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Combined 192 IgG-saporin and 5,7-dihydroxytryptamine lesions in the male rat brain: A neurochemical and behavioral study

Olivia Lehmann, Hélène Jeltsch, Christine Lazarus, Laurent Tritschler, Fabrice Bertrand, Jean-Christophe Cassel*

LN2C, UMR 7521 CNRS/Université Louis Pasteur, IFR de Neurosciences 37, 12 rue Goethe, F-67000 Strasbourg, France

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Abstract

In a previous experiment [Eur J Neurosci 12 (2000) 79], combined intracerebroventricular injections of 5,7-dihydroxytryptamine (5,7-DHT; 150 μg) and 192 IgG-saporin (2 μg) in female rats produced working memory impairments, which neither single lesion induced. In the present experiment, we report on an identical approach in male rats. Behavioral variables were locomotor activity, T-maze alternation, beam-walking, Morris water-maze (working and reference memory) and radial-maze performances. 192 IgG-saporin reduced cholinergic markers in the frontoparietal cortex and the hippocampus. 5,7-DHT lesions reduced serotonergic markers in the cortex, hippocampus and striatum. Cholinergic lesions induced motor deficits, hyperactivity and reduced T-maze alternation, but had no other effect. Serotonergic lesions only produced hyperactivity and reduced T-maze alternation. Beside the deficits due to cholinergic lesions, rats with combined lesions also showed impaired radial-maze performances. We confirm that 192 IgG-saporin and 5,7-DHT injections can be combined to produce concomitant damage to cholinergic and serotonergic neurons in the brain. In female rats, this technique enabled to show that interactions between serotonergic and basal forebrain cholinergic mechanisms play an important role in cognitive functions. The results of the present experiment in male rats are not as clear-cut, although they are not in obvious contradiction with our previous results in females.

Keywords: 5,7-DHT; 192 IgG-saporin; Basal forebrain; Beam-walking; Locomotion; Memory; Radial maze; Raphe; Motor; T-maze; Sex; Water maze

1. Introduction

Clinical observations (e.g., Bartus et al., 1985) and experimental data (e.g., Collerton, 1986; Smith, 1988) support the view that central cholinergic functions and memory processes are intimately connected. Nevertheless, recent studies based on specific cholinergic lesion techniques suggest that this view is probably too reductionistic (e.g., Dunnett et al., 1991; McAlonan et al., 1995a,b; Wrenn and Wiley, 1998). In most studies, which selectively damaged the basal forebrain cholinergic neurons with 192 IgG-saporin, a monoclonal antibody to the $p75^{NGF}$ receptor coupled to the ribosomal toxin saporin (Wiley et al., 1991), a severe depletion of cholinergic markers, was evidenced in the hippocampus and/or neocortex, but the impairment of spatial memory capabilities was modest (e.g., Baxter and Gallagher, 1996; Baxter et al., 1995; Berger-Sweeney et al., 1994; Chappell et al., 1998; Dornan et al., 1997; Leanza et al., 1996; McMahan et al., 1997; Waite et al., 1995, 1999; Walsh et al., 1995). Although cholinergic processes are considered crucial in learning and memory, other neurotransmitter systems (e.g., GABAergic, noradrenergic, serotonergic, etc.) may also participate in these functions (e.g., Decker and McGaugh, 1991; Levin et al., 1990; Wenk et al., 1987).

The functional involvement of interactions between cholinergic and serotonergic mechanisms in the brain have been reviewed (e.g., Cassel and Jeltsch, 1995; Sirviö et al., 1994; Steckler and Sahgal, 1995). One method to examine these interactions is to combine neurotoxins selective to each system, for instance 5,7-dihydroxytryptamine (5,7-DHT, a serotonergic toxin) and 192 IgG-saporin.

* Corresponding author. Tel.: +33-390-241-952; fax: +33-390-241-958.

E-mail address: jean-christophe.cassel@psycho-ulp.u-strasbg.fr (J.-C. Cassel).

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Table 1
Time-line for the series of experiments given in days after the last surgery

Variable assessed or experimental step	Time in days
Home-cage activity	14
Beam-walking test	26–30 (a.m.)
Forced T-maze alternation	26–30 (p.m.)
Morris water-maze test (reference memory)	32–36
Morris water-maze test (visible platform)	37–38
Morris water-maze test (working memory)	39–43
Reduction of body weight and radial-maze training	43–58
Radial-maze test	61–91
Sacrifice (microwave irradiation)	96, 97
Sacrifice (perfusion for AChE staining)	97

When both toxins were injected intracerebroventricularly, we found that they induced cholinergic and serotonergic depletions, as well as cognitive impairments in T-maze, radial-maze and water-maze tasks (Lehmann et al., 2000). Conversely, 5,7-DHT or 192 IgG-saporin lesions did not induce such impairments, although 192 IgG-saporin altered sensorimotor capabilities, and 5,7-DHT increased locomotor activity (Lehmann et al., 2000). These data were obtained in adult female rats. In the literature, there is evidence that cognitive capabilities (e.g., Andrews, 1996; Beatty, 1979, 1992; Ghi et al., 1999; Harrell and Parsons, 1988; but see Bucci et al., 1995; Juraska et al., 1984), strategies used to solve a problem in a given task (e.g., Kanit et al., 1998; Lebowitz and Brown, 1999; Roof and Stein, 1999; Tropp and Marcus, 2001), alterations of cognitive functions during aging (Luine et al., 1986; Lukoyanov et al., 1999), effects of pharmacological substances (Berger-Sweeney et al., 1995; Van Hest et al., 1990) or even of lesions in the brain (Lipsey and Robinson, 1986; Roof and Hall, 2000; Roof et al., 1993) may vary according to the sex. Due to sex-dependent differences in numerous aspects of behavioral performances, pharmacological sensitivity, lesion effects, cholinergic and serotonergic mechanisms, we wondered whether our findings in female rats could be replicated in males. A recent report suggested no sex dependency of the neural and behavioral effects of 192 IgG-saporin (Sherren et al., 1999), but the lesions were made in neonates (see also McGaughy and Sarter, 1999). In the present experiment, male rats were injected intracerebroventricularly with 150 μg of 5,7-DHT, 2 μg of 192 IgG-saporin or with both toxins (150 + 2 μg). Behavioral evaluations were performed in the same order and for approximately the same period durations as in the study by Lehmann et al. (2000). Thus, because basal forebrain damage induces alterations in locomotor activity and spatial as well as nonspatial working- or reference-memory performances, we measured home-cage locomotion, T-maze alternation and both water- and radial-maze performances. As intracerebroventricular 192 IgG-saporin reaches the cerebellum and, there, alters Purkinje cells (e.g., Waite et al., 1995, 1999), we also assessed sensorimotor functions using a beam-walking test. After completion of behavioral testing, all rats were sacrificed by microwave irradiation for neurochemical determinations in the cortex, the hippocampus and the striatum, which are all important targets of central cholinergic and serotonergic innervation systems. The extent of the cholinergic damage was also assessed on material stained histochemically for acetylcholinesterase (AChE).

2. Materials and methods

2.1. Subjects and design

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; permission #6212 to J-C.C. and 6714-bis to H.J., O.L., L.T., F.B. and C.L. under the former's responsibility) and international (NIH publication, No. 85-23, revised 1985) policies.

The study used 49 male Long–Evans rats (R. Janvier, France). At approximately 90 days of age (316 g ± 2), the rats were allocated randomly to one of four experimental groups constituted by sham-operated control rats (SHAM; $n = 11$), rats with only 192 IgG-saporin lesions (SAPO; $n = 12$), rats with only 5,7-DHT lesions (DHT; $n = 12$) or rats with both 5,7-DHT and 192 IgG-saporin lesions (DHT + SAPO; $n = 14$). The rats were housed singly in transparent Makrolon cages ($42 \times 26 \times 15$ cm) under a 12:12 h dark–light cycle (lights on at 0700 h), with ad libitum access to food (except during radial-maze testing) and water throughout the experiment. The colony and testing rooms were under controlled temperature (21 °C).

2.2. Surgeries

All surgical procedures were conducted under aseptic conditions, using pentobarbital anaesthesia (65 mg/kg ip). Injections into the lateral ventricles were performed stereotaxically through a 2-μl Hamilton syringe at the following coordinates (in mm): A: −0.8 (from Bregma), L: ±1.4 (from the midline), V: −4.3 (from Bregma), with the incisor bar set at the level of the interaural line (Paxinos and Watson, 1986). After each injection, the needle was left in situ for 5 min, retracted on 2 mm, and a second delay of 4 min was allowed before complete retraction.

Table 2
Experimental groups and sample size for behavioral, neurochemical and histochemical assessments

Treatment	Behavior*	Neurochemistry	Histochemistry
SHAM	11	10	1
SAPO	11	10	1
DHT	11	9	2
SAPO + DHT	13	10	3

* Except for T-maze alternation ($n = 11$, 11, 10 and 13, respectively).

All rats sustaining a 5,7-DHT lesion were pretreated with desipramine (25 mg/kg ip in saline; Sigma, St. Louis, USA) in order to protect the noradrenergic system (e.g., Björklund et al., 1975). As a control, all other rats were also injected with desipramine. Twelve rats were injected (4.5 μl/ventricle) with 150 μg of 5,7-DHT (creatinine sulphate salt; 338 μg dissolved in 20 μl of physiological saline containing 20 mg/ml ascorbic acid; Sigma). Twelve rats were lesioned using 2 μg/rat of the immunotoxin 192 IgG-saporin (1 μl/ventricle, concentration 1 μg/μl of phosphate-buffered saline). The 14 rats with combined lesions were first subjected to intracerebroventricular injections of 150 μg of 5,7-DHT and, 8 days later, received 2 μg of 192 IgG-saporin. In 11 sham-operated rats, a volume of 4.5 μl of phosphate-buffered saline ($n=6$) or saline ($n=5$) was injected into each ventricle. All rats from the SHAM, SAPO and DHT groups were subjected to a second sham-operation 8 days after their first surgery.

2.3. Behavioral studies

Behavioral studies began about 14 days after the second surgery. A time-line of the experiments is shown in Table 1. As the behavioural evaluation methods were the same as in our previous experiment, they are only briefly described herein.

2.3.1. Home-cage activity

The test was performed 14 days after the last surgery. The spontaneous activity of the rats was recorded over 24 h in their home cage (3 h of habituation, 9 h diurnal and 12 h nocturnal periods) as described in the previous study on females (Lehmann et al., 2000).

2.3.2. Beam-walking test

This test was run in the morning from the 26th to the 30th day after the last surgery. A qualitative assessment of sensorimotor coordination was performed by placing each rat on a 2.5 × 200 cm wooden beam elevated 80 cm above the floor level, divided into four virtual 50-cm segments and which was connected to the home cage. All rats were trained and tested according to the protocol described in the previous experiment (Lehmann et al., 2000). For the test, a score of 0 was given for each beam segment (50 cm) on which the rat slipped or placed its toes on the side surface. Otherwise, it was quoted 1. The overall score was calculated by adding the scores from three last runs (max-

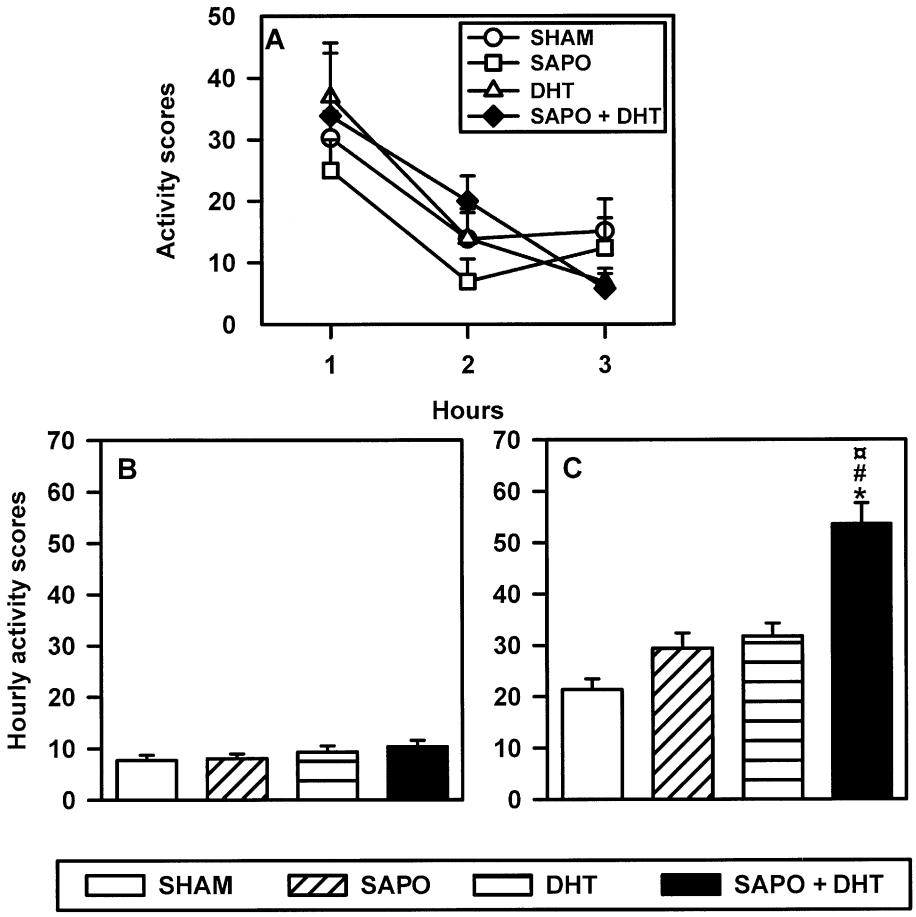

Fig. 1. Mean (±S.E.M.) hourly activity scores recorded during 3 h of habituation (A), the diurnal (B) and the nocturnal (C) period of the cycle in rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO + DHT). Statistics: significantly different from SHAM, $*P<.05$; significantly different from SAPO, $^{\#}P<.05$; significantly different from DHT, $^{¤}P<.05$.

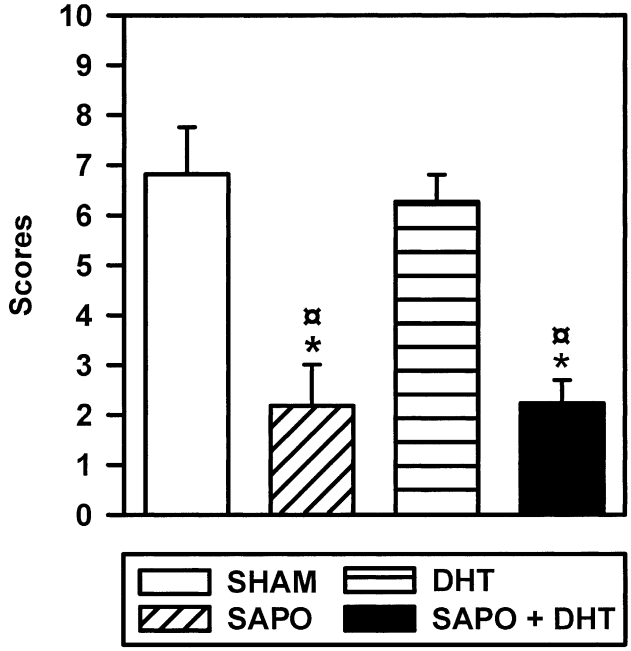

Fig. 2. Mean (±S.E.M.) motor beam-walking scores in rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO + DHT). Statistics: significantly different from SHAM, $*P<.05$; significantly different from SAPO, $¤P<.05$.

imal score = 12). The experimenter was not aware of the rat's surgical treatment.

2.3.3. Forced T-maze alternation

This test was run in the afternoon from the 26th to the 30th day after the last surgery. The apparatus and the protocol were identical to those described by Lehmann et al. (2000). Two trials (one trial consisting in a forced run followed by a test run) were given each day during 5 days, so that 10 possible alternations were tested for each rat over the 5-day period of testing. The intertrial interval on each day lasted for about 2 h.

2.3.4. Morris water maze

This test, run from the 32nd to the 43rd postsurgical day, was performed using two procedures, one placing emphasis on reference memory, the other on working memory. The apparatus and the testing protocols were identical to those described by Lehmann et al. (2000) with one exception: a test with a visible platform was performed on 2 days between reference and working memory testing.

2.3.5. Radial arm maze

Training and testing were run as described by Lehmann et al. (2000), using two identical radial mazes (half the rats on each) placed in the same room. Before training, the body weight of all rats was reduced progressively (over 10 days, starting on the 43rd day) and subsequently maintained at about 80% of the free-feeding value. Water was available ad libitum. All rats were habituated to eat the food pellets (45 mg, Noyes, distributed by Sandow Scientific, UK) in the maze on 5 consecutive days according to a training schedule described in detail by Jeltsch et al. (1994). Following training, all rats were tested for a series of 24 trials between the 61st and 91st day after the last surgery. The testing procedure was standard (Olton and Samuelson, 1976).

2.4. Neurochemical determinations

2.4.1. Tissue preparations

On the 96th or 97th day after lesion surgery (see Table 1), part of the rats from each group (SHAM: $n=10$; SAPO: $n=10$; DHT: $n=9$; SAPO + DHT: $n=10$) were sacrificed by microwave irradiation (2.0 s; 6.3 kW; Sairem, Villeurbanne, France) in order to rapidly inactivate brain enzymes such as AChE (Stavinoha et al., 1973). The brains were prepared as described by Lehmann et al. (2000). Concentration of acetylcholine (ACh), dopamine (DA), noradrenaline (NA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (Lehmann et al., 2000).

2.5. Histochemistry

The rats not sacrificed for neurochemical determinations were injected with an overdose of pentobarbital (100 mg/kg ip; Sanofi, France) and their brain processed for AChE-staining as previously described (Lehmann et al., 2000).

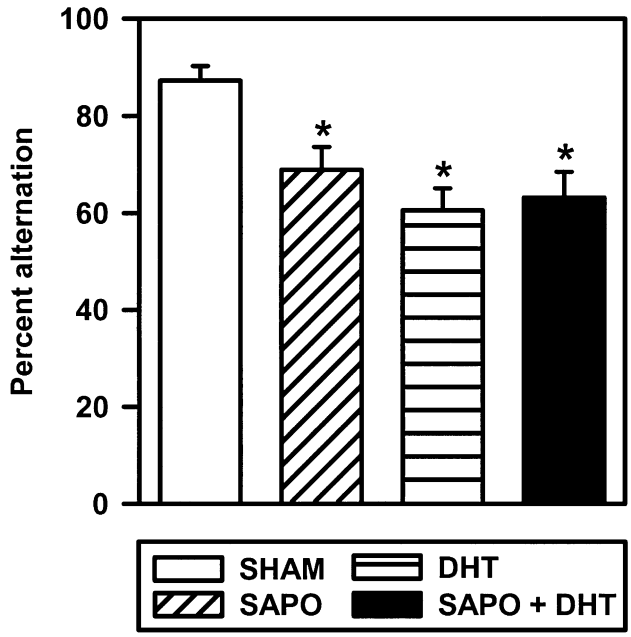

Fig. 3. Mean (±S.E.M.) percentage of alternations in the forced T-maze alternation test in rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO + DHT). Statistics: significantly different from SHAM, $*P<.05$.

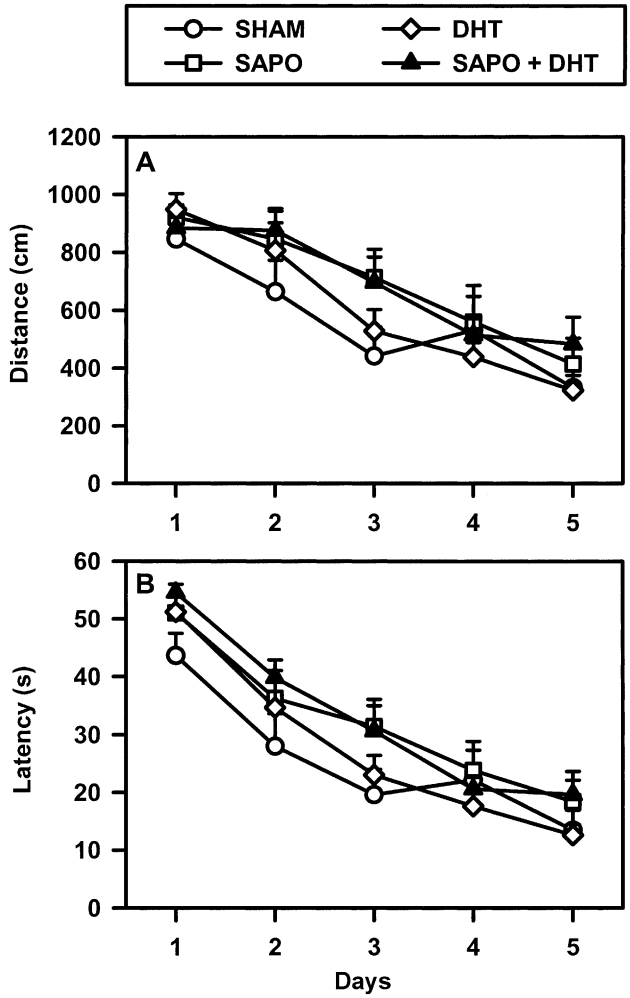

Fig. 4. Mean (± S.E.M.) distances (A) and latencies (B) to reach the platform in the water-maze test made according to a protocol placing emphasis on reference memory. Group abbreviations refer to rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO + DHT).

2.6. Statistical analysis

Due to health problems, three rats were discarded from the study (one from each lesion group). All data were analysed by analysis of variance (ANOVA) followed, where appropriate, by 2 × 2 comparisons based on the Newman–Keuls multiple range test (Winer, 1971). The number of rats in each group used for behavioral, neurochemical and histochemical determinations is shown in Table 2.

3. Results

3.1. Behavioral data

3.1.1. Home-cage activity

Data are shown in Fig. 1. ANOVA of the scores recorded during the habituation period (Fig. 1A) only showed a significant Hour effect [$F(2,84) = 16.89$, $P < .001$] due to an overall activity, which was significantly higher during the first hour than during both subsequent ones ($P < .001$). ANOVA of the diurnal activity scores (Fig. 1B) failed to show a significant Group effect [$F(3,42) = 1.19$]. ANOVA of the nocturnal activity scores (Fig. 1C) showed a significant Group effect [$F(3,42) = 8.77$, $P < .001$] due to activity levels that were significantly higher in the SAPO + DHT group as compared to each of the three other groups ($P < .01$, at least).

3.1.2. Beam-walking

Data are illustrated in Fig. 2. ANOVA showed a significant Group effect [$F(3,42) = 12.70$, $P < .001$], which was due to a significant reduction of motor performances in SAPO and SAPO + DHT rats as compared to SHAM or DHT rats ($P < .001$, in all cases).

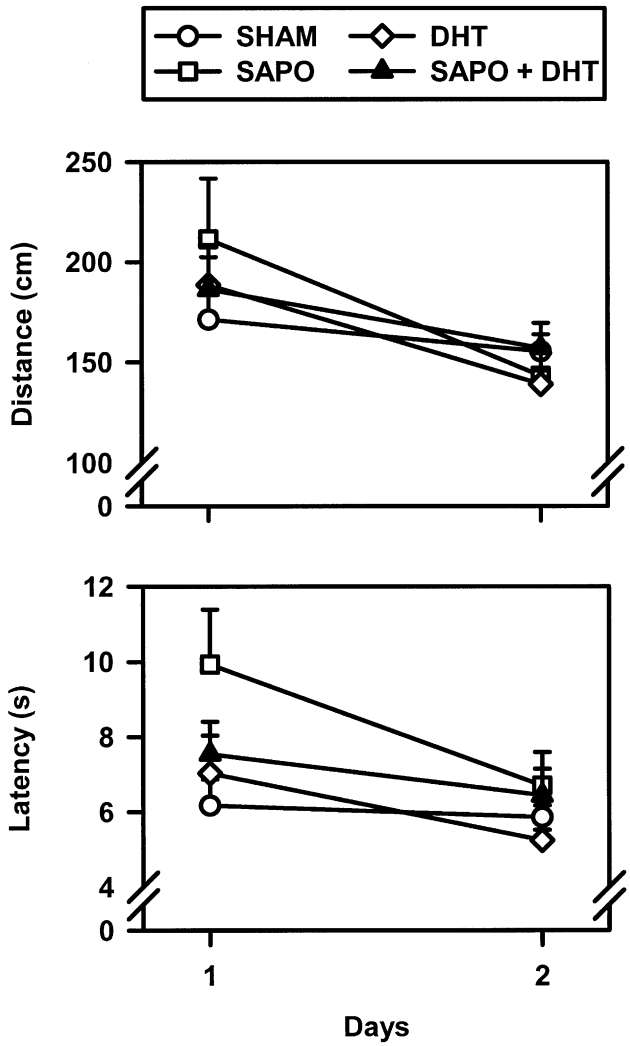

Fig. 5. Mean (± S.E.M.) distances (A) and latencies (B) to reach the platform in the visible platform water-maze test. Group abbreviations refer to rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO + DHT).

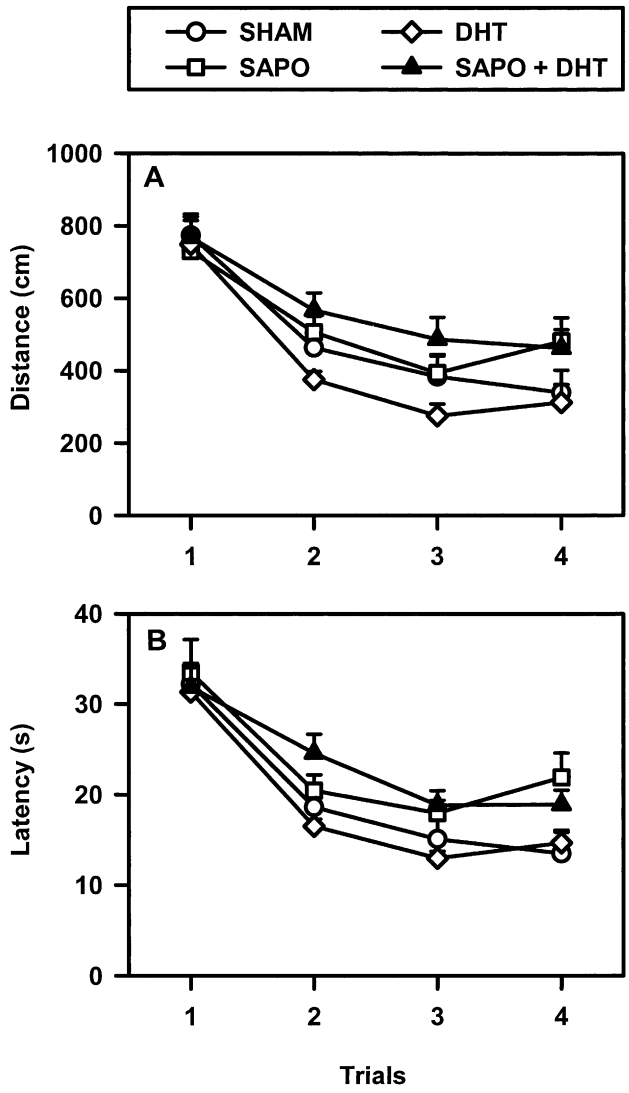

Fig. 6. Mean (±S.E.M.) distances (A) and latencies (B) to reach the platform in the water-maze test made according to a protocol placing emphasis on working memory. Group abbreviations refer to rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO+DHT).

3.1.3. Forced alternation

Data are shown in Fig. 3. One rat (group 5,7-DHT), which performed all other tasks, did not complete the alternation task. ANOVA of the alternation rates of the remaining rats showed a significant Group effect [$F(3,41) = 6.71$, $P < .01$] due to significantly lower alternation rates in all three lesion groups as compared to SHAM rats ($P < .01$, at least).

3.1.4. Water-maze test, reference memory

Data are shown in Fig. 4. ANOVA of the latencies only showed a significant Day effect [$F(4,168) = 87.59$, $P < .001$] due to overall latencies, which decreased significantly over days ($P < .05$, in all cases). ANOVA of the distances yielded a strictly comparable picture. An analysis of the overall swim velocity (cm/s) indicated that there was no significant difference among the four groups (not illustrated). Finally, in the probe trial, there was no significant Group effect on time spent or distance swum in the probe quadrant (not illustrated).

3.1.5. Water-maze test, visible platform

Data are shown in Fig. 5. ANOVA of the latencies only showed a significant Day effect [$F(1,42) = 14.19$, $P < .001$] due to overall latencies which decreased significantly from Days 1 to 2 ($P < .001$). ANOVA of the distances yielded a strictly comparable picture.

3.1.6. Water-maze test, working memory

Data are shown in Fig. 6. ANOVA of the latencies showed significant Group [$F(3,42) = 3.54$, $P < .05$] and Trial effects [$F(3,126) = 47.46$, $P < .001$], but no significant interaction [$F(9,126) = 0.96$]. The Group effect was due to overall latencies, which were significantly longer in SAPO and SAPO+DHT rats ($P < .05$), but only when compared to DHT rats. The Trial effect was due to a decrease of the overall latencies in Trials 2, 3 and 4 as compared to the first trial ($P < .001$, in all cases). As a single start place was used for all daily trials, the working memory component of the task was mainly in the comparison between Trials 1 and 2 (first matching-to-position); performances on Trials 3 and 4 rather accounted for procedural memory. ANOVA of the

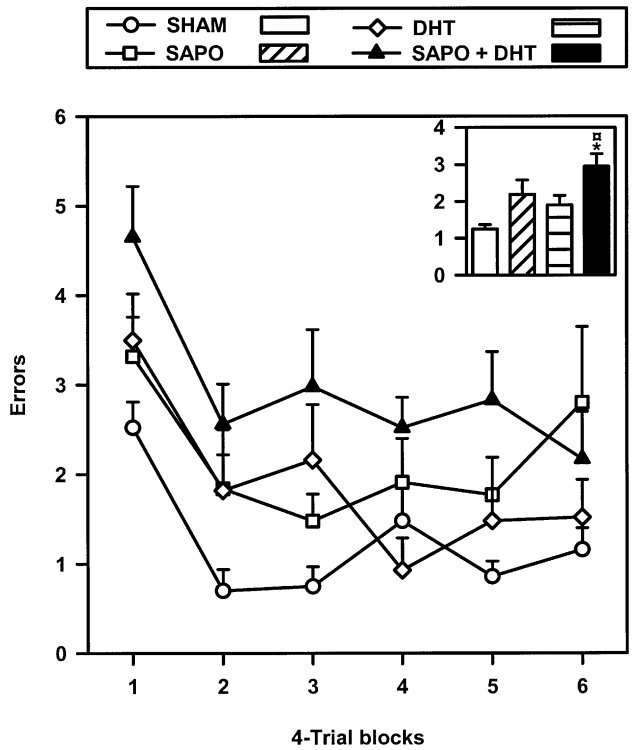

Fig. 7. Mean (±S.E.M.) number of errors in the radial-maze task. Group abbreviations refer to rats subjected to the injection of vehicle (SHAM), 2 μg of 192 IgG-saporin (SAPO), 150 μg of 5,7-DHT (DHT) or both neurotoxins (SAPO+DHT). Statistics: significantly different from SHAM, * $P < .05$; significantly different from DHT, ¤ $P < .05$.

latencies showed only a significant Trial effect [$F(1,42) = 66.21$, $P < .001$] on the first two trials and only a significant Group effect [$F(3,42) = 2.97$, $P < .05$] on the last two trials. The latter effect was due to latencies that were significantly higher in SAPO rats than in DHT rats ($P < .05$).

ANOVA of the distances yielded a comparable picture. When all four trials were considered, there were significant Group [$F(3,42) = 4.31$, $P < .01$] and Trial effects [$F(3,126) = 35.97$, $P < .001$], but no interaction [$F(9,126) = 0.69$]. The Group effect was due to overall distances that were significantly longer in SAPO + DHT rats as compared to DHT rats ($P < .01$). The Trial effect was due to a decrease of the overall distances on Trials 2, 3 and 4 as compared to Trial 1 ($P < .001$). ANOVA of performances on Trials 1 and 2 only showed a significant Trial effect [$F(1,42) = 52.01$, $P < .001$]. On Trials 3 and 4, ANOVA showed a significant Group effect [$F(3,42) = 3.42$, $P < .05$], which was due to distances that were significantly higher in SAPO + DHT rats than in DHT rats ($P < .05$). Never were the performances of rats from any lesion group significantly different from those found in SHAM rats.

An analysis of the overall swim velocity (in cm/s) indicated no significant difference amongst the four groups (not illustrated).

3.1.7. Radial maze

Data are shown in Fig. 7. ANOVA of the number of errors showed significant Group [$F(3,42) = 5.80$, $P < .01$] and Block [$F(6,210) = 12.04$, $P < .001$] effects, but no interaction. The Group effect was due to an overall number of errors, which was significantly higher in SAPO + DHT rats as compared to either SHAM ($P < .01$) or DHT rats ($P < .05$). The comparison between SAPO + DHT and SAPO rats only yielded a tendency, as was the case for SAPO rats compared to SHAM or to DHT rats ($P = .08$, in all cases). The Block effect reflected an overall number of errors, which was significantly lower in Blocks 2, 3, 4, 5 and 6 as compared to Block 1 ($P < .01$).

3.2. Neurochemical data

The concentration of DA could be determined reliably only in the striatum. Detection limits were 2 ng for ACh, 50 pg for NA and DA, 100 pg for 5-HT and 5-HIAA. All data are shown in Table 3.

3.2.1. Dorsal hippocampus

ANOVA of ACh concentrations showed a significant Group effect [$F(3,35) = 14.50$, $P < .001$] due to significant reduction in SAPO and SAPO + DHT rats as compared to SHAM ($P < .001$, in both cases) or DHT rats ($P < .01$, in both cases). There was no significant Group effect on the concentration of NA [$F(3,35) = 1.43$]. A significant Group effect was found on the concentration of 5-HT [$F(3,35) = 330.3$, $P < .001$] and 5-HIAA [$F(3,35) = 287.5$, $P < .001$].

Table 3
Mean (±S.E.M.) concentrations of ACh, NA, DA, 5-HT and 5-HIAA in rats after intracerebroventricular injections of vehicle, or SAPO, DHT or a combination of both

	ACh	NA	DA	5-HIAA	5-HT
Dorsal hippocampus					
SHAM	1.71 ± 0.18	0.17 ± 0.01	n.d.	0.27 ± 0.01	0.14 ± 0.00
SAPO	0.99 ± 0.15 *,†	0.16 ± 0.01	n.d.	0.26 ± 0.01	0.13 ± 0.00
DHT	1.83 ± 0.13	0.16 ± 0.01	n.d.	0.04 ± 0.01 *,¤	0.03 ± 0.00 *,¤
SAPO + DHT	0.67 ± 0.09 *,†	0.17 ± 0.01	n.d.	0.04 ± 0.01 *,¤	0.03 ± 0.00 *,¤
Ventral hippocampus					
SHAM	1.55 ± 0.21	0.30 ± 0.02	n.d.	0.28 ± 0.03	0.23 ± 0.01
SAPO	0.77 ± 0.13 *,†	0.28 ± 0.01	n.d.	0.31 ± 0.01	0.22 ± 0.01
DHT	1.79 ± 0.29	0.30 ± 0.02	n.d.	0.04 ± 0.00 *,¤	0.03 ± 0.00 *,¤
SAPO + DHT	0.42 ± 0.05 *,†	0.33 ± 0.02	n.d.	0.04 ± 0.01 *,¤	0.03 ± 0.00 *,¤
Frontoparietal cortex					
SHAM	0.90 ± 0.08	0.15 ± 0.00	n.d.	0.13 ± 0.00	0.12 ± 0.00
SAPO	0.70 ± 0.08 *,†	0.14 ± 0.01	n.d.	0.13 ± 0.01	0.11 ± 0.00
DHT	1.07 ± 0.09	0.15 ± 0.01	n.d.	0.04 ± 0.01 *,¤	0.05 ± 0.01 *,¤
SAPO + DHT	0.60 ± 0.04 *,†	0.17 ± 0.01 *,¤	n.d.	0.04 ± 0.01 *,¤	0.05 ± 0.00 *,¤
Striatum					
SHAM	4.58 ± 0.60	0.08 ± 0.01	1.70 ± 0.06	0.37 ± 0.01	0.19 ± 0.01
SAPO	5.39 ± 0.40	0.06 ± 0.01	1.82 ± 0.07	0.36 ± 0.03	0.18 ± 0.01
DHT	5.07 ± 0.55	0.09 ± 0.01	1.68 ± 0.09	0.17 ± 0.03 *,¤	0.09 ± 0.01 *,¤
SAPO + DHT	4.78 ± 0.29	0.08 ± 0.01	1.63 ± 0.06	0.15 ± 0.02 *,¤	0.08 ± 0.01 *,¤

n.d.: not determined.
* $P < .05$ vs. SHAM group.
† $P < .05$ vs. DHT group.
¤ $P < .05$ vs. SAPO group.

For both markers, the effect was due to a significant reduction in DHT and SAPO + DHT rats as compared to SAPO or SHAM rats ($P < .001$, in all cases).

3.2.2. Ventral hippocampus

ANOVA of ACh, NA, 5-HT and 5-HIAA concentrations showed the same effects and differences as for the dorsal hippocampus.

3.2.3. Frontoparietal cortex

ANOVA of ACh, NA, 5-HT and 5-HIAA concentrations showed the same effects and differences as for the dorsal hippocampus, except that there was a significant Group effect on the concentration of NA [$F(3,35) = 4.96$, $P < .05$] due to values, which were significantly higher in SAPO + DHT as compared to SHAM or SAPO rats.

3.2.4. Striatum

ANOVA showed no significant lesion effect on concentrations of ACh [$F(3,35) < 1$], DA [$F(3,35) = 1.36$] and NA [$F(3,35) = 2.74$]. There was a significant Group effect on the concentration of 5-HT [$F(3,34) = 33.29$, $P < .001$] and 5-HIAA [$F(3,35) = 21.07$, $P < .001$] due to significant reduction of both markers in DHT and SAPO + DHT rats as compared to SHAM ($P < .001$, in both cases) or SAPO rats ($P < .001$, in both cases).

3.3. Histochemistry

Only one SHAM and one SAPO rats were used for AChE-staining. The sections stained for AChE from the corresponding brains were comparable to the sections obtained in SHAM and SAPO rats used in subsequent experiments based on identical operations (same amount, same batch; unpublished). In the SAPO and SAPO + DHT rats, the intracerebroventricular injections of the toxin induced a depletion of AChE-positive reaction products in cortical areas (Fig. 8, compare A to B), as well as in the hippocampus, whether in *Cornu ammonis* or in the dentate gyrus (Fig. 8, compare C to D). In the striatum, the AChE-positivity was not altered or, at most, seemed weakly reduced (Fig. 8, compare A to B). These changes were of comparable extent in the SAPO rat and in the SAPO + DHT rats. Compared to the SHAM rat, DHT rats showed no detectable modification in all these regions (data not illustrated). Also, there was no clear-cut alteration of AChE-positivity in the thalamus of the SAPO or the SAPO + DHT rats (data not illustrated).

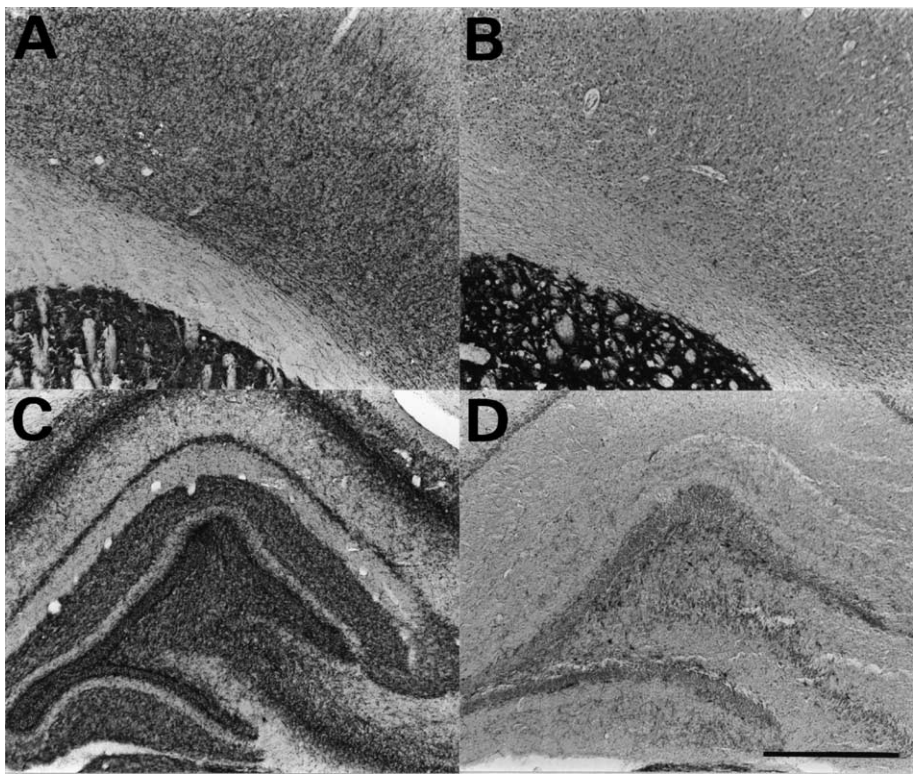

Fig. 8. Typical examples of AChE-positive staining in the frontoparietal cortex (A, B) and the dentate gyrus of rats given vehicle (left) or 192 IgG-saporin (right) injections. Examples from 5,7-DHT-lesioned rats or rats with combined injections are not shown as they were comparable to the staining observed in SHAM and SAPO rats, respectively. Scale bar = 500 μm in all photographs.

4. Discussion

After the first experiment in female rats (Lehmann et al., 2000), the present study aimed at characterizing the neurochemical and behavioral effects of lesions made with two selective toxins, 192 IgG-saporin and 5,7-DHT in male rats. The behavioral results showed that 192 IgG-saporin lesions alone reduced beam-walking scores and alternation rates, but had no effect on locomotor activity and performances in the water maze. In the radial maze, the rats with cholinergic lesions only tended to be impaired. 5,7-DHT lesions alone only impaired alternation scores. In rats given combined lesions, all deficits due to saporin lesions were apparent but, in addition, combined lesions induced nocturnal hyperactivity. It is noteworthy that radial-maze performances in SAPO + DHT rats were significantly worsened in comparison with those of SHAM and DHT rats, whereas the difference between the overall number of errors of each single lesion group and the controls failed to reach significativity. Our histochemical material showed that the decrease of AChE-positive reaction products was as dramatic in the hippocampus as in cortical regions. In particular, there was no residual staining in the dentate gyrus. Table 4 summarizes the main behavioral and histochemical effects found in the present study as compared to our former one in females (Lehmann et al., 2000).

Although our results in males and females were not obtained in the same experiment, and as neurochemical and behavioral effects of 5,7-DHT and/or 192 IgG-saporin injections were extensively discussed in our previous report on females (Lehmann et al., 2000), the discussion will focus on the main differences between both studies (see Table 4).

4.1. Histochemical and neurochemical effects

Regarding the cholinergic lesion, when taken together with data reported by Lehmann et al. (2000), the histological and neurochemical results of our present experiment do not yield the same picture. Indeed, although male and female Long–Evans rats had been subjected to virtually comparable lesion methods (2 μg 192 IgG-saporin into the lateral ventricles), a major difference was found on material stained for AChE in the dentate gyrus where males (one SAPO and three SAPO + DHT rats) showed an almost complete depletion of this marker against almost no effect in the females of our previous experiment. This difference was confirmed by neurochemical data when looking at the percent of cholinergic depletion in both studies (about 55% in the whole hippocampus of males against 40% for females). In a recent article in which 192 IgG-saporin had been injected directly into the nucleus basalis magnocellularis, McGaughy and Sarter (1999) raised the possibility that the basal forebrain cholinergic neurons of female rats might be more sensitive to 192 IgG-saporin than those of males (McGaughy and Sarter, 1999 vs. McGaughy et al., 1996). However, they also stated that the differences to which they were referring might be due to differences in the potency of the batches of 192 IgG-saporin used in males and females. As our experiments were run with different batches of 192 IgG-saporin (because of technical reasons), it cannot be excluded that the difference in the extent of the cholinergic lesion between males and females was due to variations in the potency of the batches. This possibility may be supported by the following arguments: (i) in the literature, it is recognized that the potency of 192 IgG-saporin may vary markedly between different batches (e.g., Sarter et al., 2000); (ii) we have recently compared the AChE-positivity in the cortical

Table 4
Summary of the main behavioral and histochemical effects reported by Lehmann et al. (2000) in female rats as compared to the effects found in the present study in male

Variable assessed	Male (present experiment)			Female (Lehmann et al., 2000)		
	SAPO	DHT	SAPO + DHT	SAPO	DHT	SAPO + DHT
Activity — habituation	ns	ns	ns	ns	ns	↑
Activity — diurnal	ns	ns	ns	ns	↑	↑
Activity — nocturnal	ns	ns	↑	ns	↑	↑
Beam-walking	↓	ns	↓	↓	↓	↓
T-maze alternation	↓	↓	↓	ns	ns	↓
Water maze — reference memory	ns	ns	ns	ns	ns	ns
Water maze — working memory	ns	ns	ns	ns	ns	↓
Radial maze	ns	ns	↓	ns	ns	↓
AChE-positivity — cortex	↓	ns	↓	↓	ns	↓
AChE-positivity — CA fields	↓	ns	↓	↓	ns	↓
AChE-positivity — dentate gyrus	↓	ns	↓	ns	ns	ns

Abbreviations: SAPO = injected with 192 IgG-saporin; DHT = injected with 5,7-DHT; SAPO + DHT = injected with both toxins; ns = no effect or no significant effect (as compared to SHAM); ↑ increase; ↓ decrease or impairment. In greyish: variable for which conclusions on males differ from those on females.

mantle, the hippocampus and the striatum in male and female rats subjected to intracerebroventricular injections of the same amount (2 μg) of 192 IgG-saporin taken from the same batch and could not evidence any significant difference related to the sex of the rats. It is noteworthy that the concentrations of ACh in control rats were almost twofold higher in males than in females. Several studies showed sex differences for virtually all central cholinergic markers in normal animals (see Rhodes and Rubin, 1999 for review), but these studies rather pointed to larger ACh levels in females. This difference was generally related to the estrous cycle and to hormonal levels (e.g., Hörtnagl et al., 1993). Another possibility may be that the difference in the weight of the males from the present experiment and that of females of the former one may have interfered with the quality of the neurotransmitter yield of microwave irradiation. A direct comparison of cholinergic markers in males and females in the same experiment should allow to clarify this point.

Concerning the serotonergic lesions, the variability between males and females was not as marked as for the cholinergic lesions, except in the striatum where the reduction was higher in females than in males (75% against 50%, respectively). One may nevertheless notice that basal levels of 5-HT of sham-operated male rats were always slightly higher than in their female counterparts. Again, the level of serotonergic markers may depend on the sex (e.g., Carlsson and Carlsson, 1988). This view is consistent with the fact that ovarian steroids influence serotonergic functions (e.g., Bethea et al., 1998).

Finally, as previously reported in females (Lehmann et al., 2000), the combination of 192 IgG-saporin and 5,7-DHT injections added the singular effects of the toxins. Although SAPO + DHT rats seemed to exhibit a greater cholinergic depletion than SAPO rats, mainly in the hippocampus, this difference never reached significance. It is also noteworthy that the concentration of ACh in both SAPO and SAPO + DHT rats was not reliably correlated with cognitive performances (data not illustrated).

4.2. Behavioral effects of serotonergic lesions

The comparison between our present results and the former ones (Lehmann et al., 2000) indicate possible sex-related differences in rats subjected to 5,7-DHT lesions: males were never hyperactive or impaired in the beam-walking test but were so on T-maze alternation, whereas an opposite feature was found in females.

Concerning activity, there is no consensus in the literature about the locomotor effects of serotonergic lesions: some studies reported hypoactivity (e.g., Lipska et al., 1992; Nilsson et al., 1988), others hyperactivity (Vanderwolf, 1989; Williams et al., 1990; Lehmann et al., 2000). However, it seems that levels of activity depend on the testing conditions (i.e., familiar or novel environment; e.g., Gerson and Baldessarini, 1980), as well as on the delay elapsed between lesion surgery and functional evaluations (e.g., Gately et al., 1986). Another factor of variability might be related to sex as sex-related differences have been reported on activity levels, particularly in fear- and anxiety-related tests: females showed more activity and more exploration (Beatty, 1979, 1992; Fernandes et al., 1999).

The impairment in the T-maze is not consistent with data suggesting that overall 5-HT depletions have weak effects on working memory performances (e.g., Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995, for a review), a memory component required for T-maze alternation. It is also discrepant with the report by Asin and Fibiger (1984) who showed that 5,7-DHT lesions of the median raphe had no effect on spontaneous alternation scores and with our previous results in females (Lehmann et al., 2000), but confirms a study by Williams et al. (1990) who injected 5,7-DHT directly into the fimbria-fornix. As our rats were never impaired in tasks assessing working memory, other variables related to the apparatus and protocols used (e.g., motivation, fear and/or curiosity) might account for the aforementioned discrepancy (Beatty, 1979, 1992; Fernandes et al., 1999).

Finally, as concerns spatial working and spatial reference memory performances in the radial or the water maze, our observations in 5,7-DHT rats are in line with our findings in females (Lehmann et al., 2000) and with those of previous experiments (e.g., Nilsson et al., 1988; Riekkinen et al., 1990, 1991; Jäkälä et al., 1993; Harder et al., 1996): serotonergic depletions do not, or only weakly alter such functions. It is noteworthy that rats subjected to only 5,7-DHT showed working memory performances, which, in the water maze, were significantly better than those of SAPO + DHT rats, though not better than in SHAM rats. Whether such an observation may be related to studies showing cognitive enhancement following serotonergic lesions (e.g., Altman et al., 1990; Normile et al., 1990) would deserve a particular experiment. Finally, considering the overall data related to serotonergic lesions, it is also possible that the lack of 5,7-DHT-induced effects on almost all behaviors assessed herein had something to do with plasticity phenomena already described after such types of lesions (Dugar et al., 2001; Zhou and Azmitia, 1984).

4.3. Behavioral effects of cholinergic lesions

As found in our previous experiment (Lehmann et al., 2000), intracerebroventricular injections of 192 IgG-saporin resulted in a severe beam-walking motor impairment. As suggested by Waite et al. (1999), this impairment may be due to damage of cerebellar Purkinje cells, which also express $p75^{NGF}$ receptors (Pioro and Cuello, 1988). The motor deficits might also be due to the cholinergic denervation of the frontoparietal cortex. Abdulla et al. (1994) reported that lesions in the nucleus basalis magnocellularis induced sensorimotor deficits related to cholinergic dysfunctions. This is strengthened by two other observations:

(i) scopolamine treatment produces deficits in a beam-walking test (Beiko et al., 1997); (ii) injections of 192 IgG-saporin directly into the nucleus basalis magnocellularis, a technique preventing Purkinje cell loss (e.g., Heckers et al., 1994) alters beam-walking performances (Galani et al., unpublished results obtained by part of us).

Concerning radial-maze and water-maze performances, we confirm our previous findings in females, namely that the cholinergic lesion alone is not sufficient to induce marked deficits in these tasks. The fact that the immunotoxin did not alter maze performances does not necessarily stand in contradiction with the literature. Indeed, it seems that damage to the basal forebrain cholinergic system must be extensive to induce detectable learning and memory deficits (e.g., Waite et al., 1995; Walsh et al., 1995; Wrenn and Wiley, 1998). Although the cholinergic lesion effects were obvious in the present study, they were not maximal as ACh could still be detected in the cortex and the hippocampus. It must also be noticed that the cholinergic denervation of the neocortex appears quite modest, although it was not much different from that found in females. Furthermore, that overall water maze performances were not altered might be due to the fact that our testing protocol enabled, at least in part, egocentric striatum-dependent navigation (Trials 3 and 4), and this region was almost intact. A major difference with our study on females is that males subjected to 192 IgG-saporin lesions showed a clear-cut deficit in the forced alternation task. As working memory was preserved in lesioned rats, such a deficit might be interpreted as a corollary of factors related to motivation and/or stress, which might have a larger impact in male than in female rodents (see Archer, 1975; Fernandes et al., 1999). Further studies should allow to explore this issue more directly.

4.4. Behavioral effects of combined lesions

The effects of the combination of cholinergic and serotonergic lesions in males overall confirmed our previous findings in females (Lehmann et al., 2000). In both studies, nocturnal activity was higher than in all three other groups, and radial maze performances were significantly altered only in the group with both lesions. Moreover, these results are in line with the literature on cholinergic/serotonergic interactions (e.g., Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995, for review). The hyperactivity can be compared, for instance, with the hyperlocomotor effects of a combination of muscarinic and 5-HT_{1A} receptor blockades (e.g., Bertrand et al., 2001). However, it has to be mentioned that the diurnal activity was not affected by the combined lesion in the present experiment, although it was clearly increased in the previous one. This difference might be attributed to a weaker effect of the serotonergic lesion in males. On radial-maze performances, our findings are also in line not only with experiments combining various types of lesions (e.g., Nilsson et al., 1988; Normile et al., 1990; Richter-Levin and Segal, 1991; Riekkinen et al., 1990), but also with studies combining drug treatments (e.g., Bertrand et al., 2001; Riekkinen et al., 1991): there may be a sufficient potentiation between the physiological effects of both alterations to bring about alterations of working memory performances. The combination of both lesions failed to alter water-maze performances in males, whereas it induced a deficit in females. It is also noteworthy that, in male rats, a deficit of alternation scores was found in all lesion groups, whereas, in females, such a deficit was only observed after combined lesions (Lehmann et al., 2000). Such differences suggest that the cognitive implication of the interaction between the serotonergic and the cholinergic systems is not as clear in males as in females. One way to account for these discrepancies might be to consider the differences between both studies as to the extent of the cholinergic lesions. Another explanation might be related to sex differences in the sensitivity of cholinergic and serotonergic neurotransmitter systems towards 192 IgG-saporin and 5,7-DHT, respectively, and in the involvement of these systems in male and female cognitive processes. Although such an issue would require further investigations, first of all by comparing males and females within the same experiment, the literature provides some illustrative examples suggesting that (i) the sensitivity of cognitive capabilities to manipulations such as stress or drugs may differ between males and females (e.g., Bowman et al., 2001; Slamberova et al., 2001; Wood and Shors, 1998); (ii) male and female rodents do not necessarily show identical cue utilization or cognitive strategies in solving tasks (Bimonte and Denenberg, 2000; Lebowitz and Brown, 1999; Roof and Stein, 1999; Tropp and Markus, 2001); (iii) male and female rodents do not necessarily show identical levels of performances in a spatial task (Bimonte et al., 2000; Ghi et al., 1999). Indeed, it is noteworthy that the performances recorded in the sham rats of our two experiments were better in males than in females, particularly in the radial maze where males showed fewer number of errors and quickly reached asymptotic performances as already reported (see Beatty, 1979, 1992; Harrell and Parsons, 1988; but see Juraska et al., 1984).

5. Conclusions

We confirm that the combination of lesion techniques using 192 IgG-saporin and 5,7-DHT is a satisfactory tool to produce concomitant damage to cholinergic and serotonergic neurons in the brain. While such a technique used in female rats has allowed to clearly substantiate the fact that an interaction between serotonergic and basal forebrain cholinergic mechanisms might play some important role in cognitive functions related to spatial working memory, the results of the present experiment in male rats are not as clear-cut, although they do not stand in contradiction with our previous findings in females (Lehmann et al., 2000). Thus, whether and to which extent manipulations of the

central serotonergic tone have cognitive consequences that might depend on the level of activation or damage of the septohippocampal and/or the basalocortical systems is a question deserving further investigations, perhaps in relation with possible gender-differences, and particularly because of the limited success of cholinotherapies in the case of Alzheimer's disease.

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