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# Differential modulation of the 5-$HT_4$ receptor agonists and antagonist on rat learning and memory

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**Abstract**

Recent data suggest that activation of 5-$HT_4$ receptors may modulate cognitive processes such as learning and memory. In the present study, the effects of two potent and selective 5-$HT_4$ agonists, RS 17017 [1-(4-amino-5-chloro-2-methoxyphenyl)-5-(piperidin-1-yl)-1-pentanone hydrochloride] and RS 67333 [1(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone], were studied in an olfactory associative discrimination task. The implication of 5-$HT_4$ receptors in the associative discriminative task was suggested by the following observation. Injection of a selective 5-$HT_4$ receptor antagonist RS 67532 [1-(4-amino-5-chloro-2-(3,5-dimethoxybenzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone; 1 mg/kg: i.p.] before the third training session induced a consistent deficit in associative memory during the following training sessions. This deficit was absent when the antagonist was injected together with either a specific hydrophilic 5-$HT_4$ (RS 17017, 1 mg/kg) or a specific hydrophobic (RS 67333, 1 mg/kg) 5-$HT_4$ receptor agonist. RS 67333 was more potent than RS 17017. This difference in potency certainly reflects a difference in their capacity to enter into the brain. This is also likely to be the reason why, injected alone, the hydrophobic 5-$HT_4$ agonist (RS 67333) but not the hydrophilic 5-$HT_4$ agonist (RS 17017) improved learning and memory performance. 

*Keywords:* Associative olfactory task; Learning; Long-term memory; RS 17017; RS 67333; RS 67532; 5-$HT_4$ receptors

## 1. Introduction

5-$HT_4$ receptors were first described in mouse colliculus neurons (Dumuis et al., 1988) and in guinea-pig hippocampal homogenates (Bockaert et al., 1989). The introduction of selective radioligands to label 5-$HT_4$ receptors in the brain confirmed their colliculi and hippocampal localizations, but also indicated that they have a wider distribution (for a review, see Waeber et al., 1994; Eglen et al., 1995b). The expression of 5-$HT_4$ receptors in limbic areas suggested that their possible role in learning and memory may be through the modulation of acetylcholine release in these structures (Consolo et al., 1994).

In order to study the potential role of 5-$HT_4$ receptors in learning and memory, BIMU-1, a mixed 5-$HT_4$ agonist/5-$HT_3$ antagonist, was tested on a social olfactory recognition task (Letty et al., 1997) and on olfactory associative task (Marchetti-Gauthier et al., 1997). The choice of olfactory tasks was based on high level performance obtained on learning and memory using olfactory stimuli in rats (Eichenbaum and Otto, 1993; Slotnick and Katz, 1974; Slotnick, 1993). In addition, lesions of the limbic system in rats (Eichenbaum et al., 1988; Chaillan et al., 1997) suggest that olfactory stimuli allow for better access to higher cognitive processes than stimuli using other sensory modalities. Moreover, by using the same associative discrimination olfactory task, it was possible to observe differential behavioral performance on procedural (i.e. memory of the inter-trial duration) versus reference (i.e. memory of the odor-reward associations) long-term memory (Roman et al., 1993b) similar in some respects to a dichotomy between declarative and nondeclarative long-term memory (i.e. maintain in memory after several minutes) observed in

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humans, as reported by Squire and Zola (1996). In previously reported experiments, the intraperitoneal injection of BIMU-1 mainly at 10 mg/kg was followed by a substantial improvement, firstly in reference memory, and secondly in procedural memory. Difficulty in rapidly reversing behavioral responses to previously learned associations 1 month later indicated that the BIMU-1 effect was not transient, but correlated to long-term memory. The effects of BIMU-1 were attributed to the activation of 5-$HT_4$ receptors since they were blocked by GR 125487, a high-affinity, 5-$HT_4$-specific antagonist having relatively low affinity for 5-$HT_3$ receptors (Gale et al., 1994a,b).

More recent experiments using RS 17017 [1-(4-amino-5-chloro-2-methoxyphenyl)-5-(piperidin-1-yl)-1-pentanone hydrochloride] (1 mg/kg) enhanced delayed matching performance in young and old macaques (Terry et al., 1998), and the 5-$HT_4$ receptor agonist RS 67333 [1(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone] (1 mg/kg) reversed the performance deficit produced by atropine in the Morris water maze (Fontana et al., 1997).

The aim of the present study was to further investigate the involvement of 5-$HT_4$ receptors in learning and long-term memory using our olfactory associative discrimination task. The physiological role of the 5-$HT_4$ receptor was studied using the two highly potent, selective 5-$HT_4$ receptor agonists described above, one hydrophilic (RS 17017), one hydrophobic (RS 67333) and a high affinity, 5-$HT_4$-specific antagonist (RS 67532) [1-(4-amino-5-chloro-2-(3,5-dimethoxybenzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone] (all from the Institute of Chemistry, Roche Bioscience, Palo Alto, CA) tested under the same conditions at the most efficient dose on the same behavioral task.

## 2. Methods

### *2.1. Animals*

Male adult Sprague Dawley rats (300–350 g) from the IFFA CREDO Company (L'Arbresle, France) were used in all experiments. They were individually housed and kept on a 24-h light–dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.) in a room held at a constant temperature (22°C). Each animal was handled (10 min) every day for 3 days and then deprived of water for 48 h before training.

### *2.2. Apparatus and training procedure*

The olfactory training apparatus was a rectangular box made of wire mesh (30×30×50 cm). A conical odor port (1.5 cm in diameter, 0.5 cm above the floor) was drilled horizontally through a triangular wedge of Plexiglas, mounted in one corner of the cage. A circular (1 cm diameter) water port in the shape of a well was placed directly above the odor port. Responses to the odor presentation were monitored by a photoelectric circuit. Two flashlight bulbs which could be turned on and off, as conditions required, were placed outside the cage, one on each side of the odor and water ports, 10 cm above the floor. Individual odors were delivered by forcing clean air (0.7 bars) through one of two 1000 ml Ehrlenmeyer flasks that contained 500 ml of water mixed (2‰) with one of the chemicals or natural odorants. The odor pairs used were jasmine–strawberry and rose–lemon for S+ and S−, respectively. Non-odorized air was delivered by sending air through a flask that contained only water. Odorized and clean air streams were passed individually through tubes, which were put through the back of the sound-attenuating chamber and attached to the odor port. Water was delivered using a gravity-fed system, and passed through a valve which, when opened, allowed 0.1 ml of water to be released into the water port. All experiments were conducted simultaneously in four cages to ensure training under the same conditions. Animals were trained to make two odor-reward associations. Each odor had to be associated with a specific reward, one arbitrarily designated as positive and the other as negative, using a successive Go or No-Go paradigm. The rats had to approach the odor and water ports to interrupt the light beam during the 10 s of presentation of the positive odor. Response to the odor designated as negative resulted in a 10 s presentation of non-aversive light. The water was only distributed with a response to the positive odor.

Individual trials were presented in a quasi-random fashion and either odor was presented for, at most, 10 s, if the rat did not interrupt the light beam before that time.

Each new trial was started only when the subject left the corner where the reward was distributed; if not, the trial was delayed for 10 s (cumulative time). In all cases, a new trial could not start any earlier than 15 s after the termination of either water or light delivery, or no response. A daily session was made up of 60 trials with an inter-trial interval (ITI) of 15 s. Animals were tested every day between 08:00 a.m. and 02:00 p.m.

### *2.3. Experimental design*

Twenty-seven animals were placed in four similar olfactory training apparatuses for two daily sessions. Then they were divided into seven pretreatment groups. Four animals were given either the selective 5-$HT_4$ receptor agonists RS 67533 and RS 17017, or the 5-$HT_4$ antagonist, RS 67532, 30 min prior to undergoing the third training session.

Three groups also contained four rats. They were injected with either a saline solution, a mixture of RS 67533+RS 67532, or a mixture of RS 17017+RS 67532.