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Blockade of 5-HT$_{1B}$ receptors facilitates contextual aversive learning in mice by disinhibition of cholinergic and glutamatergic neurotransmission

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Abstract

Serotonergic (5-HT) neurotransmission plays a role in learning and memory processes, but the physiological role of various receptor subtypes is not well characterised. Among these, 5-HT$_{1B}$ receptors are located as autoreceptors on 5-HT axons and heteroreceptors on non-serotonergic terminals. This study examined the role of the 5-HT$_{1B}$ receptor in one-trial aversive contextual learning using the passive avoidance (PA) task in NMRI mice. Subcutaneous administration of the 5-HT$_{1B}$ receptor agonist anpirtoline (0.1–1.0 mg/kg) before PA training impaired retention performance 24 h later. Combined administration of anpirtoline with the selective 5-HT$_{1B}$ receptor antagonist NAS-181 (0.1–1.0 mg/kg) fully blocked the impairments. Administration of NAS-181 alone dose-dependently improved PA retention performance. This facilitatory effect was blocked by subthreshold doses of both the muscarinic antagonist scopolamine (0.03 mg/kg) and the NMDA receptor antagonist MK-801 (0.03 mg/kg). NAS-181 also fully blocked the PA impairments induced by an amnesic dose of scopolamine (0.1 mg/kg), when administered prior to, but not after, scopolamine. In addition, NAS-181 attenuated PA impairments induced by MK-801 (0.3 mg/kg). These findings indicate that 5-HT$_{1B}$ receptors are activated at basal levels of 5-HT transmission. The facilitatory effect of NAS-181 involved alleviation of an inhibitory 5-HT tone mediated via 5-HT$_{1B}$ receptors on cholinergic and glutamatergic transmission. This disinhibition is expected to occur in neuronal circuits involved in contextual learning including the hippocampus and interconnected cortico-limbic regions. Blockade of brain 5-HT$_{1B}$ heteroreceptors may represent a novel therapeutic strategy for restoration of deficient cholinergic and glutamatergic neurotransmission contributing to memory disorders.

Keywords: Contextual learning; Memory; Passive avoidance; 5-HT$_{1B}$ receptor; Acetylcholine; Glutamate

1. Introduction

The serotonergic (5-HT) system is involved in a range of physiological functions including learning and memory processes (e.g. Hoyer et al., 2002; Ögren, 1985a; Sari, 2004). The ascending 5-HT projections innervate corticolimbic areas and exert a modulatory influence on several neurotransmitter systems via multiple receptor subtypes (Azmitia and Segal, 1978; Hoyer et al., 2002). The 5-HT$_{1B}$ receptor functions as a terminal autoreceptor, decreasing the release of 5-HT (Boschert et al., 1994; Engel et al., 1986). Consistently, microdialysis data support an inhibitory role of 5-HT$_{1B}$ receptors in the control of 5-HT release in the hippocampus and frontal cortex of mice and rats (Adell et al., 2001; de Groote et al., 2002, 2003; Hertel et al., 1999; Hjorth and Tao, 1991; Trillat et al., 1997). In addition, 5-HT$_{1B}$ receptors are located postsynaptically to serotonergic neurons, predominantly on axon terminals (Boschert et al., 1994). These heteroreceptors are thus in position to modulate the release of multiple neurotransmitters. Neurophysiological and neurochemical data based on basal and electrically evoked

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release of transmitters in brain slices and synaptosome preparations suggest an inhibitory role of 5-HT_{1B} receptors on the release of acetylcholine (ACh) (Bolanosjimenez et al., 1994; Cassel et al., 1995; Rutz et al., 2006; Steckler and Sahgal, 1995), glutamate (Boeijinga and Boddeke, 1996; Li and Bayliss, 1998) and GABA (Chadha et al., 2000; Johnson et al., 1992). The effects of 5-HT_{1B} receptor agonists on ACh release are inconsistent. Thus, when using microdialysis in freely moving rats, it was found that local administration of the 5-HT_{1B} receptor agonists CGS-12066B decreased extracellular levels of dorsohippocampal ACh (Izumi et al., 1994). In contrast, the 5-HT_{1B} receptor agonist CP-93,129 and the unselective 5-HT releasing compound d-norfenfluramine given locally, increased ACh in the frontal cortex (Consolo et al., 1996). Supporting a potential inhibitory role of the 5-HT_{1B} receptor on ACh outflow, systemic administration of the selective 5-HT_{1B} receptor antagonist NAS-181 (Berg et al., 1998) dose-dependently increased extracellular levels of ACh in both the frontal cortex and ventral hippocampus of awake rats (Hu et al., 2007).

The physiological role of the 5-HT_{1B} receptor in cognition is still unclear, partly due to the lack of sufficiently selective 5-HT_{1B} receptor antagonists and divergent results from studies with pharmacological interventions *vs*. 5-HT_{1B} receptor knockout (KO) mice. For instance, systemic and intrahippocampal administration of the 5-HT_{1B} receptor agonists anpirtoline and CP-93,129 impaired spatial learning in the water maze in rats (Åhlander-Lüttgen et al., 2003; Buhot et al., 1995). Anpirtoline also impaired aversive learning in the passive avoidance (PA) task in rats (Åhlander-Lüttgen et al., 2003). Consistently, the 5-HT_{1B} receptor antagonist NAS-181 did not alter spatial learning, but facilitated PA retention (Åhlander-Lüttgen et al., 2003). On the other hand, 5-HT_{1B} receptor KO mice displayed enhanced acquisition in a reference memory procedure in the Morris water maze (Malleret et al., 1999), while they were impaired in delayed working memory using the radial-arm water maze (Wolff et al., 2003). 5-HT_{1B} receptor KO mice did not display any changes in fear conditioning (Malleret et al., 1999) contrary to the facilitatory effect of NAS-181 in the PA task.

The aim of the present study was to analyse the role of the 5-HT_{1B} receptor in emotional learning in mice by using receptor-specific compounds, i.e. the 5-HT_{1B} receptor antagonist NAS-181 (Berg et al., 1998) and the 5-HT_{1B} receptor agonist anpirtoline (Schlicker et al., 1992). In view of the evidence that 5-HT_{1B} receptors serve as heteroreceptors, an important aim was to further investigate whether 5-HT_{1B} receptor function influences cholinergic and glutamatergic neurotransmission. The 5-HT_{1B} receptor may serve as a potential target for novel pharmacological strategies aiming at restoration of deficient cholinergic and glutamatergic neurotransmission contributing to cognitive disorders. The studies were performed using the PA task, a one-trial contextual learning procedure sensitive to alterations in serotonergic, cholinergic and glutamatergic transmission (Misane and Ögren, 2003; Ögren, 1985b). The PA task involves hippocampal processing and interactions with interconnected areas, including the amygdala, medial septal area and raphe nuclei (see Baarendse et al., 2008; Elvander-Tottie et al., 2006).

2. Materials and methods

2.1. Animals

Adult male NMRI mice (Scanbur, Sollentuna, Sweden) were used in all experiments. The mice were 10 weeks of age and weighed 25–30 g at the time of testing. The animals were housed in groups of 4–6 in Macrolon-3 cages (42 × 26 × 20 cm) in a temperature- and humidity-controlled environment with 12-h light/dark cycle (lights on at 7 a.m.). Standard lab pellets (Ewos R36, Ewos AB, Södertälje, Sweden) and tap water were provided *ad libitum*. All experiments were conducted in experimentally naïve animals, with all efforts made to minimise suffering and the number of animals used. The animal experiments were approved by the local Animal Ethical Committee (Stockholm Norra Djurförsöksetiska Nämnd; Approval Numbers N155/01, N48/02, N132/01), in accordance with the European Council Directive of 24 Nov 1986 (86/609/EEC).

2.2. Drugs and drug administration

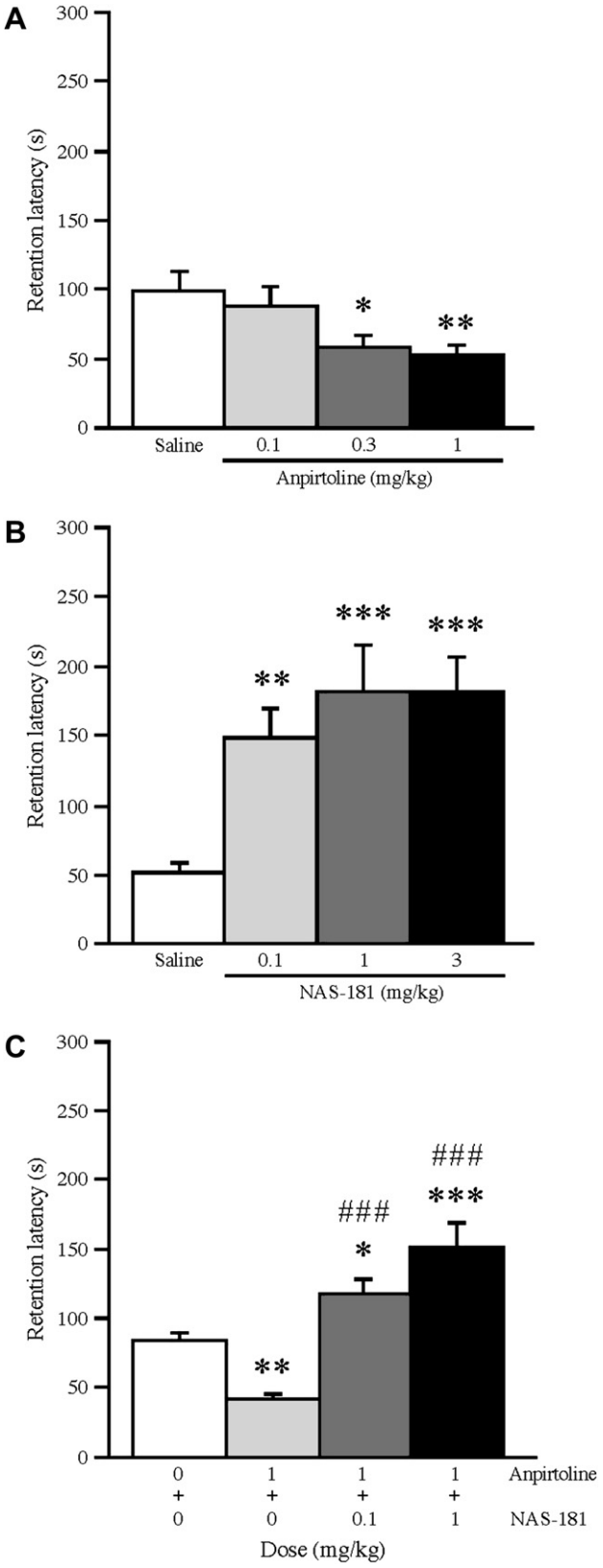
Compounds used were: NAS-181, [*R*-(+)-2-(3-morpholinomethyl-2H-chromen-8-yl) oxymethylmorpholine methanesulfonate] (AstraZeneca R&D, Södertälje, Sweden); anpirtoline hydrochloride [6-Chloro-2-[piperidinyl-4-thio]-pyridine] (Tocris, Bristol, UK); (−)-scopolamine hydrobromide (Sigma-Aldrich, St. Louis, MO); MK-801 [(5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine hydrogen maleate] (Sigma-Aldrich, St. Louis, MO). The test drugs were dissolved in sterile saline (Clean Chemical Sweden AB, Borlänge, Sweden) on the day of PA training and injected subcutaneously (s.c.) into the scruff of the neck at a volume of 4 ml/kg. Saline control groups were run concurrently. Drug administrations occurred at specific time intervals prior to training or immediately posttraining, as noted in the figure legends. The timing of NAS-181 administration was based on the observation that NAS-181 produces a maximum decrease of 5-HT synthesis in the mouse brain 30 min after injection (Stenfors et al., 2001). High doses of scopolamine or MK-801 were administered 10 min prior to training, based on findings in both rats and mice that the 5-HT_{1A} receptor antagonist NAD-299 prevents PA impairments when injected prior to, but not after, scopolamine or MK-801 (Madjid et al., 2006; Misane and Ögren, 2003). Sub-threshold doses of scopolamine or MK-801 were administered prior to NAS-181 in studies investigating mechanisms involved in 5-HT_{1B} receptor mediated effects.

2.3. Step-through passive avoidance (PA)

The contextual learning PA task was conducted as previously described (Madjid et al., 2006; Ögren, 1985b). The PA apparatus (Ugo Basile, Comerio-Varese, Italy) consisted of two compartments of identical size (10 × 16 × 18 cm) connected with a 4 × 4 cm sliding door. Both compartments had a stainless steel grid floor. The light compartment had white plastic walls and was illuminated (24 V: 5 W, 330 lx), while the dark (conditioning) compartment had black plastic walls (3 lx). Before the behavioural experiments started, the mice were habituated to the animal facilities for at least five days, followed by daily 1-min handling by the experimenter for the next five days. On the following day, behavioural testing was conducted between 10 a.m. and 3 p.m., with PA training performed on the first day and the retention test on the following day.

The animals were brought to the experimental room and allowed to acclimate in ventilated cabinets for 30–60 min prior to preexposure, followed by training and testing. Preexposure to the PA apparatus occurred 60 min before training. Mice were allowed to explore the light compartment for 60 s, with no access to the dark compartment. Thereafter, the sliding door was opened and, once the animal had crossed to the dark compartment, the door automatically closed. The animals were then allowed to explore the dark compartment for an additional 60 s. After the preexposure, animals were returned to a holding cage until administration of the test compounds as described below. This preexposure procedure has been found to reduce the latency to enter the dark compartment at the subsequent training session (Madjid et al., 2006).

For PA training, each mouse was placed in the light compartment with no access to the dark compartment. During the exploration period, rearing, defecation, and motility were observed and recorded. After 60 s, the sliding door was



opened and the latency for the mouse to step through to the dark compartment was measured as training latency. After the mouse entered the dark compartment with all four paws, the sliding door was closed and an electrical current (unconditioned stimulus, US) was delivered through the grid floor. Two different intensities of the US was used as described earlier by Madjid et al. (2006). A weak electrical current of 0.3 mA (2 s duration, scrambled current) was used to optimise detection of possibly facilitatory effects on acquisition of the PA task (see figure legends). A higher US intensity of 0.4 mA was used to increase the performance and explicitly study drug-induced impairments of PA retention. Observable reactions to the US were noted, including vocalisation and flinching. After exposure to the US, each mouse remained in the dark compartment for 30 s before being transferred to a holding cage, to prevent mixing of trained and untrained mice. After completion of training, all mice were transferred to their home cages and returned to the animal facilities.

During the retention test, 24 h after training, each mouse was placed in the light compartment with access to the dark compartment after 10 s. The retention latency to enter the dark compartment with all four paws was recorded. If an animal did not enter the dark compartment within 300 s, it was removed and assigned the maximum retention latency time of 300 s.

2.4. Statistics

The effects of the treatments on training and retention latencies were examined with a one-way analysis of variance (ANOVA), using a significance level of $P < 0.05$. For each significant F-ratio, Fisher's protected least significant difference test (Fisher's PLSD test) was used as post-hoc comparison to analyse statistical significance between individual groups. In addition, t-test analyses of training *vs*. retention latencies were performed within each treatment and control group, to determine whether animals had successfully acquired the PA task, as indicated by a significantly higher retention than training latency. Mean training latencies in all groups ranged from 15 to 30 s.

3. Results

3.1. Role of 5-HT_{1B} receptors in PA learning

3.1.1. Effects of the 5-HT_{1B} receptor agonist anpirtoline

Pretraining administration of anpirtoline dose-dependently impaired performance in the retention test 24 h after training, as shown by animals receiving the two highest doses (0.3 and 1.0 mg/kg), but not 0.1 mg/kg, displaying significantly shorter step-through latencies than control mice (ANOVA $F_{3,28} = 4.24$; $P < 0.05$; Fisher's PLSD test 0.3 mg/kg; $P < 0.05$ and 1 mg/kg $P < 0.01$) (Fig. 1A). Although anpirtoline decreased retention performance, the t-test analysis for training *vs*. retention latencies for each treatment group revealed that retention latency was significantly higher than training latency for all groups, indicating that all groups of mice had learned the task ($P < 0.001$, data not shown). No alterations related to pretraining injection of anpirtoline were detected in

Fig. 1. (A) Dose-dependent effects of the 5-HT_{1B} receptor agonist anpirtoline (0.1, 0.3 and 1 mg/kg s.c., 15 min prior to training) on PA retention of mice (US intensity during training: 0.4 mA). (B) PA retention effects of the 5-HT_{1B} receptor antagonist NAS-181 (0.1, 1 and 3 mg/kg s.c.; administered 30 min prior to training) (US: 0.3 mA). (C) Combined effects of the 5-HT_{1B} receptor antagonist and agonist (30 + 15 min prior to training, respectively) (US: 0.4 mA). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs*. saline, $^{\#\#\#}P < 0.001$ *vs*. anpirtoline 1 mg/kg. The saline + saline control groups were run concurrently in all experiments. Bars represent means + S.E.M.; $n = 8$/group.

training latency or number of rearings ($F_{3,28} = 0.08$; *n.s.*, $F_{3,28} = 0.24$; *n.s.*, respectively, data not shown).

3.1.2. Effects of the 5-HT$_{1B}$ receptor antagonist NAS-181

NAS-181 was administered in order to investigate the role of basal 5-HT$_{1B}$ receptor transmission for acquisition of the PA task. Blockade of 5-HT$_{1B}$ receptors resulted in an overall effect of treatment on retention latency ($F_{3,28} = 6.99$; $P < 0.01$), with the post-hoc analysis revealing that pretraining administration of NAS-181 facilitated PA performance (Fig. 1B). Indeed, animals treated with NAS-181 displayed significantly longer retention latencies at all three doses tested when compared to saline-treated control animals ($P < 0.01$; $P < 0.001$ and $P < 0.001$ for 0.1, 1 and 3 mg/kg, respectively). Correspondingly, *t*-test analyses showed significantly longer retention than training latencies in each group ($P < 0.001$, data not shown), indicating that all groups had acquired the task. In addition, no drug-induced changes were found for training using a one-way ANOVA ($F_{3,28} = 0.14$; *n.s.*, $F_{3,28} = 1.54$; *n.s.* for training latency and rearing, respectively, data not shown).

3.1.3. Co-administration of anpirtoline and NAS-181

This agonist-antagonist experiment addressed the question of whether the impairment of PA memory caused by anpirtoline was 5-HT$_{1B}$ receptor-dependent (Fig. 1C). One-way ANOVA of retention latencies revealed an overall difference ($F_{3,28} = 19.32$; $P < 0.001$), with anpirtoline (1 mg/kg) again decreasing retention latency ($P < 0.01$). Importantly, NAS-181 completely blocked the impairment induced by anpirtoline. Thus, animals receiving combined treatments (NAS-181 0.1 mg/kg + anpirtoline 1 mg/kg and 1 mg/kg + NAS-181 1 mg/kg + anpirtoline) showed significantly longer retention latencies than animals given anpirtoline alone ($P < 0.001$ for both treatments) (Fig. 1C). Co-administration of anpirtoline with NAS-181 0.1 or 1.0 mg/kg also increased retention latencies as compared to controls ($P < 0.05$, $P < 0.001$, respectively). Similar to the results of anpirtoline and NAS-181 administered alone, the group-wise comparison of training *vs.* retention latencies indicated that all animals had acquired the task ($P < 0.001$, data not shown) and there were no significant effects of treatment on either training latency or number of rearings (data not shown).

3.1.4. Posttraining administration of 5-HT$_{1B}$ receptor ligands

To complement the previous studies on the role of 5-HT$_{1B}$ receptor in encoding of PA learning, this experiment investigated whether the 5-HT$_{1B}$ receptor affects memory consolidation processes. As illustrated in Fig. 2, administration of either anpirtoline or NAS-181 immediately after PA training did not alter retention performance ($F_{2,21} = 0.12$; *n.s.*). The *t*-test analyses showed that retention latencies were significantly higher than training latencies in all groups ($P < 0.001$, data not shown). Taken together, these data indicate that PA learning was not affected by posttraining 5-HT$_{1B}$ receptor intervention.

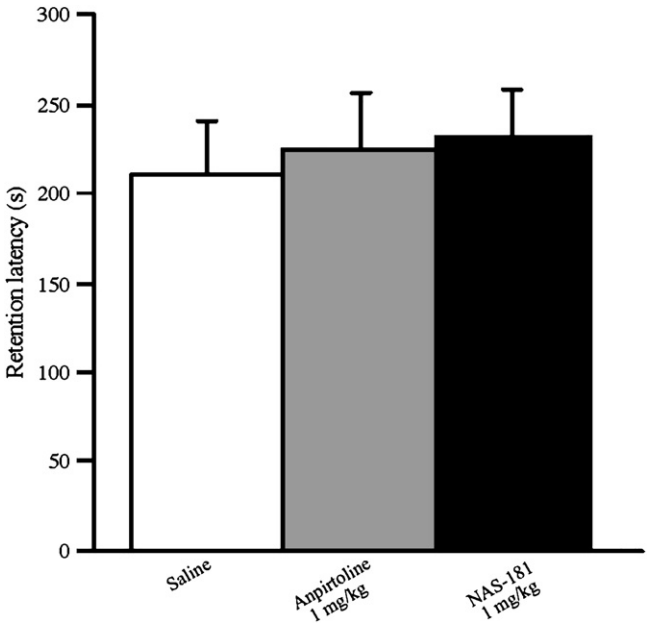
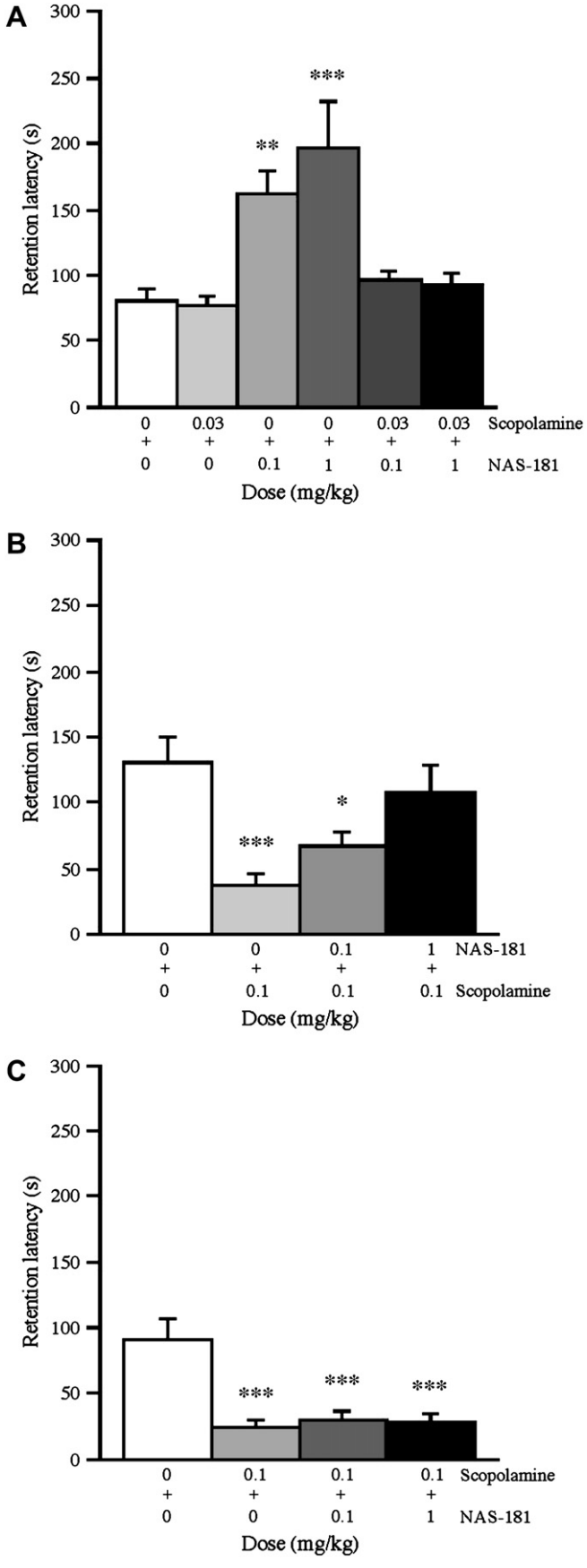


Fig. 2. PA retention performance following administration of 5-HT$_{1B}$ receptor agonist and antagonist anpirtoline and NAS-181, respectively, immediately after PA training (US: 0.4 mA). Bars represent means + S.E.M.; $n = 8$/group.

3.2. Involvement of cholinergic mechanisms in 5-HT$_{1B}$ receptor-mediated PA effects

3.2.1. Effects of a subthreshold dose of scopolamine on the effect of NAS-181

Since blockade of 5-HT$_{1B}$ receptors increases ACh efflux (Hu et al., 2007) this experiment aimed at elucidating whether pretreatment with a subthreshold dose of the muscarinic ACh receptor antagonist scopolamine could prevent the facilitation of PA memory caused by NAS-181. A one-way ANOVA of retention performance indicated an overall effect ($F_{5,30} = 8.26$; $P < 0.001$). As previously shown (Madjid et al., 2006), the 0.03 mg/kg dose of scopolamine did not affect PA performance (saline *vs.* scopolamine 0.03 mg/kg: *n.s.*). Consistent with the dose-response study (Fig. 1B), NAS-181 facilitated retention performance at 0.1 mg/kg ($P < 0.01$) and 1 mg/kg ($P < 0.001$). Notably, co-administration of scopolamine completely prevented the increase in retention latency induced by NAS-181 at 0.1 mg/kg (scopolamine + NAS-181 *vs.* NAS-181: $P < 0.05$) and 1 mg/kg scopolamine + NAS-181 *vs.* NAS-181: $P < 0.001$) and resulted in retention performance similar to that of controls (Fig. 3A). The subsequent *t*-tests indicated that retention latencies were higher than training latencies in all groups, supporting the conclusion that all groups learned the PA task ($P < 0.001$, data not shown). In addition, there were no effects of treatment on training latencies ($F_{5,30} = 0.17$: *n.s.*), but all groups displayed significantly fewer rearings than control mice ($P < 0.05$ $F_{5,30} = 3.06$ on ANOVA; post-hoc $P < 0.05$ for saline *vs.* scopolamine; $P < 0.01$ for saline *vs.* additional treatment groups, data not shown).



3.2.2. Effects of NAS-181 on an amnesic dose of scopolamine

To link a facilitatory effect of blockade of 5-HT_{1B} receptors to cholinergic transmission, the next experiments investigated whether pretreatment with NAS-181 could prevent the impairing effects on PA memory caused by a high dose (0.1 mg/kg) of scopolamine (see Madjid et al., 2006). Pretraining treatments caused significant differences in retention latencies ($F_{3,20} = 6.71$; $P < 0.01$). Post-hoc analyses indicated that scopolamine (0.1 mg/kg) impaired PA learning, since the retention latency in this group was significantly shorter than in controls ($P < 0.001$) (Fig. 3B). This impairment caused by scopolamine was completely blocked by NAS-181 1 mg/kg ($P < 0.01$), and the retention latency did not differ from that of the controls. The group receiving the combination of the lower dose of NAS-181 (0.1 mg/kg) and scopolamine did not differ from scopolamine alone. The analyses of training and retention latencies within each group further supported that NAS-181 blocked the impairing effect of scopolamine, since retention latencies were significantly longer than training latencies in all groups ($P < 0.001$, data not shown) except for scopolamine alone (training 16.2 s *vs.* retention 36.4 s, $t = -2.00$ with 10 degrees of freedom (df) *n.s.*). Statistical analysis of the training session showed no significant difference in training latency ($F_{3,20} = 0.48$, *n.s.*), with an exception of decreased rearing by scopolamine ($F_{3,20} = 6.07$; $P < 0.01$) when administered 10 min prior to training (see figure legends and compare with Section 3.2.3). Animals receiving scopolamine (0.1 mg/kg) or NAS-181 (0.1 mg/kg) + scopolamine (0.1 mg/kg) reared less often than controls ($P < 0.001$ and $P < 0.01$, respectively). The scopolamine-induced decrease of rearing was significantly attenuated by the higher dose of NAS-181 ($P < 0.05$), and returned to control level ($P = 0.18$, *n.s.*).

3.2.3. Effects of an amnesic dose of scopolamine prior to administration of NAS-181

Since pretreatment of NAS-181 was found to prevent the amnesic effects of scopolamine (Fig. 3B), an additional experiment was designed to investigate whether the impairing effect of muscarinic ACh receptor blockade could also be reversed when NAS-181 was administered after scopolamine. Analysis of the retention latencies indicated an overall effect of treatment ($F_{3,20} = 10.96$; $P < 0.001$) (Fig. 3C), and the post-hoc test showed that scopolamine 0.1 mg/kg impaired PA retention ($P < 0.001$ *vs.* saline). In contrast to the beneficial effect of NAS-181 when administered prior to scopolamine, the impairment by scopolamine could not be reversed by NAS-181 at

Fig. 3. (A) Effects of the muscarinic ACh receptor antagonist scopolamine when preinjected at a subthreshold dose (0.03 mg/kg s.c., 40 min prior to training) on the facilitatory effect of the 5-HT_{1B} receptor antagonist NAS-181 (0.1 or 1 mg/kg s.c., 30 min pretraining) on PA retention (US: 0.3 mA). (B) Effects of NAS-181 (0.1 or 1 mg/kg s.c., 20 min prior training) on impairments of PA performance induced by scopolamine (0.1 mg/kg s.c., 10 min pretraining) (US: 0.4 mA). (C) Effects of scopolamine (0.1 mg/kg s.c., 40 min pretraining) followed by NAS-181 (0.1 or 1 mg/kg s.c., 30 min pretraining) (US: 0.3 mA). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.01$ *vs.* control. Bars represent means + S.E.M.; $n = 6$/group.

either of the two doses tested (scopolamine *vs.* scopolamine + NAS-181 0.1 mg/kg *n.s.*; scopolamine *vs.* scopolamine + NAS-181 1 mg/kg *n.s.*). Consistent with the inability of NAS-181 to reverse scopolamine-mediated impairments, none of the treatment groups showed significant differences between training and retention latencies (13.0 *vs.* 24.6 s, $t = -2.12$; 13.6 *vs.* 30.2 s, $t = 2.33$; 14.7 *vs.* 27.1 s, $t = -1.66$, respectively, 10 df, *n.s.*). In contrast to the decreased rearing when scopolamine was injected 10 min prior to training (see figure legends and compare with Section 3.2.2 above), administration of scopolamine 40 min pretraining did not alter training latency ($F_{3,20} = 0.19$, *n.s.*) or rearing activity ($F_{3,20} = 2.65$, *n.s.*).

3.3. Involvement of glutamatergic mechanisms in 5-HT$_{1B}$ receptor-mediated PA effects

3.3.1. Effects of a subthreshold dose of MK-801 on the effect of NAS-181

Since the functional role of 5-HT$_{1B}$ receptors located on glutamatergic terminals (see Section 1) has not yet been identified, this experiment aimed to reveal whether the facilitatory effect of NAS-181 on PA learning involves changes in glutamatergic NMDA receptor signalling. Confirming the dose-response study (see Fig. 1B), treatment with NAS-181 prior to training resulted in significantly longer retention latencies than that of the control group (0.1 mg/kg, $P < 0.05$ and 1 mg/kg, $P < 0.001$, with an overall effect on the ANOVA; $F_{4,35} = 7.26$; $P < 0.001$). This facilitatory effect on PA retention induced by NAS-181 was prevented with a low dose of the NMDA receptor antagonist MK-801 that does not impair PA performance (see Madjid et al., 2006) ($P < 0.05$ for MK-801 0.03 mg/kg + NAS-181 0.1 mg/kg *vs.* NAS-181 0.1 mg/kg; $P < 0.001$ for MK-801 0.03 mg/kg + NAS-181 1 mg/kg *vs.* NAS-181 1 mg/kg) as shown in Fig. 4A. Analysis of training and retention latencies for each group showed significantly longer retention than training latencies for all groups ($P < 0.001$, data not shown). Analysis of the training session showed no statistically significant differences between groups on step-through latency or rearing ($F_{4,35} = 0.19$; *n.s.*, $F_{4,35} = 0.31$; *n.s.*, respectively).

3.3.2. Effects of NAS-181 on an amnesic dose of MK-801

To examine the extent to which 5-HT$_{1B}$ heteroreceptors may influence brain processing relevant to aversive contextual learning, the next set of experiments investigated whether NAS-181 can attenuate the impairment of PA learning caused by the NMDA receptor antagonist MK-801. The dose of MK-801 0.3 mg/kg was selected on the basis of previous findings that this dose produced a deficit in PA performance (Madjid et al., 2006). The analysis of the retention test displayed an overall effect of treatment ($F_{3,20} = 10.89$; $P < 0.001$) (Fig. 4B). Post-hoc analyses confirmed that pretraining administration of MK-801 severely disrupted acquisition of the PA task ($P < 0.001$). The MK-801-induced PA deficit was attenuated by NAS-181 ($P < 0.05$ for NAS-181 1 mg/kg + MK-801 0.3 mg/kg *vs.* MK-801 0.3 mg/kg; $P < 0.05$ for NAS-181 1 mg/kg + MK-801 0.3 mg/kg *vs.* saline) with no significant effect of the lower dose of NAS-181 (Fig. 4B). The beneficial effect of NAS-181 on MK-801-induced impairments was also supported by the finding that both controls and the group treated with 1 mg/kg of NAS-181 prior to MK-801 displayed significant differences in training compared to retention latencies ($P < 0.001$, data not shown), indicating that these animals had learned the PA task. In contrast, the same analyses demonstrated that mice injected with MK-801 alone or the lower dose of NAS-181 combined with MK-801 displayed non-significant effects on training compared to retention latencies (18.8 *vs.* 36.3 s,

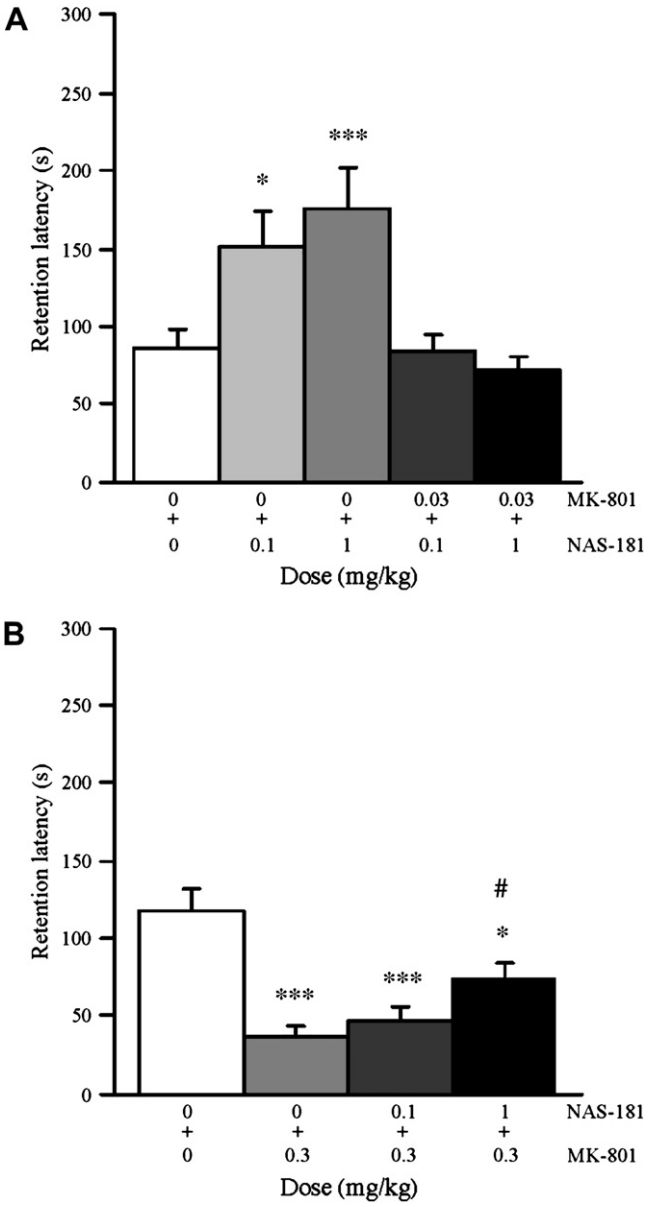


Fig. 4. (A) Effects of a subthreshold dose of the NMDA receptor antagonist MK-801 (0.03 mg/kg s.c., 40 min pretraining) on the facilitatory effect of NAS-181 (0.1 or 1 mg/kg s.c., 30 min pretraining) on PA retention (US: 0.3 mA). (B) Effects of pretreatment with NAS-181 (0.1 or 1 mg/kg s.c., 20 min pretraining) on the PA impairment caused by MK-801 (0.3 mg/kg s.c., 10 min pretraining) (US: 0.4 mA). $^{*}P < 0.05$ and $^{***}P < 0.01$ *vs.* control; $^{\#}P < 0.05$ *vs.* MK-801 (0.3 mg/kg s.c.). Bars represent means + S.E.M.; $n = 8$/group (A) and $n = 6$/group (B).

$t = -1.88$ for MK-801 0.3 mg/kg; 25.5 *vs.* 46.3 s, $t = -1.76$ for NAS-181 0.1 mg/kg + MK-801 0.3 mg/kg, with 10 df, *n.s.*). Analyses of behaviours during the training session showed no effects of treatments on training latency ($F_{3,20} = 0.40$; *n.s.*), but did reveal an overall effect in number of rearings ($F_{3,20} = 3.72$; $P < 0.05$, data not shown). In addition, NAS-181 dose-dependently blocked the reduction of rearing caused by MK-801 ($P = 0.056$ *n.s.* for NAS-181 0.1 mg/kg; $P < 0.05$ for NAS-181 1 mg/kg, data not shown).

4. Discussion

The present results demonstrate that pretraining administration of the 5-HT_{1B} receptor agonist anpirtoline impaired contextual memory performance, while the antagonist NAS-181 facilitated PA retention. The results provide evidence that 5-HT_{1B} receptor activation mediates an inhibitory influence on cognitive processing during acquisition, but not consolidation, since retention performance was not affected when anpirtoline was administered immediately posttraining. The role of the 5-HT_{1B} receptor in the encoding phase of PA learning is further supported by the absence of the facilitatory effect of NAS-181 when administered after training. These data also indicate that 5-HT_{1B} receptors mediate their action via an inhibitory action on cholinergic and glutamatergic transmission. Notably, the facilitatory effect of 5-HT_{1B} receptor blockade was prevented by subthreshold doses of either scopolamine or MK-801. Blockade of 5-HT_{1B} receptors also attenuated PA impairments induced by high amnesic doses of scopolamine and MK-801.

The physiological role of the 5-HT_{1B} receptor in learning processing has been difficult to characterise due to the lack of specific pharmacological tools (see Hoyer et al., 2002). Anpirtoline is used as a standard 5-HT_{1B} receptor agonist displaying a 50-fold higher affinity for 5-HT_{1B} receptors *vs.* 5-HT_2 receptors and a 5-fold higher selectivity compared to rat 5-HT_{1A} receptors (Schlicker et al., 1992). The effect of anpirtoline, especially at higher doses, could therefore be suspected to be partly related to stimulation of 5-HT_{1A} receptors. However, NAS-181, which has a low affinity for 5-HT_{1A} receptors (Berg et al., 1998), fully blocked the learning impairments caused by anpirtoline in both mice and rats (Åhlander-Lüttgen et al., 2003), and the decrease of 5-HT synthesis induced by anpirtoline (Stenfors et al., 2001). These data indicate a selective involvement of 5-HT_{1B} receptors in the actions of anpirtoline in the dose-range used in this study. In the view of lack of data on brain levels of the compounds, the possibility of pharmacokinetic interactions cannot be excluded. However, the compounds were injected s.c. instead of i.p. in order to reduce the risk for hepatic pharmacokinetic interactions, since i.p. administration of many drugs results in a high degree of first-passage metabolism.

The behavioural outcome of 5-HT_{1B} receptor blockade depends on the basal level of 5-HT_{1B} receptor activation by endogenously released 5-HT. These data provide functional evidence that activation of 5-HT_{1B} receptors mediates a tonic inhibitory influence on signalling in neuronal circuits important for cognitive processing. An action via 5-HT_{1B} autoreceptors probably plays a minor role in the enhancing effect of NAS-181. Theoretically, blockade of autoreceptors would increase 5-HT outflow stimulating several 5-HT receptor subtypes, notably postsynaptic 5-HT_{1A} receptors. The 5-HT_{1A} receptor has consistently been demonstrated to impair acquisition of rodents in aversive and spatial learning tasks (Carli and Samanin, 1992; Lüttgen et al., 2005; Madjid et al., 2006; Misane and Ögren, 2000; Stiedl et al., 2000b). In addition, since 5-HT_{1B} receptor antagonists do not increase extracellular levels of 5-HT (see de Groote et al., 2002, 2003; Hjorth et al., 2000), it is likely that an inhibition of 5-HT_{1B} hetero- rather than autoreceptor signalling is the main mechanism underlying the facilitatory effects of 5-HT_{1B} receptor blockade on aversive contextual learning.

The present findings are consistent with behavioural and neurochemical data in rats (Åhlander-Lüttgen et al., 2003; Buhot et al., 1995; Hu et al., 2007) indicating that the facilitatory effect of 5-HT_{1B} receptor blockade involves activation (disinhibition) of cholinergic and glutamatergic transmission. Indeed, the facilitatory effect on PA retention caused by NAS-181 was blocked by subthreshold doses of scopolamine and MK-801, while administration of NAS-181 attenuated the amnesic effect induced by high doses of scopolamine and MK-801. Although changes in memory performance in 5-HT_{1B} receptor KO mice have been attributed to changes in ACh release, indirect data from hippocampal, but not cortical, slices support this notion (Rutz et al., 2006). In the rat, the facilitatory effect on aversive learning caused by NAS-181 is blocked by the muscarinic antagonist scopolamine (Åhlander-Lüttgen et al., 2003). Moreover, NAS-181 enhanced PA retention at doses which increase extracellular levels of ACh in the ventral hippocampus and frontal cortex (Hu et al., 2007).

Hippocampal 5-HT_{1B} heteroreceptors are located in positions to control both glutamatergic and cholinergic transmission. Previous studies mainly investigated the location of 5-HT_{1B} receptors on glutamatergic neurons, i.e. terminals of CA1 pyramidal cells in major output projections to the subiculum (Ait Amara et al., 1995, 2001; Boeijinga and Boddeke, 1996; Boschert et al., 1994). The majority of the mRNA-encoding 5-HT_{1B} receptors synthesised in CA1 pyramidal cells migrate to CA1 axon terminals located in the stratum oriens and dorsal subiculum (Boeijinga and Boddeke, 1996). In addition, 5-HT_{1B} receptors have also been found to mediate a presynaptic inhibitory influence on excitatory postsynaptic potentials in local CA1/CA1 circuits (Ait Amara et al., 2001; Mlinar et al., 2003). In comparison, indirect evidence suggests that stimulation of 5-HT_{1B} receptors inhibits ACh release in hippocampal slices (Cassel et al., 1995). Despite the compelling evidence that 5-HT_{1B} receptors are located on glutamatergic rather than cholinergic terminals, 5-HT_{1B} receptors were recently found to mediate a tonic suppression of cholinergic transmission in the ventral hippocampus and frontal cortex of rats, while no detectable changes in extracellular glutamate were found (Hu et al., 2007, see above).

The finding that the subthreshold dose of scopolamine prevented the facilitatory effect of NAS-181 and that pre-treatment with NAS-181 blocked the impairing effect of the amnesic dose of scopolamine implies that these effect of scopolamine is predominantly caused by blockade of muscarinic ACh receptors

located somatodendritically on postsynaptic neurons. The postsynaptic effects of scopolamine induce memory deficits, which in many ways mimic the memory impairments seen in dementia (Bartus et al., 1982). The M_1, M_3 and M_5 receptor subtypes stimulate phospolipase C signalling pathways and are expressed on cholinergic neurons in the medial septal area/diagonal band of Broca and on glutamatergic pyramidal cells of the hippocampus (see Caulfield, 1993). In contrast, M_2 receptors are predominantly located on cholinergic terminals in the hippocampus and blockade of these receptors by scopolamine is expected to increase the release of ACh, similarly to the effect of NAS-181 itself (Hoss et al., 1990; Hu et al., 2007). It is important to note the differences between the scopolamine model and clinical dementias, involving loss of cholinergic neurons, resulting in a reduction of cholinergic transmission. This means that compounds, which act via ACh release such as cholinesterase inhibitors, or in this case 5-HT_{1B} receptor antagonists, can only attenuate the memory impairment by scopolamine by displacing scopolamine from the postsynaptic receptor sites, by sufficient increase in endogenous ACh activity. It seems likely that ACh levels must be elevated prior to the injection of scopolamine in order to have a sufficient receptor stimulation to compete with the postsynaptic blockade of the muscarinic antagonist. When given in the reversal mode, the compound can only marginally add to the elevated ACh levels produced by scopolamine itself. This explanation is supported by the observation that cholinesterase inhibitors such as tacrine and donepezil can block the memory impairments induced by scopolamine, when given prior to scopolamine (Misane and Ögren, 2003), but not when given after scopolamine (unpublished data). In previous studies, we have also shown, both in the rats and mice, that the 5-HT_{1A} receptor antagonist NAD-299 is only able to block the action of scopolamine when injected prior to but not after scopolamine (for a detailed discussion see Misane and Ögren, 2003; Madjid et al., 2006).

A similar argument for the results of the studies can be applied to the studies on MK-801. The prevention of the facilitatory effect of NAS-181 by a subthreshold dose of MK-801 is most likely mediated by postsynaptic NMDA receptors located on pyramidal dendrites and presumably partly also by presynaptically located NMDA receptors on glutamatergic and other terminals (see Duguid and Sjostrom, 2006). Hence, blockade of both terminal and postsynaptic NMDA receptors is ultimately expected to inhibit excitatory transmission and counteract the facilitatory effects of 5-HT_{1B} receptor blockade by NAS-181. Indeed, there is electrophysiological data demonstrating that stimulation of 5-HT_{1B} receptors has an inhibitory influence on glutamatergic projections, originating from cerebral cortex and innervating subcortical areas (Morikawa et al., 2000; Muramatsu et al., 1998; Svenningsson et al., 2006). The failure to observe an effect of NAS-181 on extracellular glutamate in the ventral hippocampus and frontal cortex is probably partly related to the methodological problems of measuring neuronal release of glutamate (Hu et al., 2007; see Timmerman and Westerink, 1997). In addition, since 5-HT_{1B} receptors are predominantly located in positions to regulate the release of glutamate in output neurons (see above) it is also possible that the inability to detect an effect of NAS-181 on intrahippocampal levels of glutamate could be related to a more important role for 5-HT_{1B} receptors on regulation of hippocampal output rather than intrahippocampal circuits.

The present studies in mice reinforce previous findings in rats of an inhibitory role of 5-HT_{1B} receptors in spatial and contextual learning and memory (Åhlander-Lüttgen et al., 2003; Buhot et al., 1995). Interestingly, 5-HT_{1B} receptor knockout mice were not impaired in contextual fear conditioning (Malleret et al., 1999), another hippocampus-dependent learning task (Stiedl et al., 2000a). Effects on PA performance caused by pharmacological treatments and genetic manipulations have to be carefully analysed with regard to possible effects of non-cognitive factors, such as disturbances in sensorimotor functions including locomotor activity. 5-HT_{1B} receptor mRNA is abundant in the caudate-putamen, indicating involvement in motor functions (Bruinvels et al., 1994; Cloëz-Tayarani et al., 1997). However, in the dose range used in the present study, anpirtoline and NAS-181 did not alter training latencies. The effects of 5-HT_{1B} receptor ligands on aversive learning do not appear to be secondary to alterations of locomotor activity, as shown previously for moderate doses of anpirtoline and CP-94,253 in mice (De Almeida and Miczek, 2002). This is in agreement with the findings that neither NAS-181 nor anpirtoline, independently or together, altered locomotor activity in rats (Åhlander-Lüttgen et al., 2003). Furthermore, no apparent changes were detected in reactivity to the aversive stimulus. 5-HT_{1B} receptor KO mice do not display alterations in motility in the open field, in motor coordination or in emotional reactivity in the elevated plus maze and light/dark choice tests, but show increased novel object investigation and increased pre-pulse inhibition Brunner et al., 1999; Malleret et al., 1999; Ramboz et al., 1996). On the other hand, transgenic mice overexpressing p11, resulting in increased 5-HT_{1B} receptor function, display increased open field activity and decreased immobility in the tail suspension test (Svenningsson et al., 2006). It is evident that the results from acute pharmacological 5-HT_{1B} receptor blockade and genetic modification of 5-HT_{1B} receptor function in transgenic mice differ in both the behavioural pattern and the cognitive performance. It remains to be determined in conditional KO models targeting the 5-HT_{1B} receptor whether the lack of effects in the constitutive 5-HT_{1B} receptor KO model was due to developmental feedback mechanisms resulting in counter-regulation or secondary mechanisms unrelated to direct 5-HT_{1B} receptor signalling (see Stiedl and Meyer, 2003).

In summary, the present results indicate that 5-HT transmission via the 5-HT_{1B} receptor impairs PA learning in mice. Importantly, this study provides evidence that 5-HT_{1B} receptors are activated by endogenous levels of 5-HT during contextual aversive learning. The basal inhibitory tone mediated by 5-HT_{1B} heteroreceptors appears to involve suppression of cholinergic and glutamatergic transmission. Blockade of this tonic activation of 5-HT_{1B} receptors by the antagonist NAS-181 facilitated PA performance and attenuated memory impairments caused by the muscarinic ACh receptor antagonist

scopolamine and the NMDA receptor antagonist MK-801. The 5-HT_{1B} receptor appears to modulate cognitive functions by regulation of cholinergic and glutamatergic transmissions in cortical and hippocampal circuits involved in learning and memory. These findings suggest that 5-HT_{1B} receptor antagonists may be novel drug targets in the treatment of dysfunctional neuronal transmissions underlying cognitive impairments, as observed in age-related cognitive decline and affective disorders.

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References

Adell, A., Celada, P., Artigas, F., 2001. The role of 5-HT1B receptors in the regulation of serotonin cell firing and release in the rat brain. Journal of Neurochemistry 79, 172–182.

Åhlander-Lüttgen, M., Madjid, N., Schött, P.A., Sandin, J., Ögren, S.O., 2003. Analysis of the role of the 5-HT1B receptor in spatial and aversive learning in the rat. Neuropsychopharmacology 28, 1642–1655.

Ait Amara, D., Segu, L., Naili, S., Buhot, M.-C., 1995. Serotonin 1B receptor regulation after dorsal subiculum deafferentation. Brain Research Bulletin 38, 17–23.

Ait Amara, D., Segu, L., Buhot, M.C., 2001. Region-specific decrease in 5-HT1A and 5-HT1B binding sites after intra-hippocampal ibotenic acid injections in the rat. Neuroscience Letters 310, 25–28.

Azmitia, E.C., Segal, M., 1978. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. Journal of Comparative Neurology 179, 641–668.

Baarendse, P.J., van Grootheest, G., Jansen, R.F., Pieneman, A.W., Ögren, S.O., Verhage, M., Stiedl, O., 2008. Differential involvement of the dorsal hippocampus in passive avoidance in C57bl/6J and DBA/2J mice. Hippocampus 18, 11–19.

Bartus, R.T., Dean 3rd, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–414.

Berg, S., Larsson, L.G., Renyi, L., Ross, S.B., Thorberg, S.O., Thorell-Svantesson, G., 1998. *R*-(+)-2-(3-Morpholinomethyl-2*H*-chromen-8-yl)oxymethylmorpholine methanesulfonate), a new selective rat 5-hydroxytryptamine1B receptor antagonist. Journal of Medicinal Chemistry 41, 1934–1942.

Boeijinga, P.H., Boddeke, H.W.G.M., 1996. Activation of 5-HT_{1B} receptors suppresses low but not high frequency synaptic transmission in the rat subicular cortex in vitro. Brain Research 721, 59–65.

Bolanosjimenez, F., Decastro, R.M., Fillion, G., 1994. Effect of chronic antidepressant treatment on 5-HT_{1B} presynaptic heteroreceptors inhibiting acetylcholine-release. Neuropharmacology 33, 77–81.

Boschert, U., Amara, D.A., Segu, L., Hen, R., 1994. The mouse 5-hydroxytryptamine1B receptor is localized predominantly on axon terminals. Neuroscience 58, 167–182.

Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D., Palacios, J.M., 1994. Localization of 5-HT_{1B}, 5-HT_{1D}-Alpha, 5-HT_{1E} and 5-HT_{1F}, receptor messenger-RNA in rodent and primate brain. Neuropharmacology 33, 367–386.

Brunner, D., Buhot, M.C., Hen, R., Hofer, M., 1999. Anxiety, motor activation, and maternal-infant interactions in $5HT_{1B}$ knockout mice. Behavioral Neuroscience 113, 587–601.

Buhot, M.C., Patra, S.K., Naili, S., 1995. Spatial memory deficits following stimulation of hippocampal 5-HT1B receptors in the rat. European Journal of Pharmacology 285, 221–228.

Carli, M., Samanin, R., 1992. 8-Hydroxy-2-(di-n-propylamino)tetralin impairs spatial learning in a water maze: role of postsynaptic 5-HT_{1A} receptors. British Journal of Pharmacology 105, 720–726.

Cassel, J.C., Jeltsch, H., Neufang, B., Lauth, D., Szabo, B., Jackisch, R., 1995. Downregulation of muscarinic- and 5-HT_{1B}-mediated modulation of [3H]acetylcholine release in hippocampal slices of rats with fimbria-fornix lesions and intrahippocampal grafts of septal origin. Brain Research 704, 153–166.

Caulfield, M.P., 1993. Muscarinic receptors—characterization, coupling and function. Pharmacology & Therapeutics 58, 319–379.

Chadha, A., Sur, C., Atack, J., Duty, S., 2000. The 5HT(1B) receptor agonist, CP-93129, inhibits [(3)H]-GABA release from rat globus pallidus slices and reverses akinesia following intrapallidal injection in the reserpine-treated rat. British Journal of Pharmacology 130, 1927–1932.

Cloëz-Tayarani, I., Cardona, A., Rousselle, J.C., Massot, O., Edelman, L., Fillion, G., 1997. Autoradiographic characterization of [^{3}H]-5-HT-moduline binding sites in rodent brain and their relationship to 5-HT_{1B} receptors. Proceedings of the National Academy of Sciences USA 94, 9899–9904.

Consolo, S., Arnaboldi, S., Ramponi, S., Nannini, L., Ladinsky, H., Baldi, G., 1996. Endogenous serotonin facilitates in vivo acetylcholine release in rat frontal cortex through 5-HT_{1B} receptors. Journal of Pharmacology & Experimental Therapeutics 277, 823–830.

De Almeida, R.M.M., Miczek, K.A., 2002. Aggression escalated by social instigation or by discontinuation of reinforcement (''frustration'') in mice:inhibition by anpirtoline—a 5-HT_{1B} receptor agonist. Neuropsychopharmacology 272, 171–181.

Duguid, I., Sjostrom, P.J., 2006. Novel presynaptic mechanisms for coincidence detection in synaptic plasticity. Current Opinion in Neurobiology 16, 312–322.

Elvander-Tottie, E., Eriksson, T.M., Sandin, J., Ögren, S.O., 2006. *N*-methyl-D-aspartate receptors in the medial septal area have a role in spatial and emotional learning in the rat. Neuroscience 142, 963–978.

Engel, G., Gothert, M., Hoyer, D., Schlicker, E., Hillenbrand, K., 1986. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat-brain cortex with 5-HT1B binding-sites. Naunyn-Schmiedebergs Archives of Pharmacology 332, 1–7.

de Groote, L., Olivier, B., Westenberg, H.G., 2002. Extracellular serotonin in the prefrontal cortex is limited through terminal 5-HT(1B) autoreceptors: a microdialysis study in knockout mice. Psychopharmacology (Berl) 162, 419–424.

de Groote, L., Klompmakers, A.A., Olivier, B., Westenberg, H.G., 2003. An evaluation of the effect of NAS-181, a new selective 5-HT(1B) receptor antagonist, on extracellular 5-HT levels in rat frontal cortex. Naunyn-Schmiedebergs Archives of Pharmacology 367, 89–94.

Hertel, P., Nomikos, G.G., Svensson, T.H., 1999. The antipsychotic drug risperidone interacts with auto- and hetero-receptors regulating serotonin output in the rat frontal cortex. Neuropharmacology 38, 1175–1184.

Hjorth, S., Tao, R., 1991. The putative 5-HT1B receptor agonist CP-93,129 suppresses rat hippocampal 5-HT release in vivo: comparison with RU 24969. European Journal of Pharmacology 209, 249–252.

Hjorth, S., Bengtsson, H.J., Kullberg, A., Carlzon, D., Peilot, H., Auerbach, S.B., 2000. Serotonin autoreceptor function and antidepressant drug action. Journal of Psychopharmacology 14, 177–185.

Hoss, W., Messer Jr., W.S., Monsma Jr., F.J., Miller, M.D., Ellerbrock, B.R., Scranton, T., Ghodsi-Hovsepian, S., Price, M.A., Balan, S., Mazloum, Z., et al., 1990. Biochemical and behavioral evidence for muscarinic autoreceptors in the CNS. Brain Research 517, 195–201.

Hoyer, D., Hannon, J.P., Martin, G.R., 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacology Biochemistry and Behavior 71, 533–554.

Hu, X.J., Wang, F.H., Stenfors, C., Ögren, S.O., Kehr, J., 2007. Effects of the 5-HT(1B) receptor antagonist NAS-181 on extracellular levels of

acetylcholine, glutamate and GABA in the frontal cortex and ventral hippocampus of awake rats: a microdialysis study. European Neuropsychopharmacology 17, 580–586.

Izumi, J., Washizuka, M., Miura, N., Hiraga, Y., Ikeda, Y., 1994. Hippocampal serotonin 5-HT_{1A} receptor enhances acetylcholine release in conscious rats. Journal of Neurochemistry 62, 1804–1808.

Johnson, S.W., Mercuri, N.B., North, R.A., 1992. 5-hydroxytryptamine1B receptors block the GABAB synaptic potential in rat dopamine neurons. Journal of Neuroscience 12, 2000–2006.

Li, Y.W., Bayliss, D.A., 1998. Presynaptic inhibition by 5-HT_{1B} receptors of glutamatergic synaptic inputs onto serotonergic caudal raphe neurones in rat. Journal of Physiology-London 510, 121–134.

Lüttgen, M., Elvander, E., Madjid, N., Ögren, S.O., 2005. Analysis of the role of 5-HT_{1A} receptors in spatial and aversive learning in the rat. Neuropharmacology 48, 830–852.

Madjid, N., Tottie, E.E., Lüttgen, M., Meister, B., Sandin, J., Kuzmin, A., Stiedl, O., Ögren, S.O., 2006. 5-Hydroxytryptamine 1A receptor blockade facilitates aversive learning in mice: interactions with cholinergic and glutamatergic mechanisms. Journal of Pharmacology and Experimental Therapeutics 316, 581–591.

Malleret, G., Hen, R., Guillou, J.L., Segu, L., Buhot, M.C., 1999. 5-HT1B receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. Journal of Neuroscience 19, 6157–6168.

Misane, I., Ögren, S.O., 2000. Multiple 5-HT receptors in passive avoidance: comparative studies of *p*-chloroamphetamine and 8-OH-DPAT. Neuropsychopharmacology 22, 168–190.

Misane, I., Ögren, S.O., 2003. Selective 5-HT_{1A} antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. Neuropsychopharmacology 28, 253–264.

Mlinar, B., Falsini, C., Corradetti, R., 2003. Pharmacological characterization of 5-HT_{1B} receptor-mediated inhibition of local excitatory synaptic transmission in the CA1 region of rat hippocampus. British Journal of Pharmacology 138, 71–80.

Morikawa, H., Manzoni, O.J., Crabbe, J.C., Williams, J.T., 2000. Regulation of central synaptic transmission by 5-HT_{1B} auto- and heteroreceptors. Molecular Pharmacology 58, 1271–1278.

Muramatsu, M., Lapiz, M.D.S., Tanaka, E., Grenhoff, J., 1998. Serotonin inhibits synaptic glutamate currents in rat nucleus accumbens neurons via presynaptic 5-HT_{1B} receptors. European Journal of Neuroscience 10, 2371–2379.

Ögren, S.O., 1985a. Central serotonin neurones in avoidance learning: interactions with noradrenaline and dopamine neurones. Pharmacology Biochemistry and Behavior 23, 107–123.

Ögren, S.O., 1985b. Evidence for a role of brain serotonergic neurotransmission in avoidance learning. Acta Physiologica Scandinavica 125, 1–71.

Ramboz, S., Saudou, F., Amara, D.A., Belzung, C., Segu, L., Misslin, R., Buhot, M.C., Hen, R., 1996. 5-HT1B receptor knock out—behavioral consequences. Behavioural Brain Research 73, 305–312.

Rutz, S., Riegert, C., Rothmaier, A.K., Buhot, M.C., Cassel, J.C., Jackisch, R., 2006. Presynaptic serotonergic modulation of 5-HT and acetylcholine release in the hippocampus and the cortex of 5-HT_{1B}-receptor knockout mice. Brain Research Bulletin 70, 81–93.

Sari, Y., 2004. Serotonin(1B) receptors: from protein to physiological function and behavior. Neuroscience and Biobehavioral Reviews 28, 565–582.

Schlicker, E., Werner, U., Hamon, M., Gozlan, H., Nickel, B., Szelenyi, I., Gothert, M., 1992. Anpirtoline, a novel, highly potent 5-HT_{1B} receptor agonist with antinociceptive/antidepressant-like actions in rodents. British Journal of Pharmacology 105, 732–738.

Steckler, T., Sahgal, A., 1995. The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. Behavioural Brain Research 67, 165–199.

Stenfors, C., Yu, H., Ross, S.B., 2001. Pharmacological characterisation of the decrease in 5-HT synthesis in the mouse brain evoked by the selective serotonin re-uptake inhibitor citalopram. Naunyn-Schmiedeberg's Archives of Pharmacology 363, 222–232.

Stiedl, O., Meyer, M., 2003. Cardiac dynamics in corticotropin-releasing factor receptor subtype-2 deficient mice. Neuropeptides 37, 3–16.

Stiedl, O., Birkenfeld, K., Palve, M., Spiess, J., 2000a. Impairment of conditioned contextual fear of C57BL/6J mice by intracerebral injections of the NMDA receptor antagonist APV. Behavioural Brain Research 116, 157–168.

Stiedl, O., Misane, I., Spiess, J., Ögren, S.O., 2000b. Involvement of the 5-HT1A receptors in classical fear conditioning in C57BL/6J mice. Journal of Neuroscience 20, 8515–8527.

Svenningsson, P., Chergui, K., Rachleff, I., Flajolet, M., Zhang, X., El Yacoubi, M., Vaugeois, J.M., Nomikos, G.G., Greengard, P., 2006. Alterations in 5-HT_{1B} receptor function by p11 in depression-like states. Science 311, 77–80.

Timmerman, W., Westerink, B.H.C., 1997. Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27, 242–261.

Trillat, A.C., Malagie, I., Scearce, K., Pons, D., Anmella, M.C., Jacquot, C., Hen, R., Gardier, A.M., 1997. Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT_{1B} receptors: in vivo microdialysis studies. Journal of Neurochemistry 69, 2019–2025.

Wolff, M., Benhassine, N., Costet, P., Hen, R., Segu, L., Buhot, M.C., 2003. Delay-dependent working memory impairment in young-adult and aged 5-HT1BKO mice as assessed in a radial-arm water maze. Learning & Memory 10, 401–409.