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Serotonin, locomotion, exploration, and place recall in the rat

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Abstract

Intracerebroventricular injection of 5,7-dihydroxytryptamine (5,7-DHT) led to a 90% reduction of the 5-hydroxytryptamine (5-HT) reuptake site. Behavioural symptoms were studied early (45 to 93 h) as well as late (11 to 14 days) in the postoperative period. Forty-five hours postoperatively, recall of a place navigation task in a water maze was clearly impaired in 5,7-DHT-treated animals. This impairment had disappeared by the fifth postoperative session. During the early test period, injection of scopolamine (0.5 mg/kg) or *d*-amphetamine (3.0 mg/kg) did not affect place recall of the vehicle-treated control group. In contrast, 5,7-DHT-treated animals were impaired by administration of scopolamine, but not *d*-amphetamine. During the late test period, the place recall of both groups was affected by scopolamine, but only the performance of the 5,7-DHT lesioned animals was sensitive to *d*-amphetamine. Locomotion was not severely affected at any time after 5,7-DHT treatment. The vertical hole-board test indicated that the exploratory activities of the animals were relatively unaffected by 5,7-DHT when measured 48 h postoperatively. At 14 days postsurgery, the 5,7-DHT-treated animals demonstrated an impaired habituation of the exploratory behaviour.

Keywords: 5,7-Dihydroxytryptamine; Serotonin; Water maze; Activity cage; Vertical hole-board; Neurochemical systems interaction; Brain damage recovery; Cognitive functions; Alzheimer's dementia

1. Introduction

Intracerebroventricular administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) leads to a substantial and selective loss of serotonin (5-hydroxytryptamine, 5-HT) in the brain. Serotonergic axons and axon terminals seem to be the structures mainly affected by injections of 5,7-DHT (e.g., Iijima et al., 1990). If an appropriate dosage of 5,7-DHT is selected and injection of the toxin is combined with protection of the dopaminergic and noradrenergic systems by treatment with reuptake inhibitors, an almost complete and selective depletion of serotonin from the brain of the rat can be achieved by injections into the ventricles or the raphe nuclei of the brainstem (e.g., Gately et al., 1986; Lyness and Moore, 1981; Pranzatelli and Snodgrass, 1986a; Wenk et al., 1987). A more restricted depletion of serotonin can be obtained by injection of 5,7-DHT into individual brain structures (e.g., Black and Robinson, 1985; Carter and Pycock, 1979; Lyness and Moore, 1981).

Functional studies of animals with near-total elimination of serotonin provide information about the ways in which the brain can mediate behaviour in the absence of serotonin. Although being valuable contributions to the understanding of the functions of the brain's serotonergic system, such studies are complicated by the sequence of dynamic changes that lesions of serotonergic neurons provoke. As demonstrated in studies of the consequences of the mainly cholinergic lesion produced by transection of the fimbria–fornix (e.g., Dyon-Laurent et al., 1994; Gage and Björklund, 1986; Gage et al., 1983a,b, 1984; Loy and Moore, 1977; Sara, 1989; Stenevi and Björklund, 1978), loss of the axonal terminals within one neurotransmitter system leads to a long-lasting chain of structural changes within a multitude of other neurotransmitter systems. In addition, the elimination of the presynaptic element of serotonergic synapses must be expected to provoke compensatory regulations of postsynaptic serotonergic receptors. Consequently, the profile of behavioural symptoms after injection of 5,7-DHT into the brain is likely to depend on the duration of the "recovery period" between 5,7-DHT administration and behavioural testing.

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Changes in general activity, locomotion, or exploration (Black and Robinson, 1985; Carter and Pycock, 1979; Dickson and Vanderwolf, 1990; Erinoff and Snodgrass, 1986; Gately et al., 1986; Ismailova et al., 1990; Lyness and Moore, 1981; Pranzatelli and Snodgrass, 1986a,b; Stewart et al., 1979; Vanderwolf, 1989; Vergnes et al., 1988; Williams et al., 1990) have frequently been found after administration of 5,7-DHT to the rat. As argued elsewhere (Geoffroy and Mogensen, 1988; Mogensen, 2003; Mogensen et al., 1994) comparisons across such studies are often complicated by methodological problems. The conceptual distinction between exploration and locomotion is relatively easy. The experimental analysis of these behavioural categories, however, is frequently unable to establish whether a change in behaviour reflects the purely motoric modifications associated with the concept of locomotion or an alteration in the more "cognitive" processes comprised within the concept of exploration. A distinction between these categories of behaviour would only be possible if an experiment included exploration-independent tests of locomotion and locomotion-independent tests of exploration.

Regional administration of 5,7-DHT has been found to be associated with a broad spectrum of apparent changes in locomotion and/or exploration (Black and Robinson, 1985; Carter and Pycock, 1979; Ismailova et al., 1990; Lyness and Moore, 1981; Pranzatelli and Snodgrass, 1986a; Vergnes et al., 1988; Williams et al., 1990). Even within studies in which locomotion and/or exploration have been examined after global depletion of serotonin the results are inconsistent (Dickson and Vanderwolf, 1990; Erinoff and Snodgrass, 1986; Lyness and Moore, 1981; Pranzatelli and Snodgrass, 1986a; Stewart et al., 1979; Vanderwolf, 1989). Pranzatelli and Snodgrass (1986b) demonstrated that the habituation of locomotion is impaired during the first week after administration of 5,7-DHT—subsequently habituation of locomotion normalized. In addition, Gately et al. (1986) found 5,7-DHT-provoked changes in habituation of locomotion. Three days after 5,7-DHT administration, habituation was decreased whereas the same animals overhabituated on the 11th postoperative day. The indications that the temporal pattern (habituation) of locomotion and/or exploration may be changed in rats subjected to depletion of serotonin may provide an explanation for some of the discrepancies between the abovementioned studies. As argued elsewhere (Mogensen et al., 1994), modified habituation of exploration or locomotion can easily lead to confusing experimental results if the temporal distribution of activity within a test session is not taken into account.

In the present study we decided to examine rats suffering 5,7-DHT-provoked global serotonin depletion in the "activity cage" locomotion test and the "vertical hole-board" exploration test (Iversen and Mogensen, 1988). Because the two tests are able to discriminate relatively well between exploration and locomotion (Geoffroy and Mogensen, 1988; Iversen and Mogensen, 1984, 1988; Mogensen and Divac, 1993; Mogensen et al., 1994), comparison between 5,7-DHT-associated symptoms in the two tests should allow conclusions about the relative involvement of exploration and locomotion in the behavioural changes associated with serotonin depletion. However, as discussed above, even such tests may provide misleading results if the 5,7-DHT treatment does not change a particular behaviour per se but rather modifies the temporal distribution of the behaviour, e.g., habituation. To address this issue each test was administered as a 1-h session during which data were collected during three evenly spaced periods. This procedure made it possible to evaluate whether serotonin-depleted and normal animals display similar patterns of habituation. As mentioned above, the profile of behavioural symptoms after serotonin depletion seems to depend on the length of the delay between 5,7-DHT administration and behavioural testing. Consequently, we decided to administer both the above-described tests twice: approximately 48 h after injection of 5,7-DHT and again 14 days postoperatively.

Rats with 5,7-DHT-provoked global serotonin depletion were able to acquire a place learning task in a water maze as well as normal animals (Nilsson et al., 1988; Riekkinen et al., 1990a). The serotonin depletion was, however, able to potentiate the place learning deficits associated with lesions of the septum (Nilsson et al., 1988) or the nucleus basalis magnocellularis (Riekkinen et al., 1990a). These results emphasize the importance of serotonergic/cholinergic interactions in the brain and indicate that in the serotonin-depleted rat brain, remaining neural systems are able to mediate place learning at normal levels of proficiency. Normal place learning by serotonin-depleted rats is, however, not necessarily an indication that serotonin does not participate in mediation of the performance of this task in intact animals. Only studies in which the place learning task is acquired by the intact animal and the retention of the task (place recall) is studied after serotonin depletion can establish whether the performance of a normally acquired place learning task relies on serotonergic mechanisms. To test the hypothesis that the serotonergic system contributes significantly to mediation of the performance of a place learning task if such a task has been acquired by the normal brain, we decided to test the place recall of the two experimental groups of the present study approximately 45 h after injection of 5,7-DHT. Consequently, all animals were preoperatively trained on a place learning task in a water maze until a high proficiency of task performance had been reached. The first postoperative session was the critical test for evaluation of our hypothesis. We did, however, decide to continue training and testing of the place learning task for a total of 12 postoperative sessions. If 5,7-DHT-treated animals were impaired on the first postoperative session, subsequent sessions would allow us to study the potential functional recovery of such rats.

After lesions of a structural or neurochemical system, which in the normal brain contributes significantly to mediation of place learning, the task may still be acquired to a normal level of proficiency (e.g., Mogensen et al.,

1995a,b, 2002; Wörtwein et al., 1995). In such instances, a "recovery" may be mediated by compensatory neural mechanisms. The neurochemical and/or structural identity of such compensatory neural mechanisms may be elucidated by pharmacological and/or surgical "challenges" (see Mogensen et al., 1995b), which are administered after successful task acquisition by brain-damaged animals (for further discussion, see Mogensen et al., 1995b, 2002; Wörtwein et al., 1995). We decided to address the question of serotonergic/cholinergic interactions (e.g., Nilsson et al., 1988; Riekkinen et al., 1990a; Wenk et al., 1987) in spatial cognition by administration of pharmacological challenges during early as well as late phases of the postoperative place recall tests. The cholinergic challenge chosen was administration of the muscarinergic receptor antagonist scopolamine (intraperitoneal [ip] injection at a dose of 0.5 mg/kg body weight). Furthermore, we decided to hyperactivate (and thereby functionally impair) the catecholaminergic systems by administration of the indirect catecholaminergic agonist *d*-amphetamine (ip injection at a dose of 3.0 mg/kg body weight). Scopolamine was administered on the 2nd and 9th postoperative session while *d*-amphetamine was administered on the 3rd and 12th postoperative session. We did not expect *d*-amphetamine to significantly impair the place recall of any of the experimental groups (e.g., Mogensen et al., 1995b), and although a certain level of scopolamine-induced behavioural impairment was expected in the control group (e.g., Mogensen et al., 1995b), the most substantial behavioural impairment was expected after scopolamine administration to 5,7-DHT-treated animals (e.g., Nilsson et al., 1988; Riekkinen et al., 1990a).

After completion of the behavioural procedures, quantitative receptor assays were performed on brain homogenates from the animals of both groups. The receptors studied were the serotonin reuptake site, the noradrenaline reuptake site, the serotonergic 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptors, and the β-adrenergic receptors. The serotonin reuptake site was mainly studied to evaluate the extent to which serotonergic axon terminals had been eliminated by 5,7-DHT administration, whereas the noradrenaline transporter was measured as an index of the extent to which noradrenergic terminals had been damaged by 5,7-DHT. The quantitative assays of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and β-adrenergic receptors were performed to gain information about some of the receptor regulations that might occur after a "global" depletion of serotonin.

2. Methods

2.1. Subjects

The subjects were 16 experimentally naive male Wistar albino rats weighing approximately 250 g at the beginning of the experiment. They were housed individually in cages where commercial rat chow and water were always available. The animals' living quarters were maintained on a 12-h light/dark cycle (lights on at 0600 h). The rats were divided into two experimental groups according to the quality of their preoperative performance of the place recall task (see General procedure). The animals of one experimental group received intracerebroventricular injections of 5,7-DHT ($n = 8$); the rats of the control group were subjected to intracerebroventricular administration of a "vehicle" solution ($n = 8$). Forty-eight hours prior to the initial place learning training session all animals were dyed black on the neck.

The experimental protocol was approved by the Danish National Review Committee for the use of Animal Subjects ("Dyreforsøgstilsynet"), and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Surgery

Prior to surgery each animal was pretreated with desmethylimipramine (25 mg/kg ip, 60 min before surgery) and nomifensine (10 mg/kg ip, 30 min before surgery).

Anaesthesia was achieved by ip injections of Equithesin (3.3 mg/kg) and 1% atropine sulphate (0.9 mg/kg). Surgery was performed under clean but nonsterile conditions. The intracerebroventricular injections were performed stereotactically with the aid of an injection unit. The coordinates of the injections were 1.00 mm anterior to bregma and 0.95 mm lateral to the sagittal suture (in both hemispheres). The tip of the cannula of the injection unit was lowered to an injection position 4.20 mm ventral to the dura.

Each animal of the 5,7-DHT group received 0.2 mg 5,7-DHT dissolved in 20 μl of a 0.1% aqueous solution of ascorbic acid. Ten microliters was injected into each of the lateral ventricles (at the coordinates indicated above). For the animals of the control group, 10 μl of the ascorbic acid vehicle was injected into each of the lateral ventricles.

After each intracerebroventricular injection the cannula was allowed to remain in place for at least 10 min.

2.3. Apparatuses

2.3.1. Water maze

The water maze in which training and tests of place learning and place recall tasks were performed has been described in detail previously (e.g., Mogensen et al., 1995b) and consisted of a circular water tank measuring 1.85 m in diameter by 0.50 m in height. All parameters involving time were measured in seconds, and all distances were measured in arbitrary units ("pixels").

2.3.2. Activity cage

The activity cage was a wooden, gray box with no top. The floor measuring 40 × 25 cm was free to tilt around the midpoint of the longer axis, thereby activating two microswitches situated immediately below floor level. The pressure required to activate a microswitch (when applied at

either end of the longer axis) was 8 g. The walls were 43 cm high. The cage was situated in a dark, sound-shielded chamber and solid-state equipment recorded the activity of the microswitches.

2.3.3. Vertical hole-board

One semiopaque, 8-mm-thick wall in a 25.6-cm-wide, 26.5-cm-deep, and 22.5-cm-high opaque chamber had 54 holes (diameter 0.7 cm; arranged in six horizontal and nine vertical lines). The centre-to-centre distance between holes was horizontally and vertically 2.5 cm and diagonally 3.5 cm. The top of the box also served as door to the chamber. The floor of the chamber consisted of a wiregrid. The wall containing the holes had a grid of 3-mm-wide channels imbedded in it. Each channel had an infrared light-emitting diode (LED) at one end and a photocell at the other end. The grid was arranged in such a way that each hole contained the crossing of one horizontal and one vertical line at its centre. Nose poking would break the infrared light beams of the two channels. The photocells were connected to an interface card through which the data were collected by a computer. A detailed description of this apparatus has been published separately (Iversen and Mogensen, 1988). The hole-board apparatus was placed in a sound-shielded enclosure and all testing was performed in complete darkness.

2.4. Behavioural procedures

2.4.1. General procedure

Preoperatively, all animals were subjected to 14 daily sessions of place learning in a water maze. The 15th session (on Day 15 of the experiment) served as a "preoperative retention test"—constituting the preoperative test of place recall. Two days after the preoperative place recall test all animals were operated as described. The rats were divided into the two experimental groups on the basis of their performance during the preoperative place recall test—a procedure resulting in two groups of nearly equal preoperative performance levels. Forty-five hours after the completion of surgery all animals were subjected to the initial postoperative place recall session. The second and third postoperative tests of place recall were conducted 24 and 48 h after the initial postoperative place recall session. While the preoperative sessions of place learning/place recall as well as the initial postoperative place recall session were all preceded by ip injections of saline, the second postoperative place recall session was preceded by ip injection of scopolamine (SAD, Denmark) at a dose of 0.5 mg/kg body weight, and the third postoperative place recall session was preceded by ip injection of *d*-amphetamine (Sigma, USA) at a dose of 3.0 mg/kg body weight. All subsequent place recall sessions except the 9th and 12th were preceded by ip injections of saline. The 9th postoperative place recall session was preceded by ip injection of scopolamine (SAD) at a dose of 0.5 mg/kg body weight, while the 12th postoperative place recall session was preceded by ip injections of *d*-amphetamine (Sigma) at a dose of 3.0 mg/kg body weight. All injections were given 20 min before the place recall session. All postoperative place recall sessions were separated by intervals of 24 h.

The activity cage locomotion test and the vertical hole-board exploration test were both administered twice: 48 h and 14 days after surgery. Within both experimental groups, the order in which the two tests were administered was completely balanced.

Twenty-four hours after the last behavioural test the animals were decapitated under CO_2 anaesthesia and the brains (not including the cerebellum) were removed and kept at -80 °C until analysis.

For a summary of the timeline of the general procedure, see Table 1.

During all behavioural procedures the experimenter was kept ignorant about the group to which an individual rat belonged.

2.4.2. Place learning and place recall

The behavioural procedures were similar to those described by Mogensen et al. (1995b). Briefly, each animal was given five trials (swims) per session. Each trial had as its start position one of the locations N, S, E, or W. A given start position was not allowed to be selected on more than two trials and the start positions were otherwise randomly selected. The following parameters were considered: the total swim distance, the total duration of a swim, the average speed of a swim, the mean distance to platform, the heading angle

Table 1
Summary of the timeline of the general procedure

Day	Procedures	Injections preceding place learning/ place recall
Preoperative		
1–14	Preoperative sessions 1–14 of place learning	Saline
15	"Preoperative retention test" of place recall	Saline
16	Pause	
17	Surgery	
Postoperative		
1	Pause	
2	1st postoperative place recall session, locomotion/exploration tests	Saline
3	2nd postoperative place recall session	Scopolamine
4	3rd postoperative place recall session	*d*-Amphetamine
5	4th postoperative place recall session	Saline
6	5th postoperative place recall session	Saline
7	6th postoperative place recall session	Saline
8	7th postoperative place recall session	Saline
9	8th postoperative place recall session	Saline
10	9th postoperative place recall session	Scopolamine
11	10th postoperative place recall session	Saline
12	11th postoperative place recall session	Saline
13	12th postoperative place recall session	*d*-Amphetamine
14	Locomotion/exploration tests	

error, and the percentage of the swim duration during which the animal was found in each of the four quadrants, the percentage of the swim duration during which the animal was found in each of three maze-centred annuli, and the percentage of the swim duration spent in each of three platform-centred annuli. Twenty minutes before each session of place learning/place recall all animals received an ip injection at the volume of 1.0 ml/kg body weight. Substances injected were saline, scopolamine, and *d*-amphetamine; distribution of substances across sessions is described above.

2.4.3. Activity cage

The animal was placed in the activity cage, the door of the sound-shielded chamber closed, and the 60-min recording session was immediately started. The parameters considered in this test were the latency (in seconds) to the first microswitch activation after the initiation of the session and the number of microswitch activations ("counts") during three (10 min long) time intervals: the first 10 min of the session, the interval from the 26th to the 35th min (both included), and the final 10 min of the session.

2.4.4. Vertical hole-board

The animal was placed in the hole-board apparatus; the top of the hole-board chamber and the sound-shielded enclosure were closed, and the rat was allowed 60 min of undisturbed exploration. The apparatus automatically recorded all hole visits, storing the information about duration and position of the visit. A hole visit was registered if the animal simultaneously broke both a horizontal and a vertical infrared line, thereby also indicating the position at which the visit occurred. Previous experience indicates that almost all such visits are performed by passing the nose and, in some instances, also the mouth through a hole. From the computer files containing the data the following parameters were calculated: the latency from initiation of the session to the occurrence of the first hole visit; the total number of visits; the total time spent visiting holes; the mean duration of individual visits; the number of different holes visited; the mean number of visits per visited hole; the number of visits to the sixth (lowermost) row of holes as percentage of the total number of visits (a parameter that might reflect motoric impairments via the animal's potential inability to reach higher rows); the number of short (duration <0.5 s) visits as percentage of the total number of visits; and the number of clusters of visits (a cluster of visits is defined by the temporal proximity of consecutive visits; if the intervisit time exceeds 10.0 s, the following visit constituted the first visit of the next cluster). The 60-min session was divided into three 20-min intervals and all the abovementioned parameters were analysed separately within each of these intervals.

2.5. Receptor assays

Brain membranes were prepared as follows: The brains were homogenized with an Ultraturrax homogenizer for 10 s at three fourths of maximum speed in a buffer containing 150 mM NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA) and 50 mM Tris, pH 7.5 at 0 °C. The homogenate was centrifuged and the membranes washed with the same buffer. The drained membranes were lysed and rehomogenized and lysed in buffer containing 5 mM EDTA and 5 mM

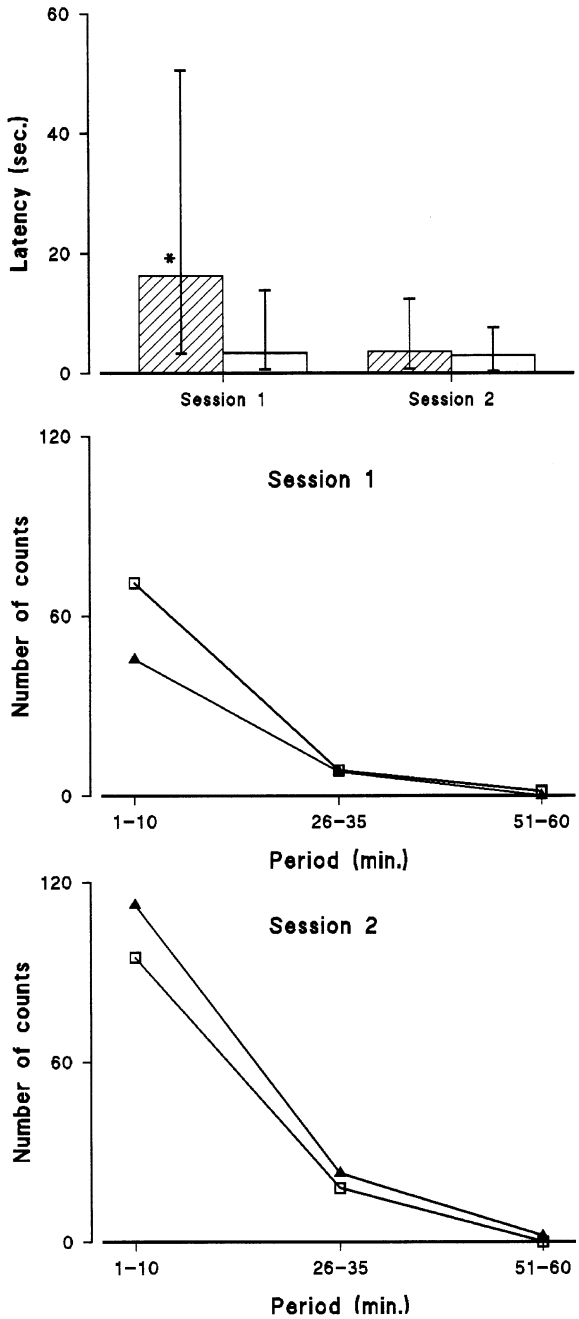

Fig. 1. Results of the activity cage tests. Upper panel: In the 5,7-DHT-treated rats, the latency to first activity count was increased 48 h but not 14 days postoperatively. Hatched bars: 5,7-DHT-treated animals. Open bars: vehicle-treated control animals. Values are given as medians (with ranges in the upper panel). * $P<.05$. Middle and lower panel: The number of activity counts accumulated during three 10-min intervals on the first (48 h) and second (14 days) postoperative sessions. Triangles: 5,7-DHT-treated animals. Squares: vehicle-treated control animals.

Tris, pH 7.5 at 0 °C. The membranes were centrifuged and washed twice with Buffer 1 containing 120 mM NaCl, 5 mM KCl, 50 mM Tris, pH 7.5. Finally, the membranes were suspended in Buffer 1 at a protein concentration of about 6 mg/ml and kept at − 80 °C until required.

Membrane protein concentrations were determined using Peterson's (1977) modification of the Lowry method. The tritiated ligands used in the receptor assays were purchased from Amersham.

Receptor binding was determined as described in Johanning et al. (1992). With respect to the 5-HT uptake site, B_{max} for [^{3}H]paroxetine binding was determined at 20 °C in a final volume of 2000 μl of Buffer 1, containing 100 μl membrane suspension and [^{3}H]paroxetine at one of six concentrations between 0.04 and 0.6 nM. Specific binding was determined by using 1 μM citalopram as the displacing agent. With respect to the 5-HT$_{1A}$ receptor, B_{max} for [^{3}H]8-OH-DPAT binding was determined at 20 °C in a final volume of 300 μl of Buffer 1 (including 5 mM $MgCl_2$) containing 50 μl membrane suspension and [^{3}H]8-OH-DPAT at one of six concentrations between 0.1 and 2.0 nM. Specific binding was determined by using 1 μM

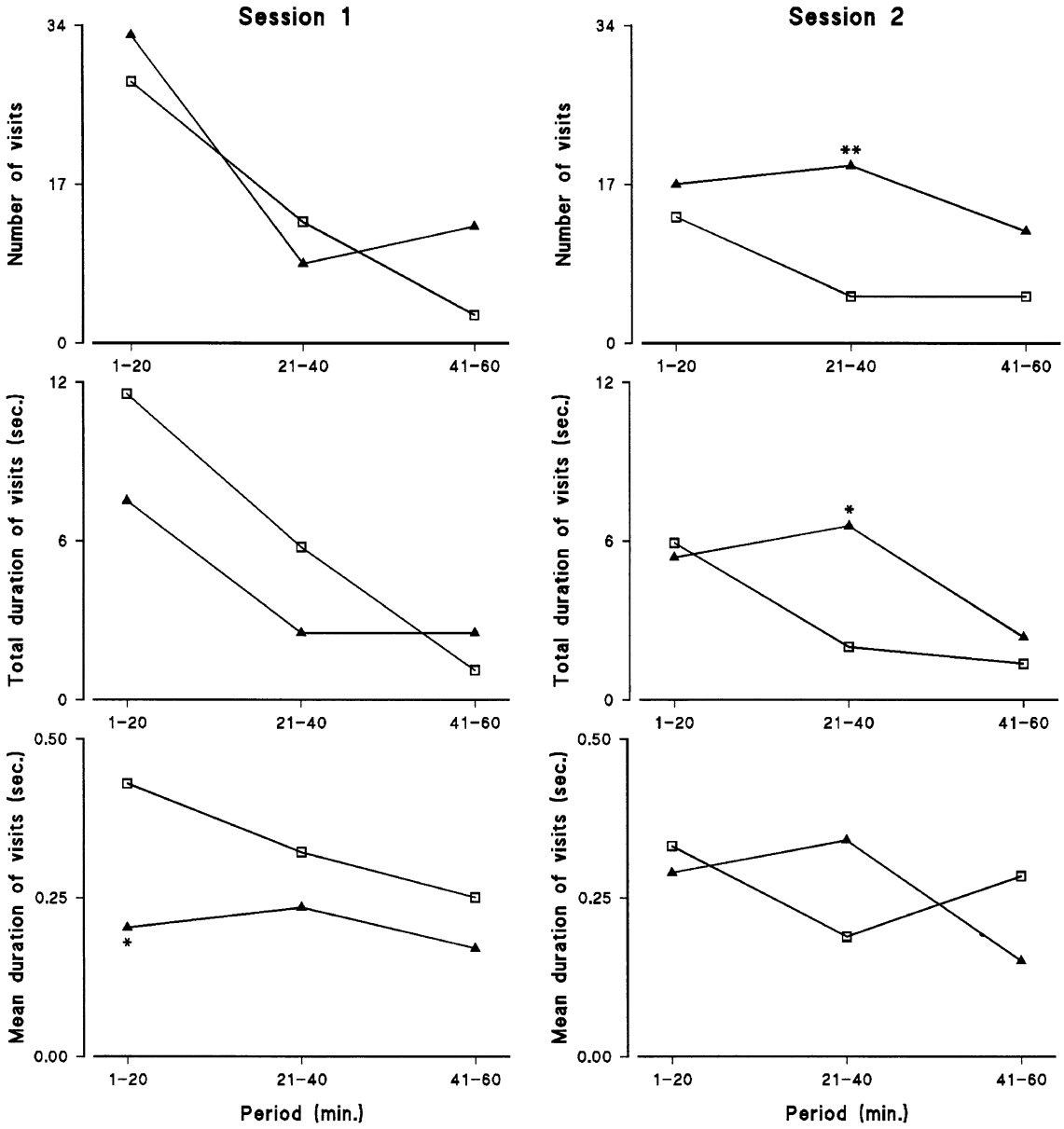

Fig. 2. Results of the vertical hole-board tests. Values are given as medians. Left column: During the first postoperative session (48 h), only the mean duration of hole visits during the first of three 20-min intervals differed significantly between 5,7-DHT-injected and vehicle-treated control animals. Right column: During the second postoperative session (14 days), both the number of hole visits and the total duration of hole visits differed between groups during the second of three 20-min intervals. Triangles: 5,7-DHT-treated animals. Squares: vehicle-treated control animals. * $P < .05$. ** $P < .01$.

buspirone as the displacing agent. With respect to the 5-HT_{1B} receptor, B_{max} for [^{3}H]5-HT binding was determined at 0 °C in a final volume of 500 μl of Buffer 1 (including 50 nM 8-OH-DPAT, 5 mM ascorbic acid, 10 μM pargyline), containing 50 μl membrane suspension and [^{3}H]5-HT at one of 10 concentrations between 0.1 and 6.0 nM. Specific binding was determined by using 1 μM RU24969 as the displacing agent. With respect to the 5-HT_{2A} receptor, B_{max} for [^{3}H]ketanserine binding was determined at 20 °C in a final volume of 300 μl of Buffer 1 (including 5 mM $MgCl_2$) containing 50 μl membrane suspension and [^{3}H]ketanserine at one of six concentrations between 0.25 and 4.0 nM. Specific binding was determined by using 10 μM mianserin as the displacing agent. With respect to the β-adrenergic receptors, B_{max} for [^{3}H]dihydroalprenolol (DHAP) binding was determined at 20 °C in a final volume of 300 μl of Buffer 1 (including 5 mM $MgCl_2$) containing 50 μl membrane suspension and [^{3}H]DHAP at one of six concentrations between 0.1 and 1.5 nM. Specific binding was determined by using 1 μM propranolol as the displacing agent. With respect to the NA uptake site, B_{max} for [^{3}H]nisoxetine binding was determined at 20 °C in a final volume of 300 μl of buffer 1 with the addition of NaCl (300 mM NaCl, 5 mM KCl, 50 mM Tris, pH 7.5), containing 50 μl membrane suspension and [^{3}H]nisoxetine at one of six concentrations between 0.2 and 4.0 nM. Specific binding was determined by using 1 μM desimipramine as the displacing agent.

2.6. Statistical analysis

Because the behavioural data could not be expected to be normally distributed nonparametric statistics were chosen for the statistical analysis. To evaluate potential effects of the administration of 5,7-DHT on place recall, separate analyses were performed for the two experimental groups comparing the performance on the last preoperative place recall session to the performance on the first postoperative place recall session (utilizing a paired design in which pre- and postoperative performances were compared within individuals) (Wilcoxon matched pairs test, two-tailed; Siegel, 1956). Potential effects of the pharmacological challenges administered on the 2nd, 3rd, 9th, and 12th postoperative place recall sessions were all evaluated by

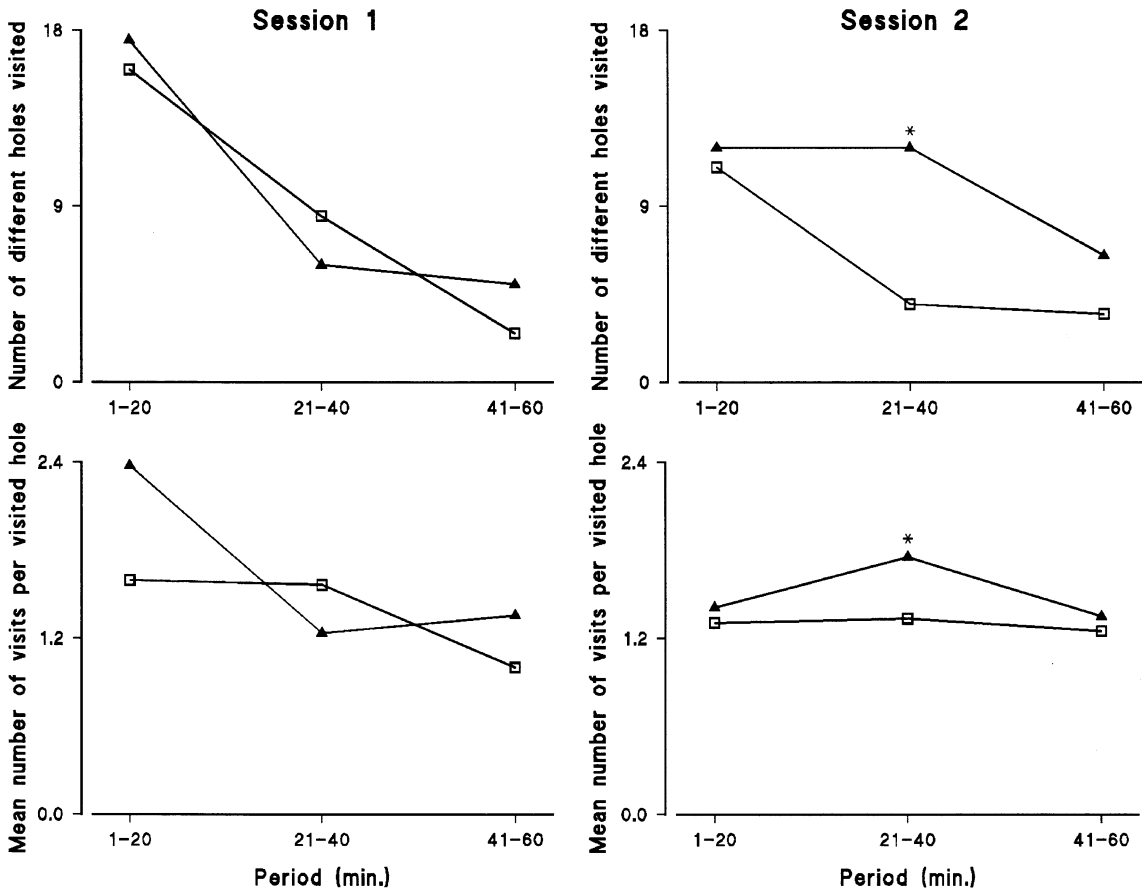

Fig. 3. Results of the vertical hole-board tests. Values are given as medians. Left column: During the first postoperative session, there were no significant differences between 5,7-DHT-treated and vehicle-treated control animals. Right column: During the second postoperative session (14 days), both the number of different holes visited and the number of visits per visited hole differed between groups during the second of three 20-min intervals. Triangles: 5,7-DHT-treated animals. Squares: vehicle-treated control animals. * $P < .05$.

within-group comparisons between performance on the relevant session of pharmacological challenge and the immediately preceding place recall session that had been preceded by saline administration. Direct comparisons between the two experimental groups (utilizing the Mann–Whitney U test, two-tailed; Siegel, 1956) were performed on three place recall sessions: the last preoperative session, the first postoperative session and the fifth postoperative session. The performance of the two experimental groups in the activity cage and vertical hole-board tests were compared using the Mann–Whitney U test, two-tailed (Siegel, 1956), for all the parameters studied.

The results of the receptor assays were analyzed by comparing the B_{max} and K_D values of the two experimental groups using t tests (two-tailed) (Winer, 1962).

3. Results

3.1. Activity cage test

The results of the two postoperative activity cage tests are illustrated in Fig. 1. The only significant group difference ($P<.05$) found occurred between the latencies to first microswitch activation of the two experimental groups on the first postoperative test (Fig. 1).

3.2. Vertical hole-board test

Aspects of the results obtained in the two postoperative vertical hole-board tests are illustrated in Figs. 2 and 3. Statistical comparisons between the two experimental groups revealed the significant group differences illustrated in Figs. 2 and 3.

3.3. Place recall

Aspects of the results from the place recall tests are illustrated in Figs. 4–6. Whereas comparison of the performance of the two experimental groups on the last preoperative and fifth postoperative place recall sessions revealed no significant group differences, a number of significant group differences were found on the first postoperative place recall session. On this session, significant group differences were found on the following parameters: the swim distance ($P<.01$), the swim dura-

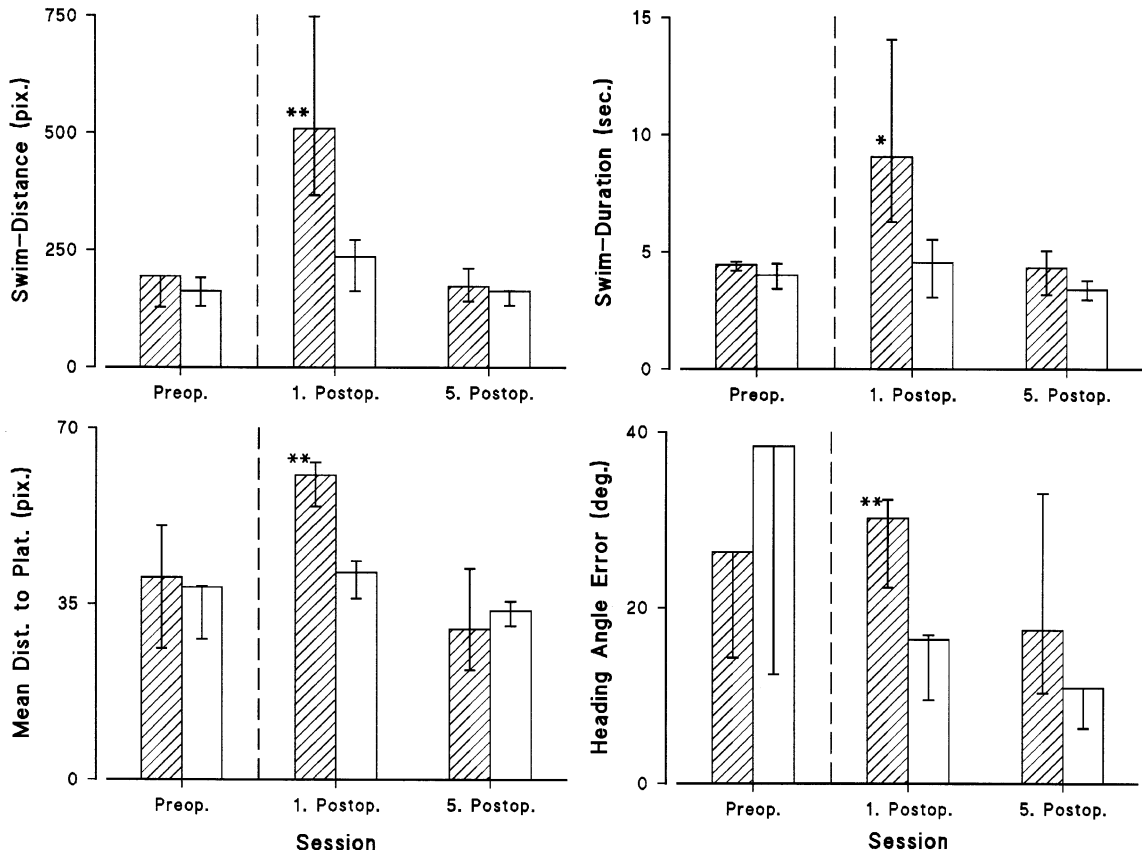

Fig. 4. Pre- and postoperative place recall. Values are given as medians with ranges. During the first postoperative place recall session, 5,7-DHT-treated animals were significantly impaired. This impairment had normalized by the fifth postoperative session. Hatched bars: 5,7-DHT-treated animals. Open bars: vehicle-treated control animals. $*P<.05$, $**P<.01$.

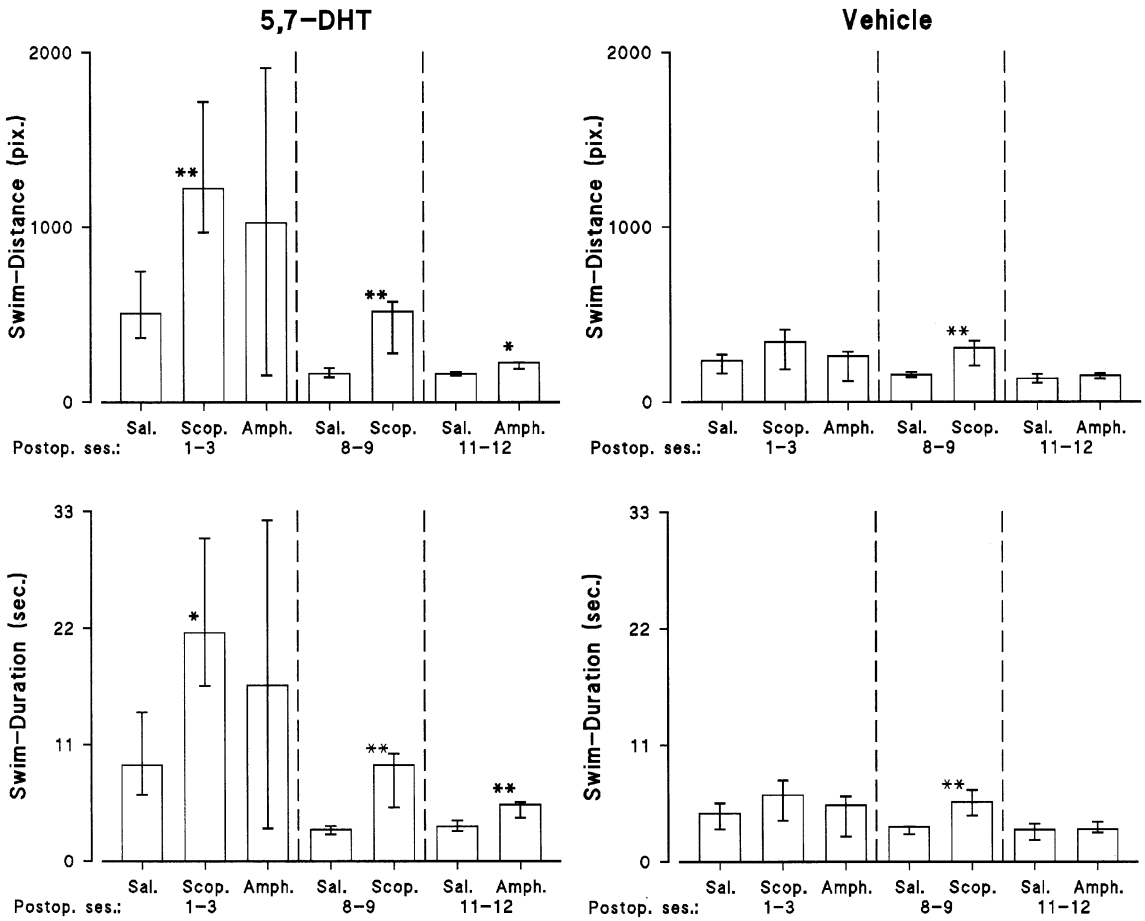

Fig. 5. The effects of scopolamine (Scop., 0.5 mg/kg. ip) and *d*-amphetamine (Amph., 3.0 mg/kg ip) on place recall. Left column: 5,7-DHT-treated animals. Right column: vehicle-treated control animals. Values are given as medians with ranges. Scopolamine affected place recall of 5,7-DHT-treated animals during early (Session 2, 72 h postoperatively) as well as late (Session 9, 10 days postoperatively) phases of testing. Scopolamine affected only the performance of the vehicle-treated control group on Session 9. *d*-Amphetamine affected only place recall of 5,7-DHT-treated animals during late phases of testing (Session 12, 13 days postoperatively) and never affected the performance of the control group. Sal.: saline. $*P<.05$, $**P<.01$.

tion ($P<.05$), the mean distance to platform ($P<.01$), the heading angle error ($P<.01$), the per-centage swim time spent in the SW quadrant ($P<.05$, the 5,7-DHT-treated animals having the higher value), the percentage swim time spent in the SE (platform-containing) quadrant ($P<.01$, the 5,7-DHT-treated animals having the lower value), the percentage swim time spent in the middle maze centred annulus ($P<.05$, the 5,7-DHT-treated animals having the lower value), the percentage swim time spent in the inner platform centred annulus ($P<.05$, the 5,7-DHT-treated animals having the lower value), and the percentage swim time spent in the outer platform centred annulus ($P<.05$, the 5,7-DHT-treated animals having the higher value).

Whereas comparisons between the last preoperative place recall session and the first postoperative place recall session of the vehicle-treated control group failed to reveal any significant surgery associated behavioural changes, similar comparisons between the immediately pre- and postoperative place recall sessions of 5,7-DHT-treated animals revealed the significant postoperative impairments, which are illustrated in Fig. 4.

The initial administration of the scopolamine challenge to the vehicle-treated control group was only associated with one significant ($P<.05$) behavioural symptom—the increased heading angle error illustrated in Fig. 6. The initial administration of the *d*-amphetamine challenge to the vehicle-treated control animals was not associated with any significant behavioural changes. The second administration of a scopolamine challenge to the vehicle-treated control group was associated with the significant symptoms illustrated in Figs. 5 and 6, as well as the following significant changes: a significantly ($P<.01$) increased percentage of the swim time spent in the NW quadrant, a significantly ($P<.01$) decreased percentage swim time spent in the SE (platform-containing) quadrant, a significantly ($P<.01$) decreased percentage swim time in the inner platform centred annulus, and a significantly ($P<.01$) increased percentage swim time in the outer platform centred annulus. The second administration of the *d*-

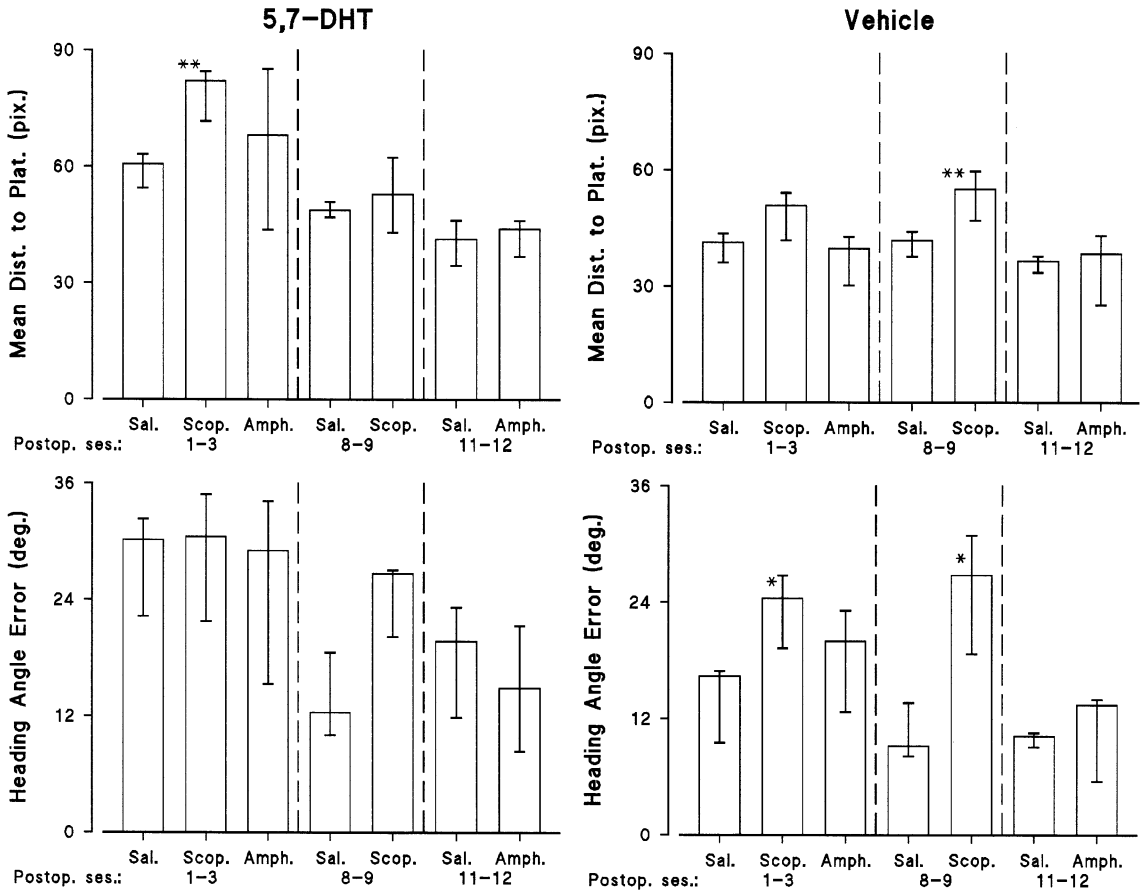

Fig. 6. The effects of scopolamine (Scop., 0.5 mg/kg ip) and *d*-amphetamine (Amph., 3.0 mg/kg ip) injection on place recall. Left column: 5,7-DHT-treated animals. Right column: vehicle-treated control animals. Values are given as medians with ranges. Scopolamine affected the quality of place recall of 5,7-DHT-treated animals during early (Session 2, 72 h postoperatively) but not late (Session 9, 10 days postoperatively) phases of testing. Scopolamine affected the quality of performance of the vehicle-treated control group during both phases of testing. *d*-Amphetamine never affected the quality of place recall of either experimental group. Sal.: saline. $*P<.05$, $**P<.01$.

amphetamine challenge to the vehicle-treated control animals was not associated with any significant behavioural changes.

The initial administration of the scopolamine challenge to the 5,7-DHT-treated group was associated with the significant behavioural changes illustrated in Figs. 5 and 6, while the initial administration of the *d*-amphetamine challenge to the 5,7-DHT-treated animals did not provoke any significant behavioural changes. The second administration of the scopolamine challenge to the 5,7-DHT-treated animals as well as the second administration of the *d*-amphetamine challenge to this experimental group were associated with the significant behavioural changes illustrated in Figs. 5 and 6.

Table 2
Results of the receptor assays

	5-HT uptake site		5-HT$_{1A}$ receptor		5-HT$_{1B}$ receptor		5-HT$_{2A}$ receptor		Beta-adrenergic receptors		Noradrenergic uptake site	
Ligand	[^{3}H]Paroxetine		[^{3}H]8-OH-DPAT		[^{3}H]5-HT		[^{3}H]Ketanserine		[^{3}H]DHAP		[^{3}H]Nisoxetine	
Displacer	Citalopram		Buspirone		RU24969		Mianserin		Propranolol		Desimipramine	
Group	5,7-DHT	Saline	5,7-DHT	Saline	5,7-DHT	Saline	5,7-DHT	Saline	5,7-DHT	Saline	5,7-DHT	Saline
B_{max} (fmol/mg protein)	49[a] (4)	471 (12)	30 (1.4)	32 (1.2)	45 (2)	52 (3)	115 (4)	119 (5)	55 (2)	53 (3)	87 (2)	92 (4)
P values	$P<.001$		NS		NS		NS		NS		NS	
K_D (nM)	0.025 (0.002)	0.033 (0.003)	1.0 (0.05)	0.9 (0.04)	0.5 (0.04)	0.6 (0.04)	1.9 (0.1)	1.7 (0.2)	0.7 (0.1)	0.7 (0.1)	2.0 (0.06)	2.1 (0.1)

[a] Values are given as mean (standard error of the mean).

3.4. Receptor assays

For the results of the receptor binding studies, see Table 2. Only the B_{max} of the serotonin reuptake site was significantly reduced ($P < .001$).

4. Discussion

The magnitude and selectivity of the 5,7-DHT-provoked lesion of the serotonergic system is emphasized by the outcome of the biochemical analyses. The elimination of 90% of the serotonin reuptake sites reflects a relatively complete lesion of the serotonergic axon terminals. The unchanged B_{max} of the noradrenaline reuptake site indicates that the lesion left the noradrenergic system intact. On the background of a 5,7-DHT-provoked 90% reduction of the B_{max} of the serotonin reuptake site, it is rather surprising to find the B_{max} of the 5-HT_{1B} receptor unaffected by the toxin, as the 5-HT_{1B} receptor is thought to act as a presynaptic autoreceptor. These results therefore suggest that the 5-HT_{1B} receptor may have additional functions, and that the majority of this receptor pool actually is not situated on serotonergic terminals. These speculations are in agreement with the distribution of 5-HT_{1B} receptor messenger RNA in the guinea pig brain (Bonaventure et al., 1998), as well as with the immunocytochemical localization of this receptor to nonserotonergic axons and endothelial cells of microvessels (Riad et al., 1998, 2000; Sari et al., 1999).

The biochemical analyses performed in the present study demonstrated the selectivity of the lesion by indicating an almost complete elimination of the serotonergic system while showing no sign of damage to the noradrenergic system. No analysis was performed within the dopaminergic system. Future studies should include such analysis to exclude the possibility of unwanted damage to dopaminergic neurons. It should, however, be remembered that at the time of 5,7-DHT injection both the dopaminergic and noradrenergic systems were protected by reuptake inhibitors.

Effects of 5,7-DHT injections were neither found on the B_{max} nor the K_D values of the studied postsynaptic receptors—neither the serotonergic 5-HT_{1A} and 5-HT_{2A} receptors nor the β-adrenergic receptors (Table 2). The present data offer only an analysis of these receptors after one postoperative survival period (21 days). Consequently, one cannot easily interpret the lack of effects of 5,7-DHT treatment upon the studied receptors. The apparent lack of postsynaptic receptor regulations is, however, rather surprising, because other manipulations that affect the availability of serotonin in the synaptic cleft are known to cause regulations of, for instance, the 5-HT_{2A} receptor (e.g., Johanning et al., 1992). Numerous serotonergic as well as nonserotonergic receptors remain to be studied and those addressed in the present experiment should be measured after a range of postoperative survival periods. It may, however, tentatively be concluded that at a point when functional results indicate that the reactive neural reorganization that is initiated by 5,7-DHT administration has progressed to such an extent that the pattern of "late" symptoms is established, there is no indication that neither the B_{max} nor the K_D of the presently studied postsynaptic serotonergic receptors are modified.

The only locomotion-associated parameter to demonstrate a significant effect of the serotonergic depletion was the latency to first count on the first activity cage session—a test which was conducted approximately 48 h postoperatively (Fig. 1). Because neither the absolute levels of locomotion nor the habituation pattern of activity in the activity cage revealed significant effects of 5,7-DHT treatment, the significantly increased latency to initiate locomotion should not necessarily be seen as an indication of a motoric impairment. It can be argued that although the number of counts in the activity cage seems to be a rather exploration-free measure of locomotor activity, the latency to initiate locomotion is potentially more influenced by the exploratory tendencies of the animal.

The vertical hole-board exploration tests indicated that even the exploratory activities of the animals were relatively unaffected by 5,7-DHT treatment when measured approximately 48 h after administration of the toxin (Figs. 2 and 3). Only the mean duration of visits during the initial 20-min period of the test session was significantly decreased by serotonin depletion. In contrast to the relatively normal locomotion, exploration—and habituation of both—48 h postoperatively, the pattern of exploratory activities measured approximately 14 days after 5,7-DHT administration differed significantly from the exploration pattern of normal animals (Figs. 2 and 3). During the second 20-min period of the vertical hole-board session administered 14 days postoperatively, the 5,7-DHT-treated animals explored the vertical hole-board significantly more than the control-treated animals. During the first and last 20-min periods of the same 1-h session, the two treatment groups did not differ significantly. The pattern of result seems to indicate that what is affected by depletion of serotonin is the habituation of exploration rather than the exploratory behaviour as such. If the 5,7-DHT-associated behavioural changes were an increase in the exploratory tendencies per se, one would have expected a hyperexploration during the initial 20-min period of the test session. Consequently, it has to be concluded that the pattern of results obtained in the present study indicates that (1) exploration rather than locomotion is affected by depletion of serotonin; (2) the 5,7-DHT-provoked modifications of exploratory behaviour reflect an impaired habituation of exploration rather than a shift in the basic level of exploratory activities; and (3) the serotonin-depletion-associated changes in habituation of exploration do not appear immediately after the lesion. As discussed in the Introduction numerous reactive processes are likely to occur after neurotoxic lesions of serotonergic axon terminals. Both the nature and time course of such reactive processes are largely unknown. Consequently, all that can presently be

stated with certainty is that although the 5,7-DHT-provoked serotonin depletion initiates the process, the serotonergic depletion per se is not sufficient to significantly impair the habituation of exploratory behaviour.

Our demonstration that habituation of exploration is impaired 2 weeks after administration of 5,7-DHT agrees with the results of Williams et al. (1990), who found a reduced habituation of exploration 16–20 days postoperatively. On the other hand, Pranzatelli and Snodgrass (1986b) and Gately et al. (1986) found impaired habituation of locomotion only within the first week after administration of 5,7-DHT. In the study by Gately et al. (1986), 5,7-DHT-treated animals were even found to overhabituate on the 11th postoperative day. On the basis of the results obtained by Pranzatelli and Snodgrass (1986b) and Gately et al. (1986), it would appear that the loss of serotonergic axon terminals may rather directly cause an overhabituation, whereas subsequent reactive processes are able to either eliminate (Pranzatelli and Snodgrass, 1986b) or reverse (Gately et al., 1986) such symptoms. On the other hand, the results of Williams et al. (1990) as well as the data from the present study indicate that the impaired habituation of exploration after administration of 5,7-DHT is the combined result of the neurotoxic elimination of serotonergic axon terminals and subsequent reactive neural processes. These discrepancies may be the result of methodological differences between studies.

On the first postoperative place recall session the task performance of the 5,7-DHT-treated rats was clearly impaired (Fig. 4). This serotonin-depletion-associated impairment of place recall did, however, disappear within a few sessions (Fig. 4). Because lesions of the central serotonergic system are able to impair the performance of an already acquired place learning task significantly, it has to be concluded that in the normal brain serotonergic mechanisms contribute significantly to the mediation of place learning and place recall. This conclusion is in apparent contrast to the results of Nilsson et al. (1988) and Riekkinen et al. (1990a), who found normal place learning in animals that had suffered serotonin depletion prior to task acquisition. As discussed in the Introduction, normal place learning in serotonin-depleted rats may indicate that either place learning is normally mediated without significant contribution from the serotonergic system or the rat brain in the absence of normal serotonergic activities can compensate for the lack of serotonergic mechanisms to such a degree that, behaviourally, a normal level of place learning is observed. Based on the present results the latter situation seems to be the scenario that most adequately can account for previous (Nilsson et al., 1988; Riekkinen et al., 1990a) and present data.

Future studies will be needed to identify the nature of the compensatory processes that are able to allow normal proficiency of place learning and place recall in serotonin-depleted rats (even in the present study normal place recall was accomplished after a number of postoperative sessions).

The importance of functional interactions between the serotonergic and cholinergic systems of the brain has previously been emphasized in the context of the mediation of place learning (e.g., Nilsson et al., 1988; Riekkinen et al., 1990a). The results obtained during the pharmacological challenges of the present study stress the degree to which the performance of a place recall task is vulnerable to simultaneous destruction or disturbance of the cholinergic and serotonergic systems. When on the second postoperative session the muscarinergic acetylcholine antagonist scopolamine (0.5 mg/kg) was administered to all animals, the performance of the normal control group suffered only marginally, whereas the 5,7-DHT-treated animals were severely impaired (Figs. 5 and 6). The subsequent administration of a similar dosage of scopolamine on the ninth postoperative session impaired the task performance of both experimental groups significantly—still, however, with a more pronounced impairment seen in the serotonin-depleted group (Figs. 5 and 6). The performance of both groups was challenged by hyperactivation of the catecholaminergic systems by administration of the indirect catecholaminergic agonist *d*-amphetamine (3.0 mg/kg) on the 3rd and 12th postoperative place recall sessions. The first of these *d*-amphetamine challenges failed to influence the performance of any group significantly. When, on the 12th postoperative place recall session, the fully recovered place recall of the 5,7-DHT-injected animals was challenged by *d*-amphetamine, a significant impairment was seen while the performance of normal animals was unaffected (Figs. 5 and 6). The impairment provoked by *d*-amphetamine in 5,7-DHT-treated animals was less pronounced than the corresponding impairment provoked by scopolamine. The performance of normal animals was never significantly affected by catecholaminergic hyperactivation. It might be argued that the outcome of our pharmacological challenges mainly demonstrates that the task performance of 5,7-DHT-injected rats is hypervulnerable to any pharmacological disturbance. Only future studies in which pharmacological challenges include the use of multiple dosages of scopolamine and *d*-amphetamine, as well as additional drugs, can clarify this point. At our present level of knowledge it is, however, tempting to speculate that the compensational neural processes, which mediate the functional recovery of place recall after lesions of the serotonergic system, receive significant contributions from the cholinergic system as well as the dopaminergic and/or noradrenergic systems of the brain. The relevance of functional interactions between the cholinergic and serotonergic systems have been emphasized by, amongst others, Nilsson et al. (1988), Riekkinen et al. (1990a), and the present study. The importance of functional interactions between the cholinergic and noradrenergic systems has been demonstrated by Decker et al. (1990) and Riekkinen et al. (1990b). In several variants of place learning tasks we have found indications of functional interactions between the cholinergic system and the catecholaminergic systems (e.g., Mogensen et al., 1995b, 2002; Wörtwein et al., 1995).

Although conclusions regarding the importance of cholinergic/serotonergic and cholinergic/noradrenergic interactions (as well as potential serotonergic/catecholaminergic interactions) in the mediation of "cognitive" tasks have to be considered preliminary at present, it is interesting to notice that the neurochemical changes seen in the brains of patients suffering Alzheimer's dementia not only include the often emphasized drastic reductions within the cholinergic system (e.g., Bowen et al., 1983; Collerton, 1986; Francis et al., 1985; Iversen et al., 1983; Perry et al., 1985), but also significant reductions of markers for the serotonergic and noradrenergic systems (e.g., Arai et al., 1984; Bondareff et al., 1982; Bowen et al., 1983; Francis et al., 1985; Gottfries et al., 1983; Iversen et al., 1983; Mann and Yates, 1983, 1986; Rasool et al., 1986; Yamamoto and Hirano, 1985). It could be imagined that the devastating cognitive impairments seen in patients suffering dementia of the Alzheimer type are not—as frequently believed—exclusively associated with disturbances within the cholinergic systems. Rather, such symptoms might be the consequences of a drastic cholinergic reduction parallelled by losses within the serotonergic and/or noradrenergic systems. While symptoms caused by a purely cholinergic degeneration might eventually be reduced or eliminated by compensatory processes, the concurrent reductions within multiple neurotransmitter defined systems may eliminate the possibility of such a functional "recovery" (Mogensen, 2003). The data obtained in the present experiment additionally hint at the possibility of cognitive reductions being associated with simultaneous disturbances within the serotonergic and catecholaminergic systems. The indirect cholinergic agonist tacrine (tetrahydroaminoacridine) is one of the relatively few drugs that have been found to somewhat improve the cognitive status of patients suffering Alzheimer's dementia (e.g., Davis et al., 1992; Farlow et al., 1992; Knapp et al., 1994; Maltby et al., 1994). In the present context it may be noted that tacrine, in addition to its indirect cholinergic agonism, seems to be able to strengthen the monoaminergic systems of the brain (Drukarch et al., 1988; Jossan et al., 1992; Robinson et al., 1989; Soininen et al., 1990; see, however, Baldwin et al., 1991).

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References

Arai H, Kosaka K, Iizuka R. Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. J Neurochem 1984;43:388–93.

Baldwin HA, De Souza RJ, Sarna GS, Murray TK, Green AR, Cross AJ. Measurements of tacrine and monoamines in brain by in vivo microdialysis argue against release of monoamines by tacrine at therapeutic doses. Br J Pharmacol 1991;103:1946–50.

Black RS, Robinson RG. Intracortical 5,7-dihydroxytryptamine depletes brain serotonin concentrations without affecting spontaneous activity. Pharmacol Biochem Behav 1985;22:327–31.

Bonaventure P, Voorn P, Luyten WH, Jurzak M, Schotte A, Leysen JE. Detailed mapping of serotonin 5-HT_{1B} and 5-HT_{1D} receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function. Neuroscience 1998;82:469–84.

Bondareff W, Mountjoy CQ, Roth M. Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus coeruleus) in senile dementia. Neurology 1982;32:164–8.

Bowen DM, Allen SJ, Benton JS, Goodhardt MJ, Haan EA, Palmer AM, et al. Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. J Neurochem 1983; 41:266–72.

Carter CJ, Pycock CJ. The effects of 5,7-dihydroxytryptamine lesions of extrapyramidal and mesolimbic sites on spontaneous motor behaviour, and amphetamine-induced stereotypy. Naunyn-Schmiedebergs Arch Pharmacol 1979;308:51–4.

Collerton D. Cholinergic function and intellectual decline in Alzheimer's disease. Neuroscience 1986;19:1–28.

Davis KL, Thal LJ, Gamzu ER, Davis CS, Woolson RF, Gracon SI, et al. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. N Engl J Med 1992;327:1253–9.

Decker MW, Gill TM, McGaugh JL. Concurrent muscarinic and β-adrenergic blockade in rats impairs place-learning in a water maze and retention of inhibitory avoidance. Brain Res 1990;513:81–5.

Dickson CT, Vanderwolf CH. Animal models of human amnesia and dementia: hippocampal and amygdala ablation compared with serotonergic and cholinergic blockade in the rat. Behav Brain Res 1990;41:215–27.

Drukarch B, Leysen JE, Stoof JC. Further analysis of the neuropharmacological profile of 9-amino-1,2,3,4-tetrahydroacridine (THA), an alleged drug for the treatment of Alzheimer's disease. Life Sci 1988;42: 1011–7.

Dyon-Laurent C, Hervé A, Sara SJ. Noradrenergic hyperactivity in hippocampus after partial denervation: pharmacological, behavioral, and electrophysiological studies. Exp Brain Res 1994;99:259–66.

Erinoff L, Snodgrass SR. Effects of adult or neonatal treatment with 6-hydroxydopamine or 5,7-dihydroxytryptamine on locomotor activity, monoamine levels, and response to caffeine. Pharmacol Biochem Behav 1986;24:1039–45.

Farlow M, Gracon SI, Hershey LA, Lewis KW, Sadowsky CH, Dolan-Ureno J. A controlled trial of tacrine in Alzheimer's disease. JAMA 1992;268:2523–9.

Francis PT, Palmer AM, Sims NR, Bowen DM, Davison AN, Esiri MM, et al. Neurochemical studies of early-onset Alzheimer's disease. Possible influence on treatment. N Engl J Med 1985;313:7–11.

Gage FH, Björklund A. Compensatory collateral sprouting of aminergic systems in the hippocampal formation following partial deafferentation. In: Isaacson RL, Pribram KH, editors. The hippocampus, vol. 3. New York: Plenum; 1986. p. 33–63.

Gage FH, Björklund A, Stenevi U. Reinnervation of the partially deafferented hippocampus by compensatory collateral sprouting from spared cholinergic and noradrenergic afferents. Brain Res 1983a;268:27–37.

Gage FH, Björklund A, Stenevi U, Dunnett SB. Functional correlates of compensatory collateral sprouting by aminergic and cholinergic afferents in the hippocampal formation. Brain Res 1983b;268:39–47.

Gage FH, Björklund A, Stenevi U. Cells of origin of the ventral cholinergic septohippocampal pathway undergoing compensatory collateral sprouting following fimbria–fornix transection. Neurosci Lett 1984;44: 211–6.

Gately PF, Segal DS, Geyer MA. The behavioral effects of depletions of brain serotonin induced by 5,7-dihydroxytryptamine vary with time after administration. Behav Neural Biol 1986;45:31–42.

Geoffroy M, Mogensen J. Differential recovery in measures of exploration/ locomotion after a single dosage of reserpine in the rat. Acta Neurobiol Exp 1988;48:263–74.

Gottfries C-G, Adolfsson R, Aquilonius S-M, Carlsson A, Eckernäs S-Å, Nordberg A, et al. Biochemical changes in dementia disorders of Alzheimer type (AD/SDAT). Neurobiol Aging 1983;4:261–71.

Iijima K, Ohtomo K, Ogawa T, Kobayashi R. The distribution of serotonin immunoreactivity in the rat locus coeruleus after intraventricular injections of either 5,6- or 5,7-dihydroxytryptamine with special reference to serotonin synthesis. Acta Histochem 1990;89:141–56.

Ismailova KhYu, Gasanov GG, Semenova TP, Bobkova NV, Nesterova IV, Gromova EA. Influence of local injection of 5,7-DHT and 6-OH-DA into the neocortex on learning and exploratory behavior of rats in the open field. Neurosci Behav Physiol 1990;20:493–9.

Iversen IH, Mogensen J. *d*-Amphetamine affects one-trial appetitive conditioning in rats. Abstract published at Association for Behavior Analysis: Tenth Annual Convention, Nashville, Tennessee; 1984.

Iversen IH, Mogensen J. A multipurpose vertical holeboard with automated recording of spatial and temporal response patterns for rodents. J Neurosci Methods 1988;25:251–63.

Iversen LL, Rossor MN, Reynolds GP, Hills R, Roth M, Mountjoy CQ, et al. Loss of pigmented dopamine-B-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type. Neurosci Lett 1983;39:95–100.

Johanning H, Plenge P, Mellerup E. Serotonin receptors in the brain of rats treated chronically with imipramine or RU24969: support for the 5-HT_{1B} receptor being a 5-HT autoreceptor. Pharmacol Toxicol 1992; 70:131–4.

Jossan SS, Adem A, Winblad B, Oreland L. Characterisation of dopamine and serotonin uptake inhibitory effects of tetrahydroaminoacridine in rat brain. Pharmacol Toxicol 1992;71:213–5.

Knapp MJ, Knopman DS, Solomon PR, Pendlebury WW, Davis CS, Gracon SI. A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. JAMA 1994;271:985–91.

Loy R, Moore RY. Anomalous innervation of the hippocampal formation by peripheral sympathetic axons following mechanical injury. Exp Neurol 1977;57:645–50.

Lyness WH, Moore KE. Destruction of 5-hydroxytryptaminergic neurons and the dynamics of dopamine in nucleus accumbens septi and other forebrain regions of the rat. Neuropharmacology 1981;20:327–34.

Maltby N, Broe GA, Creasey H, Jorm AF, Christensen H, Brooks WS. Efficacy of tacrine and lecithin in mild to moderate Alzheimer's disease: double blind trial. Br Med J 1994;308:879–83.

Mann DMA, Yates PO. Serotonin nerve cells in Alzheimer's disease. J Neurol Neurosurg Psychiatry 1983;46:96.

Mann DMA, Yates PO. Neurotransmitter deficits in Alzheimer's disease and in other dementing disorders. Hum Neurobiol 1986;5:147–58.

Mogensen J. Animal models in neuroscience. In: Hau J, van Hoosier GL, editors. Handbook of laboratory animal science. 2nd ed., vol. II. Animal models. Boca Raton (FL): CRC Press LLC; 2003. p. 95–109.

Mogensen J, Divac I. Behavioural changes after ablation of subdivisions of the rat prefrontal cortex. Acta Neurobiol Exp 1993;53:439–49.

Mogensen J, Pedersen TK, Holm S. Effects of chronic imipramine on exploration, locomotion, and food/water intake in rats. Pharmacol Biochem Behav 1994;47:427–35.

Mogensen J, Hasman A, Wörtwein G. Place learning during inhibition of nitric oxide synthase in the rat. Homeostasis 1995a;36:12–8.

Mogensen J, Wörtwein G, Hasman A, Nielsen P, Wang Q. Functional and neurochemical profile of place learning after L-nitro-arginine in the rat. Neurobiol Learn Mem 1995b;63:54–65.

Mogensen J, Christensen LH, Johansson A, Wörtwein G, Bang LE, Holm S. Place learning in scopolamine treated rats: the roles of distal cues and catecholaminergic mediation. Neurobiol Learn Mem 2002;78: 139–66.

Nilsson OG, Strecker RE, Daszuta A, Björklund A. Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. Brain Res 1988;453:235–46.

Perry EK, Curtis M, Dick DJ, Candy JM, Atack JR, Bloxham CA, et al. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. J Neurol Neurosurg Psychiatry 1985;48:413–21.

Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83:346–56.

Pranzatelli MR, Snodgrass SR. Enhanced selective 5-HT depletions in the DHT rat model: denervation supersensitivity and recovery of function. Psychopharmacology 1986a;89:449–55.

Pranzatelli MR, Snodgrass SR. Motor habituation in the DHT model: bin analysis of daytime and nocturnal locomotor activity. Pharmacol Biochem Behav 1986b;24:1679–86.

Rasool CG, Svendsen CN, Selkoe DJ. Neurofibrillary degeneration of cholinergic and noncholinergic neurons of the basal forebrain in Alzheimer's disease. Ann Neurol 1986;20:482–8.

Riad M, Tong XK, el Mestikawy S, Hamon M, Hamel E, Descarries L. Endothelial expression of the 5-hydroxytryptamine$_{1B}$ antimigraine drug receptor in rat and human brain microvessels. Neuroscience 1998;86: 1031–5.

Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, et al. Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. J Comp Neurol 2000;417:181–94.

Riekkinen P, Sirviö J, Riekkinen P. Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. Brain Res 1990a; 527:342–5.

Riekkinen P, Sirviö J, Valjakka A, Pitkänen A, Partanen J, Riekkinen P. The effects of concurrent manipulations of cholinergic and noradrenergic systems on neocortical EEG and spatial learning. Behav Neural Biol 1990b;54:204–10.

Robinson TN, De Souza RJ, Cross AJ, Green AR. The mechanism of tetrahydroaminoacridine-evoked release of endogenous 5-hydroxytryptamine and dopamine from rat brain tissue prisms. Br J Pharmacol 1989;98:1127–36.

Sara SJ. Noradrenergic–cholinergic interactions: its possible role in memory dysfunction associated with senile dementia. Arch Gerontol Geriatr 1989;(Suppl 1):99–108.

Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M, et al. Cellular and subcellular localization of 5-hydroxytryptamine$_{1B}$ receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. Neuroscience 1999;88:899–915.

Siegel S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.

Soininen H, Unni L, Shillcutt S. Effect of acute and chronic cholinesterase inhibition on biogenic amines in rat brain. Neurochem Res 1990;15: 1185–90.

Stenevi U, Björklund A. Growth of vascular sympathetic axons into the hippocampus after lesions of the septo-hippocampal pathway: a pitfall in brain lesion studies. Neurosci Lett 1978;7:219–24.

Stewart RM, Campbell A, Sperk G, Baldessarini RJ. Receptor mechanisms in increased sensitivity to serotonin agonists after dihydroxytryptamine shown by electronic monitoring of muscle twitches in the rat. Psychopharmacology 1979;60:281–9.

Vanderwolf CH. A general role for serotonin in the control of behavior: studies with intracerebral 5,7-dihydroxytryptamine. Brain Res 1989; 504:192–8.

Vergnes M, Depaulis A, Boehrer A, Kempf E. Selective increase of offen-

sive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. Behav Brain Res 1988;29:85–91.

Wenk G, Hughey D, Boundy V, Kim A, Walker L, Olton D. Neurotransmitters and memory: role of cholinergic, serotonergic, and noradrenergic systems. Behav Neurosci 1987;101:325–32.

Williams JH, Meara JR, Azmitia EC. Effects of 5,7-dihydroxytryptamine injections in the fornix–fimbria on locomotor activity in photocell cages and the open field. Behav Brain Res 1990;40:37–44.

Winer BJ. Statistical principles in experimental design. New York: McGraw-Hill; 1962.

Wörtwein G, Særup LH, Charlottenfeld-Starpov D, Mogensen J. Place learning by fimbria–fornix transected rats in a modified water maze. Int J Neurosci 1995;82:71–81.

Yamamoto T, Hirano A. Nucleus raphe dorsalis in Alzheimer's disease: neurofibrillary tangles and loss of large neurons. Ann Neurol 1985; 17:573–7.