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Low doses of 8-OH-DPAT prevent the impairment of spatial learning caused by intrahippocampal scopolamine through 5-HT$_{1A}$ receptors in the dorsal raphe

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1 We studied the effects of low doses of 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, on the impairment of spatial learning caused by scopolamine injected into the CA1 region of the dorsal hippocampus of rats performing a two-platform spatial discrimination task.

2 Bilateral injections of 4 μg (in 1 μl) of scopolamine into the CA1 region of the dorsal hippocampus 10 min before each training session impaired choice accuracy with no effect on choice latency and errors of omission.

3 Administered subcutaneously 20 min before each training session, 8-OH-DPAT 10 and 30 (but not 3) μg kg^{-1} did not modify choice accuracy but prevented the impairment by intrahippocampal scopolamine.

4 Injection of 1.0 μg (in 0.5 μl) of WAY 100635, a 5-HT$_{1A}$ receptor antagonist, into the dorsal raphe 5 min before scopolamine had no effect on choice accuracy and latency or errors of omission and did not modify the effect of scopolamine, but completely antagonized the effect of 10 and 30 μg kg^{-1} 8-OH-DPAT on scopolamine-induced impairment of choice accuracy.

5 The results confirm previous findings that stimulation of presynaptic 5-HT$_{1A}$ receptors in the dorsal raphe attenuates the deficit of spatial learning caused by blockade of cholinergic excitatory input on hippocampal pyramidal cells.

6 Drugs that stimulate presynaptic 5-HT$_{1A}$ receptors such as 5-HT$_{1A}$ receptor partial agonists may be useful in the symptomatic treatment of human memory disturbances associated with loss of cholinergic innervation to the hippocampus.

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Abbreviations: ANOVA, analysis of variance; DR, dorsal raphe; MR, median raphe; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; WAY 100635, N-{2-{4-(2-methoxyphenyl)-1-piperazinyl}-N-(2-pyridinyl)cyclohexanecarboxamide

Introduction

A series of studies in our laboratory suggest that stimulation of postsynaptic serotonin 1A (5-HT$_{1A}$) receptors in the dorsal hippocampus impairs spatial learning in rats. We first showed that 100 μg kg^{-1} 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, injected subcutaneously before each training session, selectively impaired spatial learning in a two-platform spatial discrimination task, with no effect on motor/motivational performance or non-spatial visual learning (Carli & Samanin, 1992; Carli *et al*., 1995a). The involvement of postsynaptic 5-HT$_{1A}$ receptors was suggested by the finding that the effect of 8-OH-DPAT was blocked by infusing 5-HT$_{1A}$ receptor antagonists in the CA1 region of the dorsal hippocampus but not by an intraventricular injection of 5,7-dihydroxytryptamine to destroy 5-HT neurons (Carli & Samanin, 1992; Carli *et al*., 1995a). Moreover, direct stimulation of hippocampal 5-HT$_{1A}$ receptors by locally administered 8-OH-DPAT impaired the acquisition of spatial learning and the effect was blocked by a 5-HT$_{1A}$ receptor antagonist (Carli *et al*., 1992).

Choice accuracy was impaired, though with no effect on motor/motivational performance or non-spatial visual discrimination, on infusing 3.5–7.5 μg scopolamine in the CA1 region of the dorsal hippocampus (Carli *et al*., 1997b) and the effect of scopolamine was prevented by blockade of hippocampal 5-HT$_{1A}$ receptors (Carli *et al*., 1995b,a). These findings suggest that muscarinic cholinergic and 5-HT$_{1A}$ receptors in the dorsal hippocampus have opposite functions in regulating spatial learning in rats and the loss of cholinergic excitatory input on pyramidal cells may be compensated by blockade of the inhibitory 5-HT$_{1A}$ receptors. A similar conclusion was reached by Harder *et al*. (1996) in a study in which blockade of 5-HT$_{1A}$ receptors by WAY 100635 improved the cognitive deficit caused by fornix transection in the marmoset.

There is a high density of 5-HT$_{1A}$ receptors in the nucleus raphe dorsalis (DR) where they act as presynaptic somatodendritic 5-HT receptors (Sprouse & Aghajanian, 1988). Stimulation of these receptors reduces 5-HT synthesis and release in various brain regions (Invernizzi *et al*., 1991; Bonvento *et al*., 1992) and causes changes in various forms of motivated behaviour including increased feeding (Bendotti & Samanin, 1986) and anxiolytic-like effects in various models (Handley, 1995; Griebel, 1995). Recent evidence suggests that 5-HT$_{1A}$ receptors in the DR are also involved in the regulation of spatial learning since their stimulation by administering locally 1 μg 8-OH-DPAT reversed the deficit of spatial learning caused by intrahippocampal scopolamine and the effect of 8-

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OH-DPAT was blocked by a 5-HT$_{1A}$ receptor antagonist, WAY 100635, injected into the DR (Carli *et al*., 1998).

Since low doses of 8-OH-DPAT may preferentially affect presynaptic 5-HT$_{1A}$ receptors (Hjorth & Magnusson, 1988), relatively low doses of 8-OH-DPAT should attenuate the learning deficit caused by intrahippocampal scopolamine. In line with this, a recent study showed that low doses of 8-OH-DPAT did attenuate the accuracy impairment caused by systemically administered scopolamine in an operant delayed matching to position task, while a high dose potentiated the impairment (Cole *et al*., 1994). However, this study provided no direct evidence of a role of presynaptic 5-HT$_{1A}$ receptors in the effects of low doses of 8-OH-DPAT and Stanhope *et al*. (1995), using a similar model, were unable to replicate Cole's group's findings.

The main aim of the present study was to examine the effects of low doses of 8-OH-DPAT on the acquisition of spatial learning in a two-platform spatial discrimination task routinely used in our laboratory, and on the impairment of choice accuracy by 4.0 μg scopolamine injected into the dorsal hippocampus (Carli & Samanin, 1992; Carli *et al*., 1992, 1995b, 1997b, 1998). To clarify the role of presynaptic 5-HT$_{1A}$ receptors, in one experiment WAY 100635 was infused into the DR to antagonize any effect of 8-OH-DPAT on rats' performance.

Methods

Animals

Male Crl:CD(SD)BR rats (Charles River, Italy) were housed in groups of 10 in standard laboratory conditions (temperature $20 \pm 1^{\circ}$C and 60% relative humidity) in a room with the light on from 07.00 to 19.00 h. Food and water were freely available.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national (D.L. n. 116, G.U., suppl., 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Cannula implantation and histology

The rats, anaesthetized with Equithesin (9.7 mg ml^{-1} sodium pentobarbital in saline + 42.6 mg ml^{-1} chloral hydrate in propylenglycol + 21.2 mg ml^{-1} Mg_2SO_4 in ethanol; 3.0 ml kg^{-1} i.p.), were immobilized in a Kopf stereotaxic instrument. The skin was cut and the skull cleaned for implantation of guide cannulae made of 23-gauge stainless steel tubing, 2 mm above the sites to be injected. The guide tubes were secured by acrylic dental cement anchored to three stainless steel screws fixed to the skull. To prevent clogging, 30-gauge stainless steel stylets were placed in the guide cannulae until the animals were given intracerebral injections.

A single guide cannula was implanted to give access to the dorsal raphe (DR) nucleus. It was positioned at an angle of 26° to the saggital plan to avoid damage to the sinus. The coordinates calculated from the interaural line were A, +1.1 mm; L, −1.4 mm; H, +3.2 (Paxinos & Watson, 1982). To gain access to the CA1 region of the dorsal hippocampus bilateral guide cannulae were implanted at coordinates calculated from the interaural line: A = +5.2 mm L = ±2.0 and H = +7.3 (Paxinos & Watson, 1982). Rats were simultaneously implanted with three guide cannulae (bilaterally into the CA1 region of the hippocampus and singly into the DR).

After the guide cannulae were implanted the rats were housed singly and were allowed 7 days of recovery. During the last 3 days they were adapted to the injection procedure by removing each one from its home cage and transporting it to the place where the injection was to be made. The stylets were removed and the rat was held firmly for 3 min (approximately the time needed for the injection procedure) after which the stylets were put back. On the days of acquisition training the stylets were withdrawn and replaced by injection units (30-gauge stainless steel tubing) terminating 2 mm below the tip of the guides.

On completion of each experiment the rats were killed and their brains removed and frozen on dry ice. To check the position of the cannulae tracks, 40 μm thick brain sections were cut in the coronal plane in a Cryo-cut, and the location of the infusion was verified visually. For each experiment only data from rats in which the cannulae were located in the appropriate structures were included in the results. About 95% of cannualated rats had the cannulae positioned correctly in the DR and the CA1 region of the dorsal hippocampus. Some rats were killed and their brains were removed, stored initially in formalin (4%) and then in a sucrose solution (30%). Sections 30 μm thick were cut in the coronal plane in a Cryo-cut, mounted on treated slides and stained with cresyl violet.

Apparatus

A circular 'swimming pool' was used, 1.5 m in diameter and 0.5 m high. The pool was filled to a depth of 0.29 m with water at $26 \pm 1^{\circ}$C, rendered opaque by the addition of a food dye (coffee color, Bayo, Italy). The water was changed daily. The pool was placed in the middle of a large room and was surrounded by various visual cues: a blackened window with a big white cross, a white wall with a big black cross, a long table, a door and a picture-covered wall with a rack for cages. The objects could be covered, when required, by black curtains around the maze. When open, the curtains were collected together at one corner of the room, forming another prominent visual cue. The room was lit by a 100 W light bulb in the centre of the ceiling, 2.4 m above the water surface. The light intensity at the water surface was 80 lux (measured by an Illuminometer, Mod 5200, Kyoritsu, Japan).

Two visible platforms were used. The fixed one protruded 1.5–2.0 cm above the water. Its top was square (11 × 11 cm) and made of Perspex. The second platform also protruded 1.5–2.0 cm above the water and was made of the same material but was filled with expanded polystyrene. It was 'anchored' by thread to a solid movable base on the bottom of the pool. Thus one platform was rigid and provided support, and the other sank when the rats tried to climb onto it.

Training procedure

The black curtains were drawn together to allow a full view of extra-maze cues. Rats were trained to swim to the rigid grey escape platform while avoiding the floating grey platform. For all rats, the fixed escape platform (correct) was always in the same place at the centre of one of the eight sectors. The floating platform (incorrect) was positioned over successive trials in a quasi-random sequence of eight locations around the pool, subject to the constraint that the spatial relationship between the platform and the starting position did not consistently reward either right- or left-turning tendencies.

The rats were trained with 10 trials a day for five days. A trial began with the rat being placed in the pool while held at, and facing, the side wall. Eight possible starting locations were used in quasi-random sequence across trials. A trial ended when the rat escaped onto the rigid platform, where it was allowed to sit for 15 s before being returned to a holding cage until the next trial. The rats were trained in squads of four. Inter-trial intervals were approximately 2–4 min so each rat's daily testing lasted approximately 30 min. A correct trial was one in which the rat climbed onto the rigid platform without touching the floating platform with its forepaws or snout. The occasional incident of brushing past the floating platform in passing was not considered an error. If the rat did not choose to escape onto either platform (correct or incorrect) in 60 s it was taken out of the pool and an omission error was scored.

We measured (1) the first choice in each trial (correct/incorrect), (2) the latency to escape (s), and (3) the number of omissions.

Treatment schedules

After recovery from surgery and adaptation to the injection procedure rats were allocated to different treatment groups. Scopolamine HBr (Sigma, U.S.A.), WAY 100635 (Pharmacia, Nerviano, Italy) or saline solutions were delivered at a rate of 0.5 μl min^{-1} by a Hamilton syringe mounted on a CMA/100 infusion pump (CMA Microdialysis, Stockholm, Sweden), connected by PP10 tubing to a 30-gauge stainless steel cannula (injection unit) terminating 2 mm below the tip of the guides.

Since in a previous study a dose of 1 μg but not 0.2 μg WAY 100635 infused into the DR was effective in reversing the effects of 8-OH-DPAT injected into the DR on the impairment of spatial discrimination learning caused by intrahippocampal scopolamine (Carli *et al*., 1998) a dose of 1 μg WAY 100635 was used in this study. A total of 1 μg (in 0.5 μl) WAY 100635 dissolved in saline, or 0.5 μl saline alone, was administered by a single injection into the DR over a 1-min period.

Bilateral injections of 4 μg (in 1 μl) scopolamine dissolved in saline, or 1 μl of saline alone, into the hippocampus were made over 2 min. The injection cannulae were left in place for another minute before withdrawal to allow diffusion from the tip and prevent reflux of the solution.

The number of animals in each group is given below in brackets. On each acquisition training day, the rats were injected subcutaneously (s.c.) with saline 2 ml kg^{-1} ($n=8$) or 8-OH-DPAT (RBI, Wayland, MA, U.S.A.) 10 μg kg^{-1} ($n=8$) or 30 μg kg^{-1} ($n=8$) dissolved in saline and 20 min later they received a bilateral injection of 1 μl of saline into the dorsal hippocampus (stratum radiatum of area CA1).

Other rats were injected s.c. with 2 ml kg^{-1} saline ($n=13$), 10 μg kg^{-1} ($n=8$) or 30 μg kg^{-1} 8-OH-DPAT ($n=8$), followed 15 min later by an injection of 0.5 μl saline into the DR. Three groups of eight rats each were injected s.c. with 10 or 30 μg kg^{-1} 8-OH-DPAT or saline (ml kg^{-1}) followed 15 min later by an injection of 1 μg WAY 100635 into the DR. Five minutes later all rats received bilateral injections of 4 μg scopolamine into the dorsal hippocampus.

In a second experiment on each acquisition training day rats were injected s.c. with 2 ml kg^{-1} saline ($n=8$) or 3 μg kg^{-1} of 8-OH-DPAT ($n=7$) then 20 min later they were injected bilaterally into the dorsal hippocampus with 1 μl of saline. Other rats were injected s.c. with 2 ml kg^{-1} saline ($n=8$) or 3 μg kg^{-1} of 8-OH-DPAT ($n=8$), and 20 min later they received bilateral injections of 4 μg scopolamine into the dorsal hippocampus.

Each animal received only one drug regimen given over all testing days. Ten minutes after saline or scopolamine injection into the hippocampus the rats started their daily testing.

Statistical analysis and measures

Choice accuracy of spatial discrimination was measured as the proportion of correct choices (total correct choices/total correct choices + total incorrect choices). Choice latency was defined as the time in seconds taken by the rat to swim from the starting location to either the correct or incorrect platform. For each training day the mean latency to escape was calculated for each rat (total latency/total number of trials). Errors of omission were measured as the number of failures to choose in 60 s. Trials in which the animals made errors of omission were not counted for the measurement of choice accuracy and latency.

The effects of different doses of 8-OH-DPAT s.c. plus WAY 100635 infused into the DR on the intrahippocampal scopolamine-induced deficit in accuracy (percentage of correct choices) were analysed by three-factor ANOVA (two between, one within subjects). The between-subjects factors were: 8-OH-DPAT (three levels) and WAY 100635 (two levels). There were five levels of the within-subjects factor, TIME. Significant three-way interaction was analysed separately by two-way ANOVA (8-OH-DPAT × WAY 100635) for each training day, and significant two-way interaction between 8-OH-DPAT and WAY 100635 was examined by comparing treatment group means for the five training sessions, using Tukey's test.

The effects of 3 μg kg^{-1} 8-OH-DPAT on the intrahippocampal scopolamine-induced deficit was examined by a three-way ANOVA with time as within-subjects factor and 8-OH-DPAT and scopolamine as between-subjects factors. Significant interactions between time, scopolamine and 8-OH-DPAT were followed by the appropriate two-way ANOVA and Tukey's test.

The same statistical analysis was applied to choice latencies. The percentages of correct choices and choice latencies for each rat were transformed according to the formula 2arsin[SQRT(%correct)] and $\log_{10}$ (choice latency), respectively, to normalize the distributions in accordance with the ANOVA model (Winer, 1971). The numbers of omissions during the acquisition training were analysed by ANOVA.

Analyses of variance for unbalanced data were done by the general linear model procedure (GLM). The degrees of freedom associated with the F values for the univariate tests of within-subjects effects were adjusted by the Greenhouse-Geisser (G–G) epsilon (ε). In the Results section we have reported only the adjusted Pr > F (G–G) without showing the degrees of freedom of the F values of the repeated factor time. The degrees of freedom of the F values are reported only for the between-subjects factors 8-H-DPAT, WAY 100635 and scopolamine and their interactions.

All statistical analysis was done using the SAS Institute Inc. (U.S.A.) software run on a Micro VAX 3500 computer (Digital, U.S.A.).

Results

Examination of the stained coronal sections showed that multiple injections into the hippocampus and dorsal raphe caused very limited tissue damage in the majority of rats. The degree of gliosis around the injection needle track was similar in all experimental groups. No differences were observed in the location of cannulae tracks of saline or drug injected rats.

Figure 1 shows representative photographs of the histological sections from animals which received a single injection into the DR and bilateral injections into the CA1 region of the dorsal hippocampus on each of the five training days.

Table 1 shows that the accuracy of rats on the first day of acquisition training was not appreciably different, their performance ranging from 50–60% of correct choices and mean latencies between 12 and 16 s. The accuracy of control rats and of those given 10 or 30 μg kg^{-1} s.c. of 8-OH-DPAT improved at the same rate over training, reaching about 85% of correct choices on day 5 (DPAT, $F_{2,21}=0.51$ $P=0.6$; time × DPAT, $F=1.9$ $P=0.08$; time, $F=28.1$ $P=0.0001$). Choice latencies decreased equally in all groups (DPAT, $F_{2,21}=1.3$ $P=0.3$; time × DPAT, $F=0.7$ $P=0.63$; time, $F=20.8$ $P=0.0001$) and omissions practically disappeared from day 2 of training.

Since rats given intrahippocampal saline after s.c. 8-OH-DPAT (Table 1) or 1.0 μg WAY 100635 into the DR (Carli *et al*., 1998, 1999) showed no effect on choice accuracy or latency, we examined how WAY 100635 injected into the DR modified the effects of 8-OH-DPAT on the scopolamine-induced deficit in accuracy (per cent of correct choices) only in the intrahippocampal scopolamine-injected rats.

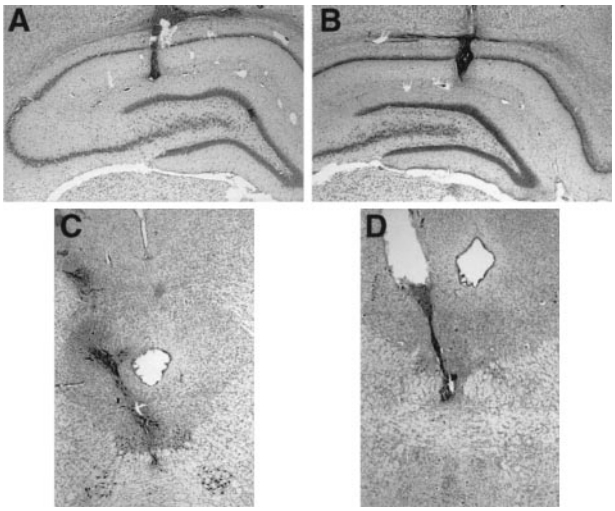

Figure 1 Representative photographs of histological sections showing the cannulae tracks and infusion sites into the CA1 region of the dorsal hippocampus (A, B) and into the corresponding dorsal raphe nucleus of the mesencephalon (C,D).

Figure 2A shows the accuracy of rats injected with 4.0 μg scopolamine in the CA1 region of the dorsal hippocampus before each acquisition session: they still showed about 60% of correct choices on the last day of training.

Overall ANOVA examining the effects of the combination of 10 or 30 μg kg^{-1} 8-OH-DPAT or saline 2 ml kg^{-1} and 1.0 μg WAY 100635 or 0.5 μl saline injected into the DR in intrahippocampal scopolamine-treated rats showed a non-significant three-way interaction (time × 8-OH-DPAT × WAY, $F=0.42$ $P=0.9$) but a significant two-way interaction between 8-OH-DPAT and WAY 100635 ($F_{2,46}=9.9$ $P=0.0003$).

The histograms in Figure 2B present the results as means ± s.e.mean of the five training sessions. Scopolamine-treated rats injected with 10 or 30 μg kg^{-1} 8-OH-DPAT made significantly more correct choices than those injected with saline ($P<0.05$; Tukey's test) and rats treated with 10 or 30 μg kg^{-1} 8-OH-DPAT + WAY 100635 in the DR had significantly worse choice accuracy than those injected with 10 or 30 μg kg^{-1} 8-OH-DPAT + saline in DR ($P<0.05$; Tukey's test). Injection of WAY 100635 into the DR by itself did not affect the choice accuracy of scopolamine-treated rats ($P>0.05$; Tukey's test).

The results on choice latency are shown in Figure 2C. In all groups choice latencies significantly declined with training (time, $F=34.5$ $P=0.0001$). Overall statistical analysis on scopolamine-treated rats showed a significant three-way interaction (time × 8-OH-DPAT × WAY, $F=2.54$ $P=0.02$). Further two-way ANOVA for each training day showed significant interactions between 8-OH-DPAT and WAY 100635 on days 3 ($F_{2,47}=10.4$ $P=0.0002$), 4 ($F_{2,47}=7.7$ $P=0.001$) and 5 ($F_{2,47}=5.4$ $P=0.008$). *Post hoc* Tukey's test indicated that on day 3 rats injected with 30 μg kg^{-1} 8-OH-DPAT + saline in the DR were significantly faster than those given s.c. saline + saline ($P<0.05$) or 10 μg kg^{-1} 8-OH-DPAT + saline in the DR ($P<0.05$). On day 3 and 4 rats injected with 30 μg kg^{-1} 8-OH-DPAT + WAY 100635 in the DR had longer choice latencies than those given 30 μg kg^{-1} 8-OH-DPAT + saline in the DR ($P<0.05$). On day 5 only rats injected with saline + WAY 100635 in the DR were significantly faster ($P<0.05$) than those given 30 μg kg^{-1} 8-OH-DPAT + WAY 100635 in the DR. WAY 100635 or 8-OH-DPAT, or the two combined, had no effect on errors of omission in intrahippocampal scopolamine-treated rats (data not shown).

Figure 3A shows the effects of 3 μg kg^{-1} 8-OH-DPAT in intrahippocampal saline- or scopolamine-treated rats. ANOVA showed a non-significant three-way interaction between

Table 1 Effects of 8-OH-DPAT (μg kg^{-1}) injected subcutaneously on the percentages of correct choices, choice latencies and number of omissions in control rats

Treatment (μg per 0.5 μl)	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*
Per cent correct choices					
Saline + saline	56.9 ± 3.8	70.2 ± 3.6	67.5 ± 5.3	85.0 ± 6.3	82.5 ± 3.7
8-OH-DPAT 10 + saline	50.1 ± 5.2	76.7 ± 6.8	80.0 ± 2.2	76.3 ± 2.6	84.0 ± 1.9
8-OH-DPAT 30 + saline	56.1 ± 4.9	62.0 ± 4.4	72.5 ± 3.4	78.7 ± 2.9	83.7 ± 2.6
Choice latencies					
Saline + saline	15.8 ± 1.3	11.9 ± 0.5	10.9 ± 0.7	9.5 ± 0.7	8.4 ± 0.6
8-OH-DPAT 10 + saline	12.3 ± 1.2	12.7 ± 1.2	9.3 ± 1.0	9.6 ± 1.6	6.8 ± 0.7
8-OH-DPAT 30 + saline	14.4 ± 2.2	14.9 ± 2.1	10.1 ± 1.3	9.6 ± 1.3	7.0 ± 0.9
Number of omissions					
Saline + saline	1.5 ± 0.5	0.4 ± 0.3	0.0	0.0	0.0
8-OH-DPAT 10 + saline	1.3 ± 0.3	0.3 ± 0.1	0.0	0.0	0.1 ± 0.1
8-OH-DPAT 30 + saline	2.1 ± 0.6	0.1 ± 0.1	0.4 ± 0.2	0.0	0.0

Means ± s.e.mean of eight rats per group. On each acquisition day 10 or 30 μg kg^{-1} 8-OH-DPAT or saline (2 ml kg^{-1}) were injected subcutaneously 20 min before saline (0.5 μl) into the dorsal raphe. Five minutes later all rats were given saline (1 μl) intrahippocampally.

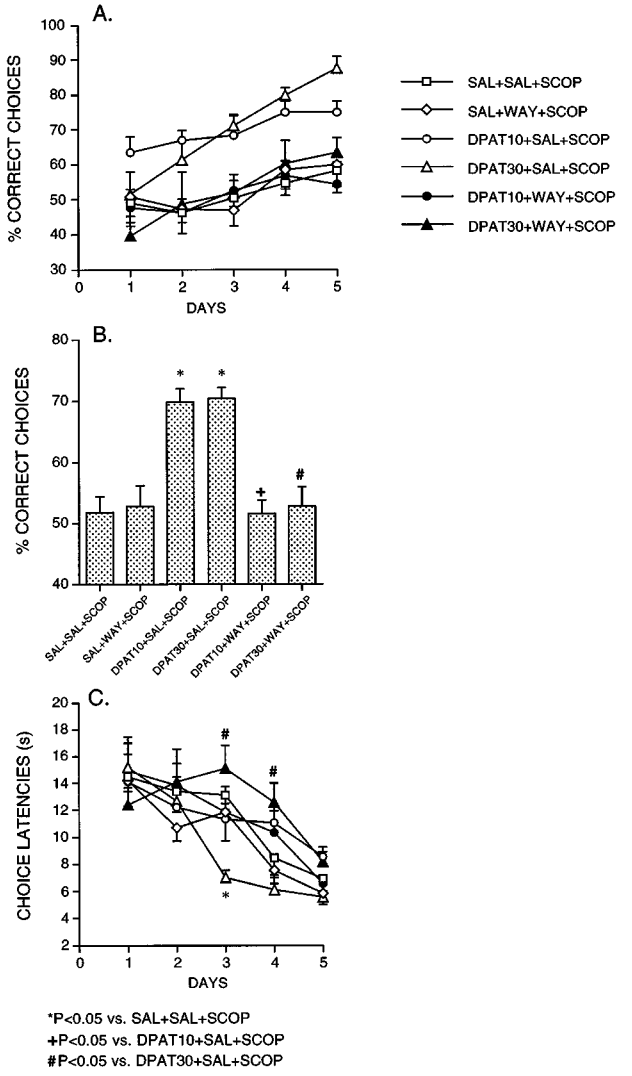

Figure 2 Effects of 1.0 μg WAY 100635 (WAY) or 0.5 μl saline (SAL) injected into the dorsal raphe (DR) of rats injected subcutaneously with saline (2 ml kg^{-1})(SAL) or 10 or 30 μg kg^{-1} 8-OH-DPAT (DPAT 10 or DPAT 30) on the percentage of correct choices (A), mean $\pm$ s.e.mean of correct choices in the five training sessions (B) and choice latencies (C). All rats received 4.0 μg scopolamine (SCOP) intrahippocampally. On each acquisition day, 8-OH-DPAT was given 15 min before WAY 100635 or saline which were injected into DR 5 min before scopolamine. The rats started their daily training session 10 min later. *$P<0.05$ vs SAL + SAL + SCOP (Tukey's test); +$P<0.05$ vs DPAT 10 + SAL + SCOP (Tukey's test); #$P<0.05$ vs DPAT 30 + SAL + SCOP (Tukey's test).

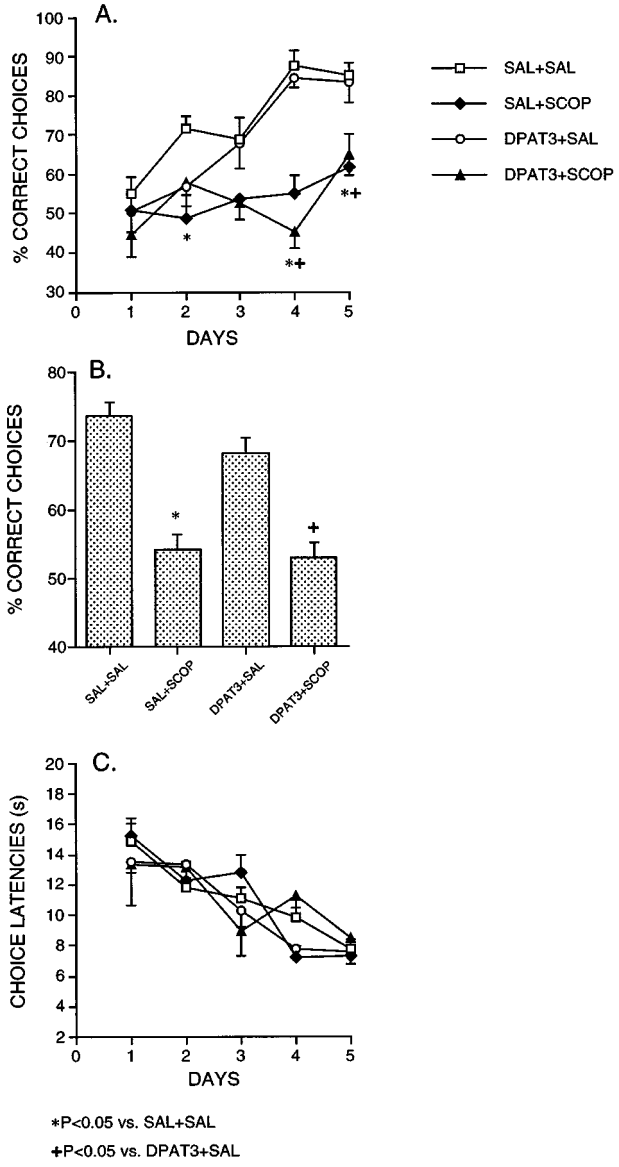

Figure 3 Effects of saline (SAL) or 3.0 μg kg^{-1} 8-OH-DPAT (DPAT 3) injected subcutaneously on the percentage of correct choices (A), mean $\pm$ s.e.mean of correct choices in the five sessions (B) and choice latencies (C) of rats given saline (SAL) or 4.0 μg scopolamine (SCOP) intrahippocampally. On each acquisition day, 8-OH-DPAT was injected 20 min before scopolamine. The rats started their daily training session 10 min later. *$P<0.05$ vs SAL + SAL (Tukey's test); +$P<0.05$ vs DPAT 3 + SAL (Tukey's test).

time, 8-OH-DPAT and scopolamine ($F=1.4$ $P=0.25$) but a significant interaction between time and scopolamine ($F=6.7$ $P=0.0002$) and a significant main effect of scopolamine ($F_{1,27}=35.8$ $P=0.0001$). The analysis did not show a significant main effect of 8-OH-DPAT ($F_{1,27}=2.4$ $P=0.13$) or its interaction with scopolamine ($F_{1,27}=1.0$ $P=0.31$). *Post hoc* Tukey's test suggested that independently of 8-OH-DPAT, rats given scopolamine in the hippocampus were worse then controls (saline + saline) on days 2, 4 and 5 (all $P<0.05$). The histograms in Figure 3B show that in five training sessions saline + scopolamine treated rats made significantly fewer correct choices ($P<0.05$; Tukey's test) and that 8-OH-DPAT 3 μg kg^{-1} did not reverse the scopolamine-induced impairment of choice accuracy ($P>0.05$; Tukey's test).

The results on choice latencies are shown in Figure 3C. Overall statistical analysis showed the drugs had no significant effect on choice latency (time $\times$ 8-OH-DPAT $\times$ scopolamine, $F=2.1$ $P=0.09$; 8-OH-DPAT $\times$ scopolamine, $F_{1,27}=0.6$ $P=0.43$). In all groups choice latencies significantly declined with training (time, $F=19.7$ $P=0.0001$). 8-OH-DPAT or scopolamine, or the two combined, had no effect on errors of omissions (data not shown).

Discussion

Ten and 30 (but not 3) μg kg^{-1} 8-OH-DPAT s.c. did not affect the acquisition of spatial learning and completely antagonized the deficit caused by intrahippocampal scopolamine. In agreement with previous findings (Carli *et al.*, 1998, 1999), 1 μg WAY 100635 injected into the DR did not affect choice accuracy and latency or errors of omission, or modified the effect of intrahippocampal scopolamine, but completely

antagonized the effect of both doses of 8-OH-DPAT on scopolamine-induced impairment of choice accuracy.

Thus presynaptic 5-HT$_{1A}$ receptors in the DR are clearly involved in the fact that low doses of 8-OH-DPAT counteract the impairment of spatial learning caused by scopolamine but are not directly involved in the mechanisms that underlie rats' performance in the two-platform spatial discrimination task (Carli *et al*., 1998). A similar suggestion was made in a previous study where 8-OH-DPAT in the DR did not modify the acquisition of spatial learning but reversed the learning deficit caused by intrahippocampal scopolamine (Carli *et al*., 1998).

Subcutaneous doses of 8-OH-DPAT (0.05, 0.1 and 1.0 mg kg^{-1}) or 8-OH-DPAT infused into the DR (10, 30 and 100 ng) have been reported to have no effect on rats' performance in a delay non-matching to sample procedure (Warburton *et al*., 1997). Stanhope *et al*. (1995) also found that a wide range of s.c. doses of 8-OH-DPAT had no effect on the percentage of correct choices in a delayed matching to position task.

On the other hand, relatively low intraperitoneal doses of 8-OH-DPAT (0.1 and 0.3 mg kg^{-1}) improved rats' performance in a model similar to that of Stanhope *et al*. (1995) and the effect was tentatively attributed to drug-induced stimulation of presynaptic 5-HT$_{1A}$ receptors (Cole *et al*., 1994). A possible explanation for the different results is that, as the authors themselves suggest, 8-OH-DPAT may have facilitated the adoption of an orienting posture during the delays in the Cole *et al*. (1994) study whereas Stanhope *et al*. (1995) used dividers to prevent animals using mediating behaviour to complete the task.

Because 8-OH-DPAT in the DR and the low doses of 8-OH-DPAT used in the present study did not affect 5-HT synthesis and release in the dorsal hippocampus (Invernizzi *et al*., 1991, 1995; Kreiss & Lucki, 1994), an alternative explanation is that serotonergic innervation of the dorsal hippocampus is selectively involved in the acquisition of spatial memory. This is suggested by the finding that 5-HT deafferentation of the hippocampus facilitated learning of a positively reinforced spatial discrimination task in the Stone maze (Altman *et al*., 1990). On the other hand, an intraventricular injection of 5,7-dihydroxytryptamine, that reduced 5-HT levels in the hippocampus by 95%, did not modify rats' performance in the two platform spatial discrimination task (Hagan *et al*., 1990; Carli & Samanin, 1992).

In view of the obvious procedural differences between the Stone maze which is based on positive reinforcement and which uses egocentric cues almost exclusively for orientation, and the two-platform discrimination task in which animals are aversively motivated and allocentric cues are essential for acquisition of the task, it would be interesting to clarify whether a selective lesion of local 5-HT innervation to the dorsal hippocampus facilitates learning in the later task.

As regards the role of the median raphe (MR), from which most 5-HT neurons innervating the dorsal hippocampus originate (Jacobs & Azmitia, 1992), 100 ng 8-OH-DPAT infused into this region improved performance accuracy in a delayed non-matching-to-position task (Warburton *et al*., 1997), but the effect was independent of delay. Infusion of 8-OH-DPAT into the MR does not facilitate the acquisition of spatial learning and actually impairs choice accuracy in the two-platform spatial discrimination task (unpublished results).

Whatever the role of the MR in spatial learning, the present results suggest that serotonergic neurons originating from the DR have no direct effect on spatial memory but do influence other more directly involved systems.

It has been suggested that enhanced anxiety may contribute to the learning deficit following hippocampal cholinergic blockade (Smythe *et al*., 1996, 1998) and systemically administered 8-OH-DPAT has shown anxiolytic-like effect in various rat models (Griebel, 1995). It is unlikely, however, that the doses used in the present study (10 and 30 μg kg^{-1}) reduced the deficit caused by scopolamine through anxiolytic activity since they had no effect on motivational indices such as choice latency and errors of omission, which one would expect to be influenced by an anxiolytic effect in an aversively motivated task. Moreover, anxiolytic drugs such as benzodiazepines impair spatial learning in a water maze (McNaughton & Morris, 1987).

Finally, the intrahippocampal dose of scopolamine in the present study (4 μg) was much lower than those used by Smythe *et al*. (1998) (15–30 μg) and even 15 μg scopolamine administered into the hippocampus did not significantly modify choice latency in the spatial discrimination task or choice accuracy and choice latency during the acquisition of a visual discrimination task (Carli *et al*., 1997b).

As regards the mechanism by which 8-OH-DPAT antagonizes the effect on intrahippocampal scopolamine, the drug enhances acetylcholine release in the rat hippocampus (Fujii *et al*., 1997) but this was seen at much higher dose (500 μg kg^{-1}) than we used, and appeared to involve activation of postsynaptic 5-HT$_{1A}$ receptors.

The entorhinal cortex provides a major cortical input to the hippocampus through a pathway using glutamate as neurotransmitter (Iijima *et al*., 1996; see Francis *et al*., 1993) and selective lesions in this area cause a selective deficit in an allocentric memory task (Holscher & Schmidt, 1994). Superficial cells of the entorhinal cortex are inhibited by serotonin (Schmitz *et al*., 1995) and are preferentially innervated by serotonergic neurons in the dorsal raphe (Jacobs & Azmitia, 1992). Low doses of 8-OH-DPAT, by stimulating 5-HT$_{1A}$ receptors in the DR, may reduce serotonin transmission in this region. The reduction of serotonergic inhibitory influence to the entorhinal cortical cells may facilitate the transfer of excitatory information from the entorhinal cortex to the hippocampus and thus compensate the loss of cholinergic input to the hippocampal pyramidal cells. This is currently under investigation in our laboratory.

The present results clearly show that low doses of 8-OH-DPAT, with a preferential effect on presynaptic 5-HT$_{1A}$ receptors, may compensate the loss of cholinergic excitatory input on pyramidal cells whereas our previous studies indicated that higher doses of 8-OH-DPAT (100–300 μg kg^{-1}) impair spatial learning by an action of 5-HT$_{1A}$ in the hippocampus (Carli & Samanin, 1992; Carli *et al*., 1992, 1995a). Together with the fact that 5-HT$_{1A}$ receptor antagonists prevent the impairment of spatial learning caused by scopolamine in the hippocampus, through local action in this region (Carli *et al*., 1995b, 1997a), partial agonists exerting presynaptic stimulatory and postsynaptic blocking action on 5-HT$_{1A}$ receptors may offer a fresh approach for the symptomatic treatment of memory disturbances in man, including Alzheimer's disease, where a close correlation has been found between the reduction in cholinergic markers in the hippocampus and behavioural deficit (Katzman *et al*., 1986).

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