Neuropsychopharmacology (2003) 28, 1642–1655

www.neuropsychopharmacology.org

Analysis of the Role of the 5-HT_{1B} Receptor in Spatial and Aversive Learning in the Rat

Maria Åhlander-Lüttgen[1], Nather Madjid[1], Pär A Schött[1], Johan Sandin[1] and Sven Ove Ögren*[,1]

[1]*Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden*

The present study examined the role of the 5-HT_{1B} receptor in learning and memory. The ability of the 5-HT_{1B} receptor agonist anpirtoline and the selective 5-HT_{1B} receptor antagonist NAS-181 to affect spatial learning in the water maze (WM) and aversive learning in the passive avoidance (PA) task were examined in the rat. Anpirtoline (0.1–1.0 mg/kg, s.c.) caused a dose-dependent impairment of learning and memory in both the WM and PA tasks. NAS-181 (1.0–10 mg/kg, s.c.) failed to alter performance of the WM task, but produced a dose-dependent (0.1–20 mg/kg) facilitation of PA retention. Furthermore, treatment with NAS-181 (10 mg/kg) fully blocked the impairment of the WM and PA performance caused by anpirtoline (1.0 mg/kg). In contrast, NAS-181 (3.0–10 mg/kg) did not attenuate the spatial learning deficit and the impairment of PA retention caused by scopolamine (0.1 mg/kg in WM task, 0.3 mg/kg in PA task, s.c.), a nonselective muscarinic antagonist. Moreover, a subthreshold dose of scopolamine (0.1 mg/kg) blocked the facilitation of PA retention induced by NAS-181 (1.0–10 mg/kg). In addition, the behavioral disturbances (eg thigmotaxic swimming and platform deflections) induced by anpirtoline and scopolamine were analyzed in the WM task and correlated with WM performance. These results indicate that: (1) 5-HT_{1B} receptor stimulation and blockade result in opposite effects in two types of cognitive tasks in the rat, and that (2) the 5-HT_{1B} antagonist NAS-181 can facilitate some aspects of cognitive function, most likely via an increase of cholinergic transmission. These results suggest that 5-HT_{1B} receptor antagonists may have a potential in the treatment of cognitive deficits resulting from loss of cholinergic transmission.

Neuropsychopharmacology (2003) **28,** 1642–1655, advance online publication, 02 July 2003; doi:10.1038/sj.npp.1300235

Keywords: 5-HT_{1B} receptors; NAS-181; anpirtoline; spatial learning; passive avoidance; hippocampus

INTRODUCTION

There is accumulating evidence for the view that 5-hydroxytryptamine (5-HT) via its multiple receptors can influence or modulate cognitive processes, such as learning and memory (Buhot *et al*, 2000; Ögren, 1985). The ascending 5-HT containing neurons, localized in the brainstem raphe nuclei, project to virtually all regions of the forebrain (Dahlström and Fuxe, 1964; Vertes, 1991; Vertes *et al*, 1999). The physiological effects of 5-HT in brain areas of innervation are mediated by seven families of 5-HT receptors, comprising at least 14 different subtypes (Hoyer and Martin, 1997). Among these, the 5-HT_{1A} receptor has so far received most attention in cognitive research (Buhot *et al*, 2000; Carli and Samanin, 1992; Misane *et al*, 1998; Schechter *et al*, 2002; Ögren, 1986). The 5-HT_{1A} receptor is localized both at the midbrain raphe nuclei cell bodies (autoreceptor), where it can regulate the firing rate of the 5-HT neurons, as well as at postsynaptic localizations (heteroreceptor) in brain regions that regulate cognitive processes including the cortex, the hippocampal formation, and the basal forebrain (Chalmers and Watson, 1991; Pompeiano *et al*, 1992; Schmitz *et al*, 1995; Sprouse and Aghajanian, 1987).

Also other 5-HT receptors, such as the 5-HT_{1B}, 5-HT_3, and 5-HT_4 subtypes, which in some aspects differ from the 5-HT_{1A} receptor in their regulatory functions (Hoyer *et al*, 1994), have been implicated in cognition (Buhot *et al*, 2000). The 5-HT_{1B} receptor, which functions as a terminal 5-HT autoreceptor, regulates the exocytic release of 5-HT in brain areas innervated by the 5-HT systems (Engel *et al*, 1986; Maura *et al*, 1986; Pineyro *et al*, 1995). In addition, this receptor also has a postsynaptic localization as a heteroreceptor, which allows for potential multiple interactions with several neurotransmitter systems. Thus, studies *in vitro* have given circumstantial evidence that the 5-HT_{1B} receptor may regulate the release of acetylcholine (Bolanos-Jiménez *et al*, 1994; Maura and Raiteri, 1986), glutamate (Li and Bayliss, 1998), GABA (Chadha *et al*, 2000), and dopamine (Sarhan *et al*, 2000). In the context of this study,

*Correspondence: Dr SO Ögren, Division of Behavioural Neuroscience, Department of Neuroscience A2:3, Karolinska Institutet, Retzius väg 8, S-171 77 Stockholm, Sweden, Tel: +46 8 7287074, Fax: +46 8 302875, E-mail: Sven.Ove.Ogren@neuro.ki.se

Received 08 January 2003; revised 09 May 2003; accepted 13 May 2003

Online publication: 15 May 2003 at http://www.acnp.org/citations/Npp05150301l/default.pdf

the possibility that the 5-HT$_{1B}$ receptor could regulate the terminal transmitter release of acetylcholine *in vivo* is of special interest. The cholinergic systems in the basal forebrain are known to play an important modulatory role in various aspects of cognitive function, such as attention, learning, and memory (Decker and McGaugh, 1991; Deutsch, 1971; Fibiger, 1991). Furthermore, the 5-HT$_{1B}$ receptor mRNA was found to be expressed in low-to-moderate concentrations in the rat septal complex of the basal forebrain, an area containing cholinergic neurons projecting to the hippocampus (Bruinvels *et al*, 1994).

Immunohistochemical and receptor autoradiographic studies have shown that the 5-HT$_{1B}$ receptor protein is distributed in brain regions regulating locomotor activity such as the basal ganglia, especially the globus pallidus and the substantia nigra and also in brain regions involved in cognitive functions, such as the dorsal subiculum of the hippocampal formation and amygdala (Bruinvels *et al*, 1993; Cloëz-Tayarani *et al*, 1997; Sari *et al*, 1999). In contrast, the 5-HT$_{1B}$ receptor mRNA has a dense distribution in, for example, the subthalamic nucleus of the basal ganglia and in the pyramidal cell layer of the CA1 area of the hippocampus, but not in the globus pallidus, substantia nigra, or dorsal subiculum (Bruinvels *et al*, 1994; Doucet *et al*, 1995). This mismatch between the distribution of the 5-HT$_{1B}$ receptor protein and its encoding mRNA supports the notion that the 5-HT$_{1B}$ receptor is localized mainly on preterminal axons or axon terminals, where it could inhibit the release of neurotransmitters via a G-protein coupled inhibition of adenylyl cyclase (Aït Amara *et al*, 2001; Boschert *et al*, 1994; Bouhelal *et al*, 1988; Riad *et al*, 2000).

The specific role of the 5-HT$_{1B}$ receptor in learning and memory has remained unclear since, until recently, no selective pharmacological tools have been available to characterize the physiological role of the 5-HT$_{1B}$ receptor *in vivo*. The development of selective compounds has been complicated by the fact that the 5-HT$_{1B}$ receptors in rodents (r5-HT$_{1B}$) and humans (h5-HT$_{1B}$) differ in 32 amino acids and that only a single amino acid gives rise to distinct pharmacological profiles (Hartig *et al*, 1996; Oksenberg *et al*, 1992). The available evidence on the role of the 5-HT$_{1B}$ receptor is mainly based on results obtained with receptor agonists. A spatial memory deficit was found following intrahippocampal administration of the 5-HT$_{1B}$ receptor agonist CP-93,129 in the rat (Buhot *et al*, 1995). However, the nonspecific 5-HT$_{1B}$ antagonist 21-009 given prior to training in the passive avoidance (PA) task induced amnesia in the domestic chick (Stephenson and Andrew, 1994). A study using 5-HT$_{1B}$ receptor knockout mice reported enhanced spatial performance in the water maze (WM), but no effect in a contextual fear-conditioning paradigm (Malleret *et al*, 1999). In view of the possibility for adaptive changes, studies with selective 5-HT$_{1B}$ receptor antagonists combined with results from conditional 5-HT$_{1B}$ receptor knockout mice will be important to provide evidence for the physiological role of the endogenous 5-HT$_{1B}$ receptor in learning and memory.

The present study examined whether blockade of 5-HT$_{1B}$ receptors *in vivo* would modify performance in two different cognitive tasks in the rat. NAS-181, [*R*-(+)-2-(3-morpholinomethyl-2*H*-chromen-8-yl)oxymethylmorpholine methanesulfonate], has been characterized as a potent and selective rat 5-HT$_{1B}$ receptor antagonist *in vitro*. The receptor-binding profile of NAS-181 showed a 13-fold preference for the rat ($K_i = 47$ nM) *vs* the bovine 5-HT$_{1B}$ receptor ($K_i = 630$ nM), and showed a very low affinity ($IC_{50} > 1000$ nM) for other serotonergic (5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_6$, 5-HT$_7$) as well as adrenergic (α_1, α_2, β), dopaminergic (D_1, D_2), and muscarinic receptors (Berg *et al*, 1998). The effects of NAS-181 on spatial learning and memory were studied using a computerized variant of the Morris WM task (Ögren *et al*, 1996). This task depends mainly on hippocampal mechanisms, which play an important role in learning and memory tasks involving spatial representation (Morris, 1981; O'Keefe, 1993). The effects of NAS-181 were also investigated using the passive avoidance (PA) task, which involves both classical (Pavlovian) fear conditioning and instrumental conditioning. The PA task is mediated by neuronal circuits in the limbic forebrain, such as hippocampus and amygdala, and is known to be modified by alterations in both serotonergic and cholinergic transmission (Bammer, 1982; Misane and Ögren, 2000; Ögren, 1985).

The behavioral effects of NAS-181 were examined 30 and 60 min after administration. NAS-181 has been demonstrated to enhance 5-HT metabolism in the rat brain in a dose-dependent manner (0.1–20 mg/kg, s.c.), with maximal effects 60 min after the injection (Stenfors *et al*, 2000). The effects of NAS-181 on performance in the WM and PA tasks as well as the ability of the compound to block or attenuate the effects of a nonselective 5-HT$_{1B}$ agonist, anpirtoline were investigated (Gothert *et al*, 1995; Schlicker *et al*, 1992; Swedberg *et al*, 1992). The serotonergic and cholinergic systems appear to interact in various aspects of learning and memory (Steckler and Sahgal, 1995). Therefore, NAS-181 was administered alone or in combination with the nonselective muscarinic antagonist scopolamine (Blokland, 1996; Bymaster *et al*, 1993). To analyze the contribution of nonspecific factors for spatial learning performance, a detailed analysis of the WM behavior was performed following treatment with anpirtoline, NAS-181, and scopolamine. In addition, since stimulation of 5-HT$_{1B}$ receptors has been reported to interfere with motor function (Cheetham and Heal, 1993; O'Neill and Parameswaran, 1997), the effects of NAS-181 and anpirtoline on locomotor activity were also studied.

MATERIALS AND METHODS

Subjects

Adult male Sprague–Dawley rats (11–12 weeks of age), weighing 280–350 g at the time of testing, were obtained from B&K Universal AB (Sollentuna, Sweden). The animals were housed in groups of four in standard plastic cages (Type IV Macrolon®) in a temperature- and humidity-controlled room with a constant 12-h light/dark cycle (lights on at 06.00 h) and free access to standard lab chow (Ewos R36, Ewos AB, Södertälje, Sweden) and tap water up to the time of experiments. Animals were allowed to habituate to the animal maintenance facilities for a period of at least 5 days before the beginning of the experiments. The cages were changed twice a week. Animal housing and all experimental procedures followed the provisions and

general recommendations of the Swedish animal protection legislation. The experimental procedures were approved by the local Animal Ethics Committee (ethical numbers 79/96, 34/98, 116/00).

Experimental Procedures

Each experiment was carried out by only one operator. Behavioral tests were conducted during the circadian cycle 09.00–16.00 h. All the behavioral experiments were carried out in experimentally naïve rats and they were used in only one type of experiment to avoid possible carryover effects. Prior to testing, the animals were brought to the experimental room (PA and WM studies) or to a room adjacent to the experimental room (motor activity study), where they were allowed to habituate for a period of 30–60 min.

Water Maze

The WM tests were performed in a circular pool located in the center of an experimental room (435 × 295 cm) surrounded by several extramaze cues, such as asymmetrically placed lamps and a black window (Ögren *et al*, 1996). These extramaze cues were kept constant throughout the testing period. The pool was made from dark gray plastic, measuring 180 cm in diameter and 45 cm in height. The tank was filled with tap water (thermostatically controlled at 22 ± 1°C) up to a height of 28 cm. The circular escape platform (15 cm in diameter) was made of dark gray plastic (exactly the same color as the water tank) and submerged 1 cm below the water surface. Previous studies have demonstrated that the platform is invisible to the rats.

Video tracking was conducted with a digital TV system (Hitachi CCTV camera, model HV-720K and an Ikegami 12-in CCTV Picture monitor, PM-127) placed on the ceiling above the center of the pool. The video camera was connected to a Victor VPCIIe computer and controlled by a computer program developed in cooperation between Epoc System and the Karolinska Institutet and monitored several parameters on-line, for example, latency to escape towards the platform, swim distance, and swim speed for each trial, and swim time and swim distance in each quadrant of the pool (Ögren *et al*, 1996). In all experiments, the animals received daily injections during the training days (days 1–5) of NAS-181, anpirtoline, scopolamine, a combination of NAS-181 and anpirtoline, a combination of NAS-181 and scopolamine or saline prior to the first training trial. The animals were trained in the pool for 5 consecutive days, four trials/day. The pool was divided into four equally sized quadrants (north, east, south, and west). On day 1, the first trial was initiated from the starting point 'west'. The starting point for the first trial was then rotated clockwise over the days of training and within training days, the starting points were rotated clockwise, one-quarter of a turn per trial (Schött *et al*, 1998). Previous data have indicated that there is no apparent difference in the rate of acquisition when using a clockwise rotation schedule of the starting points compared to the more common semirandom starting points (unpublished results). The platform remained in the center of the south-east quadrant throughout the training. On each trial, the animal was lowered gently into the water, facing the wall of the pool, and then released. At the same time, the monitoring was started by means of a mouse connected to the computer. If the animal failed to find the platform within 60 s, it was guided there by the experimenter's hand, and an extra 5 s was added to the escape latency time. The animal was allowed to rest on the platform for 30 s after each trial (intertrial time). After the last trial, the animal was placed in a drying cage and allowed to dry for at least 10 min before being returned to its experimental cage. The retention test was conducted 24 h after the last training session without the platform present (a probe trial), with all other parameters remaining the same as during training (Ögren *et al*, 1996). The rats did not receive any post-training treatment or any treatment prior to the probe trial. The following parameters were recorded: initial latency and distance to the first crossing of the virtual (former) location of the platform, swim speed, number of crossings of the virtual location of the platform, percentage of the total time spent in the quadrant of the virtual location of the platform (target quadrant). All animals were started from a position opposite to the quadrant where the platform had been located during the training sessions.

In an additional experiment, an improved computerized WM system (Water Maze Software, Edinburgh, UK) was used, which allows calculation of several parameters important for WM performance. It was of special interest to analyze the following parameters which have been related to sensorimotor disturbances, (see Cain *et al*, 1996; Whishaw *et al*, 1987): (1) thigmotaxis = the percentage of time spent swimming in the pool's peripheral annulus (within 10 cm of the walls of the pool), (2) deflection = the percentage of contacts that the rat makes with the hidden platform which results in physical displacement of its body or a change in swim direction, (3) swimover = the rat swims over the hidden platform without stopping, and (4) jump off = the rat jumps off the platform after having settled on it initially. In addition to the conventional measures for retention performance, an additional parameter was included: the percentage of total time swimming in the platform zone (a circle with a 20 cm radius from the center of the virtual position of the platform) (Cain *et al*, 1996).

Passive Avoidance

All animals were handled daily for at least 5 days before the experiments. The PA task was conducted as described earlier with minor modifications (Misane and Ögren, 2000; Ögren, 1986) using a two-compartment standard shuttle-box (25 × 50 × 25 cm) (Ugo Basile, Comerio-Varese, Italy). The two communicating (7 × 7 cm sliding door built in the separating wall) compartments were of equal size and had a stainless-steel bar floor. The right-hand compartment (shock compartment) was painted black to obtain a dark chamber, while the left-hand compartment was illuminated by a bulb (24 V; 5 W) installed on the top Plexiglas cover.

Before training, the rats were allowed to explore the lighted and dark compartments (without any electric current) for 60 s each. PA training was conducted in a single session (day 1). The animals ($n = 6$–10, for details, see figure legends) were treated with the test compounds as described below. After the selected time interval following injection (day 1), rats were placed in the lighted compart-

ment (with no access to the dark compartment) and were allowed to explore for 60 s.

During the exploration period in the PA apparatus rearing frequency, defecation, and motility were noted. When 60 s expired, the sliding door was automatically opened by pressing a pedal and the rat was allowed to cross over into the dark compartment. Once the rat had entered the dark compartment with all four paws, the sliding door was automatically closed and a weak electrical current (constant current, scrambled, duration 2 s, 0.3 or 0.4 mA) was delivered through the grid floor. Latency to cross into the dark compartment (training latency) was recorded. If a rat failed to move into the dark compartment within 300 s (cutoff latency), the door was reopened and the rat was gently moved into the dark compartment by the experimenter, where it received the foot shock. After exposure to the foot shock, the rat was allowed to stay for 30 s in the dark compartment before it was removed from the PA apparatus to its home cage.

Retention was tested 24 h after training (day 2). The animal was again placed in the lighted (safe) compartment with access to the dark compartment (within 10 s, without any shock) for a period of 300 s. The latency to enter the dark compartment with all four paws was automatically measured (retention latency). If the rat failed to enter the dark compartment within 300 s, it was removed and assigned a maximum test latency score of 300 s.

Locomotor Activity

Horizontal activity (motility and locomotion) and vertical activity (rearing) were simultaneously recorded by means of a multicage (eight cages), red, and infrared-sensitive motion detection system (Motron Products®, Stockholm, Sweden). The system is fully computerized and uses beams of infrared and red lights in combination with vertical and horizontal photocell arrays to detect movements of animals (Ögren, 1986). Horizontal movements were detected by 48 photosensors (in 4×4 cm squares covering the entire measurement area) placed in the floor of the motility meters. Motility was measured by counting all movements of a distance of 4 cm detected by the horizontal photosensors, and represents a measure of general activity. Locomotion was defined as movement between two rows of photosensors located at opposite ends of the cage floor, which is a distance of at least 32 cm. Rearing detectors (invisible infrared beams) were placed in rows, separated by 4 cm, 13 cm above the cage floor covering the entire measurement area. Following test drug injection, the animals were returned to their homecages and 20 min later the animals were placed (one animal per cage) in the Type III Macrolon® cages ($25 \times 40 \times 30$ cm; $W \times D \times H$) located on top of the motion detection system. The Macrolon III cage contained 50 ml wood shavings, which was changed between each animal. Recording was initiated immediately after the animal was brought to the cage and it lasted for a period of 30 min.

Drugs

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) and (−)-scopolamine hydrobromide (Sigma, St Louis, MO, USA) were used in the present studies. All drugs, dissolved in saline (0.9% NaCl), were injected subcutaneously (s.c.) into the scruff of the neck at a volume of 2 ml/kg. NAS-181 was administered 30 or 60 min, anpirtoline 30 min, and scopolamine 40 min prior to the first WM training trial every day during the training period. In the PA test, NAS-181 was administered 30 or 60 min, anpirtoline 30 min and scopolamine 40 min prior to training (day 1). In the locomotor test, NAS-181 or anpirtoline was administered 20 min prior to the start of recording. Saline control groups were run concurrently.

Statistics

Treatment effects were examined using a repeated-measures two-way analysis of variance (ANOVA) (WM task) or a one-way ANOVA (PA task and locomotor recordings). Differences between treatment groups within days of treatment or between days within groups were analyzed using a factorial ANOVA. For each significant F-ratio, Fisher's protected least significant difference test (Fisher's PLSD test, two-tailed) was used to analyze the statistical significance between multiple groups (Kirk, 1968). A probability level of $P<0.05$ was accepted as statistically significant.

RESULTS

Studies in the WM

Effects of the 5-HT$_{1B}$ receptor agonist anpirtoline. The first experiment addressed the issue of whether systemic injections of the 5-HT$_{1B}$ receptor agonist anpirtoline (0.3 and 1.0 mg/kg, s.c., 30 min prior to training) would modify the acquisition and retention of the WM task in a dose- and time-dependent manner. A repeated-measures analysis showed a highly significant effect of treatment on acquisition of the platform position as measured by latency ($F_{2,21}=24.22$, $P<0.001$), swim distance ($F_{2,21}=29.41$, $P<0.001$), and swim speed ($F_{2,21}=7.16$, $P<0.01$) (Figure 1a). In addition, a significant interaction between treatment and training session was found with regard to swim distance ($F_{4,8}=2.29$, $P<0.05$), but not to latency ($F_{4,8}=1.73$, NS). Analysis of escape latencies using Fisher's PLSD-test showed that the impairment induced by the 0.3 mg/kg dose diminished over the training days, the increase in escape latencies being significant only on the first 2 days (day 1: $P<0.001$, day 2: $P<0.05$). In contrast, at the 1.0 mg/kg dose, the impairment was highly significant throughout the 5 training days (Figure 1a). Swim speed was significantly increased by the 1.0 mg/kg dose on days 2–5, but not on day 1, probably due to acute treatment effects ($P<0.05$ on days 2 and 5, $P<0.01$ on days 3 and 4). It is worth noting that the increase in swim speed at the 1.0 mg/kg dose was still seen on the retention test (day 6) ($P<0.001$) (Table 1).

Analysis of retention performance, as assessed in the probe trial, revealed significant effects of treatment on latency to the first crossing of the virtual location of the platform ($F_{2,21}=4.96$, $P<0.05$), number of crossings

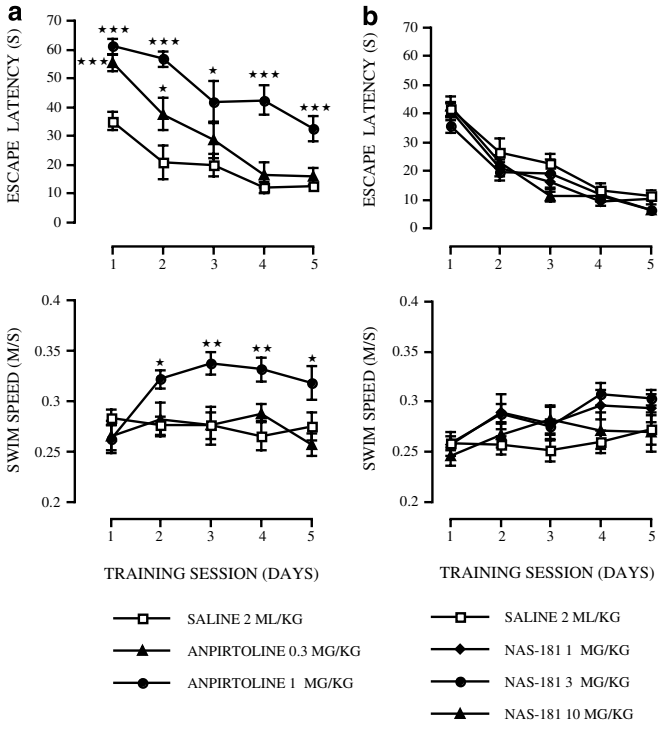

Figure 1 Dose-dependent effects of the 5-HT$_{1B}$ agonist anpirtoline (a) and the 5-HT$_{1B}$ antagonist NAS-181 (b) on the acquisition of the WM task. Anpirtoline (0.3 and 1.0 mg/kg) or NAS-181 (1, 3, and 10 mg/kg) was injected s.c. 30 min prior to training (days 1–5). The saline control groups were run concurrently. The effects of anpirtoline (a) and NAS-181 (b) on escape latency and swim speed are shown. Each value represents the mean ($\pm$ SEM) in groups of seven to eight animals ($n = 7$–8). The statistical analysis was performed by repeated-measures ANOVA. Fisher's PLSD-test was used for pairwise group comparison: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* saline. For further details, see Materials and methods.

($F_{2,21} = 4.21$, $P<0.05$), and on time spent in target quadrant ($F_{2,21} = 3.94$, $P<0.05$). Individual comparisons indicated a significant difference between the control group and the 1.0 mg/kg treatment group on latency to the first crossing ($P<0.05$), number of crossings ($P<0.01$), and time spent in target quadrant ($P<0.05$). No significant effects on retention parameters were seen after the 0.3 mg/kg dose (Table 1).

Behavioral observations during the experiment revealed that the anpirtoline-treated rats, especially at the highest dose (1.0 mg/kg), displayed hyperactivity, muscular hypertonus, and enhanced reactivity to handling with vocalizations, suggestive of increased levels of stress and anxiety. The anpirtoline-treated animals also displayed parts of the serotonin syndrome, such as flat body posture, on the platform. The behavioral changes declined somewhat over the days of training.

Effects of the 5-HT$_{1B}$ receptor antagonist NAS-181. Figure 1b shows that both the control group and the NAS-181-treated groups (1–10 mg/kg, 30 min prior to training) performed this task well and learned the position of the platform during acquisition, as indicated by decreasing escape latencies ($F_{4,27} = 90.63$, $P<0.001$) and shorter swim distances ($F_{4,27} = 76{,}65$, $P<0.001$) over training days. There was no significant effect of treatment on escape latency ($F_{3,27} = 2.09$, NS), swim distance ($F_{3,27} = 1.54$, NS), or swim speed ($F_{3,27} = 1.89$, NS) and no significant interactions between treatment and training session.

Retention was examined in a probe trial 24 h after the last training session. Treatment with NAS-181 caused no significant changes on latency to the first crossing ($F_{3,27}<1$, NS), number of crossings ($F_{3,27} = 1.02$, NS), time spent in target quadrant ($F_{3,27}<1$, NS), or swim speed ($F_{3,27} = 1.58$, NS).

Since the effects of NAS-181 on the 5-HT system have been shown to be maximal 60 min after administration (Stenfors *et al*, 2000), the effects of NAS-181 (3–20 mg/kg) administered 60 min instead of 30 min prior to testing were investigated in a separate experiment. The results obtained under these conditions did not differ from the results obtained in the experiment described above (data not shown).

Effects of combined treatment with NAS-181 and anpirtoline. This experiment addressed the issue of whether pretreatment with NAS-181 would modify the impairment of WM performance induced by anpirtoline. A repeated-measures ANOVA demonstrated significant overall treatment effects with regard to escape latency ($F_{2,29} = 66.73$, $P<0.001$) and swim distance ($F_{2,29} = 91.73$, $P<0.001$). Pretreatment with NAS-181 (10 mg/kg, 60 min prior to testing) completely blocked the anpirtoline-induced impairment, since the anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg combination did not differ from saline (NS for all parameters) (Figure 2). The performance of the rats given the combined treatment of NAS-181 and anpirtoline also differed from anpirtoline-treated rats (anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg *vs* anpirtoline 1.0 mg/kg) with regard to escape latency ($P<0.001$ days 1–5), swim distance ($P<0.05$ day 1, $P<0.001$ days 2–5), and swim speed

Table 1 Impairment of WM retention by the 5-HT$_{1B}$ Receptor Agonist Anpirtoline is Attenuated by Concurrent Treatment with the Antagonist NAS-181

Treatment	Latency to first crossing (s)	Swim speed (m/s)	Number of crossings	Time spent in target quadrant (% of total swim time)	Number of animals
Saline	18.2 $\pm$ 5.3	0.273 $\pm$ 0.009	3.2 $\pm$ 0.5	38.0 $\pm$ 2.6	$n = 16$
Anpirtoline 0.3 mg/kg	22.8 $\pm$ 6.6	0.274 $\pm$ 0.009	2.5 $\pm$ 0.6	34.0 $\pm$ 1.9	$n = 8$
Anpirtoline 1.0 mg/kg	46.7 $\pm$ 6.1**	0.307 $\pm$ 0.009*	1.1 $\pm$ 0.3**	25.2 $\pm$ 4.2**	$n = 8$
Anpirtoline 1.0 mg/kg+NAS-181 10 mg/kg	14.9 $\pm$ 5.4$^{\#\#}$	0.288 $\pm$ 0.007	2.9 $\pm$ 0.4$^{\#}$	26.8 $\pm$ 3.0*	$n = 8$

NAS-181 was administered s.c. 60 min and anpirtoline 30 min prior to training (days 1–5). Retention was examined in a probe trial 24 h after the last training day. Latency to the first crossing of the virtual location of the platform, number of crossings of the virtual location of the platform, and percentage of swim time in the target quadrant were measured. $^{*}P<0.05$, $^{**}P<0.01$ *vs* saline. $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* anpirtoline 1.0 mg/kg. For further details, see Materials and methods.

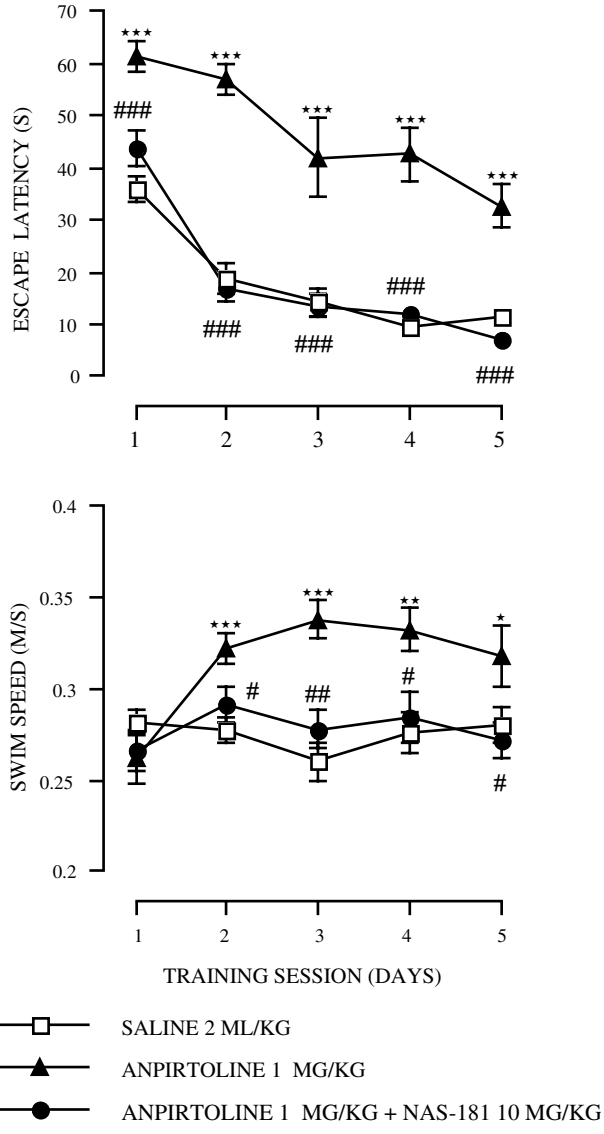

Figure 2 Effects of the interaction of NAS-181 with anpirtoline on the acquisition of the WM task. NAS-181 (10 mg/kg) was injected s.c. 60 min and anpirtoline (1.0 mg/kg) 30 min prior to training (days 1–5). The effects of the combination of anpirtoline and NAS-181 on escape latency and swim speed are shown ($n=8$ for the treatment groups, $n=16$ for the saline control group). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* saline, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs* anpirtoline.

($P<0.05$ days 2, 4, and 5, $P<0.01$ day 3). In addition, the combined administration of NAS-181 + anpirtoline blocked the impairment in the retention test; latency to the first crossing (anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg *vs* saline: NS; anpirtoline 1.0 mg/kg *vs* anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg: $P<0.01$), and number of crossings (anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg *vs* saline: NS; anpirtoline 1.0 mg/kg *vs* anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg: $P<0.05$), but failed to reverse the reduction of time spent in the target quadrant (anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg *vs* saline $P<0.05$, anpirtoline 1.0 mg/kg *vs* anpirtoline 1.0 mg/kg + NAS-181 10 mg/kg: NS) (Table 1). The swim speed in the retention test did not significantly differ between the saline control group and the ampirtoline 1.0 mg/kg + NAS-181 1.0 mg/kg treated group.

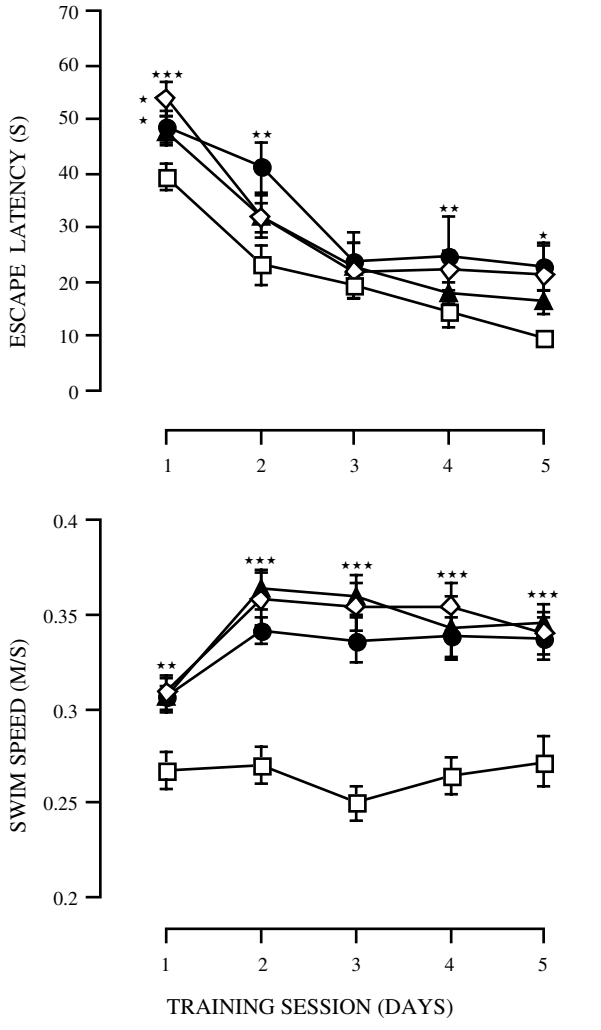

Figure 3 Effects of the interaction of NAS-181 with scopolamine on the acquisition of the WM task. NAS-181 (3 or 10 mg/kg) was injected s.c. 30 min and scopolamine (0.1 mg/kg) 40 min prior to training (days 1–5). The effects of the combination with scopolamine and NAS-181 on escape latency and swim speed are shown ($n=8$ for the treatment groups, $n=12$ for the saline control group). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* saline. In the swim speed graph, the level of significance indicated by stars, $^{**}P<0.01$ and $^{***}P<0.001$, refers to all three treatment groups for the corresponding day.

Effects of combined treatment with NAS-181 and scopolamine. The nonselective muscarinic antagonist scopolamine (0.1 mg/kg, 40 min prior to training) produced a highly significant impairment of the acquisition of the WM task (Figure 3) confirming previous data (Ögren *et al*, 1996). The purpose of this experiment was to examine if NAS-181 (3 or 10 mg/kg, 30 min prior to training) would modulate the impairment of WM performance induced by scopolamine. A repeated-measures analysis demonstrated signifi-

cant overall treatment effects on escape latency ($F_{3,32} = 5.24$, $P < 0.01$) and on swim distance ($F_{3,32} = 11.72$, $P < 0.001$). *Post hoc* analyses revealed that the scopolamine-treated rats exhibited significantly longer escape latencies ($P < 0.05$ day 5, $P < 0.01$ days 2, 4, $P < 0.001$ day 1, NS day 3), longer swim distances ($P < 0.001$ days 1–2, $P < 0.05$ day 4, $P < 0.01$ day 5, NS day 3), and faster swim speed ($P < 0.01$ day 1, $P < 0.001$ days 2–5) than the saline-treated control group. However, the performance of the groups treated with NAS-181 in combination with scopolamine did not significantly differ from the group receiving scopolamine alone (NS for all parameters) (Figure 3). Scopolamine slightly impaired memory retention, indicated by a tendency to increase latency to the first crossing and to decrease time spent in target quadrant ($P = 0.08$ and 0.09, respectively) and a significant decrease in the number of platform crossings ($P < 0.01$). Coadministration of NAS-181 did not affect the scopolamine-induced impairment of retention (Table 2). Swim speed had returned to normal at the retention test, 24 h after the last drug injection.

In a complementary experiment, scopolamine-treated rats (0.1 mg/kg, 40 min prior to training) received pretreatment with NAS-181 (10 mg/kg, 60 min prior to training) to examine if temporal effects of NAS-181 could play a role in the interaction with scopolamine on 5-HT$_{1B}$ receptor function (Stenfors *et al*, 2000). The pattern of results obtained under these conditions was identical with the results from the experiment reported above (data not shown).

Analysis of sensorimotor effects caused by anpirtoline, NAS-181, and scopolamine

In a separate experiment, a detailed behavioral analysis was performed after treatment with either anpirtoline (1.0 mg/kg, s.c., 30 min prior to training), NAS-181 (10 mg/kg, s.c., 30 min), or scopolamine (0.1 or 0.3 mg/kg, s.c., 40 min) using additional behavioral parameters such as thigmotaxic swimming, deflections, and percentage of swim time in platform zone. The pattern of results using our standard parameters was in good agreement with the results obtained in the experiments reported above. Notably, the impairment caused by anpirtoline was in the same range as the impairment caused by scopolamine 0.1 mg/kg. A repeated-measures analysis showed a highly significant overall treatment effect on acquisition of the platform position as measured by latency ($F_{4,35} = 13.42$, $P < 0.001$), swim distance ($F_{4,35} = 59.69$, $P < 0.001$), and swim speed ($F_{4,35} = 45.36$, $P < 0.01$). *Post hoc* analyses showed that treatment with anpirtoline and scopolamine produced a highly significant impairment of acquisition measured by latency (anpirtoline and scopolamine 0.3 mg/kg: $P < 0.001$, scopolamine 0.1 mg/kg: $P < 0.01$), swim distance ($P < 0.001$ for all three treatment groups), and swim speed ($P < 0.001$ for all three treatment groups), whereas treatment with NAS-181 did not significantly affect any of these parameters (Figure 4). Furthermore, the increase in latency after treatment with anpirtoline and the two doses of scopolamine was not attenuated over training days. In addition, the percentage of time swimming within a distance of 10 cm from the walls of the pool (thigmotaxic swimming) was analyzed. During the acquisition period, there was an overall treatment effect of thigmotaxic swimming ($F_{4,35} = 28.08$, $P < 0.001$), which was significantly increased by treatment with anpirtoline ($P < 0.05$) and the highest dose of scopolamine (0.3 mg/kg) ($P < 0.001$). A factorial analysis showed that anpirtoline only significantly increased this behavior on day 1 ($P < 0.05$), whereas scopolamine (0.3 mg/kg) significantly increased thigmotaxic swimming during the entire training period ($P < 0.001$ days 1–5) (Figure 5). Treatment with NAS-181 did not significantly affect thigmotaxic swimming. The percentage of contacts with the platform being deflections, a parameter reflecting sensorimotor disturbances, was also measured during acquisition. The control and NAS-181 treatment groups did not exhibit any deflections, unlike treatment with anpirtoline and scopolamine, which induced this behavior. Analysis of platform contacts being deflections over the days of training indicated a significant treatment effect ($F_{4,35} = 7.24$, $P < 0.001$) and the two scopolamine groups differed significantly from the control group (0.1 mg/kg: 23.6% deflections, $P < 0.01$, 0.3 mg/kg: 28.5% deflections, $P < 0.001$). Moreover, deflections were seen throughout the training period. In contrast, only occasional swimovers were observed (once in the anpirtoline group and once in the scopolamine 0.3 mg/kg group). In addition, a jump off was only noted once in the anpirtoline group.

Retention was examined in a probe trial 24 h after the last training session. At the retention test, there was still a highly significant overall treatment effect for thigmotaxic swimming ($F_{4,35} = 27.21$, $P < 0.001$), which was significant for anpirtoline ($P < 0.05$) and the two doses of scopolamine (0.1 mg/kg: $P < 0.01$, 0.3 mg/kg: $P < 0.001$) (Figure 5). No significant overall treatment effects were seen for the latency to the first crossing, swim speed, or number of crossings. In contrast, there was a significant overall treatment effect of the time spent in the target quadrant ($F_{4,35} = 4.86$, $P < 0.01$), which was significantly decreased by

Table 2 Effects of Concurrent Treatment with NAS-181 and the Muscarinic Antagonist Scopolamine on WM task retention

Treatment	Latency to first crossing (s)	Number of crossings	Time spent in target quadrant (% of total swim time)	Number of animals
Saline	13.1 ± 3.1	3.8 ± 0.6	37.6 ± 3.7	$n = 12$
Scopolamine 0.1 mg/kg	30.8 ± 8.7	1.8 ± 0.6**	29.5 ± 4.3	$n = 8$
Scopolamine 0.1 mg/kg+NAS-181 3 mg/kg	37.6 ± 7.9*	1.0 ± 0.3***	21.8 ± 2.0**	$n = 8$
Scopolamine 0.1 mg/kg+NAS-181 10 mg/kg	28.0 ± 9.9	1.6 ± 0.4**	25.5 ± 2.1*	$n = 8$

Scopolamine was administered s.c. 40 min, and NAS-181 30 min, prior to training (days 1–5). Retention was examined in a probe trial 24 h after the last training day. Latency to the first crossing of the virtual location of the platform, number of crossings of the virtual location of the platform, and percentage of swim time in the target quadrant were measured. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs* saline. For further details, see Materials and methods.

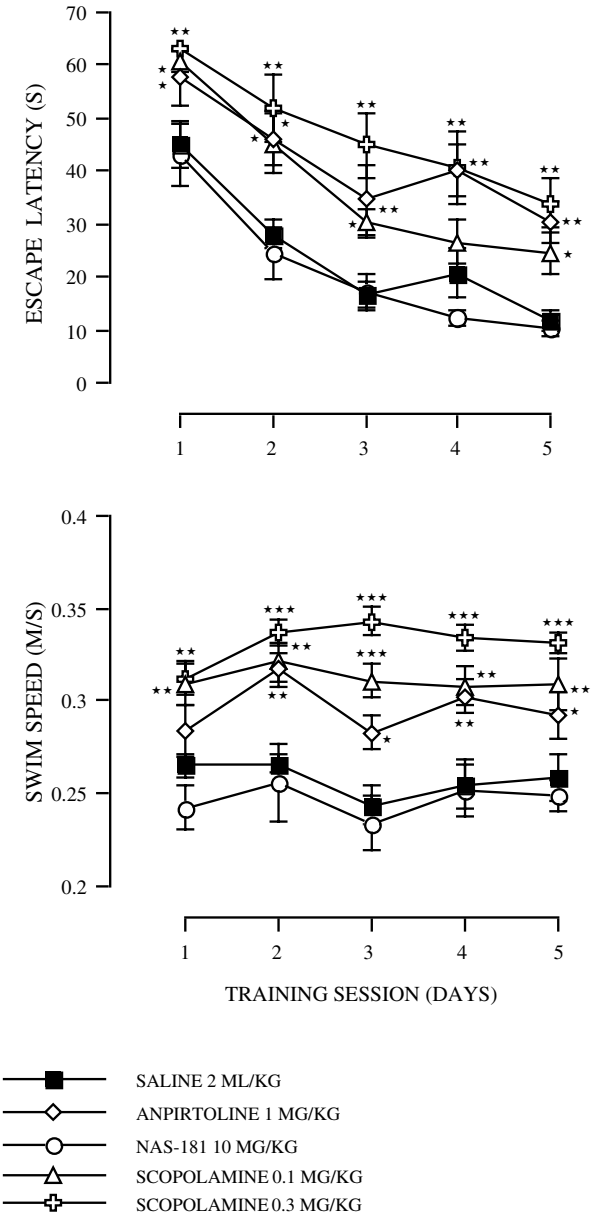

Figure 4 Effects of treatment with anpirtoline (1 mg/kg, injected s.c. 30 min prior to training) NAS-181 (10 mg/kg, 30 min) or scopolamine (0.1 and 0.3 mg/kg, 40 min) on escape latency and swim speed during the acquisition period (days 1–5) in the WM task. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs* saline ($n=8$).

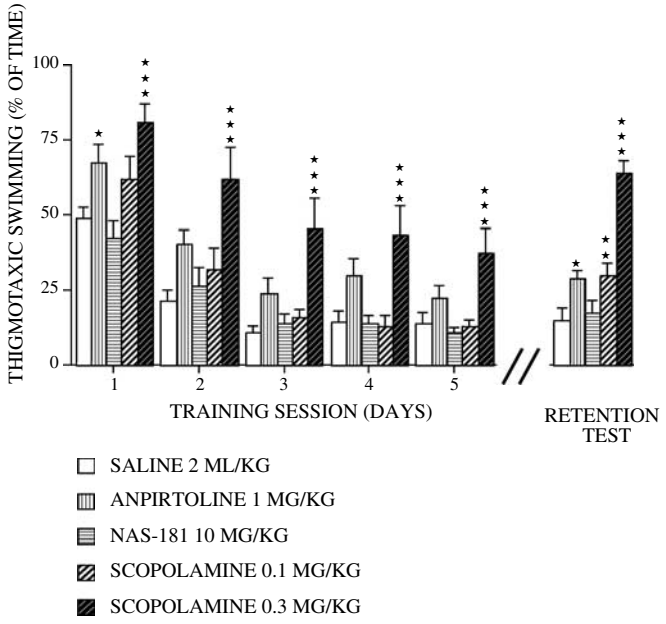

Figure 5 Effects of treatment with anpirtoline (1 mg/kg, injected s.c. 30 min prior to training) NAS-181 (10 mg/kg, 30 min) or scopolamine (0.1 and 0.3 mg/kg, 40 min) on thigmotaxic swimming during the acquisition period (days 1–5) and the retention test (a probe trial 24 h after the last training trial) in the WM task. Thigmotaxic swimming was defined as percentage of total swim time spent within a distance of 10 cm from the walls of the pool. *$P<0.05$, ***$P<0.001$ *vs* saline ($n=8$).

treatment with anpirtoline ($P<0.05$) and scopolamine (0.1 mg/kg: $P<0.05$, 0.3 mg/kg: $P<0.01$) in agreement with our previous retention data. In addition, there was an overall treatment effect of the percentage of time spent in the target platform zone ($F_{4,35}=8.33$, $P<0.001$), which was also significantly decreased by treatment with anpirtoline ($P<0.01$) and scopolamine (0.1 mg/kg: $P<0.01$, 0.3 mg/kg: $P<0.001$).

Studies in the PA Apparatus

Effects of anpirtoline and NAS-181. The retention latencies (day 2, tested 24 h after training) in the saline-treated control groups were four to five times longer than the training latencies, indicating that the animals had learned the task well (Figure 6a, b). Anpirtoline (0.1–1.0 mg/kg, s.c., 30 min prior to training) caused a dose-related impairment of PA retention ($F_{3,28}=5.90$, $P<0.01$), indicated by a decrease in the retention latencies. *Post hoc* analysis showed a significant effect at the 0.3 mg/kg dose ($P<0.01$ *vs* saline) and the 1.0 mg/kg dose ($P<0.01$ *vs* saline) (Figure 6a). In contrast, NAS-181 (0.1–20 mg/kg, s.c., 30 min prior to training) caused a dose-dependent increase of PA retention latencies ($F_{5,36}=3.90$, $P<0.01$) with a significant effect from the 1.0 mg/kg to the 20 mg/kg dose ($P<0.01$ *vs* saline), (Figure 6b).

The training latencies were not affected by anpirtoline or NAS-181 treatment. Behavioral observations were performed during the exploration period in the light compartment for 60 s. No significant changes were found in rearing frequencies following anpirtoline and NAS-181 in comparison to saline-treated animals (data not shown).

Effects of combined treatment with NAS-181 and anpirtoline. Based on the dose–response experiment and the results from the WM task, the 1.0 mg/kg dose of anpirtoline was used in the subsequent interaction study with NAS-181. Pretreatment with NAS-181 (1.0 and 10 mg/kg, 60 min prior to training) dose-dependently attenuated the impairment of PA retention induced by anpirtoline (1.0 mg/kg, 30 min), ($F_{4,27}=20.90$, $P<0.01$) with a significant effect at the 10 mg/kg dose of NAS-181 (NAS-181 10 mg/kg + anpirtoline 1.0 mg/kg *vs* anpirtoline 1.0 mg/kg: $P<0.01$) (Figure 7). The combined treatment of NAS-181 and anpirtoline did not significantly affect the training latencies (data not shown).

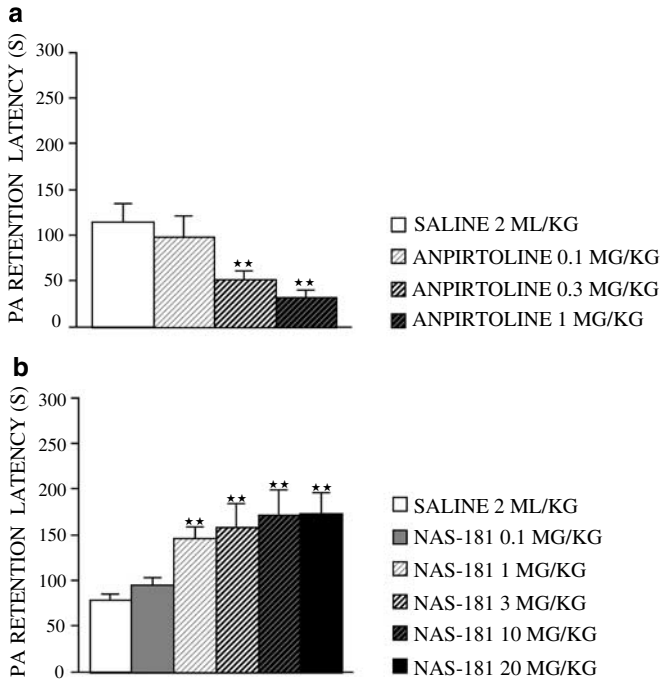

Figure 6 Dose-dependent effects of anpirtoline (a) or NAS-181 (b) on PA retention latencies. NAS-181 (0.1, 1.0, 3.0, 10, and 20 mg/kg) or anpirtoline (0.1, 0.3, and 1.0 mg/kg) was injected s.c. 30 min before the training session (before the exposure to the inescapable foot shock). The saline control group was run concurrently. The retention test was performed 24 h later. Vertical bars represent means (± SEM) of retention latencies ($n = 8$). $^{**}P < 0.01$ *vs* saline.

Effects of combined treatment with NAS-181 and scopolamine. Treatment with scopolamine (0.3 mg/kg, s.c., 40 min prior to training) caused a significant impairment of PA retention latencies ($F_{4,28} = 15.10$, $P < 0.01$) (Figure 8a), as previously demonstrated (Misane and Ögren, 2003). The scopolamine-induced impairment of PA retention was not reversed by NAS-181 (0.1–10 mg/kg, 30 min prior to training) (NAS-181 0.1 mg/kg + scopolamine 0.3 mg/kg *vs* scopolamine 0.3 mg/kg: NS; NAS 181 1.0 mg/kg + scopolamine 0.3 mg/kg *vs* scopolamine 0.3 mg/kg: NS; NAS-181 10 mg/kg + scopolamine 0.3 mg/kg *vs* scopolamine 0.3 mg/kg: NS), (Figure 8a). Moreover, pretreatment of the rats with a subthreshold dose of scopolamine (0.1 mg/kg, s.c., 40 min prior to training), which had no significant effect by itself on PA retention, completely blocked the increase of PA retention caused by NAS-181 (scopolamine 0.1 mg/kg + NAS-181 1.0 mg/kg *vs* NAS-181 1.0 mg/kg: $F_{5,35} = 11.9$, $P < 0.01$; scopolamine 0.1 mg/kg + NAS-181 10 mg/kg *vs* NAS-181 10 mg/kg: $P < 0.01$) (Figure 8b).

Locomotor Activity Studies

Effects of NAS-181 and anpirtoline and their combination. NAS-181 (3–20 mg/kg), anpirtoline (1.0 mg/kg) or their combinations (anpirtoline 1.0 mg/kg and NAS-181 1.0 or 10 mg/kg) were administered s.c. 20 min prior to the initiation of the recording of the locomotor activity in rats that were not habituated to the locomotor cages. Under this condition, control animals display high exploratory activity during the first 20 min of recording. Neither NAS-181, anpirtoline, nor their combinations produced any statistically significant changes in the accumulated rearing, motility, or locomotion counts of the locomotor activity during the 30 min recording period (data not shown).

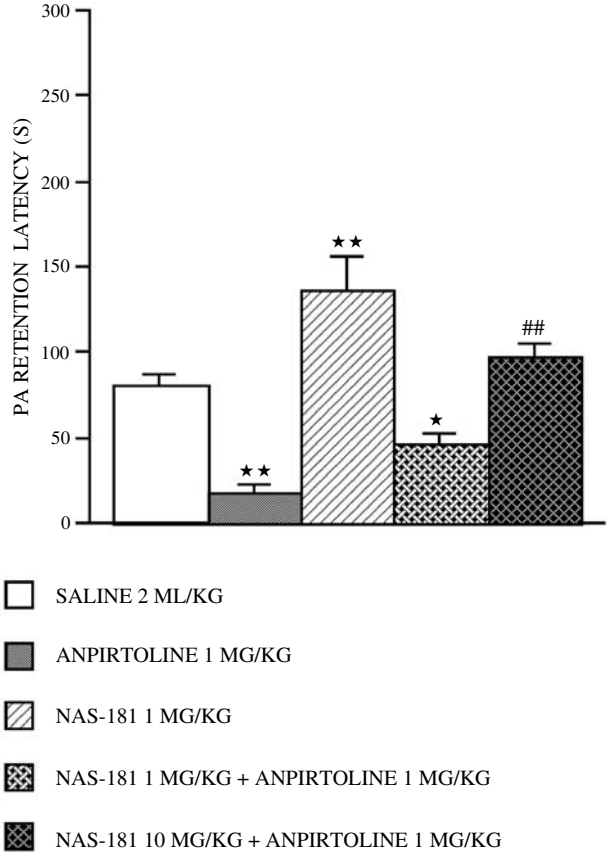

Figure 7 Effects of NAS-181, anpirtoline and their combinations on PA retention latencies. NAS-181 (1 and 10 mg/kg, 60 min) and anpirtoline (1.0 mg/kg, 30 min) were injected s.c. before the training session. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* saline, $^{\#\#}P < 0.01$ *vs* anpirtoline ($n = 6$–8).

DISCUSSION

The purpose of the present study was to examine the potential role of the 5-HT$_{1B}$ receptor in learning and memory using the WM and the PA tasks. The results show that systemic administration of the selective 5-HT$_{1B}$ receptor antagonist NAS-181 did not significantly affect spatial acquisition or memory in the WM task in young male rats, while it enhanced memory retention in the PA task. NAS-181 also completely blocked the impairment of performance in both the WM and the PA task induced by systemic administration of the 5-HT$_{1B}$ receptor agonist anpirtoline. Moreover, the facilitatory effect of NAS-181 on PA retention was blocked by a subthreshold dose of the muscarinic antagonist scopolamine. These results indicate that systemic administration of NAS-181 can facilitate some aspects of cognitive performance, possibly at least partly via enhancement of cholinergic neurotransmission.

The relationship between 5-HT$_{1B}$ receptor stimulation/blockade and learning performance has remained unclear (see Introduction). This is probably due to the use of

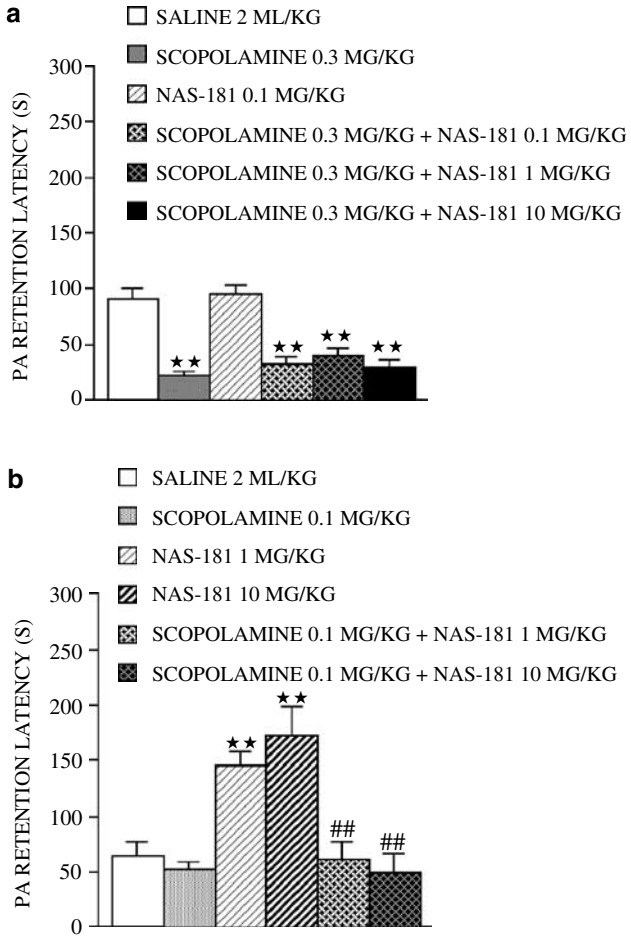

Figure 8 Effects of NAS-181, the nonselective muscarinic antagonist scopolamine and their combinations on PA retention latencies. (a) A high dose of scopolamine (0.3 mg/kg, 40 min) and NAS-181 (0.1, 1.0, and 10 mg/kg, 30 min) was injected s.c. before the training session. (b) A subthreshold dose of scopolamine (0.1 mg/kg, 40 min) and NAS-181 (1.0 and 10 mg/kg, 30 min) was injected s.c. before the training session. $^{**}P<0.01$ *vs* saline, $^{\#\#}P<0.01$ *vs* the corresponding NAS-181 treatment group ($n=6$–10).

nonspecific 5-HT$_{1B}$ ligands or even mixed 5-HT$_{1B}$ agonists/antagonists (Meneses, 2001; Meneses *et al*, 1997; Stephenson and Andrew, 1994). The facilitatory effect on PA retention seen after systemic administration of NAS-181 suggests a role for the 5-HT$_{1B}$ receptor in aversive learning. In contrast, NAS-181 failed to modulate performance in the WM task, although studies on 5-HT$_{1B}$ receptor knockout mice reported enhanced spatial acquisition (Malleret *et al*, 1999). There are several possible explanations for the discrepant results obtained in the two tasks. The difference could partly be due to different study designs. In the PA task, a weak current (0.3–0.4 mA) with a short duration (2 s) was used in order to allow demonstration of memory facilitation. Under this condition, the control group had a short mean retention latency in the range of 70–90 s, which is far below 300 s (the cutoff latency). This contrasts with the WM task, in which young, unimpaired rats acquire the task very rapidly, making it difficult to demonstrate drug-induced facilitation of performance. This conclusion is also supported by the failure of acetylcholinesterase inhibitors such as donepezil to facilitate WM acquisition and retention in young, unimpaired rats, while a facilitatory effect on memory retention is observed in the PA task (Misane and Ögren, 2003; Rogers and Hagan, 2001; Van der Staay *et al*, 1996). Acetylcholinesterase inhibitors are usually able to attenuate memory deficits induced pharmacologically, for example by scopolamine, in the WM and PA tasks (Braida *et al*, 1996; Cheng *et al*, 1996; Misane and Ögren, 2003). It also seems likely that performance in the two learning tasks is differentially regulated neurochemically, as indicated by studies with acetylcholinesterase inhibitors.

Recent results with the selective 5-HT$_{1A}$ antagonist, robalzotan, suggest that an increase of extracellular acetylcholine with about 200% in the hippocampus is sufficient to enhance PA retention but not WM performance in young, unimpaired rats (Misane and Ögren, 2003). There are also possible differences in the degree of tonic stimulation of the 5-HT$_{1B}$ receptor in different brain areas that might contribute to the differential effects of NAS-181 on PA and WM performance. In order for a 5-HT$_{1B}$ receptor antagonist to have an effect *in vivo*, the 5-HT$_{1B}$ receptors in the brain area(s) mediating the behavioral functions must probably be under basal tonic stimulation by 5-HT. Interestingly, *in vivo* data indicate that 5-HT$_{1B}$ antagonists only slightly increase the extracellular levels of 5-HT (no significant increase in the rat, +50% in the guinea-pig) in the hippocampus (Hervás *et al*, 2000; Roberts *et al*, 1999). In contrast, local application of a 5-HT$_{1B}$ antagonist induced a seven-fold increase of extracellular levels of 5-HT in the amygdalopiriform cortex (Kikvadze and Foster, 1995). This suggests a stronger tonic stimulation of terminal 5-HT$_{1B}$ autoreceptors in the amygdalopiriform cortex compared to that in the hippocampus. The amygdalopiriform cortex is a part of the amygdaloid complex, which projects to other nuclei of the amygdala, the entorhinal cortex, and hippocampus, brain areas implicated in PA performance (von Bohlen und Halbach and Albrecht, 2002). Both the 5-HT$_{1B}$ receptor mRNA and protein have been demonstrated to be present in the amygdala (Bruinvels *et al*, 1994; Cloëz-Tayarani *et al*, 1997), suggesting a potential role of 5-HT$_{1B}$ receptors in the amygdala in the regulation of PA performance.

The results from the present study are in agreement with previous studies indicating that stimulation of 5-HT$_{1B}$ receptors can impair performance in various learning and memory paradigms (Buhot *et al*, 1995; Malleret *et al*, 1999; Meneses, 2001; Meneses *et al*, 1997). Treatment with the selective 5-HT$_{1B}$ receptor agonist anpirtoline was found to cause a dose-dependent impairment of performance seen in both the WM and PA tasks. This is in agreement with the impairment seen in the radial maze after intrahippocampal injections (CA1 field of the dorsal hippocampus) with the selective 5-HT$_{1B}$ receptor agonist CP-93,129 (Buhot *et al*, 1995). CP-93,129 has been reported to show a more than 150-fold higher affinity for 5-HT$_{1B}$ ($K_i=8$ nM) *vs* 5-HT$_{1A}$ ($K_i=1500$ nM) or 5-HT$_2$ ligand-binding sites (Macor *et al*, 1990). In comparison, anpirtoline only shows a five-fold preference for the rat 5-HT$_{1B}$ receptor ($K_i=28$ nM) *vs* the rat 5-HT$_{1A}$ ($K_i=150$ nM) and a 50-fold preference *vs* the 5-HT$_2$ ($K_i=1.49$ μM) receptor (Schlicker *et al*, 1992). Furthermore, anpirtoline has been shown to act as a 5-HT$_3$ antagonist in both binding assays ($pK_i=7.53$) and

behavioral studies (Gothert *et al*, 1995). This implies that anpirtoline, at least at high doses, could stimulate the 5-HT$_{1A}$ receptor, evidenced by the observation that animals treated with anpirtoline displayed parts of the serotonin syndrome (flat body posture) during the WM task (see Results section). The serotonin syndrome has been shown to result from postsynaptic 5-HT$_{1A}$ receptor activation (Tricklebank *et al*, 1984). Moreover, stimulation of (postsynaptic) 5-HT$_{1A}$ receptors has consistently been shown to impair learning and memory in the WM and PA tasks (Carli and Samanin, 1992; Carli *et al*, 1993; Misane *et al*, 1998; Ögren, 1985), which could contribute to the cognitive deficits caused by anpirtoline. However, pretreatment with NAS-181 (10 mg/kg) completely blocked both the impairment of WM acquisition/retention and PA retention caused by anpirtoline. This suggests that the effects of anpirtoline on cognition are mediated by stimulation of the 5-HT$_{1B}$ receptor. This is consistent with biochemical studies showing that NAS-181 can fully block the decrease in 5-HT synthesis induced by anpirtoline in the mouse (Stenfors *et al*, 2001).

Since the compounds were always administered prior to training, the results obtained could be due to changes in noncognitive factors such as disturbances in sensorimotor processing or changes in locomotor activity. It is notable that the 5-HT$_{1B}$ receptor is densely expressed in the striatum, an area important for locomotor function (Cloëz-Tayarani *et al*, 1997). However, neither NAS-181, anpirtoline nor their combinations significantly affected locomotor activity measured in locomotor activity boxes or scored in the PA apparatus, indicating a minor role of the 5-HT$_{1B}$ receptor in forward locomotion. This contrasts with results obtained with the 5-HT$_{1A}$/5-HT$_{1B}$ receptor agonist RU 24969 that increases locomotor activity in rodents (Cheetham and Heal, 1993; O'Neill and Parameswaran, 1997). However, anpirtoline modified neither locomotion in mice (0.125–2.0 mg/kg) (de Almeida and Miczek, 2002) nor forward locomotion at the dose range (0.6–1.25 mg/kg) used in the present study in rats, although it significantly increased forward locomotion at higher doses (2.5–5.0 mg/kg) (O'Neill and Parameswaran, 1997). Although anpirtoline did not affect locomotion measured in the locomotor activity boxes, the 1.0 mg/kg dose of anpirtoline increased swim speed in the WM apparatus on training days 2–5 and also on the retention test. Thus, the discrepancy between measures of motor activity and swim speed probably reflects differential, context-dependent effects of the 5-HT$_{1B}$ receptor on locomotor systems. Since similar results have been seen after compounds that impair WM performance such as scopolamine (Ögren *et al*, 1996), it is possible that the increase in swim speed partly reflects the failure to acquire the task, probably due to persistent swimming along the perimeter of the maze, for example, thigmotaxic behavior. Control rats unable to learn the spatial task usually have a high swim speed combined with thigmotaxic swimming (to be published). Thigmotaxis, which is usually defined as a measure of fear or anxiety in rats (Treit and Fundytus, 1988), has also been proposed to interfere with the ability to use efficient navigational strategies in the WM (Cain *et al*, 1996; Whishaw *et al*, 1987). However, anpirtoline only increased thigmotaxic swimming on training day 1, whereas the increased escape latency was seen throughout the five training sessions. This indicates that the impaired WM acquisition and swim speed cannot simply be explained by persistent thigmotaxic behavior. Moreover, the low frequency of deflections and the lack of swimovers suggest that drug-induced sensorimotor disturbances only marginally contribute to the poor acquisition after 1 mg/kg of anpirtoline. These results, taken together, suggest that the limited behavioral disturbance (swim hyperactivity) induced by the 1 mg/kg dose of anpirtoline probably does not contribute to the poor acquisition scores.

The 5-HT$_{1B}$ receptor functions mainly as a terminal autoreceptor as well as a heteroreceptor in several neurochemical systems (see Introduction), while its role as a somatodendritic receptor seems to be marginal, at least in the rat (Adell *et al*, 2001; Evrard *et al*, 1999). However, it seems possible that NAS-181, by blocking terminal 5-HT$_{1B}$ autoreceptors, could increase 5-HT release, resulting in the stimulation of several 5-HT receptor subtypes, including the 5-HT$_{1A}$ receptors. However, the available evidence from microdialysis in awake rats shows that 5-HT$_{1B}$ receptor blockade does not result in an increase in extracellular 5-HT levels, at least not in the dorsal hippocampus (Hervás *et al*, 2000). Thus, the results with NAS-181 are probably not related to an increase of 5-HT release. Moreover, the facilitatory effects of NAS-181 on PA retention are difficult to reconcile with an increase in 5-HT release and subsequent stimulation of 5-HT$_{1A}$ receptors. Both systemic and intrahippocampal (CA1 region) administration of a 5-HT$_{1A}$ receptor agonist (8-OH-DPAT) consistently impair acquisition of spatial memory and PA retention (Carli and Samanin, 1992; Carli *et al*, 1993; Misane *et al*, 1998), deficits attributable to the activation of postsynaptic 5-HT$_{1A}$ receptors.

Theoretically, the blockade of 5-HT$_{1B}$ receptors could influence (enhance) acetylcholine release, if 5-HT$_{1B}$ receptors exert a tonic inhibitory action on cholinergic neurons (see Introduction). Based on this consideration, we studied whether NAS-181 could influence the cognitive deficits induced by the nonselective muscarinic antagonist scopolamine. Administration of NAS-181 could neither reverse the impairment of spatial learning nor the PA retention deficit induced by scopolamine (0.1 mg/kg in the WM study, 0.3 mg/kg in the PA study). However, a subthreshold dose of scopolamine (0.1 mg/kg), which by itself did not impair PA retention, significantly blocked the facilitation of PA retention induced by NAS-181. This indicates that the facilitatory effects of NAS-181 on PA retention may involve enhancement of cholinergic transmission. Unlike the effects of acetylcholinesterase inhibitors (Braida *et al*, 1996; Cheng *et al*, 1996; Misane and Ögren, 2003), the effects of 5-HT$_{1B}$ receptor blockade on cholinergic transmission does not appear to be sufficient to attenuate the deficit induced by a high dose of scopolamine in the PA task. The conclusion based on the studies with a subthreshold dose of scopolamine, that the facilitatory effects of NAS-181 in the PA task is mediated by enhanced cholinergic transmission, is supported by recent microdialysis results. NAS-181 given at the 1–10 mg/kg doses was shown to strongly elevate extracellular acetylcholine levels in the cortex and hippocampus in a dose-dependent manner (unpublished data). This agrees with a previous microdialysis study reporting

that the 5-HT$_{1B}$ agonist CGS-12066B decreased acetylcholine levels in the dorsal hippocampus of freely moving rats (Izumi *et al*, 1994). *In vitro* studies have also indicated that 5-HT could decrease acetylcholine release in hippocampal synaptosomes, probably via terminal 5-HT$_{1B}$ heteroreceptors located on cholinergic axons (Maura and Raiteri, 1986).

Scopolamine is often used as a model for drug-induced memory deficits (Blokland, 1996). In human volunteers, the pattern of cognitive impairment caused by scopolamine mimics, in some aspects, the cognitive symptomology seen in Alzheimer's disease (Ebert and Kirch, 1998), which is partly associated with the degeneration of cholinergic neurons in the basal forebrain resulting in a reduction of cholinergic neurotransmission in the cortex and the hippocampal formation (Shinotoh *et al*, 2000; Whitehouse *et al*, 1982). However, systemically administered scopolamine is not an ideal model of Alzheimer's disease, since it acts on multiple cholinergic systems, which also play a role in attention, sensory processing, and locomotion (Blokland, 1996; Gold, 1995). In fact, in the present WM study, we showed that treatment with scopolamine already at a low dose (0.1 mg/kg) could interfere with sensorimotor processing evidenced by induction of deflection behavior. Moreover, scopolamine increased thigmotaxic swimming during acquisition (0.3 mg/kg dose) and retention (0.1 and 0.3 mg/kg), a behavior that interferes with the use of efficient navigational strategies in the WM. This agrees with previous results, showing that scopolamine at a high dose (1 mg/kg) increased thigmotaxic swimming and deflections (Saucier *et al*, 1996). However, the low scopolamine dose (0.1 mg/kg) used in this study did not significantly alter thigmotaxic behavior during acquisition, indicating that the poor acquisition seen after scopolamine could not be due to increased thigmotaxic behavior. Taken together, scopolamine induces behavioral disturbances, which at the 0.3 mg/kg dose included increased swim speed (hyperactivity), thigmotaxic swimming, and deflections off the platform. These behavioral disturbances were clearly less pronounced at the 0.1 mg/kg dose, but the sensorimotor impairments may at least partly contribute to the poor spatial learning seen at this low dose. NAS-181, which did not induce any apparent behavioral disturbances, failed to block the behavioral disturbance (increased swim speed) caused by scopolamine. This could partly explain the inability of NAS-181 to block the spatial impairment caused by scopolamine. Thus, the efficacy of a 5-HT$_{1B}$ receptor antagonist, which acts as a modulator of central cholinergic transmission, could be underestimated in the scopolamine model. It will therefore be important to further analyze the effects of 5-HT$_{1B}$ receptor antagonists in animal models that better reflect the cholinergic deficits seen in dementia.

CONCLUSIONS

The present results demonstrate for the first time that 5-HT$_{1B}$ receptor stimulation and blockade results in opposite effects on cognitive functions. Moreover, 5-HT$_{1B}$ receptors probably located on cholinergic terminals, appear to be under tonic activation by serotonin *in vivo*. The 5-HT$_{1B}$ receptor antagonist NAS-181 appears to facilitate some aspects of cognitive performance, most likely via an increase of cholinergic transmissions in brain regions important for cognitive functions. This implies that 5-HT$_{1B}$ receptor antagonists might be of value for the treatment of age-related memory decline and human neurodegenerative diseases such as Alzheimer's disease, characterized by reductions of cholinergic transmission in cortico-limbic brain regions.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Medical Research Council (MFR; project No K97-14X-11588-02B), Karolinska Institutets fonder, and Alzheimerfonden. We thank Carina Stenfors (AstraZeneca R&D Södertälje, Sweden) for the supply of NAS-181.

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. *J Neurochem* **79**: 172–182.

Aït Amara D, Segu L, Buhot MC (2001). Region-specific decrease in 5-HT1A and 5-HT1B binding sites after intra-hippocampal ibotenic acid injections in the rat. *Neurosci Lett* **310**: 25–28.

Bammer G (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: a review and some new results. *Neurosci Biobehav Rev* **6**: 247–296.

Berg S, Larsson LG, Renyi L, Ross SB, Thorberg SO, Thorell-Svantesson G (1998). R-(+)-2-(3-Morpholinomethyl-2H-chromen-8-yl)oxymethylmorpholine Methanesulfonate), a new selective rat 5-hydroxytryptamine1B receptor antagonist. *J Med Chem* **41**: 1934–1942.

Blokland A (1996). Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Rev* **21**: 285–300.

Bolanos-Jiménez F, Manhaes de Castro R, Fillion G (1994). Effect of chronic antidepressant treatment on 5-HT1B presynaptic heteroreceptors inhibiting acetylcholine release. *Neuropharmacology* **33**: 77–81.

Boschert U, Amara DA, Segu L, Hen R (1994). The mouse 5-hydroxytryptamine1B receptor is localized predominantly on axon terminals. *Neuroscience* **58**: 167–182.

Bouhelal R, Smounya L, Bockaert J (1988). 5-HT1B receptors are negatively coupled with adenylate cyclase in rat substantia nigra. *Eur J Pharmacol* **151**: 189–196.

Braida D, Paladini E, Griffini P, Lamperti M, Maggi A, Sala M (1996). An inverted U-shaped curve for heptylphysostigmine on radial maze performance in rats: comparison with other cholinesterase inhibitors. *Eur J Pharmacol* **302**: 13–20.

Bruinvels AT, Landwehrmeyer B, Gastafson EL, Durkin MM, Mengod G, Branchek TA *et al* (1994). Localization of 5-HT1B, 5-HT1Dalfa, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropharmacology* **33**: 367–386.

Bruinvels AT, Palacios JM, Hoyer D (1993). Autoradiographic characterisation and localisation of 5-HT1D compared to 5-HT1B binding sites in rat brain. *Naunyn Schmiedeberg's Arch Pharmacol* **347**: 569–582.

Buhot MC, Martin S, Segu L (2000). Role of serotonin in memory impairment. *Ann Med* **32**: 210–221.

Buhot MC, Patra SK, Naili S (1995). Spatial memory deficits following stimulation of hippocampal 5-HT1B receptors in the rat. *Eur J Pharmacol* **285**: 221–228.

Bymaster FP, Heath I, Hendrix JC, Shannon HE (1993). Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. *J Pharmacol Exp Ther* **267**: 16–24.

Cain DP, Saucier D, Hall J, Hargreaves EL, Boon F (1996). Detailed behavioral analysis of water maze acquisition under APV or

CNQX: contribution of sensorimotor disturbances to drug-induced acquisition deficits. *Behav Neurosci* **110**: 86–102.

Carli M, Samanin R (1992). 8-Hydroxy-2-(di-*n*-propylamino)tetralin impairs spatial learning in a water maze: role of postsynaptic 5-HT1A receptors. *Br J Pharmacol* **105**: 720–726.

Carli M, Tatarczynska E, Cervo L, Samanin R (1993). Stimulation of hippocampal 5-HT1A receptors causes amnesia and anxiolytic-like but not antidepressant-like effects in the rat. *Eur J Pharmacol* **234**: 215–221.

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. *Br J Pharmacol* **130**: 1927–1932.

Chalmers DT, Watson SJ (1991). Comparative anatomical distribution of 5-HT1A receptor mRNA and 5-HT1A binding in rat brain—a combined *in situ* hybridisation/*in vitro* receptor autoradiographic study. *Brain Res* **561**: 51–60.

Cheetham SC, Heal DJ (1993). Evidence that RU 24969-induced locomotor activity in C57/B1/6 mice is specifically mediated by the 5-HT1B receptor. *Br J Pharmacol* **110**: 1621–1629.

Cheng DH, Ren H, Tang XC (1996). Huperzine A, a novel promising acetylcholinesterase inhibitor. *Neuroreport* **8**: 91–101.

Cloëz-Tayarani I, Cardona A, Rousselle JC, 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-HT1B receptors. *Proc Natl Acad Sci USA* **94**: 9899–9904.

Dahlström A, Fuxe K (1964). Evidence for the existence of monoamine neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. *Acta Physiol Scand* **62**: 1–55.

de Almeida RMM, Miczek KA (2002). Aggression escalated by social instigation or by discontinuation of reinforcement ('frustration') in mice: inhibition by anpirtoline—a 5-HT1B receptor agonist. *Neuropsychopharmacology* **272**: 171–181.

Decker MW, McGaugh JL (1991). The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* **7**: 151–168.

Deutsch JA (1971). The cholinergic synapse and the site of memory. *Science* **174**: 788–794.

Doucet E, Pohl M, Fattaccini CM, Adrien J, El Mestikawy S, Hamon M (1995). *In situ* hybridization evidence for the synthesis of 5-HT1B receptor in serotonergic neurons of anterior raphe nuclei in the rat brain. *Synapse* **19**: 18–28.

Ebert U, Kirch W (1998). Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur J Clin Invest* **28**: 944–949.

Engel G, Göthert 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 Arch Pharmacol* **332**: 1–7.

Evrard A, Laporte AM, Chastanet M, Hen R, Hamon M, Adrien J (1999). 5-HT1A and 5-HT1B receptors control the firing of serotonergic neurons in the dorsal raphe nucleus of the mouse: studies in the 5-HT1B knock-out mice. *Eur J Neurosci* **11**: 3823–3831.

Fibiger HC (1991). Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends Neurosci* **14**: 220–223.

Gold PE (1995). Modulation of emotional and nonemotional memories: same pharmacological system, different neuroanatomical systems. In: McGaugh JL, Weinberger NM, Lynch G (eds) *Brain and Memory: Modulation and Mediation of Neuroplasticity*. Oxford University Press: New York. pp 40–74.

Gothert M, Hamon M, Barann M, Bonisch H, Gozlan H, Laguzzi R *et al* (1995). 5-HT3 receptor antagonism by anpirtoline, a mixed 5-HT1 receptor agonist/5-HT3 receptor antagonist. *Br J Pharmacol* **114**: 269–274.

Hartig PR, Hoyer D, Humphrey PPA, Martin GR (1996). Alignment of receptor nomenclature with the human genome: classification of 5-HT1B and 5-HT1D receptor subtypes. *Trends Pharmacol Sci* **17**: 103–105.

Hervás I, Queiroz CMT, Adell A, Artigas F (2000). Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. *Br J Pharmacol* **130**: 160–166.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ *et al* (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* **46**: 157–203.

Hoyer D, Martin G (1997). 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacology* **36**: 419–428.

Izumi J, Washizuka M, Miura N, Hiraga Y, Ikeda Y (1994). Hippocampal serotonin 5-HT1A receptor enhances acetylcholine release in conscious rats. *J Neurochem* **62**: 1804–1808.

Kikvadze I, Foster GA (1995). Action-potential-dependent output of 5-hydroxytryptamine in the anaesthetized rat amygdalopiriform cortex is strongly inhibited by tonic 5-HT1B-receptor stimulation. *Brain Res* **692**: 111–117.

Kirk RE (1968). *Experimental Design: Procedures for the Behavioural Sciences*. Brooks/Cole Publishing Company: Belmonte.

Li YW, Bayliss DA (1998). Presynaptic inhibition by 5-HT1B receptors of glutamatergic synaptic inputs onto serotonergic caudal raphe neurones in rat. *J Physiol (Lond)* **510**: 121–134.

Macor JE, Burkhart CA, Heym JH, Ives JL, Lebel LA, Newman ME *et al* (1990). 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolol(3,2-b)pyrid-5-one: a potent and selective serotonin (5-HT1B) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J Med Chem* **33**: 2087–2093.

Malleret G, Hen R, Guillou JL, Segu L, Buhot MC (1999). 5-HT1B receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. *J Neurosci* **19**: 6157–6168.

Maura G, Raiteri M (1986). Cholinergic terminals in rat hippocampus possess 5-HT1B receptors mediating inhibition of acetylcholine release. *Eur J Pharmacol* **129**: 333–337.

Maura G, Roccatagliati E, Raiteri M (1986). Serotonin autoreceptor in the rat hippocampus: pharmacological characterization as a subtype of the 5-HT1 receptor. *Naunyn Schmiedeberg's Arch Pharmacol* **334**: 323–326.

Meneses A (2001). Could the 5-HT1B receptor inverse agonism affect learning consolidation? *Neurosci Biobehav Rev* **25**: 193–201.

Meneses A, Terrón JA, Hong E (1997). Effects of the 5-HT receptor antagonist GR127935 (5-HT1B/1D) and MDL100907 (5-HT2A) in the consolidation of learning. *Behav Brain Res* **89**: 217–223.

Misane I, Johansson C, Ögren SO (1998). Analysis of the 5-HT1A receptor involvement in passive avoidance in the rat. *Br J Pharmacol* **125**: 499–509.

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

Misane I, Ögren SO (2003). Selective 5-HT1A antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. *Neuropsychopharmacology* **28**: 253–264.

Morris RGM (1981). Spatial localization does not require the presence of local cues. *Learn Motiv* **12**: 239–260.

O'Keefe J (1993). Hippocampus, theta, and spatial memory. *Curr Opin Neurobiol* **3**: 917–924.

O'Neill MF, Parameswaran T (1997). Ru24969-induced behavioural syndrome requires activation of both 5HT1A and 5HT1B receptors. *Psychopharmacology* **132**: 255–260.

Ögren SO (1985). Evidence for a role of brain serotonergic neurotransmission in avoidance learning. *Acta Physiol Scand* **125**(Suppl. 544): 1–71.

Ögren SO (1986). Analysis of the avoidance learning deficit induced by the serotonin releasing compound *p*-chloroamphetamine. *Brain Res Bull* **16**: 645–660.

Ögren SO, Kehr J, Schött PA (1996). Effects of ventral hippocampal galanin on spatial learning and on *in vivo* acetylcholine release in the rat. *Neuroscience* **75**: 1127–1140.

Oksenberg D, Marsters SA, O'Dowd BF, Jin H, Havlik S, Peroutka SJ *et al* (1992). A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT1B receptors. *Nature* **360**: 161–163.

Pineyro G, Castanon N, Hen R, Blier P (1995). Regulation of [^{3}H]5-HT release in raphe, frontal cortex and hippocampus of 5-HT1B knock-out mice. *Neuroreport* **7**: 353–359.

Pompeiano M, Palacios JM, Mengod G (1992). Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. *J Neurosci* **12**: 440–453.

Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X *et al* (2000). Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J Comp Neurol* **417**: 181–194.

Roberts C, Boyd DF, Middlemiss DN, Routledge C (1999). Enhancement of 5-HT1B and 5-HT1D receptor antagonist effects on extracellular 5-HT levels in the guinea-pig brain following concurrent 5-HT1A or 5-HT re-uptake site blockade. *Neuropharmacology* **38**: 1409–1419.

Rogers DC, Hagan JJ (2001). 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology* **158**: 114–119.

Sarhan H, Grimaldi B, Hen R, Fillion G (2000). 5-HT1B receptors modulate release of [^{3}H]dopamine from rat striatal synaptosomes: further evidence using 5-HT moduline, polyclonal 5-HT1B receptor antibodies and 5-HT1B receptor knock-out mice. *Naunyn Schmiedebergs Arch Pharmacol* **361**: 12–18.

Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M *et al* (1999). Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience* **88**: 899–915.

Saucier D, Hargraeves EL, Boon F, Vanderwolf CH, Cain DP (1996). Detailed behavioral analysis of water maze acquisition under systemic NMDA or muscarinic antagonism: nonspatial pretraining eliminates spatial learning deficits. *Behav Neurosci* **110**: 103–116.

Schechter LE, Dawson LA, Harder JA (2002). The potential utility of 5-HT1A receptor antagonists in the treatment of cognitive dysfunction associated with Alzheimer's disease. *Curr Pharm Des* **8**: 139–145.

Schlicker E, Werner U, Hamon M, Gozlan H, Nickel B, Szelenyi I *et al* (1992). Anpirtoline, a novel, highly potent 5-HT1B receptor agonist with antinociceptive/antidepressant-like actions in rodents. *Br J Pharmacol* **105**: 732–738.

Schmitz D, Empson RM, Heinemann U (1995). Serotonin reduces inhibition via 5-HT1A receptors in area CA1 of rat hippocampal slices *in vitro*. *J Neurosci* **15**: 7217–7225.

Schött PA, Bjelke B, Ögren SO (1998). Distribution and kinetics of galanin infused into the ventral hippocampus of the rat: relationship to spatial learning. *Neuroscience* **83**: 123–136.

Shinotoh H, Namba H, Fukushi K, Nagatsuka S, Tanaka N, Aotsuka A (2000). Progressive loss of cortical acetylcholinesterase activity in association with cognitive decline in Alzheimer's disease: a positron emission tomography study. *Ann Neurol* **48**: 194–200.

Sprouse JS, Aghajanian GK (1987). Electrophysiological responses of serotonergic dorsal raphé neurons to 5-HT1A and 5-HT1B agonists. *Synapse* **1**: 3–9.

Steckler T, Sahgal A (1995). The role of serotonergic–cholinergic interactions in the mediation of cognitive behaviour. *Behav Brain Res* **67**: 165–199.

Stenfors C, Hong Y, Ross SB (2000). Enhanced 5-HT metabolism and synthesis rate by the new selective r5-HT1B receptor antagonist, NAS-181 in the rat brain. *Neuropharmacology* **39**: 553–560.

Stenfors C, Yu H, Ross SB (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 Arch Pharmacol* **363**: 222–232.

Stephenson RM, Andrew RJ (1994). The effects of 5-HT receptor blockade on memory formation in the chick: possible interactions between beta-adrenergic and serotonergic systems. *Pharmacol Biochem Behav* **48**: 971–975.

Swedberg MD, Shannon HE, Nickel B, Goldberg SR (1992). D-16949 (anpirtoline): a novel serotonergic (5-HT1B) psychotherapeutic agent assessed by its discriminative effects in the rat. *J Pharmacol Exp Ther* **263**: 1015–1022.

Treit D, Fundytus M (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol Biochem Behav* **31**: 959–962.

Tricklebank MD, Forler C, Fozard JR (1984). The involvement of subtypes of the 5-HT1A receptor and catecholaminergic system in the behavioural response to 8-hydroxy-2-(di-*n*-propylo-amino)tetralin in the rat. *Eur J Pharmacol* **106**: 271–282.

Van der Staay J, Hinz VCH, Schmidt BH (1996). Effects of Metrifonate, its transformation product dichlorvos and other organophosphorus and reference cholinesterase inhibitors on Morris water maze in young adult rats. *J Pharmacol Exp Ther* **278**: 697–709.

Vertes RP (1991). A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J Comp Neurol* **313**: 643–668.

Vertes RP, Fortin WJ, Crane AM (1999). Projections of the median raphe nucleus in the rat. *J Comp Neurol* **407**: 555–582.

von Bohlen und Halbach O, Albrecht D (2002). Reciprocal connections of the hippocampal area CA1, the lateral nucleus of the amygdala and cortical areas in a combined horizontal slice preparation. *Neurosci Res* **44**: 91–100.

Whishaw IQ, Mittleman G, Bunch ST, Dunnett SB (1987). Impairment in the acquisition, retention and selection of navigation strategies after medial caudate-putamen lesions in rats. *Behav Brain Res* **24**: 125–138.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**: 1237–1239.