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Spatial memory deficits following stimulation of hippocampal 5-HT_{1B} receptors in the rat

Marie-Christine Buhot [a,*], Sarat K. Patra [b], Saïd Naïli [a]

[a] *Centre National de la Recherche Scientifique, Laboratoire de Neurosciences Comportementales et Cognitives, C.N.R.S. URA 339, Université de Bordeaux I, Avenue des Facultés, 33405 Talence Cedex, France*

[b] *Centre for Advanced Study in Psychology, UTKAL University, Bhubaneswar 751 004, Orissa, India*

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Abstract

In this study we examined a possible contribution of serotonin (5-hydroxytryptamine, 5-HT) to spatial memory performance in the rat. Rats were trained to run in a radial maze in a manner that involved two kinds of memory function, i.e. working memory and reference memory. They received intrahippocampal microinjections of a 5-HT_{1A} [8-hydroxy-2-(di-*n*-propylamino)tetralin or 8-OH-DPAT], or a 5-HT_{1B} [3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one or CP-93,129] receptor agonist, and a muscarinic receptor antagonist (scopolamine). 8-OH-DPAT (5 $\mu g/\mu l$), like injections of saline, induced no change in performance levels. In contrast, rats suffered an impairment in both reference and working memory following injection of scopolamine (10 $\mu g/\mu l$). CP-93,129 induced a higher frequency of reference memory errors than of working memory errors at the intermediate (10 $\mu g/\mu l$) and higher doses (16 $\mu g/\mu l$). Thus, the stimulation of 5-HT_{1B} receptors in the CA1 field of the dorsal hippocampus impairs the performance of rats in a spatial learning task.

Keywords: 5-HT_{1A} receptor; 5-HT_{1B} receptor; Hippocampus; Scopolamine; Spatial memory; (Microinjection)

1. Introduction

The rodent hippocampus has been demonstrated repeatedly as the key cerebral structure involved in spatial learning and memory (e.g. O'Keefe and Nadel, 1978; Buhot et al., 1991; Jaffard and Meunier, 1993; Jarrard, 1993; Schmajuk et al., 1993).

Much evidence suggests that hippocampal functions are controlled by the septo-hippocampal cholinergic system (e.g. Brito et al., 1983; Kelsey and Vargas, 1993; Marighetto et al., 1993). Treatment with centrally active forms of anticholinergic drugs, such as scopolamine or atropine, have similar effects as hippocampal lesions on certain kinds of spatial memory (e.g. Buresova et al., 1986; Buhot et al., 1989b).

Brain serotonin (5-hydroxytryptamine, 5-HT) may contribute also to mechanisms underlying learning and memory. However, studies with conflicting results showed how serotonin depletion or specific lesions of serotonin neurons, are either ineffective (e.g. Asin et al., 1985; Ricaurte et al., 1993), enhance (e.g. Altman et al., 1990), or impair (e.g. Lipska et al., 1992) learning and memory. By contrast, 5-HT reuptake blockers, which enhance central 5-HT activity, have also been described as either inducing memory facilitation (e.g. Flood and Cherkin, 1987) or impairing it (e.g. Jaffard et al., 1991). Finally, raphe grafts into the hippocampus ameliorated spatial memory deficits induced by combined 5-HT-cholinergic deficiencies (e.g. Richter-Levin and Segal, 1989).

However these studies generally did not differentiate between types and sub-types of 5-HT receptors. Only 5-HT_3 receptor antagonists were demonstrated as clearly improving learning and memory or antagonizing the effects of anticholinergic- or age-related memory loss in rodents (e.g. Barnes et al., 1990). The 5-HT_{1A}

* Corresponding author. C.N.R.S., URA 339, Laboratoire de Neurosciences Comportementales et Cognitives, Université de Bordeaux I, Avenue des Facultés, 33405 Talence Cedex, France. Tel. (33) 56 84 87 42, fax (33) 56 84 87 43.

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receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), injected via a peripheral route, was found to impair performance in spatial memory (Winter and Petti, 1987) and in a passive avoidance task (Carli et al., 1992b), but to increase exploratory activity and habituation (Buhot et al., 1989a).

Little, if anything, appears to be known about the contribution of 5-HT_{1B} receptors to cognitive functions.

The hippocampal formation receives modulatory influences from the serotonergic system, in particular through type 5-HT_{1A} somatodendritic receptors which occur at a high concentration in the hippocampus, and indirectly via autoreceptors (e.g. Marcinkiewicz et al., 1984). In contrast, type 5-HT_{1B} receptors, much less studied until now, are located mainly on axon terminals (e.g. Boschert et al., 1994) where they generally inhibit neurotransmitter release. This cellular function suggests that 5-HT_{1B} receptors may play a critical role. They interact with the septo-hippocampal cholinergic system through receptors situated on (medial) septal axon terminals (Maura and Raiteri, 1986). Furthermore, we recently described 5-HT_{1B} receptors in the hippocampal stratum oriens layer, likely located on the proximal portion of axons of the CA1 pyramidal neurons, and in the dorsal subiculum where they are located on CA1 axon terminals (Aït Amara et al., 1995). In the hippocampus, 5-HT_{1B} receptors might thus modulate the release of neurotransmitters, in particular: of serotonin itself via autoreceptors, of acetylcholine via septal terminal receptors and probably of glutamate via these 5-HT_{1B} receptors located on CA1 pyramidal axons.

In the present study, we investigated the effects of intra-hippocampal injection of selective 5-HT_{1A} and 5-HT_{1B} receptor agonists on working and reference memory. Rats were equipped with intracerebral cannulas for injecting drugs into the CA1 field of the dorsal hippocampus, and were then subjected to a classical spatial learning task. The 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was used as a 5-HT_{1A} receptor agonist, and the 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one (CP 93,129) as a 5-HT_{1B} receptor agonist. Scopolamine, a muscarinic receptor antagonist, was used as a 'positive' control drug, and 0.9% NaCl as injection control.

2. Materials and methods

2.1. Animals

Male Long-Evans black-hooded rats ($n = 18$) were purchased from a commercial laboratory animal supplier (Elevage Janvier, Le Geniest-St-Isles, France). Upon receipt, they were individually housed in standard laboratory cages (37.5 × 25.5 × 16 cm), and maintained with natural lighting in a temperature-controlled room (20°C ± 2), adjacent to the experimental room. They had continuous access to food and water, except for the few days preceding learning and until the end of the experiment. They were approximately 60 days of age with an average body weight of 300 g at the time of surgery.

2.2. Surgery and histology

Surgery was performed before training the animals in order to avoid post-surgical disturbance. The general surgical and histological procedures have been described previously (Poucet and Buhot, 1994). The rats were implanted bilaterally with 23-gauge stainless steel guide cannulas aimed at the dorsal hippocampus, at the following coordinates relative to bregma: A-P = −3.8 mm; L = ±2 mm (Paxinos and Watson, 1982). At each location, a cannula was lowered vertically. Both cannulas were cut so that their tips attained a depth of 1.2 mm from the top of the skull and were 0.7 mm above the corpus callosum, at the level of the hippocampal CA1 field. The rats were subjected to behavioral testing 10 days later.

Serial sections of brains (40 μm in thickness) were stained with cresyl violet and microscopically examined for verification of the tracks of the cannulas.

2.3. Apparatus

The apparatus was an elevated (50 cm above the floor) radial maze, made of stainless steel. It was composed of eight alleys or arms (100 cm in length, 10 cm in width, bound by 1-cm raised edges) radiating from an octogonal central platform (30 cm on each side). The apparatus was placed in a large experimental room (5 m × 5 m) which was decorated with pictures. A radio diffused music which drowned outside noises and provided an additional environmental cue. Small food cups, containing a small piece of cheese, were positioned at the distal ends of each arm. Four out of the eight cups were covered by a wire mesh that prevented the food being eaten. The other four cups were uncovered thus allowing the animals to eat the contents.

2.4. Behavioral procedures

Handling

Each animal was handled daily for 10 min from receipt through a 10-day post-surgery recovery period.

Pre-training

Pre-training began 10 days post-operatively when the animals had recovered their pre-surgery weight and

appeared healthy. Their daily diet was then reduced to a level which maintained them at 85% of their free-feeding weight. They were familiarized for 5 days with the cheese to be used as the reinforcer during the experiment. During the following 5 days, animals were trained to run for food on an elevated runway. They were submitted to three trials a day, each consisting of being placed at one end of an elevated runway and allowed to run to the opposite end to get a piece of cheese placed into a foodcup.

Training

Animals were trained for the task before the injection phase until they had reached a certain level of performance which, during the 'pharmacological phase', might either improve or deteriorate, depending on the drug injected.

The rats were subjected to one trial a day during the first 3 weeks, and then to two trials a day, with an inter-trial interval of about 15 min. Before each trial, the cups were baited with fresh pieces of cheese of equal size. The four arms containing accessible rewards were chosen randomly for each subject from a set of eight possible patterns (see also details in Poucet and Buhot, 1994). At the completion of its two daily sessions, the subject was returned to its cage and received its daily diet. The test order of the subjects was designed randomly.

The animals had to learn to get food optimally, i.e. to avoid: (1) visits to the arms never baited (always in the same position with regard to the surroundings), and (2) returns to an already visited arm, i.e. whithout food. These 'un-reinforced' runs were considered as 'errors' in reference (1), and in working (2) memory, respectively.

The duration of the training phase was 4 weeks (5 days a week). During the last week of training, animals were submitted to a simulated injection, i.e. 15 min before the first trial they were habituated to be gently, but firmly, maintained in a cloth while the injection procedure was applied (see below), except that no drug was injected. The training phase was followed immediately by the pharmacological phase.

Pharmacological phase

Drugs used. Scopolamine (scopolamine hydrochloride, Sigma), a cholinergic muscarinic receptor antagonist was used at a dose (10 $\mu g/\mu l$) which was previously found to affect selectively spatial behavior (Brito et al., 1983).

The 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, RBI), a specific agonist of 5-HT_{1A} receptors, was used at a dose (5 $\mu g/\mu l$) which was found to affect optimally the rats' acquisition and performance in a spatial discrimination task (Carli et al., 1992a) and in a passive avoidance task (Carli et al., 1993) when injected into the CA1 region of the dorsal hippocampus.

The 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]-pyrid-5-one or CP 93,129, a highly specific 5-HT_{1B} receptor agonist (Macor et al., 1990), was used at doses of 5, 10 and 16 $\mu g/\mu l$. At 16 $\mu g/\mu l$, this compound, injected into the paraventricular medial hypothalamus, was found to reduce food intake more efficiently than 5-HT itself (Macor et al., 1990). This novel compound shows the best selectivity for 5-HT_{1B} receptors ($K_i = 8$ for 5-HT_{1B}, $K_i = 1500$ for 5-HT_{1A} binding sites), compared to other drugs used as 5-HT_{1B} receptors agonists in behavioral studies, such as, for example, RU 24969, which also has high affinity for 5-HT_{1A} receptors ($K_i = 4$ for 5-HT_{1B}, $K_i = 16$ for 5-HT_{1A} binding sites). The 8-OH-DPAT is a highly specific 5-HT_{1A} receptor agonist ($K_i = 6$ for 5-HT_{1A} binding sites, $K_i = 12000$ for 5-HT_{1B}) (see Boulenguez et al., 1995).

The drugs were dissolved in 0.9% NaCl solution and 1 μl of this saline solution was used for control injections.

Injection procedures. Animals were gently restrained while stylets were removed. Two injection needles (30-gauge) were then inserted into the guide cannulas, using a brace that permitted their tips to protrude 1–1.4 mm below the opening of the guides, i.e. in the CA1 field of the hippocampus. Each injection needle was connected to a 10-μl Hamilton syringe via 50-cm-long polyethylene tubing (PE-20, Intramedic), both syringes being placed into a Harvard syringe pump (Model No. 22). An air bubble was placed in the tubing (graduated in μl) so that the movement of the meniscus allowed control of the amount of drug delivered. The different solutions were injected bilaterally in a volume of 1 μl per site during 2 min, an additional 2-min waiting period ensured better diffusion of the drug. The injection needles were then removed and replaced by the stylet, and the rat was replaced in its cage.

Pharmacological protocol. The pharmacological phase began after the animals had completed the training phase; it lasted 6 weeks, each animal was submitted to two trials a day (5 days a week). Rats were injected 15 min before their first daily trial. They were either injected or not injected on alternate days so that the minimum time between two injections was 48 h. All drugs were administered twice to each animal. For any particular compound, the two injections were given during the first half (first 3 weeks) and the second half (last 3 weeks) of the pharmacological phase, so as to neutralize any training effect. The CP-93,129 was used only once at each dose, but given at three equal intervals; the high dose was given first, the low dose next, and the intermediate dose last.

2.5. Data collection and analysis

The sequence of arm entries was noted, and the number of working and reference memory errors was calculated. The time required to complete a trial was also recorded.

The training phase was analysed on the basis of total number of errors, whatever their type (working and reference memory errors).

Given the fact that it was decided to conduct the pharmacological phase while the animals had not yet completed the learning phase, the analysis of the results was performed so as to allow the observation of the occurrence of positive as well as of negative changes in performance following the various treatments. For that purpose, two main analyses were conducted. First, any performance on an 'injection day' was compared to performance on the prior non-injection day in order to assess any treatment or injection effect. The Student's *t*-test for paired comparisons was applied to these data.

Furthermore, in order to evaluate the effect of active (drug) treatments compared to control (saline) injections, the measure of performance (or error score) was calculated as the number of errors recorded during injection trials minus the number of errors recorded during non-injection trials on the preceding day. Since no differences were found with regard to the time-course of the pharmacological phase (first half versus second half), the data were pooled so that, for each animal, a score averaged for four trials (2 trials × 2 days of injection for each compound) was obtained (averaged on two trials for the CP-93,129 compound). Variance analyses (StatView II, Abacus Concepts) were calculated for these data for all conditions of injection, with treatment (saline, scopolamine, 8-OH-DPAT, CP-93,129, 5μg/μl, 10 μg/μl and 16 μg/μl) and error type (i.e. reference and working memory errors) as the two main factors.

One variable, rarely taken into account, is the time spent in performing the task. It was decided to determine trial duration as an additional measure of performance, as well as a more general indication of the influence of the compounds tested on locomotion, emotional state, decision making, and motivation. Thus, the mean duration of a trial under each pharmacological condition was calculated and statistically evaluated with reference to the data obtained with non-treated animals on the preceding day.

3. Results

3.1. Histology

Two animals lost their cannulas before the end of the experiment and one had its cannulas in much too anterior a position; the data from these animals were therefore discarded from the behavioral data analyses. The cannula placement of the other 15 animals was correct. The tracks of the cannulas were positioned symmetrically just above the CA1 field of the hippocampus, ending in the overlying cortical area without damaging the underlying tissue (corpus callosum). Dye that was injected into the brains of some live animals via cannulas was found to be confined to the dorsal part of the hippocampus, occupying an area less than 1.5 mm in diameter (see Boulenguez et al., 1995, for diffusion tests).

3.2. Behavioral findings

Training and post-injection period

Fig. 1 shows the mean total number of errors recorded during the training period and the post-injection period. An analysis of variance (one-way ANOVA for repeated measures) was performed on the data. It revealed a major effect of the period of learning ($F(3,42) = 23.93$, $P < 0.0001$). This was further confirmed by Scheffé-tests applied on paired comparisons which indicated significant (at $P < 0.05$) differences in performance for the following comparisons: period 1 vs. period 2, period 1 vs. period 3, period 1 vs. post-injection period, period 2 vs. post-injection period. Finally, the post-injection period was characterized by the best performance (with one error as the mean value), indicating thus a lack of long-term non-specific impairment which might have occurred with time or following multiple injections.

Pharmacological phase

Overall performance. The first analysis was performed on the error scores, whatever the type of the errors. Histograms of the upper panel of Fig. 2 represent the

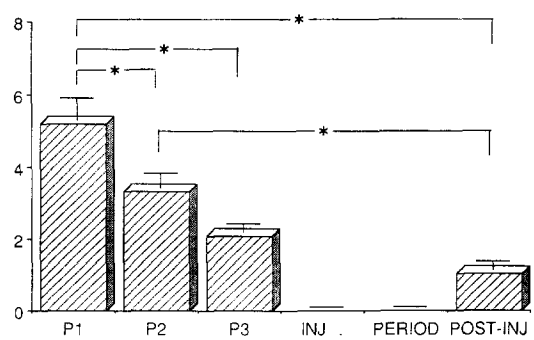

Fig. 1. Performances during the training period, period 1 (P1) to period 3 (P3) and the post-injection period (POST-INJ) as measured by the total number of errors (means ± S.E.) per trial (on the ordinate). Each value corresponds to the average of four trials. Period 1 refers to trials 1–4, period 2 refers to trials 9–12 and period 3 refers to trails 17–20. * $P < 0.05$.

error scores obtained following the different treatments (saline, scopolamine, 8-OH-DPAT, CP-93,129 at doses of 5 μg/μl, 10 μg/μl and 16 μg/μl) as given by the difference in number of errors between injection and non-injection trials.

A significant increase in the number of errors, when compared with the non-injection data, was found only following scopolamine and the CP-93,129 treatment at the highest dose [saline: $t(14) = 0.67$, NS; scopolamine: $t(14) = 3.21$, $P < 0.01$; 8-OH-DPAT: $t(14) = 0.77$, NS; CP-93,129 at 5 μg/μl: $t(14) = 1.61$, NS; CP-93,129 at 10 μg/μl: $t(14) = 1.30$, NS; CP-93,129 at 16 μg/μl: $t(14) = 3.45$, $P < 0.01$]. At the intermediate dose (10 μg/μl) of CP-93,129 there was an intermediate level of performance (average number of errors, at 5 μg/μl, $m = -0.57 \pm 0.35$; at 10 μg/μl, $m = +0.82 \pm 0.63$, and at 16 μg/μl, $m = 3.07 \pm 0.89$), suggesting a dose-dependent effect.

An analysis of variance of the error scores revealed a significant effect of treatment ($F(5,70) = 6.78$, $P < 0.0001$), but no effect of subject ($F(14,70) < 1$). Additional paired comparisons revealed in particular that, compared to saline injections, significant increases in error scores occurred following injection of scopolamine ($F(1,14) = 13.98$, $P < 0.005$) and the higher dose (16 μg/μl) of CP-93,129 ($F(1,14) = 9.07$, $P < 0.01$), but not following the other treatments (8-OH-DPAT, $F(1,14) < 1$; CP-93,129 at 5 μg/μl, $F(1,14) < 1$; CP-93,129 at 10 μg/μl, $F(1,14) = 2.24$, NS). The increase in error scores following scopolamine treatment also differed significantly from the effect of 8-OH-DPAT ($F(1,14) = 12.99$, $P < 0.005$) and CP-93,129 at 5 μg/μl ($F(1,14) = 18.59$, $P < 0.001$), but not at 10 μg/μl ($F(1,14) = 2.68$, NS), nor at 16 μg/μl ($F(1,14) = 1.11$) of the same compound. A dose-dependent effect of CP-93,129 was suggested by the following paired comparisons: CP-93,129 at 5 μg/μl vs. CP-93,129 at 10 μg/μl, $F(1,14) = 3.31$, NS; CP-93,129 at 5 μg/μl vs. CP-93,129 at 16 μg/μl, $F(1,14) = 10.87$, $P < 0.01$; CP-93,129 at 10 μg/μl vs. CP-93,129 at 16 μg/μl, $F(1,14) = 4.5$, $P = 0.05$.

Working and reference memory errors. Bottom panels of Fig. 2 show the mean error scores in reference (left-hand histograms) and working (right-hand histograms) memory, separately.

First, analyses of variance (one-way analyses for repeated measures) were conducted for reference memory errors and working memory errors, separately.

Reference memory errors: the analysis of variance conducted on the reference memory error scores revealed a significant effect of treatment ($F(5,70) = 5.45$, $P < 0.001$), but no effect of subject ($F(14,70) < 1$). Further paired comparisons showed that, compared to saline injections, significant increases in reference memory error scores were observed following scopolamine ($F(1,14) = 15.13$, $P < 0.005$) and the higher dose (16 μg/μl) of CP-93,129 ($F(1,14) = 6.81$, $P < 0.05$), but not following 8-OH-DPAT ($F(1,14) < 1$) or CP-93,129 at 5 μg/μl ($F(1,14) < 1$); nevertheless with the

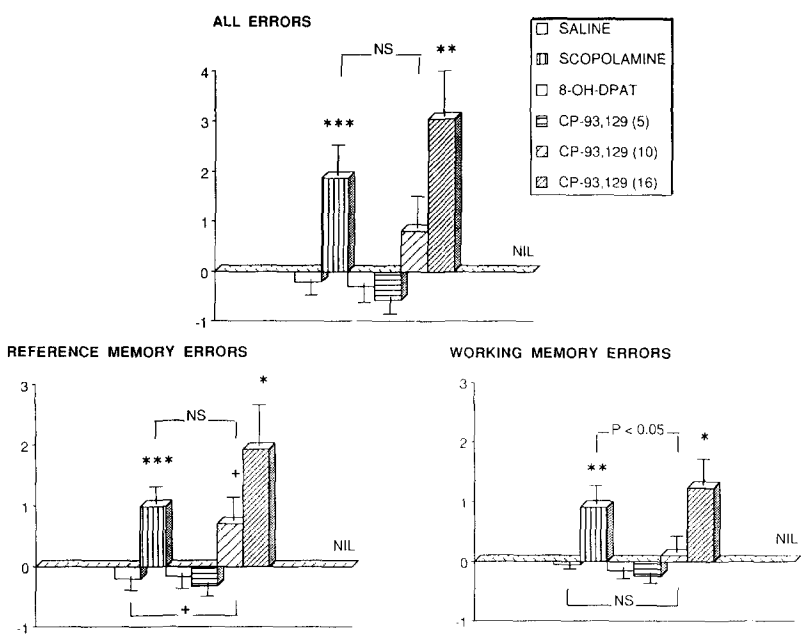

Fig. 2. Performances during the pharmacological phase. Difference in number of errors per trial (mean ± S.E. on the ordinate) between the day with injection and the preceding day without injection (NIL = non-injection level). (5), (10), (16): doses (μg/μl) used. Upper panel: all errors; lower panels: reference (left) and working (right) memory errors. Stars above histograms indicate significant differences in error scores with saline treatment. *** $P < 0.005$, ** $P < 0.01$, * $P < 0.05$, + $P = 0.06$.

CP-93,129 at 10 $\mu g/\mu l$, this comparison approached significance ($F(1,14) = 4.13$, $P = 0.06$). Furthermore, the increase in reference memory error scores following scopolamine treatment differed significantly from the effect of 8-OH-DPAT ($F(1,14) = 16.36$, $P < 0.005$) and CP-93,129 at 5 $\mu g/\mu l$ ($F(1,14) = 18.15$, $P < 0.001$), but not at 10 $\mu g/\mu l$ ($F(1,14) < 1$), nor at 16 $\mu g/\mu l$ ($F(1,14) = 1.58$) of this drug. The dose-dependent effect of CP-93,129 was indicated for the reference memory errors by further paired comparisons (CP-93,129 at 5 $\mu g/\mu l$ vs. CP-93,129 at 10 $\mu g/\mu l$, $F(1,14) = 5.03$, $P < 0.05$; CP-93,129 at 5 $\mu g/\mu l$ vs. CP-93,129 at 16 $\mu g/\mu l$, $F(1,14) = 8.01$, $P < 0.05$; CP-93,129 at 10 $\mu g/\mu l$ vs. CP-93,129 at 16 $\mu g/\mu l$, $F(1,14) = 2.62$, NS).

Working memory errors: the analysis of variance conducted on the working memory error scores revealed a significant effect of treatment ($F(5,70) = 5.39$, $P < 0.001$), but no effect of subject ($F(14,70) < 1$). The paired comparisons of the effect of the different treatments with control (saline) injections, also showed significant increases in working memory error scores following the administration of scopolamine ($F(1,14) = 10.51$, $P < 0.01$) and the higher dose (16 $\mu g/\mu l$) of CP-93,129 ($F(1,14) = 6.28$, $P < 0.05$), but not following the administration of 8-OH-DPAT ($F(1,14) < 1$), CP-93,129 at 5 $\mu g/\mu l$ ($F(1,14) = 1.91$, NS) or CP-93,129 at 10 $\mu g/\mu l$ ($F(1,14) < 1$). The increase in working memory error scores following treatment with scopolamine differed significantly from the effect of 8-OH-DPAT ($F(1,14) = 8.41$, $P < 0.05$), CP-93,129 at 5 $\mu g/\mu l$ ($F(1,14) = 12.5$, $P < 0.005$) and at 10 $\mu g/\mu l$ ($F(1,14) = 7.74$, $P < 0.05$), but not at 16 $\mu g/\mu l$ ($F(1,14) < 1$) of this drug. A dose-dependent effect of CP-93,129 was suggested for the working memory errors by further paired comparisons (CP-93,129 at 5 $\mu g/\mu l$ vs. CP-93,129 at 10 $\mu g/\mu l$, $F(1,14) = 1.11$, NS; CP-93,129 at 5 $\mu g/\mu l$ vs. CP-93,129 at 16 $\mu g/\mu l$, $F(1,14) = 9.62$, $P < 0.01$; CP-93,129 at 10 $\mu g/\mu l$ vs. CP-93,129 at 16 $\mu g/\mu l$, $F(1,14) = 4.65$, $P < 0.05$).

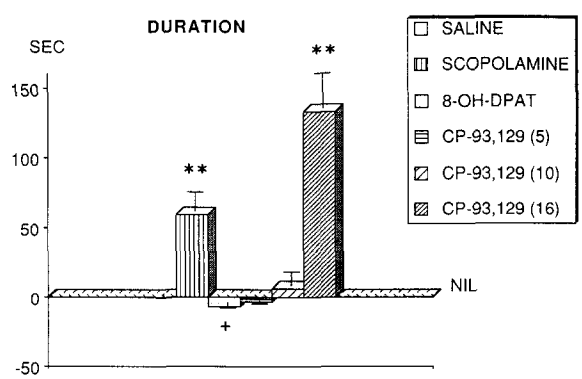

Fig. 3. Mean trial duration during the pharmacological phase. Difference in trial duration (mean ± S.E. on the ordinate) between the day with injection and the preceding day without injection (NIL = non-injection level). (5), (10), (16): doses ($\mu g/\mu l$) used. Significant difference at $^{**}P < 0.001$, $^{+}P = 0.058$.

A two-way analysis of variance was applied to the entire data, combining both types of errors, with treatment (saline, scopolamine, 8-OH-DPAT, CP-93,129 at doses 5, 10 and 16 $\mu g/\mu l$) and error type (reference memory vs. working memory errors) as the two main factors. The analysis revealed a significant effect of treatment ($F(5,140) = 10.23$, $P < 0.0001$), but no significant effect of error type ($F(1,28) < 1$), nor of treatment × error type interaction ($F(5,140) < 1$). Independent analyses were conducted for each treatment to assess any particular error type effect. These analyses revealed a lack of error type effect in all treatment groups except for the CP-93,129 at dose 10 $\mu g/\mu l$ ($F(1,14) = 8.06$, $P < 0.05$).

Trial duration. Fig. 3 shows the mean trial duration for each pharmacological treatment. Statistical analyses (using Student's t-test) were conducted on the data, separately for each treatment, by comparison with the data collected on the preceding non-injection days. They revealed a lack of change in trial duration for saline ($t(14) = 0.005$, NS), CP-93,129 at 5 $\mu g/\mu l$ ($t(14) = 1.0$, NS), and CP-93,129 at 10 $\mu g/\mu l$ ($t(14) = 0.61$, NS), a significant increase for scopolamine ($t(14) = 4.31$, $P < 0.001$), and CP-93,129 at 16 $\mu g/\mu l$ ($t(14) = 5.32$, $P < 0.001$), and a decrease approaching the significance level for 8-OH-DPAT ($t(14) = -2.07$, $P = 0.058$).

4. Discussion

Results from the training period indicated that animals improved their performance with time and continued to do so until the post-injection period which was characterized by an asymptotic level of performance. This allays any misgivings regarding overall disturbance that might result from multiple injections with various drugs.

The performance of rats injected with the 8-OH-DPAT did not differ from that following either no injection or saline injections, showing that 8-OH-DPAT had no effect on spatial memory, at the dose of 5 $\mu g/\mu l$ employed. There was an increase in running speed which apparently did not disturb performance.

Intrahippocampal treatment with scopolamine (10 $\mu g/\mu l$) resulted in similar working and reference memory impairments. This result agrees with previous findings (Blockland et al., 1992; Brito et al., 1983), and also with those that indicated both working and reference memory deficits following hippocampal lesions (e.g. Gage, 1985), but contrasts with other reports which showed specific working memory impairments following hippocampal dysfunction (e.g. Olton et al., 1979).

Septal inactivation (induced by a local injection of lidocaine) was found to induce an increased number of working memory errors, an effect that appeared greater than that observed for reference memory errors (Poucet and Buhot, 1994). The medial septum being the neural structure of origin of the septo-hippocampal pathway, may be involved in the first stage of memory formation. Following treatment with scopolamine, both working and reference memory were impaired. In this case, the target receptors (post-synaptically with regard to septal afferences) are situated at a further stage of memory formation.

Spatial performance in the radial maze was significantly impaired at the higher concentration of the 5-HT_{1B} receptor agonist, CP-93,129; the impairment was dose-dependent. In a study by O'Connor et al (1990), systemic injections of RU 24969 (1 mg/kg) were found to have an inhibitory effect on excitatory synaptic transmission in the hippocampus (CA1 field) of alert rats, a finding which constitutes a possible explanation for our data showing a dose-dependent impairment in spatial memory following intra-hippocampal CP 93,129 injections.

Nevertheless, while working memory and reference memory errors appeared closely similar following scopolamine injections, a tendency to make more reference memory errors was observed following the 5-HT_{1B} receptor agonist treatment. This result has to be analysed in the light of the cellular distribution of the receptors in the vicinity of the injection site. Thus, 5-HT_{1B} receptors, located on septal terminals, inhibit acetylcholine release (Maura and Raiteri, 1986), which, in consequence, reduces the efficacy of the septo-hippocampal cholinergic pathway. This mechanism of action is compatible with the impairment in spatial memory resulting from intra-hippocampal treatment with the 5-HT_{1B} receptor agonist, and also with the similar effect obtained following treatment with the muscarinic receptor antagonist scopolamine. Part of our results, i.e. those showing also working memory deficits following injection with the highest dose of CP-93,129, might be explained by this mechanism of action. Hippocampal 5-HT_{1B} autoreceptors, located on raphe's axon terminals, might also control the release of 5-HT itself, thus indirectly contributing to the modulatory effect of serotonin in the hippocampus. The fact that reference (long-term) memory errors were found more frequent than working (short-term) memory errors at the intermediate dose of CP-93,129 suggests another mechanism, i.e. the activation of those 5-HT_{1B} receptors located in the stratum oriens layer, i.e. on the proximal portion of axons of the CA1 pyramidal neurons (Aït Amara et al., 1995). When activated, these receptors may exert their inhibitory influence on the output of CA1 cells, i.e. probably on the glutamate release in the subiculum, and thus modify the subiculum's main function which is to transfer information (especially spatial information: see Sharp and Green, 1994), and even filter information (see Gray's hypothesis (Gray, 1984) of the subiculum as a 'comparator') to be encoded in cortical areas where they are available in long-term memory (Morris et al., 1990).

Thus, these results add new information on certain neurobiological mechanisms which may underly the function of the hippocampus, and its related structures (e.g. medial septum, subiculum), in the coding of spatial information within a continuous extended time frame (Kesner et al., 1988; Marighetto et al., 1993).

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