration of (+)-WAY-100135 (1.0–10.0 mg/kg sc) enhanced elements of resident offensive behaviour at 2.5 and 5.0 mg/kg but reduced such behaviour at 10.0 mg/kg. In comparison, resident defensive postures remained unchanged except for a significant increase in defensive sideways behaviour at 10.0 mg/kg. These effects were accompanied by reduced rearing behaviour across the dose range tested. Attend/approach behaviour was significantly reduced at the lowest, but increased at the highest, doses tested. Such results may reflect response competition rather than concomitant motor impairment. Given the dynamic behavioural interactions occurring in this paradigm, the increased offensive behaviour of the resident mice leads to enhanced defence and counter-attack by the intruder conspecifics. The results are discussed with reference to the current literature concerning the behavioural effects of other 5-HT_{1A} antagonists.

(+)-WAY-100135 Social behavior Agonistic behavior 5-HT_{1A} antagonist Ethological analysis

INTRODUCTION

CONSIDERABLE research has focused on the influences of 5-HT_{1A} receptors on rodent agonistic behavior (e.g., 2,3,4, 11,28,29,33). While such studies have typically employed 8-OH-DPAT as the selective agonist (24) for investigating the functional role(s) of the 5-HT_{1A} receptor in agonistic behavior, selective antagonists are also required to fully characterise and describe the functional role(s) of any receptor subtype (13).

Initial attempts to develop a 5-HT_{1A} receptor antagonist resulted in compounds which were either non-selective with respect to the 5-HT_{1A} receptor, e.g., spiperone (15), or possessed β-adrenoreceptor affinity, e.g., propranolol and pindolol (35). Subsequently, ligands possessing high-affinity for 5-HT_{1A} receptors, e.g., NAN-190 (17) and BMY7378 (37) have been proposed as selective 5-HT_{1A} receptor antagonists. More recently, however, these compounds have been demonstrated to display both antagonist properties at postsynaptic 5-HT_{1A} receptors and agonist-like effects at presynaptic somatodendritic 5-HT_{1A} autoreceptors (13). The agonist activity of such compounds, therefore, inhibit raphe neuronal firing (23,36) and consequently attenuate 5-HT release at axon terminals (19,34).

Fletcher et al. (12,13) described a novel phenylpiperazine derivative, (±)-WAY-100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide dihydrochloride), which demonstrates high affinity and selectivity for the 5-HT_{1A} receptors. The compound has been reported as having no intrinsic influence on raphe cell firing in vivo, whilst antagonising the inhibition of firing induced by *8-hydroxy-2-(di-n-propylamino)tetralin* (8-OH-DPAT) (20). In addition, the interaction of (±)-WAY-100135 with the 5-HT_{1A} receptor was

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stereoselective; the (+)-enantiomer being significantly more active in binding, functional and behavioural studies.

While (±)-WAY-100135 has no intrinsic agonist activity at somatodendritic 5-HT_{1A} receptors (13,32), this compound antagonised both 8-OH-DPAT-induced hypothermia in mice and 8-OH-DPAT-induced hyperphagia in rats (13). Germane to the current study, in terms of the possible link between agonistic behaviour and anxiety (2-5), are reports that (+)-WAY-100135 produced anxiolytic-like effects in the mouse light-dark test (7) and mouse elevated plus-maze (31). By contrast, (+)-WAY-100135 did not demonstrate an anxiolytic-like effect in either the rat safety signal withdrawal procedure of conflict (9) or rat elevated plus-maze (6). The reports that (+)-WAY-100135 exerts an anxiolytic-like effect in mouse, but not rat, anxiety tests may reflect a species difference in the pharmacology of the 5-HT_{1A} receptor between mice and rats (6).

Given our previous findings for the effects of pindobind 5-HT_{1A} (2), (−)-pindolol and SDZ 216-525 (3) on murine agonistic behavior, it was of interest to compare such data with results obtained for the more selective 5-HT_{1A} antagonist (+)-WAY-100135, as observed in the resident-intruder paradigm. The resident-intruder (isolation-induced aggression) paradigm, mainly represents offensive aspects of agonistic behavior in the resident mouse. However, where the intruder conspecific is also examined, defensive aspects of agonistic behavior are also represented (21).

MATERIALS AND METHODS

Subjects and Procedures

One hundred adult male albino mice of the BKW strain, weighing between 25-35 g from Queens University Belfast Medical Biology Centre Breeding stock were used. Four weeks prior to testing the mice were randomly allocated to resident or intruder status. Residents were individually caged (cage size 30 × 15 × 13 cm) and intruders housed with siblings in groups of approximately ten (cage size 44 × 28 × 13 cm). Throughout the 4 wk prior to testing, all animals were given fresh bedding weekly, with food and water available ad libitum. All subjects were maintained in a temperature-controlled room (24 ± 1°C), in which a 12 h reversed light/dark cycle was operative (lights on 2400 h).

Behavioural testing took place in the residents home cage. Food and water were removed from test cages for the duration of encounters. Resident-intruder encounters were recorded on tape by a Panasonic Saticon colour video camera (model WVP200E) with low light facility. The test cages were illuminated by two 60 W "angle-poise" lamps during social encounters. Tape analysis was carried out using a Panasonic video recorder, a VDU, an IBM portable computer (model 5155 640 K) and a tractor printer.

(+)-WAY-100135 was dissolved in physiological saline, which also served as drug-vehicle control. All injections were performed subcutaneously (sc) in a volume of 10 ml/kg thirty minutes prior to behavioural testing. Doses were selected on the basis of previous investigations (13). Animals used were both drug and experimentally naive. The experimenter remained blind to the conditions until data analysis was complete.

All testing was carried out during the dark phase under red light. Isolated animals were weighed, marked for recognition and randomly assigned to dose treatment groups. Only isolated resident mice received drug treatments. Intruder mice were then introduced into the home cages of the residents and the ensuing 10 min encounters recorded on videotape for later analysis. Five experimental conditions were used (n pairs in each condition = 10); saline, 1.0, 2.5, 5.0 and 10.0 mg/kg (+)-WAY-100135.

Measures

Behavioural analysis was similar to previously detailed procedures (2-5,30). Briefly, videotapes were analysed using direct keyboard inputs to the microcomputer which had been programmed (18) to produce data output in the form of frequency and real-time duration of behavioural elements (Table 1).

Statistical Analysis

Given the non-parametric nature of the data, results for each behavioural element were analysed using Kruskal-Wallis one-way analysis of variance across treatment groups (degrees of freedom = 4 in all cases). Where significant variations in the data were identified, further comparisons (with control group) were performed by Mann-Whitney *U*-tests. The frequency and duration measures for each behavioural element are expressed as medians, with semi-quartile ranges, for each treatment group.

RESULTS

Drug-Treated Resident Mice (Table 2)

Non-social behaviour. Kruskal-Wallis analysis revealed no significant variation in the frequency or duration of cage exploration, maintenance and digging behaviours across treatment groups (Hs $\leq$ 6.03, $p > 0.05$ in all cases). However, significant H values were identified for rearing frequency ($H = 14.6$, $p < 0.05$) and duration ($H = 19.35$, $p < 0.05$). Post hoc Mann-Whitney procedure indicated significant decreases in rearing frequency at 2.5 mg/kg ($U = 5.5$, $p < 0.002$), 5.0 mg/kg ($U = 12$, $p < 0.02$) and 10.0 mg/kg ($U = 13$, $p < 0.02$). Rearing duration decreased at 1.0 mg/kg ($U = 20$, $p < 0.05$), 2.5 mg/kg ($U = 4$, $p < 0.002$), 5.0 mg/kg ($U = 13$, $p < 0.02$) and 10.0 mg/kg ($U = 7$, $p < 0.02$).

Social behaviour. Kruskal-Wallis analysis revealed significant variation in the frequency of attend/approach behaviour ($H = 14.52$, $p < 0.05$) but failed to identify any significant variation in the duration of this behavioural element or indeed in the frequency and duration measures for any of the other behavioural elements of social behaviour (Hs $\leq$ 9.40, $p > 0.05$ in all cases). Post hoc analysis revealed that the frequency of attend/approach behaviour was significantly decreased fol-

TABLE 1

BEHAVIORAL ELEMENTS (GROUPED ACCORDING TO MOTIVATIONAL CATEGORY) USED TO EXAMINE THE EFFECTS OF (+)-WAY-100135 ON MURINE BEHAVIOR

Category	Elements
Nonsocial	Cage exploration, rearing maintenance, digging
Social	Nasogenital, nasonasal, nonspecific partner investigation, follow, attend/approach, stretched/attend
Offensive	Aggressive groom, tail rattle, offensive sideways, offensive upright, chase, bite-attack
Defensive	Evade, defensive sideways, defensive upright, submissive upright, frozen crouch

TABLE 2

EFFECTS OF (+)−WAY-100135 (1.0–10.0 mg/kg) ON BEHAVIORS DISPLAYED BY RESIDENT MICE

Behaviours		Vehicle	1.0 mg/kg	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	*H*-Values
Nonsocial							
Cage exploration	f	30 (25.5–31.5)	26 (23–30)	26 (24–28.5)	28.5 (26–35.5)	30 (26–32.5)	5.45
	d	225.38 (160.21–313.34)	334.68 (216.22–366.15)	226.19 (193.85–268.53)	292.95 (198.45–340.97)	315.43 (262.11–340.77)	6.03
Rearing	f	9.5 (8.5–13.5)	7.5 (2–11.5)	4.5 (2–6,5)‡	5 (2–6)†	6 (4–8)†	14.6*
	d	34.4 (28.01–48.2)	18.05 (6.05–24.69)*	6.32 (2.49–12.34)‡	6.33 (4.23–10.66)†	12.75 (7.27–17.12)†	19.35*
Maintenance	f	3 (2–5.5)	2.5 (0.5–3)	1.5 (1–2.5)	2 (1–4)	3.5 (1.5–6)	5.11
	d	13.35 (6.83–33.36)	17.13 (0.96–23.43)	6.18 (2.28–10.13)*	15.51 (7.77–29.62)	15.63 (4.72–28.84)	5.12
Digging	f	0 (0–0.5)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	2.07
	d	0 (0–1.16)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	2.13
Social							
Nasogenital	f	5.5 (2–8)	2 (1–5.5)	3.5 (0.5–5.5)	2.5 (1–3.5)	1.5 (1–3)†	5.18
	d	24.91 (5.76–29.25)	6.89 (2.03–24.78)	13.4 (0.55–25.91)	5.32 (1.18–16.68)	5.84 (2–10.23)	3.51
Nasonasal	f	4 (2.5–5.5)	3 (1.5–4)	2 (1–3)*	2 (1–3)*	2 (0.5–3.5)	7.33
	d	12.86 (6.25–20.39)	11.4 (3.61–12.27)	4.41 (1.75–8.12)	5.62 (2.44–17.71)	5.39 (1.21–6.36)†	6.81
Nonspecific investigation	f	21 (11–24.5)	11.5 (6–15.5)	16 (4–19.50)	6.5 (3–11)†	13.5 (7.5–14.5)	7.69
	d	98.53 (52.59–113.3)	62.38 (35.32–97.07)	113.37 (17.99–141.13)	37.59 (9.76–80.55)	61.23 (35.02–77.01)	3.97
Follow	f	8.5 (5.5–10)	2 (0–8)	4.5 (1–7)	2.5 (1.5–3.5)†	3.5 (1–9)	6.42
	d	18.39 (11.48–20.14)	4.99 (0–16.67)	7.34 (3.72–16.14)	4.51 (2.01–8.82)†	5.53 (1.45–24.71)	5.69
Attend/approach	f	10 (7.5–13)	5.5 (4–9.5)*	10 (6.5–10.5)	16.5 (12–20)*	11.5 (7–13.5)	14.52*
	d	27.28 (18.66–34.38)	12.63 (9.5–26.72)	24.2 (17.64–28.18)	40.87 (26.78–50.57)	27.37 (16.37–35.95)	9.40
Stretch/attend	f	1.5 (1–2)	2 (0.5–3.5)	2.5 (0.5–4.5)	3 (1.5–4)	2.5 (1.5–3)	2.90
	d	5.16 (1.58–5.67)	6.91 (1.26–8.44)	9.77 (0.66–11.78)	7.25 (2.25–10.58)	5.26 (3.78–8.30)	0.93

(Table continued on next page.)

TABLE 2

CONTINUED

Behaviours		Vehicle	1.0 mg/kg	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	*H*-Values
Offensive							
Aggressive groom	f	1.5 (0.5-2)	0 (0-0.5)†	1.5 (0-3)	0 (0-0.5)	0 (0-0)*	10.36*
	d	4.52 (1.71-10.84)	0 (0-0.93)†	3.3 (0-8.38)	0 (0-2.25)	0 (0-0)*	9.18
Tail rattle	f	1 (0-3.5)	0 (0-0)	8 (5-12)†	2.5 (0.5-4)	3 (0-5.5)	17.35*
	d	2.21 (0-7.86)	0 (0-0)	14.64 (9.61-29.97)*	4.88 (0.66-5.64)	4.75 (0-12.93)	15.96*
Offensive sideways	f	2.5 (1-6.5)	3.5 (0.5-5.5)	7.5 (4-20.5)	15.5 (11-17.5)†	8.5 (5.5-13)*	18.54*
	d	8.42 (2.87-24.22)	8.33 (1.45-19.54)	35.88 (11.81-99.96)†	48.49 (32.99-56.33)*	27.96 (19.84-39.92)†	17.24*
Offensive upright	f	1.5 (0-4)	1 (0.5-1.5)	5 (0.5-6)	3.5 (1-6.5)	1 (0-3)	7.57
	d	3.45 (0-11.16)	3.4 (0.6-5.16)	16.23 (3.56-20.92)	8.1 (3.32-18.87)	4.55 (0-9.94)	7.41
Chase	f	3 (0.5-5)	5 (0-7)	5 (1.5-5.5)	3.5 (1.5-4.5)	0.5 (0-3)	5.41
	d	5.66 (0.9-10.04)	13.52 (0-24.11)	10.37 (2.79-16.25)	7.24 (2.96-12.13)	1.62 (0-8.19)	4.78
Bite attack	f	3 (0-5)	0.5 (0-2.5)	4.5 (2-5.5)	5 (0.5-6)	1 (0-3)	7.13
	d	13.18 (0-30.62)	3.21 (0-21.3)	17.04 (8.96-35.21)	23.82 (0.99-32.55)	6.85 (0-11.87)	6.04
Defensive							
Evade	f	1 (0-3)	3.5 (0-7)	3 (0.5-3.5)	3 (1-4)	6 (1-7.5)	4.21
	d	2.66 (0-8.75)	11.87 (0-17.81)	5.84 (0.85-8.41)	6.03 (2.32-9.38)	14.29 (2.65-22.5)	4.06
Defensive sideways	f	0 (0-1)	2 (0-4)	1.5 (0-3.5)	2.5 (0-5.5)	4.5 (1.5-6.5)†	10.10*
	d	0 (0-1.77)	7.9 (0-13.55)	7.79 (0-19.15)	11.38 (0-17.91)	18.49 (2.67-24.75)†	9.04
Defensive upright	f	0 (0-0)	0 (0-1)	0 (0-1.5)	0 (0-1)	1.5 (0-2)	5.46
	d	0 (0-0)	0 (0-2.55)	0 (0-5.58)	0 (0-3.52)	4.08 (0-11.7)	5.65
Submissive upright	f	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
	d	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
Frozen crouch	f	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	
	d	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	

Data expressed as medians (upper to lower quartiles) for frequency (f) and duration (d). Significant values refer to Mann-Whiney comparisons with vehicle.
*$p < 0.05$; †$p < 0.02$; ‡$p < 0.002$.

lowing treatment with (+)-WAY-100135 at 1.0 mg/kg ($U = 23$, $p < 0.05$) but significantly increased following treatment with 5.0 mg/kg ($U = 23$, $p < 0.05$).

Offensive behaviour. Significant variations in the data were identified for the frequency of aggressive grooming behaviour ($H = 10.36$, $p < 0.05$) and the frequency and duration of both tail rattling (Hs = 17.35 and 15.96, respectively, $p < 0.05$ in both cases) and offensive sideways behaviour (Hs = 18.54 and 17.24, respectively, $p < 0.05$ in both cases). Kruskal–Wallis analysis failed to identify any significant variation in the duration of aggressive grooming behaviour or in the frequency and duration measures for any of the other behavioural elements of offensive behaviour (Hs ≤ 9.18, $p > 0.05$ in all cases). Post hoc Mann–Whitney comparisons revealed a significant decrease in the frequency of aggressive grooming behaviour at both 1.0 mg/kg ($U = 17.5$, $p < 0.02$) and 10.0 mg/kg ($U = 19.5$, $p < 0.05$). Tail rattling increased in frequency ($U = 18$, $p < 0.02$) and duration ($U = 23$, $p < 0.05$) at 2.5 mg/kg. Offensive sideways increased in frequency at 5.0 mg/kg ($U = 11$, $p < 0.02$) and 10.0 mg/kg ($U = 20.5$, $p < 0.05$), while the duration measure of this behaviour increased at 2.5 mg/kg ($U = 22$, $p < 0.05$), 5.0 mg/kg ($U = 16$, $p < 0.02$) and 10.0 mg/kg ($U = 23$, $p < 0.05$).

Defensive behaviour. Kruskal–Wallis analysis revealed significant variation in the frequency of defensive sideways behaviour ($H = 10.10$, $p < 0.05$) but failed to identify any significant variation in the duration of this behaviour or in the frequency and duration measures for any of the other behavioural elements of defensive behaviour (Hs ≤ 9.04, $p > 0.05$ in all cases). Further post hoc comparisons indicated a significant increase in the frequency ($U = 10.5$, $p < 0.02$) of defensive sideways behaviour following treatment with (+)-WAY-100135 at 10.0 mg/kg.

Intruder Mice (Table 3)

Non-social behaviour. Kruskal–Wallis analysis revealed significant variation in the frequency of cage exploration ($H = 19.09$, $p < 0.05$), the duration of rearing ($H = 15.24$, $p < 0.05$) and the frequency and duration of digging behaviour (Hs = 17.96 and 14.82, respectively, $p < 0.05$ in both cases) but failed to reveal any significant variation in cage exploration duration, rearing frequency, or in the frequency and duration measures for any of the other behavioural elements of non-social behaviour (Hs ≤ 6.16, $p > 0.05$ in all cases). Post hoc analysis indicated that intruder mice exhibited a significant decrease in the frequency of cage exploration ($U = 9$, $p < 0.002$) and duration of rearing behaviour ($U = 6$, $p < 0.002$) following treatment of the resident mice with (+)-WAY-100135, 5.0 mg/kg. The frequency and duration of digging behaviour of the intruder mice were both decreased following treatment of the resident mice with (+)-WAY-100135 at 2.5 mg/kg (Us = 16.5 and 19.0, respectively, $p < 0.02$ in both cases), 5.0 mg/kg (Us = 11.5 and 15.5, respectively, $p < 0.02$ in both cases) and 10.0 mg/kg (duration only, $U = 22$, $p < 0.05$).

Social behaviour. Kruskal–Wallis analysis failed to reveal any significant variation in the frequency and duration measures for any of the behavioural elements of social behaviour (Hs ≤ 8.18, $p > 0.05$ in all cases) of the intruder mice following treatment of the resident mice with (+)-WAY-100135.

Offensive behaviour. Kruskal–Wallis analysis failed to reveal any significant variation in the frequency and duration measures for any of the behavioural elements of offensive behaviour (Hs ≤ 6.02, $p > 0.05$ in all cases) of the intruder mice following treatment of the resident mice with (+)-WAY-100135.

Defensive behaviour. Kruskal–Wallis analysis failed to reveal any significant variation in the frequency and duration measures for any of the behavioural elements of defensive behaviour (Hs ≤ 9.38, $p > 0.05$ in all cases) of the intruder mice following treatment of the resident mice with (+)-WAY-100135.

DISCUSSION

The change in the behavioural profile exhibited by male resident mice induced by acute sc treatment with (+)-WAY-100135 in this study contrasts markedly with the reported effects of other 5-HT$_{1A}$ antagonists (2,3,33) on murine social and agonistic behavior. Unlike pindobind 5-HT$_{1A}$ (2), (−)-pindolol and SDZ 216-525 (3), (+)-WAY-100135 significantly increased tail rattle and offensive sideways behaviours in the dose range 2.5–5.0 mg/kg. These drug-induced behavioural changes abated at 10.0 mg/kg. A parallel, dose-related, increase in defensive sideways behaviour was recorded, reaching statistical significance at 10.0 mg/kg. Attend/approach behaviour was significantly reduced at 1.0 mg/kg but significantly enhanced at 5.0 mg/kg, whilst decreases in rearing behaviour were noted across the dose range tested. The lack of effect of (+)-WAY-100135 on the chase and bite attack elements of resident mice offensive behaviour suggests that the increased attend/approach, tail rattle and offensive sideways behaviours may reflect enhanced threat behaviour directed toward the intruder conspecific. The reduction in rearing behaviour induced by (+)-WAY-100135 may also reflect response competition. In other words, since offensive behaviours were increased, then the frequency and duration of other elements consequently decreased (2–5). In particular, the total duration of rearing and social behaviour was markedly reduced at the same doses of (+)-WAY-100135 where the total duration of the offensive behaviours was increased.

One consistent finding for pindobind 5-HT$_{1A}$, (−)-pindolol, SDZ 216-525 and (+)-WAY-100135 is the lack of evidence for concomitant motoric impairment (2,3). In the case of (+)-WAY-100135 this result is in agreement with previous studies which indicated that this compound did not influence murine locomotor activity up to a dose of 30 mg/kg (7).

A comparison of the effects of (+)-WAY-100135 and SDZ 216-525 on murine offensive behavior illustrates that whilst the former compound enhanced threat elements in the dose range 2.5–5.0 mg/kg, the latter decreased offensive elements at a dose of 1.0 mg/kg (3). In addition, the same study (3) showed that SDZ 216-525 attenuated defensive behaviours consistent with an anxiolytic effect. In the present study, however, the level of defensive behaviour of the saline-treated resident mice was too low for any further anxiolytic-like attenuation of this behaviour to be observed. Therefore, no conclusions regarding the potential anxiolytic activity of (+)-WAY-100135, which have been suggested by some studies in the mouse (7,31), but not the rat (6,9), may be drawn from the data presented here.

In comparison, an increase in defensive behaviour is generally associated with anxiogenesis (5). For example, in our previous study (5) treatment of resident mice with the anxiogenic compound CGS 12066B induced a pronounced increase in the frequency and duration of most of the defensive elements. However, (+)-WAY-100135 induced a comparatively small, albeit dose dependent, increase in the frequency of only one

TABLE 3

BEHAVIOR OF UNTREATED INTRUDERS AS A FUNCTION OF DRUG STATE OF RESIDENT MICE (1.0-10.0 mg/kg (+)-WAY-100135)

Behaviors		Vehicle	1.0 mg/kg	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	*H*-Values
Nonsocial							
Cage exploration	f	35.5 (32-40)	38 (36-39.5)	31 (24.5-35)	30 (27-30.5)‡	36 (30-38)	19.09*
	d	329.86 (282.65-391.37)	359.61 (316.88-384.81)	310.41 (213.6-337.8)	323.36 (232.21-332.25)	331.56 (260.26-351.66)	6.14
Rearing	f	12 (9-14)	12 (8-16)	9 (6-11)	8 (7-10.5)	10.5 (8-14.5)	6.16
	d	26.8 (20.56-35.18)	25.63 (16.68-30.91)	15.37 (7.54-22.08)	11.67 (8.35-16.55)‡	19.32 (11.54-25.21)	15.24*
Maintenance	f	3 (2-3)	3 (2.5-4)	3 (1.5-5)	2 (2-3)	2 (1-5)	2.32
	d	15.48 (8.92-20.3)	22.53 (11.28-42.87)	22.67 (9.41-29.91)	34.75 (14.8-44.49)	30.19 (15.71-37.55)	4.17
Digging	f	14 (6-18.5)	8.5 (8-9.5)	4.5 (3-6.5)†	4 (1.5-5)†	5 (3-9.5)	17.96*
	d	53.9 (18.03-83.09)	37.15 (25.69-44.37)	16.19 (6.38-29.98)†	12.99 (6.32-23.26)†	23.06 (8.33-33.22)*	14.82*
Social							
Nasogenital	f	0.5 (0-2.5)	1 (0-2)	1 (0-2.5)	0.5 (0-1)	1 (0-1.5)	1.26
	d	0.99 (0-7.42)	3.37 (0-5.56)	3.13 (0-8.38)	0.57 (0-2.77)	2.1 (0-4.36)	1.33
Nasonasal	f	2 (1-4)	4 (2-4.5)	1.5 (0.5-3.5)	3 (0-4)	2 (1-3)	3.95
	d	4.8 (1.72-8.58)	9.23 (4.29-19.43)	6.92 (0.49-9.72)	7.83 (0-15.36)	7.24 (1.64-9.02)	2.35
Nonspecific investigation	f	5.5 (2-8)	4 (3.5-8.5)	5 (2-8.5)	4 (1-4.5)	3.5 (2-5.5)	2.89
	d	16.33 (5.97-22.22)	12.97 (6.41-20.44)	18.51 (4.21-46.55)	18.44 (1.5-27.63)	11.61 (3.39-16.34)	1.84
Follow	f	0 (0-0.5)	1 (0-1)	0.5 (0-1.5)	0.5 (0-1.5)	1 (0-1.5)	1.58
	d	0 (0-1.29)	1.97 (0-2.52)	1.78 (0-3.62)	0.46 (0-2.41)	1.88 (0-2.93)	1.61
Attend/approach	f	4 (3-6.5)	10.5 (4-11.5)	7.5 (4-12)	9.5 (4.5-12)	8.5 (3.5-11.5)	5.29
	d	7.84 (5.66-15.53)	21.65 (7.58-27.05)	16.7 (11.08-26.64)	19.26 (9.65-26.31)	19.24 (7.32-25.18)	4.74
Stretch/attend	f	1 (0-1.5)	1 (0-2)	2.5 (1-3)*	3.5 (1-4)*	2 (1-3.5)	8.18
	d	2.82 (0-4.52)	2.39 (0-3.46)	5.21 (1.86-8.17)	8.24 (3.21-12.41†	4.03 (1.58-6.46)	8.16

Offensive							
Aggressive groom	f	0 (0–0)	0 (0–0)	0 (0–0.5)	0 (0–0)	0 (0–0)	4.44
	d	0 (0–0)	0 (0–0)	0 (0–1.72)	0 (0–0)	0 (0–0)	4.81
Tail rattle	f	0.5 (0–1)	1.5 (0–3.5)	1.5 (0–6)	2 (0–5)	4.5 (0–8)	4.27
	d	1.04 (0–2.12)	3.78 (0–7.2)	2.69 (0–9.71)	4.82 (0–12.58)	10.55 (0–14.75)	4.57
Offensive sideways	f	0 (0–4)	1.5 (0–3)	3 (0–6)	2.5 (0–5.5)	4 (0.5–7)	3.72
	d	0 (0–11.08)	4.34 (0–8.10)	7.34 (0–17.19)	6.93 (0–15.90)	12.57 (1.83–28.02)	3.14
Offensive upright	f	0.5 (0–1.5)	0.5 (0–1.5)	1.5 (0–3)	1.5 (0–2.5)	1 (0–1)	1.15
	d	2.11 (0–6.82)	1.26 (0–5.08)	4.03 (0–11.37)	2.65 (0–8.01)	3.07 (0–4.47)	0.42
Chase	f	0 (0–3)	1.5 (0–3.5)	1.5 (0–3)	0.5 (0–2)	2 (0–3.5)	1.22
	d	0 (0–8.15)	3.14 (0–9.5)	2.57 (0–8.27)	0.63 (0–6.08)	2.76 (0–9.16)	1.14
Bite attack	f	0 (0–1)	0 (0–1)	2 (0–4)	2.5 (0–3)	1 (0–2)	5.94
	d	0 (0–2.62)	0 (0–3.94)	11.27 (0–20.66)	14.65 (0–17.9)	3.37 (0–8.1)	6.02
Defensive							
Evade	f	3.5 (1.5–5.5)	2 (0.5–5)	5 (2.5–9.5)	6.5 (3.5–8)	5 (1–6.5)	3.37
	d	7.79 (3.48–14.84)	10.35 (2.33–13.87)	10.74 (4.78–20.61)	13.84 (5.19–20.93)	10.73 (2.63–15.52)	0.81
Defensive sideways	f	2.5 (1–7)	2 (0.5–4)	9 (3–12.5)	7.5 (4–10)	6.5 (4–11.5)	7.99
	d	7.13 (2.54–42.99)	6.65 (1.12–12.85)	36.62 (13.48–50.22)	27.49 (11.88–45.99)	35.06 (12.71–38.59)	7.30
Defensive upright	f	0.5 (0–3)	1 (0–3)	3.5 (1.5–10)*	4 (2–5.5)	4 (2–4.5)	8.61
	d	3.73 (0–14.16)	4.05 (0–9.79)	10.67 (4.58–68.33)	11.67 (6.4–29.80)	12.12 (6.01–16.32)	7.19
Submissive upright	f	0 (0–0)	0 (0–0)	0 (0–1)	2 (0–5)	0 (0–0.5)	8.73
	d	0 (0–0)	0 (0–0)	0 (0–1.73)	9.88 (0–46.36)	0 (0–2.74)	9.38
Frozen crouch	f	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	3.06
	d	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	3.06

Data expressed as medians (upper to lower quartiles) for frequency (f) and duration (d). Significant values refer to Mann-Whitney comparisons with vehicle.
*$p < 0.05$; †$p < 0.02$; ‡$p < 0.002$.

element of defensive behaviour (i.e., defensive sideways). This relatively minor modification of defensive behaviour should not be interpreted as an increase in murine anxiety.

The enhanced threat behaviour of the resident mice induced by (+)-WAY-100135 appears to be reflected by concomitant changes in the behavioural profile exhibited by the drug-free intruder animals. Intruder mice showed reduced cage exploration and rearing behaviour when the resident mice exhibited the maximal drug-induced increase in offensive sideways behaviour following treatment with (+)-WAY-100135, 5.0 mg/kg. The digging behaviour of intruder mice was also decreased when (+)-WAY-100135 was administered to the resident mice across the dose range 2.5–10.0 mg/kg. It would appear, therefore, that intruder mice responded to the increased resident offensive behaviour by engaging, to some degree, in defensive and possibly counter-offensive behaviours. Such a strategy may account for the reduction in non-social elements as intruders select appropriate behaviours in response to those of the residents. Furthermore, these behavioural changes contrast with the enhancement of non-social elements reported for intruder animals examined in our studies on pindobind 5-HT_{1A} (2), (−)-pindolol and SDZ 216-525 (3). These earlier results suggest that intruder mice exhibit increased exploratory behaviour and reduced defensive postures, when encountering less aggressive resident mice (2,3). If the changes in the behavioural profiles of drug-free intruder mice obtained in the pindobind 5-HT_{1A}, (−)-pindolol, SDZ 216-525 and (+)-WAY-100135 studies are considered collectively, then the behavioural profile of intruder animals is clearly dependent upon whether drug-treatment attenuates or, as in the case of (+)-WAY-100135, enhances the offensive behaviour of the resident mice.

It is of interest to compare the possible mechanisms whereby (+)-WAY-100135 and SDZ 216-525 exert their respective influences on murine agonistic behavior. The mediation of (+)-WAY-100135 induced effects on agonistic behavior may reflect antagonistic properties of this compound at presynaptic somatodendritic 5-HT_{1A} autoreceptors (32), postsynaptic 5-HT_{1A} receptors (13), or α_1-adrenoceptors (22). SDZ 216-525 has been reported to behave as a partial agonist at presynaptic somatodendritic 5-HT_{1A} autoreceptors (22,27) and as an antagonist at α_1-adrenoceptors (32). If (+)-WAY-100135 behaved purely as an antagonist at central 5-HT_{1A} receptors (27,32) then the increased offensive behaviour, as reported in this study, would be consistent with the opposite effects obtained with the 5-HT_{1A} receptor partial agonist SDZ 216-525 on agonistic behavior (3). Thus the ability of acute treatment with 5-HT_{1A} receptor agonists and partial agonists to reduce the aggressive behaviour of mice (2–4,11,28,29,33) and rats (10,26) is probably due to agonist activity at postsynaptic 5-HT_{1A} receptor sites. These observations imply that the enhanced threat behaviour of resident mice induced by acute treatment with (+)-WAY-100135 is probably due to antagonist activity at postsynaptic 5-HT_{1A} receptors. The moderate attenuation of the enhanced threat behaviour, observed following treatment with (+)-WAY-100135, 10.0 mg/kg, may be due to reduced postsynaptic 5-HT_{1A} receptor blockade as a consequence of enhanced terminal 5-HT release due to blockade of the presynaptic somatodendritic 5-HT_{1A} receptor-mediated negative feedback system (16). Since (+)-WAY-100135 and SDZ 216-525 both possess α_1-adrenoceptor antagonist activity (22,32), then such pharmacological activity is unlikely to be responsible for the contrasting effects of these two compounds on offensive behaviour.

Although (+)-WAY-100135 was previously found to be without effect on hippocampal 5-HT release (32), Fornal et al. (14) have recently reported that the compound exerted a modest suppression of serotonergic neuronal activity in the dorsal raphe nucleus. This finding indicates that (+)-WAY-100135 may possess weak partial agonist activity, rather than antagonist activity, at presynaptic somatodendritic 5-HT_{1A} autoreceptors (25). If (+)-WAY-100135 possesses partial agonist activity at 5-HT_{1A} receptors akin to SDZ 216-525, then an alternative explanation for the contrasting effects on murine offensive behavior is required. Of particular interest is the fact that (+)-WAY-100135 has been shown to significantly increase extracellular levels of noradrenaline (NE) in the hippocampus (32). Activation of α_2-adrenoceptors located on the terminals of 5-HT neurons in rat brain inhibits 5-HT release (8). A similar mechanism activated by increased NE levels in murine brain would subsequently exert an inhibitory effect on the release of 5-HT from 5-HT neurons, resulting in enhanced aggressive behaviour (1) and possible interference with the putative anxiolytic-like effects mediated by 5-HT (9).

In conclusion, present data indicate that acute treatment with (+)-WAY-100135 enhances murine threat behavior. Such results have not been reported in previous studies in mice employing other 5-HT_{1A} receptor partial agonists or antagonists. In addition, no conclusions regarding the potential anxiolytic-like activity of this compound may be drawn from this study.

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