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Psychopharmacology (2004) 175: 37–46
DOI 10.1007/s00213-004-1783-0

ORIGINAL INVESTIGATION

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Intraseptal injection of the 5-HT_{1A}/5-HT_7 agonist 8-OH-DPAT and working memory in rats

Received: 22 May 2003 / Accepted: 5 January 2004 / Published online: 11 March 2004
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Abstract *Rationale:* In rats, 5-HT_{1A} receptors are present in the septal region, e.g. on cholinergic neurons of the medial septum, where they might be a substrate for cognitively relevant interactions between cholinergic and serotonergic systems. *Objective:* The present experiment assessed the effects of the stimulation of septal 5-HT_{1A} receptors on spatial working memory. *Methods:* Stimulation of septal 5-HT_{1A} receptors was carried out by infusions targetting the medial septum of the 5-HT_{1A}/5-HT_7 receptor agonist 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT; 0.5 or 4 µg). Spatial memory was assessed in a water maze using a protocol placing emphasis on spatial working memory. The location of the hidden platform was changed every day and performance was assessed on two consecutive trials each day. *Results:* In comparison to vehicle injections, the intraseptal infusion of 4 µg 8-OH-DPAT impaired performance significantly: rats treated with 8-OH-DPAT exhibited increased distances to reach the hidden platform on both trials 1 and 2. Rats infused with 0.5 µg showed similar changes that failed to be significant. Such effects were not observed when the platform was visible. *Conclusions:* These results extend those of a previous experiment which showed that intraseptal injections of 8-OH-DPAT impaired spatial reference memory. Based on the characteristics of the observed deficits, it is suggested that the 8-OH-DPAT-induced impairment, rather than being only the result of a true alteration of working memory, might reflect a more global cognitive deficiency in which alteration of general memory capacities may be biased by disrupted search strategies/exploration and/or dysfunctions of attention.

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Keywords 5-HT · receptor · 8-OH-DPAT · Acetylcholine · Anxiety · Attention · Hippocampus · Medial septum · Serotonin · Spatial

Introduction

The central serotonergic system contributes to cognitive processes such as learning and memory (for review, see Meneses 1998, 1999; Buhot et al. 2000). Among the multiple serotonergic receptors, several lines of evidence show that the 5-HT_{1A} receptor subtype is implicated in cognitive processes, and particularly in spatial learning. For example, systemic injection of the 5-HT_{1A}/5-HT_7 receptor agonist, 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT), impairs spatial memory performance in both the water maze (Carli et al. 1995; Riekkinen et al. 1995; Kant et al. 1996, 1998) and the radial maze (Winter and Petty 1987). Moreover, the implication of 5-HT_{1A} receptors in learning and memory seems to involve an interaction with central cholinergic mechanisms, as suggested by several studies (Riekkinen et al. 1994, 1995; Carli et al. 1997, 1998, 1999; Bertrand et al. 2001; Lazaris et al. 2003). Concerning this issue, the septal region is of particular interest. This structure (i) is implicated in memory, (ii) contains neurons that provide the hippocampus with the major part of its cholinergic innervation (Von Cramon and Muller 1998), (iii) is also the target of a serotonergic innervation originating in the raphe nuclei (Milner and Veznedaroglu 1993; Acsady et al. 1996), and (iv) shows a high density of 5-HT_{1A} binding sites (Pazos and Palacios 1985; Chalmers and Watson 1991), some of which are located on cholinergic neurons (Kia et al. 1996). All these elements make the septal region a potential neuroanatomical substrate for 5-HT_{1A}-mediated interactions between cholinergic and serotonergic systems.

Nevertheless, the cognitive implication of the 5-HT_{1A} receptors of this structure is not well known. For instance, septal injections of 8-OH-DPAT increase maternal aggressive behaviour (De Almeida and Lucion 1997), influence

anxiety (De Almeida et al. 1998; Menard and Treit 1998; Micheau and Van Marrewijk 1999) and induce antidepressant-like effects (Martin et al. 1990, 1991; Schreiber and De Vry 1993). Concerning spatial memory, we previously showed that intraseptal injections of 8-OH-DPAT impaired reference-memory performance in the water maze (Bertrand et al. 2000). Our hypothesis to account for this finding was that stimulation of 5-HT_{1A} receptors in the septal region might have contributed to reduce the hippocampal cholinergic tone. Insofar as spatial working memory may be more sensitive to hippocampal cholinergic dysfunction than spatial reference memory, as documented by studies using pharmacological (Wirsching et al. 1984; Beatty and Bierley 1985; Lydon and Nakajima 1992; Varvel et al. 2001) or selective lesion approaches (Wrenn and Wiley 1998; Lehmann et al. 2000), the present study focused on the effects induced by injections of 8-OH-DPAT targetting the medial septum on spatial working-memory performance in the Morris water maze.

Materials and methods

Experimental procedures

All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (council directive no. 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; authorization no. 67-14 bis to H. J., no. 67-101 to S.S. and no. 6212 to J.C.C., F.B., R.G. and C.L. under their responsibility) and international (NIH publication, no. 86023, revised 1985) laws and policies.

Animals and design

This study was conducted on young adult Long-Evans male rats (Centre d'Elevage R. Janvier, Le Genest St-Isle, France). They arrived at the laboratory one week before surgery and were kept in individual transparent Makrolon cages (42×26×15 cm) in rooms that were maintained on a 12:12 h dark-light cycle (lights on at 0700 hours) under controlled temperature (21±1°C). The rats were housed with ad libitum access to food and water throughout the experiment. They were randomly allocated to one of three groups, abbreviated CSF, DPAT0.5 and DPAT4 hereafter (see below for details).

The study was conducted in two experiments. In the first one, 27 rats (nine CSF, nine DPAT0.5, nine DPAT4) were tested using a hidden platform, while in the second experiment, 23 other rats (nine CSF, six DPAT0.5 and eight DPAT4) were tested with a visible platform. Separate sets of rats were used for the hidden and visible platform protocols because, based on our experience, we consider six microinjections per rat a maximum as regards adverse, and thus non specific, effects around the injection site.

Surgery

One week before behavioural testing, all rats underwent surgical implantation of a stainless steel guide cannula (length 12 mm; outer diameter 0.40 mm) under aseptic conditions. They were anaesthetised with pentobarbital (0.75 mg/kg IP; Sanofi Santé Animale, Libourne, France). The guide cannula, targetting the MS area, was implanted at the following stereotaxic coordinates (in mm from bregma: A +0.8; L ±1.1; DV –5.5, with the incisor bar set at 3.3 mm below the interaural line; according to Paxinos and Watson 1998). The cannula was implanted with an angle of 10° from the sagittal plane, in order to avoid any injury to the sagittal sinus. Because the microinjector needle (outer diameter 0.28 mm) was 1 mm longer than the guide cannula, the tip of the latter was left 1 mm above the MS area. The guide cannula was kept in place by acrylic dental cement tightly fixed to the skull by stainless steel screws. At the end of surgery, a stainless steel dummy was placed in the guide cannula. After surgery, animals were allowed to recover from anaesthesia under a warm lamp before being replaced in the home cages.

Working memory assessment

Apparatus

Spatial working memory was assessed in a Morris water maze 1 week after surgery. The apparatus consisted of a circular pool (diameter 160 cm; height 60 cm), half-filled with water (~21 C) made opaque with powdered milk. A circular platform (diameter 11 cm), made of transparent plastic, was placed in the pool, 1 cm underneath the water surface and out of view from the rat. For each trial, the rat was released from the side of the pool, facing the wall, and was given 60 s to reach the submerged platform. When the rat found the platform, it was left there for 10 s before the next trial was started. When the rat did not find the platform within 60 s, the experimenter placed it on the platform for 10 s before the next trial was run. Using a video-tracking system (Noldus, Wageningen, The Netherlands), the latency to reach the platform and the distance swum by the rat were recorded for each trial. The time spent in a virtual thigmotaxis zone (10 cm large annulus at the border of the pool) and the distance swum therein, as well as the time spent, the distance swum and the number of crossings above a 30-cm diameter virtual zone corresponding to the location of the platform on the previous day, were also recorded and analysed.

In this testing procedure, the rats had to transfer into working memory new incoming information that needed to be remembered for a specific testing day during a short period of time. On the next day, this information had become irrelevant because the platform was placed in a new location each day. All rats were given two trials on each day, starting from different points on the wall of the pool. Importantly, the two daily starting points were equidistant from the platform. The different configurations used are shown in Fig. 1.

Time-line of water-maze testing

The water-maze testing lasted for 12 days. On day 0, animals underwent a pre-training session, during which no treatment was given. This session consisted of two 60-s trials with a visible platform, in order to habituate the rats to the water-maze conditions. From day 1 to day 6, the rats underwent a Test session during which they were injected with 8-OH-DPAT or, as a control, the vehicle. From day 7 to day 12, the rats underwent a Control session during which they underwent no injection prior to testing. The daily configurations of the water maze were the same as the ones used for the Test session. Rats from the different drug groups were tested according to a random order that was repeated on each day of testing. This control session was run to verify that the rats were eventually able to perform the working-memory task in absence of any drug treatment and also that the repeated 8-OH-DPAT injections produced no lasting alterations of behaviour.

For the second experiment with the water-maze testing protocol using a visible platform, the rats underwent a pre-training session, which consisted in two 60-s trials with a visible platform, during which no treatment was given. For the six following days, the platform remained visible and the rats underwent a Test session during which they were subjected to injections. The different locations of the platform and the starting points used during this session were the same as for the previous experiment (Fig. 1; Test session).

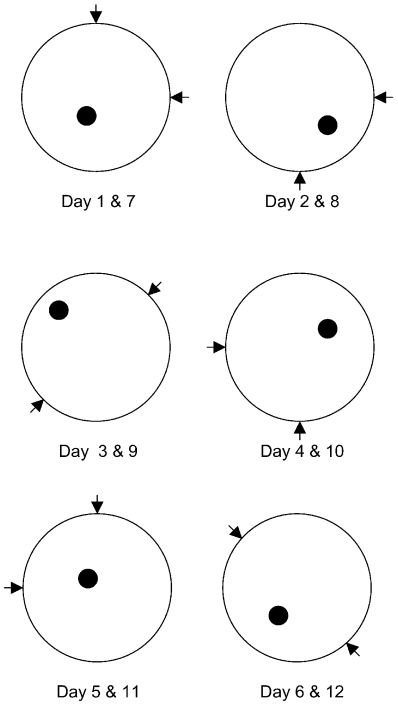

Fig. 1 Working-memory procedure. Location of the platform on the different testing days (*black circles*) and of corresponding starting points (*arrows*). Each configuration was used once for the Test session (days 1–6), and once again for the Control session (days 7–12). From day 1 to 6 (Test session), or day 7 to 12 (Control session), the starting points on trials 1 and 2 were different but equidistant from the platform

Drug and control treatments

During the Test session, the rats underwent different pharmacological treatments. CSF rats (n=18) received an intraseptal microinjection of 0.5 µl sterile artificial cerebrospinal fluid (Harvard Apparatus, Les Ulis, France). DPAT0.5 (n=15) and DPAT4 (n=17) rats received an intraseptal microinjection of 0.5 and 4 µg 8-OH-DPAT (Sigma Aldrich, St Quentin-Fallavier, France), respectively, dissolved in 0.5 µl sterile artificial cerebrospinal fluid. Microinjections were performed 10 min before the first trial was started. They were made over 1 min, and the microinjection needle was left in situ for 1 min to allow diffusion of the drug before retraction of the cannula. Solutions of 8-OH-DPAT were prepared freshly each day.

Histological verifications

After completion of behavioural testing, each rat was given an overdose of sodium pentobarbital (100 mg/kg). When the rats were deeply anaesthetised, 1 µl of methylene blue was microinjected through the cannula. The rats were then transcardially perfused with 60 ml of phosphate-buffered 4% paraformaldehyde (pH 7.4; 4°C), the brain was extracted, post-fixed for 4 h, transferred into a 0.1 M phosphate-buffered 25% sucrose solution for about 36–40 h. Coronal sections (30 µm) were cut on a freezing microtome and collected onto gelatin-coated slides. The sections were dried at room temperature and stained with cresyl violet for histological verification of microinjection sites. Rats with microinjection sites located outside the MS area were discarded from the analysis.

Statistical analyses

The different parameters measured during testing, whether with a visible or a hidden platform, were analysed using two-way ANOVAs, that considered the Trial (1, 2) as a within-subject factor and the Treatment (CSF, DPAT0.5, DPAT4) as a between-subject factor. When appropriate, two-by-two comparisons were performed using the Newman-Keuls multiple range test. The Test session and the Control session were analysed separately.

Results

Histology

The distribution of the injection sites in the CSF, DPAT0.5 and DPAT4 rats are shown on Fig. 2. On a rostro-caudal axis, the injection sites extended approximately from +1.2 to 0.2 mm from bregma (according to Paxinos and Watson 1998), with 41 rats out of 50 having their injection site

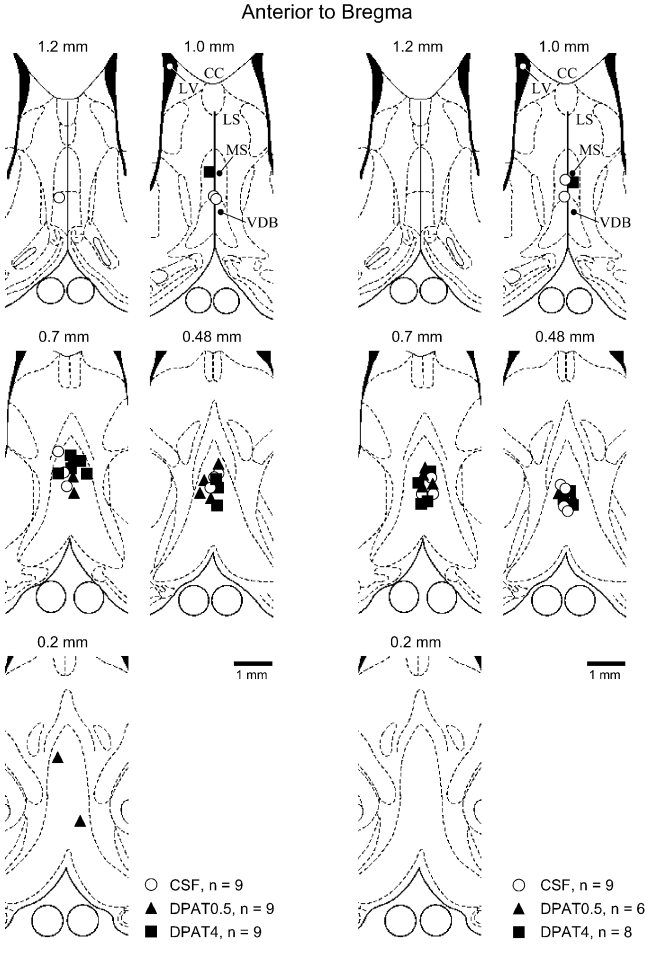

Fig. 2 Histological verification. Schematic representation of the injection sites on coronal sections through the medial septum of rats tested either with the hidden platform (*left*) or with the visible one (*right*); coordinates are in mm from bregma (Paxinos and Watson 1998). Each symbol represents the location of the tip of the microinjection needle in one rat. *CC* corpus callosum; *LS* lateral septum; *LV* lateral ventricle; *MS* medial septum; *VDB* vertical limb of diagonal band of Broca

located between 0.7 mm and 0.48 mm from bregma. Localization of the injection sites appeared homogenous among the three groups. Generally, a slight gliosis was observed in the area located immediately at the tip of the microinjection needle, and very limited damage was observed around the guide cannula track.

Working-memory assessment

Test session

Escape distances, latencies and swim speeds Data are shown in Fig. 3. ANOVA of the mean escape distances (Fig. 3A) showed significant effects of factors Treatment [$F(2,24)=4.8$, $P<0.05$] and Trial [$F(1,24)=20.3$, $P<0.001$], but no significant interaction between them [$F(2,24)=0.8$]. The Treatment effect was due to significantly impaired performance in DPAT4 rats, the distances being significantly increased in these rats as compared to CSF rats ($P<0.05$). The Trial effect was due to a significant improvement of the overall level of performance on trial 2 as compared to trial 1 ($P<0.05$).

ANOVA of the mean latencies (Fig. 3B) showed a significant Trial effect [$F(1,24)=43.6$, $P<0.001$], but there was neither a significant Treatment effect [$F(2,24)=1.7$], nor a significant interaction between both factors [$F(2,24)=0.9$]. The Trial effect was due to significant improvement of the overall level of performance on trial 2 as compared to trial 1 ($P<0.05$).

ANOVA of the average swimming speeds (Fig. 3C) showed significant Treatment [$F(2,24)=5.1$, $P<0.05$] and Trial effects [$F(1,24)=27.4$, $P<0.001$], but the interaction between the two factors was not significant. The Treatment effect was due to the fact that DPAT0.5 and DPAT4 rats were swimming significantly faster than CSF rats ($P<0.05$ in each case). The Trial effect was due to an overall swimming speed that increased significantly from trial 1 to trial 2.

Thigmotaxis Data are shown in Fig. 4. ANOVAs of the mean distances swum (Fig. 4A) and of the time spent (Fig. 4B) in the thigmotaxis zone showed a significant Trial effect [$F(1,24)=31.0$ and 36.9, respectively, $P<0.01$], but there was neither a significant Treatment effect [$F(2,24)=2.0$ and 1.1, respectively], nor a significant interaction between both factors [$F(2,24)=0.1$ and 0.01, respectively]. The Trial effect was due to a significant reduction of thigmotaxis, the distance and the time spent in the thigmotaxis zone decreasing significantly in all groups on the second trial as compared to the first one ($P<0.05$ in all cases).

Returns to the platform location on the previous day Data are shown in Fig. 5. ANOVA of the mean distances swum in the zone where the platform was located on the previous day (Fig. 5A) failed to show a significant Treatment effect [$F(2,24)=1.2$], but showed significant Trial [$F(1,24)=9.5$, $P<0.01$] and interaction effects [$F(2,24)=5.0$, $P<0.01$]. The Trial effect was due to a significant decrease of the distance swum in this virtual zone on trial 2 as compared

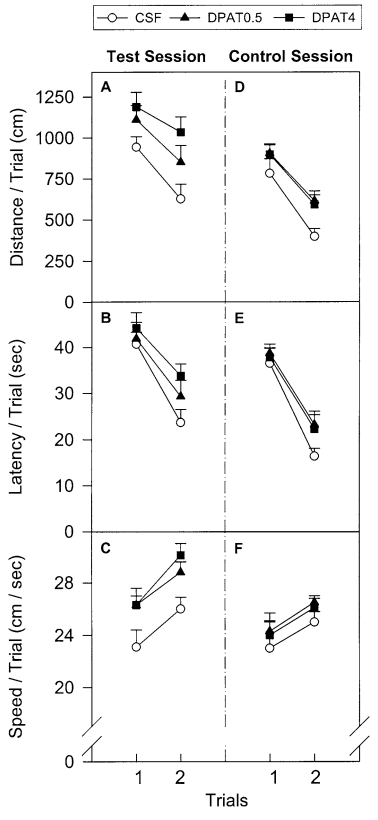

Fig. 3 Working memory, global performance. Mean (+SEM) distances (**A**, **D**), latencies (**B**, **E**), and swimming speeds (**C**, **F**) to reach the platform on trials 1 and 2 during the Test session (**A**, **B**, **C**) and the Control session (**D**, **E**, **F**). The platform was hidden

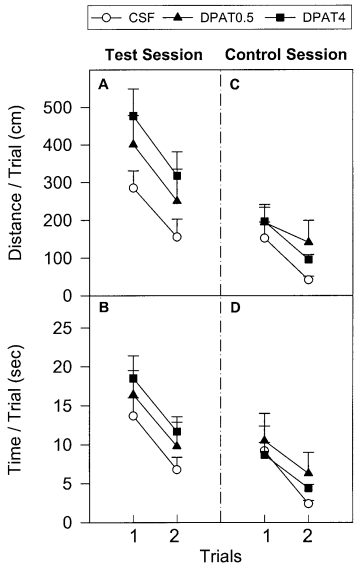

Fig. 4 Working memory, thigmotaxis. Mean (+SEM) distances swum (**A**, **C**) and time spent (**B**, **D**) in the thigmotaxis zone on trials 1 and 2 during the Test session (**A**, **B**) and the Control session (**C**, **D**). The platform was hidden

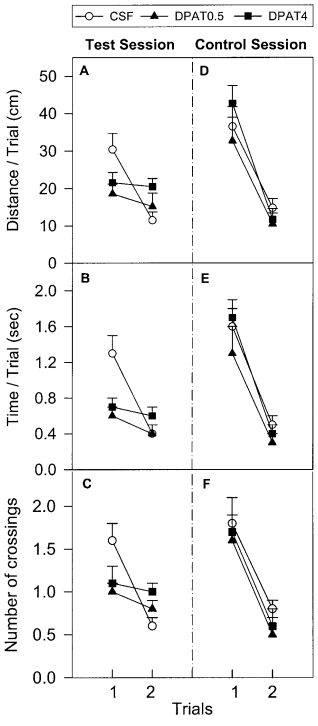

Fig. 5 Working memory, returns to platform's location on the previous day. Mean (+SEM) distances swum to (**A**, **D**), time spent in (**B**, **E**), and number of crossings above the area where the platform was located on the previous day (**C**, **F**) on trials 1 and 2 during the Test session (**A**, **B**, **C**) and the Control session (**D**, **E**, **F**). The platform was hidden

to trial 1 ($P<0.05$). The interaction effect can be explained by the fact that CSF rats swam significantly less distances in this zone on trial 2 as compared with trial 1 ($P<0.01$), while the distance swum by DPAT0.5 and DPAT4 rats remained relatively stable (linear trend analysis showed a significant decrease from trial 1 to trial 2 only in CSF rats, their linear trend being significantly different from that of DPAT0.5 ($P=0.016$), and DPAT4 rats ($P=0.008$), which did not differ significantly from each other, p=0.75). In other words, during their first trial, CSF rats were searching for the platform on the place where it was located on the previous day, whereas DPAT0.5 and DPAT4 did not. However, it is noteworthy that on trial 1, the distance swum in this zone was not significantly longer in CSF rats as compared to both other groups.

ANOVA of the time spent in this virtual zone (Fig. 5B) showed significant effects of factors Treatment [$F(2,24)=4.3$, $P<0.05$], Trial [$F(1,24)=16.9$, $P<0.001$], and of the interaction [$F(2,24)=7.2$, $P<0.01$]. The Treatment effect was due to the fact that CSF rats spent significantly more time in the zone where the platform was located on the previous day as compared to DPAT0.5 rats ($P<0.05$), but only tended to do so in comparison to DPAT4 rats ($P=0.06$). The Trial effect was due to a significant decrease of the time spent in this zone on trial 2 as compared to trial 1. The interaction effect can be explained as for the distances. This explanation was confirmed by trend analysis, the linear trend of CSF rats being significantly different from that of DPAT0.5 ($P=0.005$) and DPAT4 rats ($P=0.001$), which did not significantly differ from each other ($P=0.66$).

ANOVA of the number of crossings on the area where the platform was located on the previous day (Fig. 5C) yielded a picture comparable to that found for distances: the Trial effect was significant [$F(1,24)=13.6$, $P<0.01$] and the interaction effect was also significant [$F(2,24)=6.7$, $P<0.01$], but the Treatment effect was not. Regardless of the treatment, the Trial effect was mainly due to a significant decline of the overall number of crossings from trial 1 to trial 2. The interaction effect can be interpreted as previously and was confirmed by a trend analysis, the linear trend of CSF rats being significantly different from that of DPAT0.5 ($P=0.004$), and DPAT4 rats ($P=0.003$), which did not significantly differ from each other ($P=0.88$).

Control session

Escape distances, latencies and swim speeds Data are shown in Fig. 3. The ANOVA of the mean escape distances (Fig. 3D) showed significant effects of factors Treatment [$F(2,24)=3.5$, $P<0.05$] and Trial [$F(1,24)=42.5$, $P<0.001$], but no significant interaction [$F(2,24)=0.3$]. The Treatment effect was due to significantly impaired performance in DPAT4 rats, the distances being significantly increased in these rats as compared to CSF rats ($P<0.05$). The Trial effect was due to a significant improvement of the overall level of performance on trial 2 as compared to trial 1.

The ANOVA of the mean latencies (Fig. 3E) showed a significant Trial effect [$F(1,24)=123.2$, $P<0.001$], but there was neither a significant Treatment effect [$F(2,24)=0.9$], nor a significant interaction between both factors [$F(2,24)=1.0$]. The Trial effect was due to significant improvement of performance on trial 2 as compared to trial 1.

The ANOVA of the average swimming speeds (Fig. 3F) yielded a comparable picture: only the Trial effect was significant [$F(1,24)=27.5$, $P<0.01$], and it was due to an overall swimming speed that increased significantly from trial 1 to trial 2.

Thigmotaxis Data are shown in Fig. 4. ANOVA of the mean distances swum (Fig. 4C) and of the time spent (Fig. 4D) in the thigmotaxis zone showed a significant Trial effect [$F(1,24)=19.9$ and 17.4, respectively, $P<0.01$], but there was neither a significant Treatment effect [$F(2,24)=1.1$ and 0.4, respectively], nor a significant interaction between both factors [$F(2,24)=0.8$ and 0.5, respectively]. The Trial effect was due to a significant reduction of the distance swum in the thigmotaxis zone on trial 2 as compared to trial 1.

Returns to the platform location on the previous day Data are shown in Fig. 5.

ANOVA of the mean distances swum (Fig. 5D), of the time spent (Fig. 5E) and of the number of crossings on the area where the platform was located on the previous day (Fig. 5F) showed a significant Trial effect [$F(1,24)=44.7$,

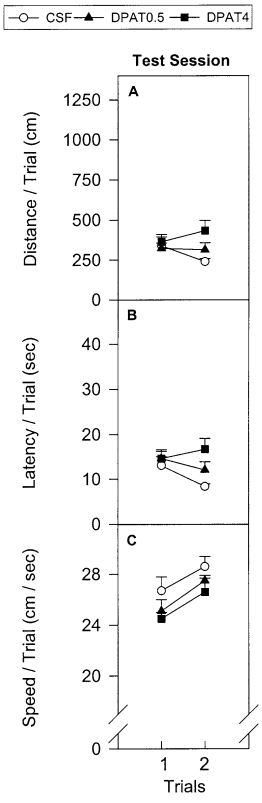

Fig. 6 Visible platform, global performance. Mean (+SEM) distances (**A**), latencies (**B**), and swimming speeds (**C**) to reach the platform on trials 1 and 2 during the Test session

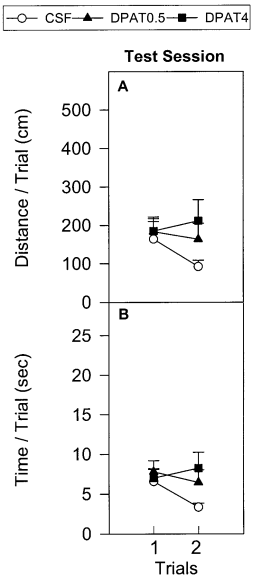

Fig. 7 Visible platform, thigmotaxis. Mean (+SEM) distances swum (**A**) and time spent (**B**) in the thigmotaxis zone on trials 1 and 2 during the Test session

53.1 and 43.7, respectively, $P<0.01$], but neither a significant Treatment effect [$F(2,24)=0.9$, 1.3 and 1.2, respectively], nor a significant interaction between both factors [$F(2,24)=0.7$, 0.6 and 0.8, respectively]. The Trial effect was due to significant improvement of the overall level of performance on trial 2 as compared to trial 1.

Visible platform

Escape distances, latencies and swim speeds Data are shown in Fig. 6. There was no significant Treatment [$F(2,20)<3.0$], Trial [$F(1,20)<2.0$] or interaction effect [$F(2,20)<3.0$], whether on distances swum (Fig. 6A) or latencies (Fig. 6B) to reach the platform. The ANOVA of the average swimming speeds (Fig. 6C) only showed a significant Trial effect [$F(1,20)=14.7$, $P<0.01$], which was due to a significant increase of the overall swimming speed from trial 1 to trial 2.

Thigmotaxis Data are shown in Fig. 7. There was no significant Treatment [$F(2,20)<2.0$], Trial [$F(1,20)<2.0$] or interaction effect [$F(2,20)<2.0$], whether on distances swum (Fig. 7A) or time spent (Fig. 7B) in the thigmotaxis zone.

Discussion

The present results show that intraseptal microinjections of the 5-HT_{1A}/5-HT_7 agonist 8-OH-DPAT induced a deficit in a water-maze task assessing working memory. In comparison to vehicle-treated rats, rats injected with 4 µg 8-OH-DPAT swam faster and exhibited increased distances to reach the platform. Rats injected with 0.5 µg showed similar changes that failed to be significant. Such effects were not observed when the platform was visible. These observations extend previous ones showing that intraseptal injections of 8-OH-DPAT altered spatial reference memory in the water maze (Bertrand et al. 2000).

While DPAT4 rats were clearly impaired, the overall aspect of their impairment raises important questions: did this impairment reflect a genuine deficit of spatial working memory? Did it correspond to a more general deficit of learning abilities? Was it due to a non-mnemonic effect, as for example modification of anxiety or an indirect sensorimotor consequence?

Sensorimotor effects

The literature is quite poor concerning motor effects of 5-HT_{1A} receptor activation in the septal region. However, observations such as the impairment found in DPAT4 rats, which appeared on distances and not on latencies, indirectly support the hypothesis that the deficit was not due to a sensorimotor bias. Although such a deficit pattern may seem contradictory (but can be explained by the increased swimming speeds), it is important to underline that in the water maze, distances are parameters depicted as rather insensitive to sensorimotor perturbations, in contrast to latencies (Lindner et al. 1998). Furthermore, and this is our principal argument, when testing was performed with a visible platform, no impairment was observed in rats microinjected with 8-OH-DPAT. Along the same line, locomotor activity was not affected by intraseptal 8-OH-DPAT microinjections in mice tested in an elevated plus maze or an 8-arm radial maze (Micheau and Van Marrewijk 1999). The swimming speed was increased in treated rats. This modification is at variance

with previous results showing that swimming speed was not affected by intraseptal 8-OH-DPAT microinjections (Bertrand et al. 2000). Hyperactivity was also noticed in a microdialysis bowl after systemic injections of 8-OH-DPAT, but this effect was not observed when 8-OH-DPAT was retrodialysed directly into the medial septum (Bertrand et al., unpublished observations). Still in rats, Schreiber and De Vry (1993) interpreted the increase of mobility observed in a forced swimming test as related to an antidepressant effect of the drug. In view of these somewhat conflicting data, further studies are necessary to get a clearer idea on the locomotor effects of intraseptal 8-OH-DPAT injections.

Effects on anxiety

As stated previously and although reported results are controversial, intraseptal injections of 8-OH-DPAT may influence anxiety. Micheau and Van Marrewijk (1999) reported that intraseptal injections of 1 μg 8-OH-DPAT tended to produce an anxiogenic-like effect in an elevated plus maze in mice. They also provided arguments indicating that these anxiogenic effects may facilitate memory performance. In rats, a comparable effect was found in females at a dose of 0.5 μg, but nothing was observed when the dose was lower (0.2 μg) or higher (2 μg) (De Almeida et al. 1998). Additionally, in males, Menard and Treit (1998) found no effect in the elevated plus maze, but an anxiolytic-like effect was observed in the shock-probe burying test (Menard and Treit 1998). In the present study, there was no significant difference between 8-OH-DPAT-treated and control rats on distance swum and time spent in the vicinity of the pool walls, a behavioural pattern usually termed thigmotaxis and which, among other behavioural variables, is thought to reflect fear and anxiety in the water maze (Hodges 1996). Nevertheless, our present data do not enable a direct assessment of the possibility that the 8-OH-DPAT-induced effects are originating on a modification of the anxiety level.

Cognitive effects

Several observations in DPAT4 rats support the hypothesis of disabilities affecting attention or exploration. For instance, DPAT4 rats swam generally longer distances to reach the platform on first trials than controls, while even ignoring the platform location. Such behaviour might reflect a deficit of non-mnemonic processes, possibly of attention or exploration strategies. Concerning attention, different authors suggested that spatial perturbations induced by septal lesions may reflect deficits of attention processes rather than a pure memory deficiency (McAlonan et al. 1995; Brandner and Schenk 1998). In addition, 8-OH-DPAT administered systemically was shown to reduce performance in tasks assessing attention processes (Carli and Samanin 2000; Nakamura and Kurasawa 2000). Such data provide arguments regarding the possibility that, at the highest dose, 8-OH-DPAT has altered attention capabilities and thereby impaired water-maze performance on the first, and most probably also on the second trials.

Another possibility concerns the exploration strategies. During the first trials, one optimal strategy relies on the exploration of all possible places in the pool until finding the platform. Indeed, within each of their first trials, rats may remember the places already visited in order to maximise the yield of their searching displacements. This strategy might place a high demand on both attention and within-trial memory. In our control rats, such a search strategy seems to occur on the first trial of a testing day. Indeed, during the first trial, these rats spent a longer time on the place where the platform was located on the previous day as compared to DPAT0.5 and DPAT4 rats. This behaviour, which is probably not based on working memory (the delay separating two successive sessions being of about 24 h), suggests that normal rats (i) use a search strategy differing from that of 8-OH-DPAT-treated rats, and (ii) are able to remember the platform location from one day to the next. Interestingly, it seems that the intraseptal injections of 8-OH-DPAT has induced forgetting of the place where the platform was located on the previous day. This finding is strengthened by the fact that during the control session, when testing was continued without prior drug injections, rats from the DPAT4 group performed almost like controls as regards the former location of the platform (i.e. on the previous day). Given that 8-OH-DPAT-treated rats exhibited a capacity to remember the location of the platform on the second trial in the working-memory version of the test, it may be postulated that the activation of septal 5-HT_{1A} receptors has compromised some aspects of information consolidation after the two trials of a given day. Alternatively, it is also possible that 8-OH-DPAT injected on the next day right before the first trial has interfered with a retrieval process. In other words, drug-treated rats may have found the platform and used the information of the new platform location during the second trial to reach it more directly, but the strategy by which they acquired this new information during the first trial was different from that used by CSF rats. Thus, the retrieval process to be altered in this case concerns the place where the platform was located on the previous day, which CSF-treated rats seem to remember while drug-treated rats do not. Further experiments analysing these two possibilities, perhaps with behavioural tests that enable a clear separation of both memory operations seem required. However, data in the literature suggest that systematically administered 8-OH-DPAT impairs both operations (acquisition and retention) in a passive avoidance task (Carli et al. 1992b; Riekkinen 1994) as well as in other tasks (Meneses and Terron 2001). Interestingly, when injected into the septal region, 8-OH-DPAT may impair passive avoidance consolidation (Lee et al. 1992), suggesting that the forgetting of the platform location of the previous day may be the result of interference with a consolidation process rather

than with retrieval. Further experiments seem required to progress on this issue.

A last point deserving discussion concerns the fact that part of the differences between 8-OH-DPAT-treated and CSF-treated rats were still observed during the control session (days 7–12), when testing was continued without drug injections. Although less pronounced than during the drug session, these differences might reflect structural or lasting functional alterations related to the repeated drug injections. Upon histological verifications, evidence for limited gliosis or weak damage around the injection site could be found, but this gliosis or damage was also observed in CSF-treated rats. Thus, the possibility that the repeated drug injections have induced functional alterations accounting for the negative behavioural "after-effects" appears more conceivable. It is not possible to propose a mechanism for these 5-HT_{1A} receptor-mediated consequences. However, it may be interesting to mention that in an earlier experiment, in which rats were treated semi-chronically with SR57746A, a neurotrophic compound having 5-HT_{1A} agonist properties (Fournier et al. 1993), it was found that cognitive performances assessed in a water maze were still altered several weeks and even months after drug administrations had been suspended (Coizet, Kelche and Cassel, unpublished data).

Neural basis of the 8-OH-DPAT effect

Regarding the location of our injection sites, it cannot be excluded that the effects we observed could be due to diffusion of 8-OH-DPAT towards the lateral septum, a structure located next to the medial septum, containing 5-HT_{1A} receptors (Pazos and Palacios 1985) and probably implicated in spatial memory (Farber 1996). Indeed, given the injections conditions (a volume of 0.5 µl injected at a speed of 1 µl/min), it is highly probable that a small amount of the drug diffused over 300–500 µm from the injection site. One has nevertheless to keep in mind that the maximal concentration of the drug was near the tip of the cannula. Intraseptal 8-OH-DPAT injections were found to impair retention of passive avoidance (Lee et al. 1992), but knowledge about cognitive effects of such administrations remains very sparse, and nothing is described concerning spatial memory. Previous studies found adverse effects of systemically administered 8-OH-DPAT on various memory tasks (Winter and Petti 1987; Carli and Samanin 1992; Carli et al. 1995; Riekkinen et al. 1995; Kant et al. 1996, 1998). At least, part of these negative effects of 8-OH-DPAT seems to involve postsynaptic 5-HT_{1A} receptors located in the hippocampus, as (i) intrahippocampal injections of 8-OH-DPAT impair memory performance (Carli et al. 1992a; Ohno et al. 1993; Warburton et al. 1997), and (ii) memory impairments induced by systemic treatment with 8-OH-DPAT can be reversed by intrahippocampal administration of WAY 100135, a 5-HT_{1A} antagonist (Carli et al. 1992a). Thus, together with a former study (Bertrand et al. 2000), the present results suggest that activation of 5-HT_{1A} receptors in the septal region may also alter memory performance.

As stated in the Introduction, some of the cholinergic neurons of the medial septum projecting to the hippocampus receive serotonergic inputs from the raphe (Milner and Veznedaroglu 1993) and express 5-HT_{1A} receptors (Kia et al. 1996). Moreover, 5-HT_{1A} receptors are negatively coupled to adenylyl cyclase (Hoyer and Martin 1997) and in vitro, inhibit the activity of, at least, dorsolateral septal neurons (Van den Hooff and Galvan 1992). Therefore, regarding the critical role of the septohippocampal cholinergic system in memory processes (Wirsching et al. 1984; Beatty and Bierley 1985; Lydon and Nakajima 1992; Wrenn and Wiley 1998; Lehmann et al. 2000; Varvel et al. 2001), the impairments we observed might be related to a reduced cholinergic tone in the hippocampus. However, it is noteworthy that even large cholinergic lesions in the medial septum and the diagonal band of Broca do not induce dramatic effects on working memory in the water maze (Lehmann et al. 2002). Furthermore, intraseptal retrodialysis of 8-OH-DPAT failed to modify the release of acetylcholine in the hippocampus (Bertrand et al., unpublished)

Thus, other hypotheses may be proposed. For instance, different authors suggested that serotonergic fibres originating in the raphe nuclei and innervating the medial septum may exert an inhibitory influence on the rhythmical firing of septal neurons (Assaf and Miller 1978; Kinney et al. 1996; Leranth and Vertes 1999). This rhythmical firing is thought to be one of the "pacemakers" for the hippocampal theta rhythm (for review, see Vertes and Kocsis 1997), and has been linked to mnesic processes (O'Keefe 1993; Vinogradova 1995). Therefore, although the type of serotonergic receptors involved in the serotonin-mediated inhibition of medial septal neurons firing is yet unknown, it is possible that intraseptal injections of 8-OH-DPAT interacted with the rhythmical firing of septal neurons, leading to desynchronisation of hippocampal activity, and thus to memory disturbance or to a more general disorganisation of behaviour in the water maze.

A role for GABAergic neurons, which are modulated by serotonin, is also possible (Alreja 1996). Further studies, perhaps relying upon electrophysiological techniques and taxing cholinergic and GABAergic neurotransmission characteristics after an intraseptal injection of 8-OH-DAPT seem to be required to analyse these issues.

In conclusion, the present study shows that the stimulation of 5-HT_{1A} receptors in the septal region of rats by local injections of 8-OH-DPAT induces a complex, in some respects dose-dependent, pattern of deficits in a water-maze task designed to assess spatial working memory. Based on the characteristics of the observed deficits, it is suggested that the 8-OH-DPAT-induced impairment, rather than being only the result of a true alteration of working memory, might reflect a more global cognitive deficiency in which perturbations of general memory capacities may be mixed with attention dysfunctions, alterations of search strategies or modification of anxiety. Future studies should help to clarify this issue by

using tests that measure these different aspects of cognition more selectively, but one may suggest that activation of 5-HT_{1A}/5HT_7 receptors in the septal region results in alteration of the organisation of behaviour requiring a cognitive investment.

Acknowledgements The authors would like to thank wholeheartedly O. Bildstein and O. Egesi for the excellent technical assistance in the animal care. They also acknowledge E. Lilly for providing a post-doctoral fellowship to F. Bertrand and research funds to the laboratory.

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