



Effects of MDL 73005 on water-maze performances and locomotor activity in scopolamine-treated rats

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Abstract

The stimulation of 5-HT_{1A} receptors in the raphe or their blockade in the hippocampus can reduce cognitive deficits induced by blockade of muscarinic receptors in the hippocampus. We investigated the effects of MDL 73005 (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methylamino) ethyl]-8-azaspiro[4,5] decane-7,9-dione methyl sulphonate), an agonist at 5-HT_{1A} somatodendritic autoreceptors and an antagonist at postsynaptic 5-HT_{1A} receptors in rats treated systemically with scopolamine. Spatial memory was assessed in a water maze using protocols testing reference and working memory. Home cage locomotor activity was also determined. Working memory and locomotor activity were evaluated before and after *para*-chlorophenylalanine (pCPA) treatment. Scopolamine produced a weak impairment of reference memory at 0.5 mg/kg, and a more pronounced impairment of working memory at 0.25 and 0.5 mg/kg. MDL 73005 alone (2 mg/kg, ip) had no effect, but prevented the memory impairments induced by 0.25 mg/kg of scopolamine. Scopolamine induced hyperlocomotion. MDL 73005 alone did not affect locomotor activity, but exacerbated the hyperlocomotion induced by 0.5 mg/kg of scopolamine. pCPA did not abolish the effects of MDL 73005, suggesting that these effects were not due to an action at presynaptic receptors, or even that they involved receptors other than serotonergic ones (e.g., D₂). In conclusion, MDL 73005 is able to antagonise moderate spatial memory dysfunctions induced by systemic muscarinic blockade. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

The serotonergic system takes part in cognitive processes, partly through an interaction with cholinergic mechanisms (e.g., Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995).

Abbreviations: 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; DOPAC, 3,4-dihydroxyphenylacetic acid; HBr, hydrobromide; HPLC, high-performance liquid chromatography; MBr, methylbromide; HVA, homovanillic acid; MDL 73005, 8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methylamino) ethyl]-8-azaspiro[4,5] decane-7,9-dione methyl sulphonate; NA, noradrenaline; NAN-190, 1-(2-methoxyphenyl)-4-(2-phtalimido)butylpiperazine; NMDA, *N*-methyl-D-aspartate; pCPA, *para*-chlorophenylalanine; WAY 100135, *N*-*tert*-butyl-3-(4-[2-methoxyphenyl]-1-piperazinyl)-2-phenylpropamide; WAY 100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide

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Among the different serotonergic receptors involved in cognition (Buhot, 1997; Meneses, 1999), 5-HT_{1A} receptors might be implicated in spatial learning and memory. For instance, systemic treatment with the specific 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), impairs performances in various spatial learning/memory tasks (water maze: Carli and Samanin, 1992; Carli et al., 1995a; Kant et al., 1996, 1998; radial maze: Winter and Petti, 1987; Helsley et al., 1998). Conversely, systemic treatment with WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide) or 100135 (*N*-*tert*-butyl-3-(4-[2-methoxyphenyl]-1-piperazinyl)-2-phenylpropamide), two specific 5-HT_{1A} antagonists, prevents the impairment of water-maze performances caused by blockade of hippocampal muscarinic (Carli et al., 1995b, 1997) or *N*-methyl-D-aspartate (NMDA) (Carli et al., 1999) receptors.

Regarding their anatomical distribution and functional characteristics (e.g., Barnes and Sharp, 1999), 5-HT_{1A} receptors that are somatodendritic can be divided into

two main groups. One comprises the receptors present in the midbrain raphe nuclei (Pazos and Palacios, 1985; Chalmers and Watson, 1991). They are considered to operate mainly as presynaptic autoreceptors: their activation inhibits both raphe serotonergic cell firing (Sprouse, 1991; Millan et al., 1993) and serotonin (5-HT) release in projection areas (Hjorth and Magnusson, 1988; Hutson et al., 1989). The other group comprises heteroreceptors, i.e., present on neurons that are not serotonergic, found in projection areas of the raphe nuclei, including structures such as the hippocampus and the septal region (Pazos and Palacios, 1985; Chalmers and Watson, 1991). There, as in other brain regions, these receptors are considered postsynaptic modulatory receptors (e.g., Buhot, 1997; Barnes and Sharp, 1999).

Interestingly, the effects on spatial memory of treatments with 5-HT_{1A} ligands seem to depend on which of both types of receptors is concerned. Indeed, spatial memory impairments induced by systemic treatment with 8-OH-DPAT persist in 5-HT-depleted rats (Carli and Samanin, 1992) and are antagonised by intrahippocampal delivery of WAY 100135 (Carli et al., 1995b), suggesting an involvement of postsynaptic 5HT_{1A} receptors. Also, when infused into the hippocampus (Carli et al., 1992) or the septal region (Bertrand et al., 2000), 8-OH-DPAT impairs water-maze performances. Conversely, when infused into the dorsal raphe, it reverses the spatial learning impairment caused by intrahippocampal scopolamine (Carli et al., 1998). These data suggest that stimulation of presynaptic or blockade of postsynaptic 5-HT_{1A} receptors has beneficial effects on spatial memory impairments. The 5-HT_{1A} ligand, MDL 73005 (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methylamino) ethyl]-8-azaspiro[4,5] decane-7,9-dione methyl sulphate), has been characterised as having agonist properties at 5-HT_{1A} autoreceptors (termed presynaptic hereafter) in the raphe nuclei, and antagonist properties at postsynaptic receptors. Indeed, this compound, like 8-OH-DPAT (a specific and well-characterised 5-HT_{1A} agonist), induces presynaptic 5-HT_{1A} receptor-mediated effects, such as inhibition of dorsal raphe cell firing (Sprouse, 1991; Millan et al., 1993; Gobert et al., 1995) and of 5-HT release in the hippocampus (Gartside et al., 1990). Simultaneously, it antagonises postsynaptic 5-HT_{1A} receptor-mediated responses elicited by 8-OH-DPAT, such as spontaneous tail flicks, flat body posture, decrease of body temperature (Moser et al., 1990; Millan et al., 1993), or increase of ACTH secretion (Gartside et al., 1990). Moreover, MDL 73005 may act as an antagonist at 5-HT_{1A} receptors on hippocampal CA1 pyramidal neurons (Van den Hooff and Galvan, 1991).

The present experiment investigated the effects of systemic treatment with MDL 73005 on spatial learning/memory deficits induced by muscarinic blockade. The dose of MDL 73005 chosen was 2 mg/kg ip, as it was close to the ID₅₀ of 8-OH-DPAT-induced responses (about 1.5 mg/kg; Millan et al., 1993). Spatial learning and memory were

tested using a water maze, first according to a reference memory protocol, second according to a “working memory” protocol, the latter involving two spatial memory components operating almost concomitantly, i.e., allocentric orientation and egocentric navigation. The capabilities in the “working memory” protocol were evaluated before and after inhibition of 5-HT synthesis by *para*-chlorophenylalanine (pCPA) injections in order to assess the relative implication of pre- vs. postsynaptic 5-HT_{1A} receptors in the effects of MDL 73005. The effects of the drugs were additionally assessed on locomotor activity.

2. Materials and methods

2.1. Animals

All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (Council Directive 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; permissions 6212 to J.-C.C. and 67-14bis to H.J., O.L., R.G., C.L., and F.B. under the former's responsibility) and international (NIH Publication no. 86-23, revised 1985) laws and policies.

The study used 89 3-month-old Long–Evans male rats (CERJ, France). The rats were housed in individual, transparent Makrolon cages (42 × 26 × 15 cm³). Food and water were available *ad libitum*. The colony and testing rooms were maintained in a 12:12 h light–dark cycle (lights on at 7:00 a.m.) under controlled temperature (22°C). The rats were randomly allocated to one of eight groups, abbreviated CTRL, CTRL + pCPA, MDL, MSCO, SCO1, SCO2, MDL + SCO1, and MDL + SCO2 hereafter (see below for details).

2.2. Behavioral testing

2.2.1. Timing of the experiment

The experiment started on Day 1. Spatial reference memory was assessed from Days 1 to 5. Locomotor activity was then measured the first time on Day 6 (Session 1). Spatial working memory was assessed from Days 8 to 11 (Session 1). After 1 day of rest, the rats were injected with pCPA on Days 13–15. Spatial working memory was again assessed from Days 16 to 19 (Session 2). Locomotor activity was finally measured on Day 21 (Session 2), and all rats were sacrificed on Day 22 or 23 for neurochemical determinations.

2.2.2. Spatial memory

Spatial reference memory and spatial working memory were assessed in a Morris water maze. The apparatus consisted of a large circular pool (Ø 160 cm), half-filled with water (temperature 20°C) made opaque with powdered

milk. A circular platform (\emptyset 11 cm), made of transparent plastic, was lowered 1 cm underneath the surface of the water; it was invisible for the rat. In each trial, the rat was placed at the edge of the pool, facing the wall, and, by using extra-maze visual cues, had to find the platform to escape from the water. For both reference and working memory procedures, the rats underwent four trials on each testing day. Each trial lasted a maximum of 60 s, after which the rat that did not find the platform was placed on it by the experimenter. Between two consecutive trials, the rats were allowed to stay for 15 s on the platform. With the help of a computerised video tracking system (Noldus, the Netherlands), escape latencies and distances swam between the starting point and the platform were recorded. Rats from the different experimental groups were tested according to a random order that was repeated on each day of testing.

In the reference memory procedure, the rats had to translate into memory the stable information present through all trials. Reference-memory testing lasted for five consecutive days. During the 19 first acquisition trials, the platform remained at the same place (Fig. 1a), and the rats started each time from a different starting point (Day 1: N–E–S–W; Day 2: SE–N–SW–SE; Day 3: SW–NE–W–E; Day 4: W–E–S–N; Day 5: NE–SW–N). For the 20th trial (last trial of Day 5, i.e., probe trial), the platform was removed and the rat was released from S. Time spent and distance swam within each quadrant of the pool (Q1, Q2, Q3, Q4, with Q3 being the probe quadrant, i.e., where the platform was located during acquisition trials; see Fig. 1) were determined.

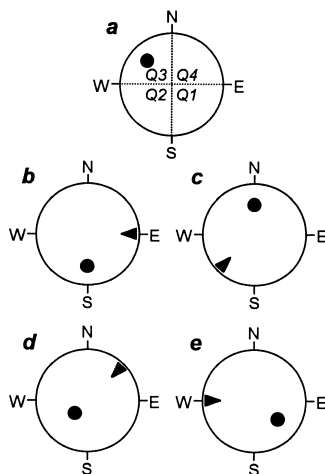


Fig. 1. Schematic representation of the water maze and the position of the platform (filled circle) and the virtual separation lines delimiting the four quadrants (Q1, Q2, Q3, Q4) in the testing protocols used to assess reference memory over 5 days [(a) same platform position each day] or working memory over 4 days [(b)–(e) platform position and start point changed each day]. For patterns (b)–(e), the place where the rat was released is indicated by the arrowhead. Pre-pCPA working memory testing was made using sequence “b–c–d–e,” while post-pCPA testing used sequence “e–d–b–c” for the placement of the platform and the release points.

In the “working memory” procedure, the rats had to translate into memory new incoming information that needed to be remembered for a specific testing day during a short period, and that became irrelevant on the next day. Working memory testing lasted for four consecutive days, with four consecutive trials given each day. On each day, the configuration of the water maze (starting point and platform positions) remained constant, but changed from one day to the next (Fig. 1b–e). From the first to the fourth day of testing, the configuration of the water maze was b–c–d–e (see Fig. 1) for the first session (Days 8–11), and e–d–b–c (see Fig. 1) for the second session (Days 16–19) run after pCPA treatment. As, on one specific day, the starting point and the goal remained the same through all daily trials, the performances of the rats with this protocol may reflect two components of spatial memory: the allocentric spatial working memory, mainly through the differences of the scores between the first and the second trials, but also possibly nonspatial strategies to search for the platform and egocentric spatial orientation.

2.2.3. Locomotor activity

Spontaneous locomotor activity was recorded in the home cages in a testing room with the same light and temperature conditions as the colony room. Each cage was traversed by two infrared light beams targeted on two reflectors, 4.5 cm above the floor level and 28 cm apart. The number of displacements from one extremity of the cage to the other, defined as successive interruptions of the infrared light beams, was monitored by a computer. The rats were placed in the testing room 16 h before injection of the drugs and recording in order to habituate to the room conditions. Activity recording lasted for 6 h, from 11:00 a.m. to 5:00 p.m.

2.3. Drug treatments

CTRL ($n=9$) and CTRL+pCPA ($n=12$) rats were injected with saline (NaCl 0.9%). MDL rats ($n=12$) were injected with MDL 73005 (2 mg/kg). MSCO rats ($n=12$) were injected with scopolamine methylbromide (MBr, 0.5 mg/kg), a derivative of scopolamine that poorly crosses the blood–brain barrier and has essentially peripheral effects. SCO1 rats ($n=11$) were injected with scopolamine hydrobromide (HBr, centrally active) at a low dose (0.25 mg/kg). MDL+SCO1 rats ($n=9$) received both scopolamine HBr at a low dose (0.25 mg/kg) and MDL 73005 (2 mg/kg). SCO2 rats ($n=12$) were injected with scopolamine HBr at a high dose (0.5 mg/kg). MDL+SCO2 rats ($n=12$) received both scopolamine HBr at a high dose (0.5 mg/kg) and MDL 73005 (2 mg/kg).

The drug solutions were prepared freshly on each day in saline. Injections were performed intraperitoneally. The animals treated with no or only one drug (i.e., CTRL, CTRL+pCPA, MSCO, MDL, SCO1, and SCO2) received

a saline injection in place of the additional drug(s). Thus, all rats were injected at two occasions before the behavioral evaluations. For the spatial memory procedures, on each day, scopolamine MBr and HBr (or saline) were injected 30 min, and MDL 73005 (or saline) was injected 15 min before the beginning of the test. For the locomotor activity measurement, scopolamine MBr and HBr (or saline) were injected 15 min, and MDL 73005 (or saline) was injected about 2 min before recording was started.

Working memory testing was interrupted for 3 days over which the rats were injected daily with pCPA, a tryptophan hydroxylase inhibitor, at a dose of 500 mg/kg/day. The pCPA was suspended in a 0.5% arabic gum solution (in saline), prepared freshly every day. CTRL rats did not receive pCPA, but only vehicle in order to control for possible pCPA-induced effects.

MDL 73005 was kindly provided by Hoechst Marion Roussel (Bridgewater, NJ, USA). All other drugs were purchased from Sigma-Aldrich (St. Quentin-Fallavier, France).

2.4. Monoamine determination

One or 2 days after the last locomotor activity test, the rats were sacrificed by microwave irradiation (2.0 s; 6.3 kW; Sairem, Villeurbanne, France) in order to rapidly inactivate brain enzymes (Stavinoha et al., 1973). After decapitation, the brain was extracted and dissected on a cold plate in order to extract the olfactory bulbs, the striatum, the frontoparietal and occipital cortices, and the hippocampus, which was separated into a dorsal (septal pole) and a ventral (temporal pole) portion. The left and right structures from each rat were pooled, weighed, and kept at -80°C until the neurochemical determinations. The tissue samples were prepared by homogenisation in 1 N formic acid/acetone (18/8.5, v/v). Concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), noradrenaline (NA), 5-HT, and 5-hydroxyindolacetic acid (5-HIAA) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system consisted of an ESA liquid chromatography pump

(ESA, Bedford, UK) coupled to an ESA Coulochem II detector (ESA, Chelmsford, USA) equipped with a 5014 high-performance analytic cell (ESA, Bedford, UK). The detector potential at the analytic cell was set at +0.4 V. The HPLC analysis was performed on a C18 Spherisorb ODS2 reverse-phase column (5 μm pore size, \emptyset 4.6 mm, 25 cm long). The mobile phase consisted of 0.1 M NaH_2PO_4 , pH=3, containing 0.1 mM EDTA, 1.7 mM 1-octane sulfonic acid sodium salt, and 10% acetonitrile. The flow rate was 1 ml/min. Concentrations of the different compounds were determined with a data analysis software (Baseline 810, Waters) and were expressed in picograms per microgram of microwaved tissue.

2.5. Statistical analyses

The behavioral and neurochemical data were analysed with a one-way analysis of variance (ANOVA) that considered the treatment factor (for swim speeds and for monoamine concentrations) or a two- or a three-way ANOVA that considered, in addition, one or two repeated-measure factors (day of test for acquisition in the reference memory procedure, quadrant for probe trial performances, trial and session numbers for working memory procedure, hour of observation, and session number for the locomotor activity measurement). The ANOVA was followed, when appropriate, by multiple two-by-two comparisons using the Newman–Keuls test (Winer, 1971). CTRL and CTRL+pCPA rats were considered as a single group for analyses of reference memory performances (no pCPA treatment was given at this stage of the experiment).

3. Results

3.1. Swim speed during the water-maze testing

Mean swim speeds during water-maze testing are shown in Table 1. ANOVA of the swim speeds during the acquisition and probe trials of reference memory testing, and during

Table 1
Swim speeds during water-maze testing

	CTRL (n=9)	CTRL+pCPA (n=12)	MSCO (n=12)	MDL (n=12)	SCO1 (n=11)	MDL+SCO1 (n=9)	SCO2 (n=12)	MDL+SCO2 (n=12)
Acquisition	26.0±0.5 ^a		26.0±0.5	26.4±0.7	31.4±0.8* [#]	31.4±0.5* [#]	32.7±1.1* [#]	32.2±0.8* [#]
Probe trial	28.8±0.5 ^a		28.7±0.8	30.2±0.9	34.0±1.0* [#]	33.5±1.4* [#]	35.9±1.2* [#]	35.6±1.2* [#]
Working memory 1	26.9±0.7	27.1±0.9	27.9±0.4	28.3±0.8	33.5±0.6* [#]	32.5±0.7* [#]	33.5±0.7* [#]	33.2±0.6* [#]
Working memory 2	27.6±0.5 [#]	29.7±0.6*	32.0±0.6	31.2±0.7	33.5±0.9* [#]	34.2±1.1* [#]	34.2±0.6* [#]	34.4±0.4* [#]

Data are expressed as means±S.E.M. during the acquisition and probe trials of the reference memory testing procedure, and during the two sessions of working memory testing procedure. Group abbreviations refer to rats that received pCPA between both working memory testing sessions and which were given an injection of saline (CTRL+pCPA), scopolamine MBr (MSCO: 0.5 mg/kg), MDL 73005 (MDL: 2 mg/kg), scopolamine HBr (SCO1: 0.25 mg/kg; SCO2: 0.5 mg/kg), or a combination of MDL 73005 and the low (MDL+SCO1) or the high (MDL+SCO2) dose of scopolamine HBr. CTRL rats were not subjected to pCPA treatment, but were given a saline injection before testing.

^a CTRL and CTRL+pCPA rats collapsed (see Materials and Methods for detail).

* Significantly different from CTRL, $P<.05$.

[#] Significantly different from CTRL+pCPA, $P<.05$.

each session of working memory testing (pre- and post-pCPA) showed an overall effect of the treatment in all cases [$F(7,81) = 16.64, 9.29, 19.33, \text{ and } 11.44$, respectively, $P < .001$ in each case]. This effect was due to the fact that rats receiving scopolamine HBr (SCO1, SCO2, MDL + SCO1, MDL + SCO2) swam faster than CTRL and CTRL rats to be treated with pCPA ($P < .05$ in all cases). The rats receiving scopolamine HBr swam also significantly faster than MDL and MSCO rats during the acquisition and probe trials of the reference memory test, as well as during the pre-pCPA assessment of working memory ($P < .01$ in each case). During the post-pCPA assessment of working memory, the swim speed of MDL and MSCO rats was still slower than in the rats given scopolamine HBr, but this difference was no longer significant.

3.2. Reference memory assessment

As they were subjected to exactly the same treatment at this stage of the experiment, CTRL and CTRL + pCPA rats were considered a single control group for analysis and data representation.

3.2.1. Acquisition trials

Distances and escape latencies during acquisition in the reference memory procedure (5-day evolution and global mean during acquisition) are shown in Figs. 2 and 3, respectively.

On the distances, ANOVA showed overall Treatment [$F(6,82) = 8.89, P < .001$] and Day [$F(4,328) = 60.35, P < .001$] effects. No significant Treatment \times Day interaction was found [$F(24,328) = 1.23$]. The Treatment effect can be explained by the observation that SCO2 and MDL + SCO2

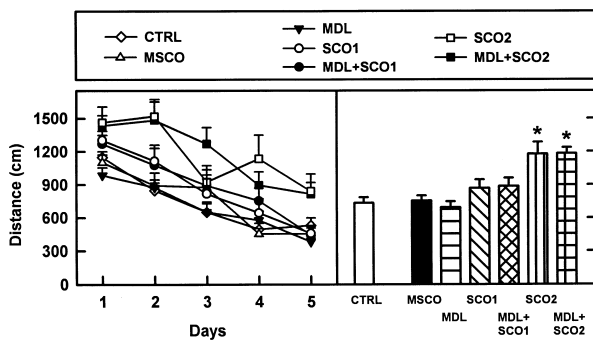


Fig. 2. Mean (+S.E.M.) distances to reach the platform in the water-maze test assessing reference memory capabilities. The left part of the figure shows the mean performances on each day. The right part of the figure illustrates the mean performances with all days collapsed (i.e., the group effect in statistical analyses). Group abbreviations refer to rats that received an injection of saline (CTRL), scopolamine MBr (MSCO: 0.5 mg/kg), MDL 73005 (MDL: 2 mg/kg), scopolamine HBr (SCO1: 0.25 mg/kg; SCO2: 0.5 mg/kg), or a combination of MDL 73005 and either the low dose (MDL+SCO1) or the high dose (MDL+SCO2) of scopolamine HBr. Statistical analyses, overall group effect: * significantly different from CTRL and MSCO, $P < .05$.

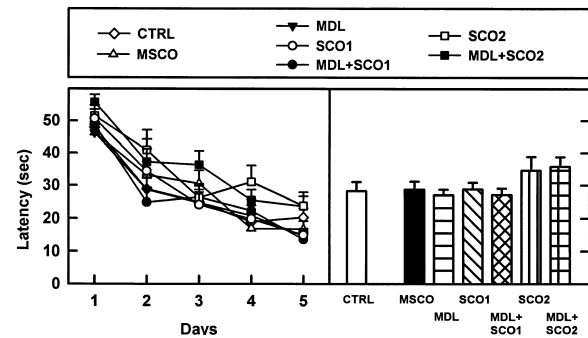


Fig. 3. Mean (+S.E.M.) latencies to reach the platform in the water-maze test assessing reference memory capabilities. The left part of the figure shows the mean performances on each day. The right part of the figure illustrates the mean performances with all days collapsed (i.e., the group effect in statistical analyses). Group abbreviations as in Fig. 2.

rats presented overall distances that were significantly longer than in the five other groups ($P < .05$ in all cases). The differences between the mean distances of the five latter groups were not significant. The Day effect was due to a global improvement of performances of the rats over the 5 days of testing. Indeed, global distances decreased significantly from day to day ($P < .01$ in each case).

On escape latencies, ANOVA showed an overall Day effect [$F(4,328) = 88.89, P < .001$], but neither a significant Treatment effect [$F(6,82) = 2.14$] nor a significant Treatment \times Day interaction [$F(24,328) = 0.81$]. The Day effect was due, as for distances, to an overall decrease of the escape latencies over the 5 days of testing ($P < .05$ in each case).

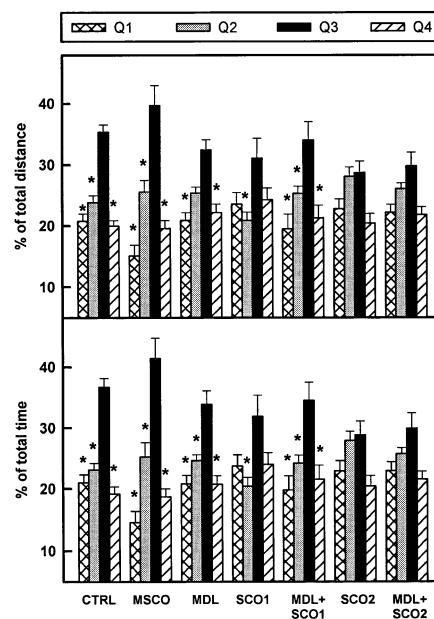


Fig. 4. Mean (+S.E.M.) percentage of distances swam (top) and time spent (bottom) in the four quadrants of the pool during the probe trial. Group abbreviations as in Fig. 2. Statistical analyses, Treatment \times Quadrant interaction: * significantly different from Q3, $P < .05$.

3.2.2. Probe trial

The distances swam and the time spent in each of the four quadrants of the pool during the probe trial are shown in Fig. 4.

Analysis was first performed on the performances in the sole probe quadrant. On the distances swam in the probe quadrant, ANOVA failed to show a significant Treatment effect [$F(6,82)=0.649$, $P=.69$]. On the time spent in the probe quadrant, ANOVA showed a significant Treatment effect [$F(6,82)=2.766$, $P<.05$], which was due to a significantly longer time spent by the MSCO rats in the probe quadrant as compared to SCO2 and MDL+SCO2 rats ($P<.05$ in each case).

As the groups treated with scopolamine HBr exhibited higher swim speeds, which might reflect a sensorimotor bias that could lead to misinterpretation of the data, analysis was also performed on the repartition of the distances swam and the time spent in each of the four quadrants, according to a Treatment \times Quadrant design.

On the distances swam in the different quadrants, ANOVA showed significant Treatment [$F(6,82)=9.68$, $P<.001$] and Quadrant [$F(3,246)=53.93$, $P<.001$] effects, as well as a significant Treatment \times Quadrant interaction [$F(18,246)=2.24$, $P<.01$]. The Treatment effect reflects the differences in the swim speed mentioned above. Indeed, as all rats were tested during a fixed time (60 s) and as SCO1, MDL+SCO1, SCO2, and MDL+SCO2 rats swam faster than CTRL, MSCO, and MDL rats, those of the former four groups swam an overall distance that was significantly longer than that in the other three groups ($P<.05$ in all cases). The Quadrant effect was due to overall distances that were significantly longer in Quadrant 3, i.e., the probe quadrant, compared to each of the three other quadrants ($P<.001$ in all cases), as well as in Quadrant 2 (next to the probe quadrant and the starting point) compared to Quadrants 1 and 4 ($P<.01$ in both cases). The Treatment \times Quadrant interaction can be explained by the fact that the repartition of the distances swam in the different quadrants was not equivalent in all treatment groups. In particular, we noticed that CTRL and MSCO rats swam a distance in the probe quadrant which was significantly longer than in each of the three other ones ($P<.001$ in all cases). In MDL rats, the distance swam in the probe quadrant was significantly longer than that swam in Quadrants 1 and 4 ($P<.05$ in each case). In SCO2 and MDL+SCO2 rats, there was no significant difference on the distance swam in the four quadrants. Interestingly, whereas SCO1 rats were moderately impaired (the distance in the probe quadrant being significantly different only from that swam in Quadrant 2, $P<.05$), MDL+SCO1 rats showed a distance in the probe quadrant that was significantly longer than that swam in each of the three other quadrants ($P<.05$ in all cases). On the time spent in the different quadrants, ANOVA showed a significant Quadrant effect [$F(3,246)=56.12$, $P<.001$] and a significant Treatment \times Quadrant interaction [$F(18,246)=2.64$, $P<.001$].

The Quadrant effect was due to the fact that the time spent in Quadrant 3, i.e., the probe quadrant, was significantly longer than that spent in each of the three other quadrants ($P<.001$ in each case), and also to the fact that the time spent in Quadrant 2 was significantly longer than that spent in Quadrants 1 and 4 ($P<.01$ in each case). The Treatment \times Quadrant interaction can be explained by the fact that the repartition of the time spent in the different quadrants was not equivalent in the seven treatment groups. CTRL, MDL, and MSCO rats spent a significantly longer time in the probe quadrant than in either quadrant ($P<.05$ in all cases). SCO2 and MDL+SCO2 rats spent a time that was not significantly different among the four quadrants. Interestingly, whereas SCO1 rats were moderately impaired (the time spent in the probe quadrant being significantly different only from that in Quadrant 2, $P<.05$), MDL+SCO1 rats showed a time spent in the probe quadrant which was significantly longer than in each of the other three quadrants ($P<.05$ in each case).

3.3. Working memory assessment

Distances and escape latencies (four-trial evolution and global mean) during the two working memory sessions,

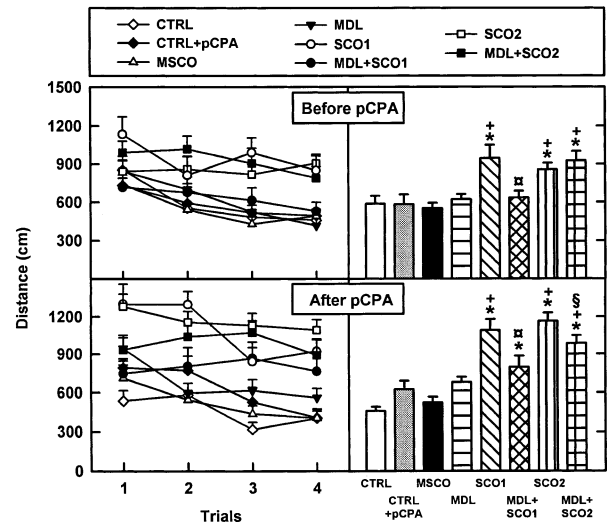


Fig. 5. Mean (+S.E.M.) distances to reach the platform in the water-maze test assessing working memory capabilities before (top) and after (bottom) pCPA treatment. The left part of the figure shows the mean performances on each trial averaged over the four testing days. The right part of the figure illustrates the mean performances with all trials collapsed (i.e., the group effect in statistical analyses). Group abbreviations refer to rats that had received pCPA between both testing sessions and which were given an injection of saline (CTRL+pCPA), scopolamine MBr (MSCO: 0.5 mg/kg), MDL 73005 (MDL: 2 mg/kg), scopolamine HBr (SCO1: 0.25 mg/kg; SCO2: 0.5 mg/kg), or a combination of MDL 73005 and the low (MDL+SCO1) or the high dose (MDL+SCO2) of scopolamine HBr. CTRL rats were not subjected to pCPA treatment, but were given a saline injection before testing. Statistical analyses: * significantly different from CTRL and MSCO, $P<.05$; + significantly different from CTRL+pCPA, $P<.05$; circle with four lines, significant effect of MDL vs. SCO1, $P<.05$; § significant effect of MDL vs. SCO2, $P<.05$.

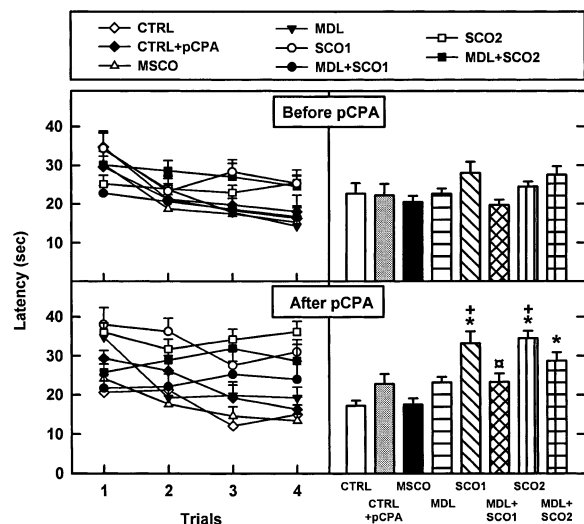


Fig. 6. Mean (+S.E.M.) latencies to reach the platform in the water-maze test assessing working memory capabilities before (top) and after (bottom) pCPA treatment. The left part of the figure shows the mean performances on each trial averaged over the four testing days. The right part of the figure illustrates the mean performances with all trials collapsed (i.e., the group effect in statistical analyses). Group abbreviations as in Fig. 5. Statistical analyses: * significantly different from CTRL and MSCO, $P < .05$; + significantly different from CTRL + pCPA, $P < .05$; circle with four lines, significant effect of MDL vs. SCO1, $P < .05$.

before and after pCPA treatment, are shown in Figs. 5 and 6, respectively.

On the distances, ANOVA showed significant Treatment [$F(7,81) = 16.30$, $P < .001$], Trial [$F(3,243) = 25.53$, $P < .001$], and Session [$F(1,81) = 8.18$, $P < .01$] effects, and also a significant Treatment \times Session interaction [$F(7,81) = 2.93$, $P < .01$]. No significant Treatment \times Trial [$F(21,243) = 1.58$], Trial \times Session [$F(3,243) = 0.61$], and Treatment \times Trial \times Session [$F(21,243) = 1.16$] interactions were found. The Treatment effect was due to distances swam by CTRL, CTRL + pCPA, MDL, and MSCO rats, which were significantly shorter than those of SCO1, SCO2, and MDL + SCO2 rats ($P < .005$ in all cases). Interestingly, in MDL + SCO1 rats, the distances were significantly shorter than those found in SCO1 rats ($P < .001$), and did not differ significantly from those found in control rats. The Trial effect reflects an overall decrease of the distances over the four trials of the test. Indeed, the distances swam on Trial 1 were significantly longer than on the other three trials ($P < .001$ in all cases), and those on Trial 2 were significantly longer than on the last two trials ($P < .01$ in both cases). The Session effect may be explained by the fact that overall distances swam during the second session were greater than during the first session. The Treatment \times Session interaction may be explained by an overall increase of the distances swam by the rats treated with scopolamine HBr during the second session (see Fig. 5). This difference was significant for the distances swam by SCO2 rats (Session 1 vs. Session 2, $P < .01$). Consequently, whereas distances swam by MDL + SCO2

rats did not differ from those of SCO2 rats during Session 1, they were significantly shorter during Session 2 ($P < .05$). Importantly, the distances of CTRL and CTRL + pCPA rats were not significantly different between both sessions, suggesting that neither the treatment with pCPA nor the repetition of the working memory procedure influenced the performances of the rats.

Concerning escape latencies, ANOVA showed significant Treatment [$F(7,81) = 16.30$, $P < .001$] and Trial [$F(3,243) = 32.64$, $P < .001$] effects, and significant Trial \times Session [$F(3,243) = 2.86$, $P < .05$], Treatment \times Trial [$F(21,243) = 3.18$, $P < .001$], and Treatment \times Session [$F(7,81) = 4.30$, $P < .001$] interactions. There was neither a significant Session effect [$F(1,81) = 2.86$] nor a significant Treatment \times Trial \times Session interaction [$F(21,243) = 1.03$]. The Treatment effect was due to the fact that escape latencies of CTRL, MDL, and MSCO rats, which did not differ from each other, were significantly shorter than those of SCO1, SCO2, and MDL + SCO2 rats ($P < .05$ in all cases). The escape latencies of CTRL + pCPA rats were intermediate, as they did not differ significantly from those of CTRL rats or those of SCO1, SCO2, and MDL + SCO2 rats. Interestingly, the escape latencies of MDL + SCO1 rats did not differ from those of CTRL rats and were significantly shorter than those of SCO1 rats ($P < .05$). The Trial effect reflected an overall decrease of the escape latencies over trials. Indeed, the escape latencies on Trial 1 were significantly longer than on either trial ($P < .001$ in each case). On Trial 2, escape latencies were longer than on the two last trials ($P < .05$ in both cases). It is noteworthy that the decrease of escape latencies over trials was more important during Session 1 (-35%) than during Session 2 (-20%), a difference which may explain the Trial \times Session interaction. The Treatment \times Trial interaction may be explained by the differences among the groups in the improvement of the escape latencies over the four trials. For instance, whereas the decrease of the escape latencies between Trials 1 and 4 was quite important in CTRL, CTRL + pCPA, MSCO, and MDL rats (-33% , -49% , -41% , and -51% , respectively), that in SCO1, SCO2, and MDL + SCO2 rats was much weaker (-28% , $+1\%$, and -5% , respectively). It is noteworthy that the improvement of the escape latencies of the MDL + SCO1 rats was small (-9%), but the animals started already on Trial 1 with small latencies. The Treatment \times Session interaction may mainly reflect an important increase of the latencies of the SCO1 and SCO2 rats during the second session (Session 1 vs. Session 2, $P < .05$). As a consequence, when the comparisons were made within each session, the beneficial effect of MDL 73005 in MDL + SCO1 rats was confirmed statistically only in the second session. Importantly, as for distances, the escape latencies of CTRL and CTRL + pCPA rats were not significantly different between both sessions, suggesting that neither the treatment with pCPA nor the repetition of the working memory procedure affected the performances of the rats.

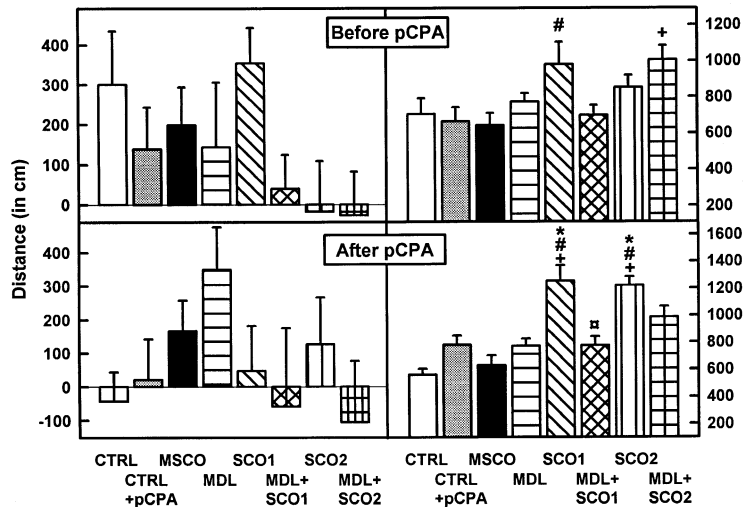


Fig. 7. Mean (+S.E.M.) differences between Trials 1 and 2 (left) and mean with the two trials collapsed (i.e., the group effect in statistical analyses, right) of distances to reach the platform in the water-maze test assessing working memory capabilities before (top) and after (bottom) pCPA treatment. Group abbreviations as in Fig. 5. Statistical analyses: * significantly different from CTRL; + significantly different from CTRL+pCPA, $P < .05$; # significantly different from MSCO, $P < .05$; circle with four lines, significant effect of MDL vs. SCO1, $P < .05$.

Additionally, analysis was performed on distances and escape latencies on the first and second trials, which account more specifically for the working memory dimension of the test. Differences between Trials 1 and 2 and global means are shown in Figs. 7 and 8.

On the distances, ANOVA showed significant Treatment [$F(7,81) = 10.52, P < .001$], Trial [$F(1,81) = 9.26, P < .01$], and Session [$F(1,81) = 5.51, P < .05$] effects, and also a significant Treatment \times Session interaction [$F(7,81) = 2.86, P < .05$]. No significant Treatment \times Trial [$F(7,81) = 1.34$], Trial \times Session [$F(1,81) = 1.42$], and Treatment \times Trial \times Session [$F(7,81) = 1.06$] interactions were found. The Treatment effect was due to distances swam by CTRL,

CTRL+pCPA, MDL, MSCO, and MDL+SCO1 rats that were significantly shorter than those of SCO1, SCO2 and MDL+SCO2 rats ($P < .01$ in all cases). The Trial effect reflects an overall decrease of the distances between Trials 1 and 2. The Session effect may be explained by the fact that overall distances swam during the second session were greater than during the first session. The Treatment \times Session interaction may be explained by an overall increase of the distances swam by the rats treated with scopolamine HBr during the second session (see Fig. 7). This difference was significant for the distances swam by SCO2 rats (Session 1 vs. Session 2, $P < .01$), and tended to reach significance for SCO1 rats (Session 1 vs. Session 2,

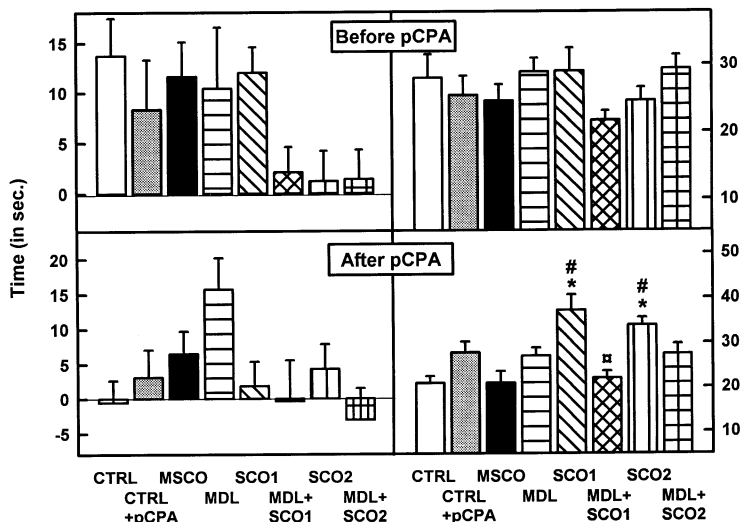


Fig. 8. Mean (+S.E.M.) differences between Trials 1 and 2 (left) and mean with the two trials collapsed (i.e., the group effect in statistical analyses; right) of escape latencies in the water-maze test assessing working memory capabilities before (top) and after (bottom) pCPA treatment. Group abbreviations as in Fig. 5. Statistical analyses: * significantly different from CTRL; + significantly different from CTRL+pCPA, $P < .05$; # significantly different from MSCO, $P < .05$; circle with four lines, significant effect of MDL vs. SCO1, $P < .05$.

$P=.056$). As a consequence, the Treatment \times Session interaction may be explained by the fact that major differences among treatments were found during Session 2. Indeed, during Session 1, only the distances of SCO1 rats differed significantly from those of MSCO rats ($P<.05$), and the distances of MDL+SCO2 rats were significantly different from those of MSCO and CTRL+pCPA rats ($P<.05$ in each case). In contrast, during Session 2, SCO1 and SCO2 rats swam significantly longer distances than CTRL, CTRL+pCPA, MSCO, and MDL rats ($P<.001$ in all cases). In addition, SCO1 rats swam a significantly longer distance than MDL+SCO1 rats. Such an effect of MDL 73005 was not found in MDL+SCO2 rats, conversely to what was found with the analysis performed on all four trials (see above). Importantly, the distances of CTRL and CTRL+pCPA rats were not significantly different between both sessions, suggesting that neither the treatment with pCPA nor the repetition of the working memory procedure influenced the performances of the rats.

Concerning escape latencies, ANOVA showed significant Treatment [$F(7,81)=3.09$, $P<.001$] and Trial [$F(1,81)=27.33$, $P<.001$] effects, and significant Trial \times Session [$F(1,81)=4.32$, $P<.05$], Treatment \times Trial [$F(7,81)=2.31$, $P<.001$], and Treatment \times Session [$F(7,81)=3.67$, $P<.01$] interactions. There was neither a significant Session effect [$F(1,81)=0.41$] nor a significant Treatment \times Trial \times Session interaction [$F(7,81)=1.34$]. The Treatment effect was due to the fact that the escape latency of SCO1 rats was longer than those of MSCO and MDL+SCO1 rats ($P<.05$ in each case). The Trial effect reflected an overall decrease of the escape latencies between Trials 1 and 2. It is noteworthy that the decrease of escape latencies between the first two trials was more important during Session 1 (-25%) than during Session 2 (-12%), a difference which may explain the Trial \times Session interaction. Therefore, global escape latencies on the Trial 2 were significantly longer during Session 2 than during Session 1 ($P<.05$). The Treatment \times Trial interaction may be explained by the differences among the groups in the improvement of the escape latencies between the first two trials. For instance, whereas the decrease of the escape latencies between Trials 1 and 2 was quite important in CTRL, CTRL+pCPA, MSCO, and MDL rats (-24% , -24% , -28% , and -41% , respectively), that in SCO1, SCO2, and MDL+SCO2 rats was weaker (-19% , -9% , and $+3\%$, respectively). It is noteworthy that the improvement of the escape latencies of the MDL+SCO1 rats was small (-4%), but the animals started already on Trial 1 with small latencies. The Treatment \times Session interaction may mainly reflect an important increase of the latencies of the rats treated with only scopolamine HBr during the second session in comparison to the first. This increase was significant for SCO1 rats (Session 1 vs. Session 2, $P<.05$). As a consequence, when the comparisons were made within each session, the beneficial effect of MDL 73005 in MDL+SCO1 rats

was confirmed statistically only in the second session. Importantly, as for distances, the escape latencies of CTRL and CTRL+pCPA rats were not significantly different between both sessions, suggesting that neither the treatment with pCPA nor the repetition of the working memory procedure affected the performances of the rats.

3.4. Spontaneous locomotor activity

Mean locomotor activity scores during each session are shown in Fig. 9.

ANOVA showed overall Treatment [$F(7,81)=20.58$, $P<.001$], Hour of observation [$F(5,405)=203.95$, $P<.001$], and Session [$F(1,81)=34.26$, $P<.001$] effects, as well as significant Treatment \times Session [$F(7,81)=5.23$, $P<.001$], Treatment \times Hour [$F(35,405)=16.74$, $P<.001$], Hour \times Session [$F(4,405)=24.44$, $P<.001$], and Treatment \times Hour \times Session [$F(35,405)=3.46$, $P<.001$] interactions. The Treatment effect may be explained by the differences in locomotor activity among different groups. The lowest overall locomotor activity was found in CTRL, CTRL+pCPA, MSCO, and MDL rats. This activity was significantly lower than that found in SCO1, SCO2, and MDL+SCO1 rats ($P<.01$ in each case). MDL 73005 seemed to exacerbate the scopolamine HBr-induced hyperactivity since MDL+SCO2 rats were much more active than the rats from all other groups ($P<.001$ in each case). The Hour effect reflects an important decrease of the overall locomotor activity over the 6 h of recording. Indeed, during the first and second hours, the overall activity levels were significantly higher than during the four subsequent hours ($P<.001$ in all cases). The Session effect and the Treatment \times Session interaction may be explained by an increase

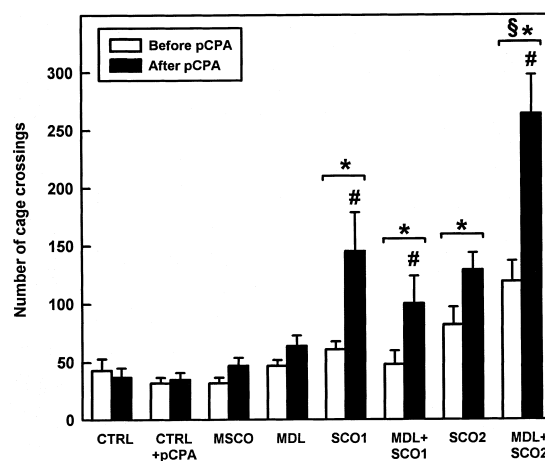


Fig. 9. Mean (+S.E.M.) locomotor activity scores before (empty bar) and after (filled bar) pCPA treatment. Group abbreviations as in Fig. 5. Statistical analyses, group effect: * significantly different from CTRL, CTRL+pCPA and MSCO, $P<.05$; § significantly different from SCO1, SCO2, MDL+SCO1, $P<.05$ (pre- and post-pCPA collapsed); Session effect: # post-pCPA significantly different from pre-pCPA within the same group, $P<.05$.

of the overall activity levels after the pCPA treatment that was essentially due to the scores of the rats treated with scopolamine HBr. This increase was significant for SCO1, MDL + SCO1, and MDL + SCO2 rats ($P < .05$ in all cases), and tended to reach significance for SCO2 rats ($P = .10$). In contrast, no significant change was observed after pCPA treatment for the other groups, especially for the CTRL + pCPA rats. Also, the activity of CTRL rats that

did not receive pCPA was not affected between both sessions, suggesting that the repetition of recording did not affect the spontaneous activity. The Treatment \times Hour, Hour \times Session, and Treatment \times Hour \times Session interactions may be explained by the fact that all the rats were hyperactive in the first 2 h of observation, but the level of this hyperactivity depended on both the treatment and the session (see above).

Table 2
Monoamine concentrations in the different brain structures examined

	CTRL (n=9)	CTRL + pCPA (n=12)	MSCO (n=12)	MDL (n=12)	SCO1 (n=11)	MDL + SCO1 (n=9)	SCO2 (n=12)	MDL + SCO2 (n=12)
<i>Occipital cortex</i>								
5-HT	597 ± 98	241 ± 38*	233 ± 42*	225 ± 15*	192 ± 13*	243 ± 42*	223 ± 14*	180 ± 13*
5-HIAA	341 ± 65	113 ± 29*	131 ± 40*	91 ± 11*	73 ± 8*	79 ± 24*	66 ± 4*	57 ± 3*
DA	198 ± 38 [†]	278 ± 50	329 ± 34*	257 ± 49	157 ± 17 [†]	151 ± 22 [†]	160 ± 13 [†]	139 ± 6 [†]
DOPAC	128 ± 4	142 ± 17	145 ± 12	143 ± 11	130 ± 9	118 ± 7	135 ± 10	115 ± 8
HVA	71 ± 5	87 ± 16	113 ± 9	84 ± 13	48 ± 5 ^{†, #}	46 ± 5 ^{†, #}	48 ± 4 ^{†, #}	46 ± 3 ^{†, #}
NA	370 ± 40	319 ± 18	283 ± 16	314 ± 17	301 ± 17	324 ± 21	304 ± 23	289 ± 16
<i>Frontoparietal cortex</i>								
5-HT	765 ± 65	195 ± 10*	217 ± 13*	190 ± 12*	192 ± 14*	240 ± 56*	195 ± 7*	184 ± 14*
5-HIAA	532 ± 53	109 ± 8*	157 ± 11*	116 ± 10*	94 ± 9*	112 ± 27*	87 ± 3*	91 ± 8*
DA	980 ± 106	901 ± 125	1025 ± 124	992 ± 88	1161 ± 148	787 ± 71	1012 ± 78	988 ± 80
DOPAC	484 ± 58	371 ± 45	522 ± 80	435 ± 53	404 ± 50	289 ± 24	349 ± 37	358 ± 31
HVA	213 ± 20	181 ± 9	198 ± 10	187 ± 10	203 ± 24	150 ± 17	178 ± 11	178 ± 13
NA	506 ± 40	447 ± 26 [†]	558 ± 39 [#]	454 ± 31	381 ± 26 [†]	390 ± 29 [†]	369 ± 11 [†]	370 ± 15 [†]
<i>Dorsal hippocampus</i>								
5-HT	965 ± 60	272 ± 25*	340 ± 23*	290 ± 17*	224 ± 10*	323 ± 90*	252 ± 21*	223 ± 14*
5-HIAA	1023 ± 102	201 ± 16*	251 ± 20*	216 ± 14*	174 ± 9*	241 ± 74*	182 ± 13*	174 ± 9*
DA	373 ± 93	294 ± 33	421 ± 42	313 ± 41	305 ± 74	219 ± 20	271 ± 22	267 ± 25
DOPAC	268 ± 39	376 ± 50	408 ± 60	328 ± 39	236 ± 25 [†]	224 ± 23 [†]	265 ± 35 [†]	218 ± 15 [†]
HVA	151 ± 21 [†]	182 ± 25	212 ± 22*	159 ± 14	118 ± 4 [†]	113 ± 8 [†]	126 ± 9 [†]	128 ± 12 [†]
NA	534 ± 26	467 ± 30	517 ± 23	488 ± 36	404 ± 14 [†]	451 ± 28	403 ± 18 [†]	398 ± 12 [†]
<i>Ventral hippocampus</i>								
5-HT	1285 ± 112	259 ± 28*	348 ± 21*	280 ± 19*	208 ± 14*	335 ± 123*	227 ± 23*	211 ± 17*
5-HIAA	1160 ± 119	197 ± 20*	300 ± 22*	221 ± 18*	156 ± 13*	223 ± 71*	150 ± 10*	149 ± 6*
DA	278 ± 17	261 ± 26	212 ± 15	229 ± 21	257 ± 18	279 ± 30	278 ± 19	257 ± 15
DOPAC	258 ± 25	220 ± 13	272 ± 44	201 ± 10	193 ± 13	202 ± 13	213 ± 21	212 ± 12
HVA	78 ± 9	87 ± 13	87 ± 20	83 ± 13	105 ± 6	109 ± 4	113 ± 9	107 ± 4
NA	845 ± 38 ^{†, #}	699 ± 43 ^{*, †}	826 ± 27 [#]	759 ± 36	620 ± 27 [†]	674 ± 39 [†]	630 ± 36 [†]	640 ± 22 [†]
<i>Striatum</i>								
5-HT	1207 ± 152	366 ± 28*	333 ± 35*	386 ± 35*	356 ± 26*	474 ± 90*	402 ± 30*	375 ± 22*
5-HIAA	1396 ± 187	251 ± 26*	285 ± 26*	262 ± 19*	231 ± 16*	328 ± 90*	244 ± 16*	243 ± 13*
DA	4712 ± 363 ^{†, #}	6327 ± 322*	7364 ± 367*	5925 ± 471 ^{*, †}	4189 ± 275 ^{†, #}	4110 ± 215 ^{†, #}	4626 ± 215 ^{†, #}	4615 ± 207
DOPAC	2841 ± 185	2838 ± 172	3263 ± 274	2961 ± 217	2212 ± 265	2291 ± 70	2247 ± 300	2321 ± 227
HVA	1351 ± 95	1170 ± 70	1381 ± 95	1248 ± 110	1200 ± 98	1311 ± 110	1223 ± 101	1279 ± 66
NA	363 ± 40	405 ± 55	384 ± 25	413 ± 68	324 ± 26	345 ± 23	374 ± 32	449 ± 84
<i>Olfactory bulbs</i>								
5-HT	854 ± 55	464 ± 210*	548 ± 93*	329 ± 47*	303 ± 49*	422 ± 93*	338 ± 53*	246 ± 38*
5-HIAA	432 ± 39	185 ± 63*	194 ± 31*	125 ± 28*	121 ± 37*	94 ± 37*	125 ± 23*	95 ± 14*
DA	1110 ± 347	750 ± 203	509 ± 103	337 ± 47	775 ± 210	484 ± 81	1433 ± 257 [†]	856 ± 209
DOPAC	592 ± 72	543 ± 155	371 ± 36	401 ± 55	489 ± 83	361 ± 60	554 ± 51	451 ± 109
HVA	282 ± 56	183 ± 37	148 ± 19	189 ± 51	187 ± 36	156 ± 30	235 ± 33	267 ± 99
NA	751 ± 103	698 ± 166	652 ± 79	559 ± 73	552 ± 61	487 ± 59	574 ± 79	506 ± 54

Data are expressed as means ± S.E.M. (in picograms per microgram of microwaved tissue). Group abbreviations as in Table 1.

* Significantly different from CTRL, $P < .05$.

[†] Significantly different from MSCO, $P < .05$.

[#] Significantly different from CTRL + pCPA, $P < .05$.

3.5. Monoamine determination

Mean concentrations of monoamines in the different structures assessed are shown in Table 2. Analysis was performed individually for each monoamine/metabolite in each structure.

On the concentrations of serotonergic markers (5-HT and its metabolite, 5-HIAA), a significant effect of the treatment was found in all structures (Table 2). Indeed, whatever structure was considered, the rats treated with pCPA (CTRL+pCPA, MSCO, MDL, SCO1, MDL+SCO1, SCO2, MDL+SCO2) presented a massive reduction of serotonergic markers in comparison to the rats of the CTRL group, which did not receive pCPA ($P < .001$ in all cases; see Table 2). This reduction reached 60–70% in the olfactory bulbs and in the various cortical regions, and 70–80% in both regions of the hippocampus and in the striatum.

Significant effects of the treatment were also found on the concentrations of catecholaminergic markers in some structures (Table 2). These effects were mainly due to a reduction of catecholamine concentrations in the rats treated with scopolamine HBr (SCO1, MDL+SCO1, SCO2, MDL+SCO2) as compared to the rats that received scopolamine MBr (MSCO), and, to a lesser extent, to those of the CTRL+pCPA group ($P < .05$). In the occipital cortex, DA and HVA concentrations were significantly reduced in all groups treated with scopolamine HBr ($P < .05$). In the frontoparietal cortex and the ventral hippocampus, NA concentration was also significantly reduced in all these groups ($P < .05$). In the dorsal hippocampus, NA, DOPAC, and HVA concentrations were significantly reduced in all these groups except the MDL+SCO1 group ($P < .05$). Finally, in the striatum, DA concentration was significantly reduced in all groups treated with scopolamine HBr, as well as in the MDL and CTRL groups ($P < .05$).

4. Discussion

The present experiment assessed the effects of MDL 73005 given systemically on Morris water-maze performances and on locomotor activity in rats pretreated with scopolamine. As compared to scopolamine MBr or saline, the centrally active scopolamine (1) weakly but significantly impaired reference memory; (2) impaired working memory more markedly; and (3) induced locomotor hyperactivity in the home cage. MDL 73005 had no effect by itself, but reduced the moderate impairment elicited by the low dose of scopolamine in the water-maze tasks and potentiated the locomotor effect of the high dose of scopolamine. These effects were still present, in some respect even exacerbated, after pCPA treatment.

The treatment with pCPA produced an important, though not complete, 5-HT depletion, as indicated by a 60–80% reduction of 5-HT and 5-HIAA concentrations in all brain structures examined. This observation suggests that the

beneficial effects of MDL 73005 were not due to an action of the compound at only the presynaptic 5-HT_{1A} receptors, which, when stimulated, decrease the 5-HT tone in projection areas, an effect also induced by pCPA. On the one hand, if the effect of MDL 73005 on the scopolamine-induced deficits was due to an action as an agonist at presynaptic 5-HT_{1A} receptors, pCPA should have produced an effect similar to the one induced by MDL 73005, and perhaps should have potentiated the latter. If so, any other 5-HT receptor, but the 5-HT_{1A} subtype, might be involved at the postsynaptic level. Our data show that pCPA did not mimic the effects of MDL 73005; pCPA even exacerbated some of the behavioral effects of scopolamine. If, on the other hand, the effect of MDL 73005 on the deficits produced by scopolamine was due to an action as an antagonist at postsynaptic 5-HT_{1A} receptors, it is more difficult to conclude something from the pCPA approach. Indeed, in the latter case, pCPA would not necessarily interact negatively with the effect of MDL 73005 (and we observed that it did not), although there still exists a theoretical possibility that pCPA also mimics the effects of MDL 73005 (which was not the case). Nevertheless, the low levels of 5-HT after pCPA treatment are in favour of an action involving the antagonist property of MDL 73005, as one may consider that the competition ratio between the endogenous neurotransmitter and the exogenous drug was displaced in favour of the latter by pCPA treatment. Therefore, the fact that MDL 73005 still produced beneficial effects after pCPA does not exclude that it could have acted at postsynaptic 5-HT_{1A} receptors. Alternatively, as MDL 73005 exhibits properties of a D₂ dopaminergic antagonist (Gobert et al., 1995), the effects found after pCPA treatment might be the result of an action at D₂ dopaminergic receptors. It is noteworthy that, in the second working memory session that was run after pCPA treatment, MDL 73005 was found to significantly reduce the distances in rats treated with the high dose of scopolamine, an observation that could be interpreted as reflecting an enhancement of the effects of MDL 73005 in 5-HT-depleted rats. Such a view requires some qualification as the treatment with pCPA potentiated the deleterious effects of scopolamine, a result which is in line with previous findings (Harder et al., 1996; Beiko et al., 1997). Thus, the pCPA-induced potentiation of the effects of scopolamine might have created an experimental condition where MDL 73005-induced effects may have been more easily detectable.

Concerning catecholaminergic markers, it is remarkable that (1) pCPA treatment has increased the concentration of DA in the striatum (see CTRL vs. CTRL+pCPA); and (2) the groups treated with scopolamine HBr (and pCPA) exhibited levels of DA that were lower than in the other groups. Similar results were found for HVA in the occipital cortex. As such changes were not observed in rats treated with scopolamine MBr, they are probably a consequence of a central action of the anti-muscarinic drug. So far, we do not know how to account for these changes, but it is possible that repeated blockade of muscarinic receptors

has altered the catecholaminergic tone by direct or indirect mechanisms. It is known that systemic treatment with scopolamine, which has no effect by itself on cortical dopamine release, suppresses the veratrine-evoked release of DA in the frontal cortex (Liu and Kato, 1996), as well as that induced in the striatum by treatment with clozapine (Meltzer et al., 1994). Bymaster et al. (1993) have observed that acute treatment (systemic) with scopolamine decreased the level of DOPAC in the striatum, an observation confirming another report by Rivest and Marsden (1992). Similar findings were obtained in the hippocampus and the frontal cortex (Memo et al., 1988). Finally, in humans, systemic administration of scopolamine decreases the striatal binding of a D₂-dopamine receptor antagonist, an observation indicative of an increased dopamine release in response to muscarinic blockade (Dewey et al., 1993). These few examples do not provide any clear explanation to account for our present observations, but they are at least in line with the idea that muscarinic blockade may interact with the catecholaminergic tone in some brain regions.

Also, in MSCO rats, some catecholaminergic markers were found to be higher, always significantly in comparison to rats treated with scopolamine HBr, than in other groups. This was the case for DA and HVA in the occipital cortex; NA in the frontoparietal cortex; DOPAC, HVA, and NA in the dorsal hippocampus; NA in the ventral hippocampus; and DA in the striatum. It is very difficult to account for these changes in the brain as scopolamine MBr is considered to exert essentially peripheral effects. It is clear that further studies are necessary to replicate these observations and, subsequently, to understand the involved mechanisms.

Scopolamine-induced hyperlocomotion was due to the central action of the drug as it was not observed with scopolamine MBr. Such results have been extensively described in the literature (e.g., Sipos et al., 1999). Interestingly, whereas it had no significant effect by itself, the pCPA-induced 5-HT depletion enhanced the hyperlocomotor effects of scopolamine. This result is in line with a putative inhibitory role of the serotonergic system on locomotor activity (Fibiger and Campbell, 1971). Moreover, along the same line, while ineffective by itself at the dose used, MDL 73005 also potentiated the hyperlocomotor effects of the high dose of scopolamine. If one considers that the level of locomotor activity may be related to the dopaminergic tonus, particularly in the striatum (e.g., Staton and Solomon, 1984; Kuczenski and Segal, 1989), two hypotheses can be proposed to account for these observations. First, although the literature is very controversial as concerns the role of 5-HT_{1A} receptors in the control of striatal DA metabolism, a few studies suggest that there may exist a 5-HT_{1A} receptor-mediated inhibition of synthesis of striatal DA (e.g., Johnson et al., 1993, 1996). Therefore, the 5-HT_{1A} antagonist properties of MDL 73005 may have contributed to elevate striatal DA activity in an amount sufficient to further increase locomotor activity under the influence of muscarinic blockade. Sec-

ond, MDL 73005 has been shown to have potential antagonist properties at D₂ dopaminergic autoreceptors, an action that is also marked by an increased striatal DA tone (Gobert et al., 1995). This D₂ antagonist property of MDL 73005 might have been all the more perceptible in scopolamine-treated rats, as their decreased striatal DA concentrations might have accounted for an increased sensitisation of a D₂ autoreceptor-mediated inhibition of DA release. This needs to be demonstrated. Nevertheless, each of these effects was insufficient to alter the locomotor activity per se, as increased locomotion was observed only in the rats given scopolamine at the highest dose. Because the literature is very controversial as to the role of 5-HT in the regulation of locomotor activity, it is difficult to give a clear-cut interpretation of these results. For instance, it appears that a 5-HT_{1A} agonist (i.e., 8-OH-DPAT) injected systemically induces hyperactivity (e.g., Wilkinson et al., 1994) and potentiates the effects of scopolamine on locomotor activity (own unpublished observations). In the present study, the scopolamine-induced hyperactivity was potentiated by MDL 73005, as well as by the pCPA-induced inhibition of 5-HT synthesis. Further studies are required to address this question more accurately.

Our results show that scopolamine increases the swim speed in the water maze and the locomotor activity in the rats' home cages. Therefore, one might consider that the deleterious effects of scopolamine on memory performances in the water maze could be the consequence of a sensorimotor bias, rather than a genuine effect upon cognition. Also, as a consequence of the former, it might be that the beneficial effects of MDL 73005 could be due to an action of the compound on the sensorimotor impact of scopolamine. This hypothesis seems unlikely for at least two reasons. First, the effects of scopolamine and MDL 73005 were always observed on distances, in a lesser degree on latencies or time (in the probe trial); in the water maze, distance is generally considered to be poorly sensitive to sensorimotor alterations (Lindner et al., 1998). Second, whereas MDL 73005 did not attenuate the scopolamine-induced increase of the swim speed in the water maze or the locomotor activity in the home cage, it actually improved the spatial memory performances in the rats treated with the low dose of scopolamine.

An important point to mention is the way MDL 73005 improved performances of the rats treated with the low dose of scopolamine in our "working memory" protocol. Indeed, the control rats exhibited a common pattern of learning in a working memory test, with an important decrease of escape distances and latencies between Trials 1 and 2. In contrast, the rats treated with scopolamine presented a weaker decrease of their escape distances and latencies over the four trials, especially during the second session. The MDL+SCO1 rats exhibited better overall performances, but there was no amelioration from the first to the second trial. This result suggests that the improvement of the performances of these rats was less a matter of spatial

allocentric working memory than a matter of change in spatial orientation strategy. As mentioned in the Materials and Methods, the performances of the rats in our “working memory” protocol may involve two spatial memory components, i.e., spatial allocentric orientation and egocentric navigation relying upon nonspatial strategies to search for the platform (route learning). Therefore, the better performances of MDL+SCO1 rats could reflect a compensation of the scopolamine-induced deficit of allocentric spatial orientation by an improvement of egocentric navigation, enabling rats to search more efficiently for the platform. Such a shift in the spatial navigation strategy might reflect an action of MDL 73005 on the striatum. Indeed, this structure is involved in egocentric spatial navigation in the water maze (e.g., Whishaw et al., 1987; McDonald and White, 1994; Devan et al., 1996). MDL 73005 may have acted through its 5-HT_{1A} properties, since 5-HT_{1A} compounds may modulate the striatal dopaminergic activity (Nissbrandt et al., 1992; Kreiss and Lucki, 1994; Johnson et al., 1993, 1996; Santiago et al., 1998). Alternatively, MDL 73005 may have acted through its dopaminergic D₂ antagonist properties (Gobert et al., 1995; see also discussion of locomotor effects), since intrastriatal administration of sulpiride, a D₂ antagonist, has been found not only to improve memory processes (Setlow and McGaugh, 2000) but also to modify the strategy that rats display to find the platform in the Morris water maze (Setlow and McGaugh, 1999). Nevertheless, such a hypothesis would need in-depth analysis with further experiments designed at discriminating (1) allocentric orientation vs. egocentric navigation and (2) hippocampus vs. striatum involvement.

5. Conclusion

Our study has shown that MDL 73005 is able to improve performances in two versions of a Morris water maze in rats treated with a low dose of scopolamine. As these effects were still present following 5-HT depletion induced by pCPA treatment, it is likely that they were not due to an action of MDL 73005 at only presynaptic 5-HT_{1A} autoreceptors, but rather at postsynaptic ones, or even at other receptors such as D₂ dopaminergic ones. Our results also confirm the important role that central interactions between cholinergic and other neurotransmitter systems, such as the serotonergic and the dopaminergic ones, play in the regulation or modulation of spatial navigation processes. As it was given systemically in a paradigm of general cholinergic dysfunction, MDL 73005 and similar compounds might be one of the noncholinergic tools used for treating moderate cognitive dysfunctions related to alterations of central cholinergic neurotransmission. As such, it might be of interest as regards the treatment of cognitive alterations found in early Alzheimer’s disease, particularly because MDL 73005 seems to work when delivered by a way more appropriate for clinical use than intracerebral injections or other types of

invasive approaches. Further studies should be undertaken in order (1) to investigate dose–response relationships in models of muscarinic blockade (2 mg/kg MDL 73005 being effective on the deficits produced by 0.25 mg/kg, but not 0.5 mg/kg scopolamine); (2) to assess whether MDL 73005 or compounds with similar properties have a therapeutic potential also in paradigms of selective cholinergic lesions (e.g., with 192 IgG saporin) or in aged rodents showing cognitive dysfunctions; and (3) to investigate the effects of D₂ receptors ligands using behavioral approaches identical to the ones used in the present experiment.

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