ELSEVIER

Brain Research Bulletin, Vol. 49, No. 4, pp. 263–272, 1999

0361-9230/99/$–see front matter

PII S0361-9230(99)00058-1

Intrahippocampal grafts containing cholinergic and serotonergic fetal neurons ameliorate spatial reference but not working memory in rats with fimbria-fornix/ cingular bundle lesions

Elise Balse,[1] Christine Lazarus,[1] Christian Kelche,[1] Hélène Jeltsch,[1] Rolf Jackisch[2] and Jean-Christophe Cassel[1*]

[1]LN2C, UMR 7521, CNRS/Université Louis Pasteur, Strasbourg, France; [2]Institut für Pharmakologie und Toxikologie der Universität Freiburg, Neuropharmakologisches Labor, Freiburg, Germany

[Received 26 November 1998; Revised 26 February 1999; Accepted 13 March 1999]

ABSTRACT: Three-month-old Long-Evans female rats sustained aspirative lesions of the dorsal septohippocampal pathways and, 2 weeks later, received intrahippocampal suspension grafts containing cells from the mesencephalic raphe, cells from the medial septum and the diagonal band of Broca, or a mixture of both. Lesion-only and sham-operated rats were used as controls. All rats were tested for locomotor activity 1 week, 3 and 5 months after lesion surgery, for spatial working memory in a radial maze from 5 to 9 months, and for reference and working memory in a water tank during the 9th month after lesioning. Determination of hippocampal concentration of acetylcholine, noradrenaline, and serotonin was made after completion of behavioral testing. Compared to sham-operated rats, all rats with lesions, whether grafted or not, exhibited increased levels of locomotor activity and made more errors in the radial maze. The lesioned rats were also impaired in the probe trial (30 first seconds) of the water-tank test made according to a protocol requiring intact reference memory capabilities. While rats with septal or raphe grafts were also impaired, the rats with co-grafts showed performances not significantly different from those of sham-operated rats. With a protocol requiring intact working memory capabilities, all lesioned rats, whether grafted or not, were impaired in the water-tank test. In the dorsal hippocampus of lesion-only rats, the concentration of acetylcholine and serotonin was significantly reduced. In rats with septal grafts or co-grafts, the concentration of acetylcholine was close to normal, as was that of serotonin in rats with raphe grafts or co-grafts. These results confirm previous findings showing that co-grafts enabled the neurochemical properties of single grafts to be combined. Data from the water-tank test suggest that cholinergic and serotonergic hippocampal reinnervations by fetal cell grafts may induce partial recovery of spatial reference, but not working memory capabilities in rats.

KEY WORDS: Cholinergic, Co-grafts, Fimbria-fornix, Hippocampus, Lesion, Locomotor activity, Memory, Radial maze, Serotonergic, Transplants, Water tank.

INTRODUCTION

Experimental lesions of the dorsal septohippocampal pathways (fimbria-fornix and cingular bundle) have often been used to investigate structural and functional effects of intrahippocampal grafts rich in fetal cholinergic neurons (e.g., [8,18,49]). Such grafts were shown to provide the denervated hippocampus with a new cholinergic innervation and to induce neurochemical, electrophysiological, and behavioral effects demonstrating their functionality (e.g., [8,49]). However, while these grafts mainly exert cholinergic effects in the hippocampus, the lesions also damage a significant part of the gamma-aminobutyric acid (GABA)ergic, noradrenergic, and serotonergic hippocampal afferents. In agreement with Nilsson et al. [35], we have reported that co-grafting cell preparations rich in cholinergic or serotonergic neurons allowed to foster concomitant recovery of cholinergic and serotonergic markers in the hippocampus [14,15]. Using electrolytic lesions restricted to the fimbria and the dorsal fornix, we showed that intrahippocampal co-grafts rich in cholinergic and serotonergic neurons were able to normalize spatial reference memory performance in the probe trial of a water-tank test [31]. This effect was not found when the cell preparations rich in cholinergic or serotonergic neurons were performed as single grafts. Interestingly, in this study, single grafts and co-grafts failed to produce any significant effect on spatial working memory performances assessed in a radial maze. Jeltsch et al. [31] hypothesized that co-grafts of cholinergic and serotonergic neurons affected dysfunctions of spatial reference memory but not of spatial working memory.

Two important questions were left open in the study by Jeltsch et al. [31]. First, as the lesions of the dorsal septohippocampal pathways only altered the fimbria and the dorsal fornix, it was not clear whether the behavioral improvement observed in co-grafted rats was due only to the co-grafts or to an interaction between both types of grafted cells and undamaged hippocampal afferents coursing in the cingular bundle. Second, as reference memory was

* Address for correspondence: Dr. Jean-Christophe Cassel, LN2C, UMR 7521, CNRS/Université Louis Pasteur, 12 rue Goethe, F-67000 Strasbourg, France. Fax: +33-388-358442; E-mail: jean-christophe.cassel@psycho-ulp.u-strasbg.fr

assessed in the water tank and working memory in the radial maze, it was not clear whether the effects induced by the co-grafts were specific to a type of memory or to a type of task. This issue is of particular importance, as the water-tank and the radial-maze tests are different in several aspects, although both are supposed to measure spatial learning/memory performances. For instance, motivation is aversive and learning occurs rapidly in the water tank, whereas, in the radial maze, motivation is appetitive and learning occurs slowly [24].

Therefore, in the present experiment which investigated various effects of co-grafts rich in cholinergic and serotonergic neurons in rats with aspiration lesions of the cingular bundle and the fimbria-fornix, two major goals were defined. First, we wanted to verify whether co-grafts are able to produce beneficial effects after lesions that also damage the supracallosal component of the dorsal septohippocampal pathways. Second, if so, we wanted to verify whether these effects were actually specific to spatial reference memory in the water-tank task by using an additional testing protocol sensitive to disruption of working memory. As a complementary approach, spatial working memory was also assessed (in the radial maze) under treatment with the acetylcholinesterase inhibitor physostigmine (0.05 mg/kg, intraperitoneally [i.p.]), the 5-HT_{1B} antagonist 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolol[1,2a]quinoxaline (CGS 12066A, 1 mg/kg, i.p.), the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT, 0.1 mg/kg, i.p.), and the 5-HT_{1A} antagonist n-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide (WAY 100635, 0.2 mg/kg). These drugs were used because previous studies had shown that the release of hippocampal acetylcholine is regulated by presynaptic 5-HT_{1B} heteroreceptors [9,10,47] and extrahippocampal 5-HT_{1A} receptors [27,52]. It is also known that physostigmine may improve cognitive functions in rats with septal cell grafts placed into a fimbria-fornix lesion cavity [34].

The present experiment used adult Long-Evans female rats, which were subjected to aspirative lesions of the dorsal septohippocampal pathways. Sham-operated rats were used as a first control group. Two weeks after lesion surgery, a first subgroup of rats received intrahippocampal suspension grafts of fetal cells from the mesencephalic raphe (rich in serotonergic neurons), a second subgroup received grafts of cells from the medial septum/diagonal band of Broca region, and a third subgroup received a mixture of both cell preparations. Lesion-only rats were used as a second control. All rats were tested for locomotor activity in their home cage, spatial working memory in a radial maze, and spatial working and reference memory in a water tank. They were sacrificed by head exposure to focused microwave irradiation, and concentration of acetylcholine, noradrenaline, and serotonin was determined in the hippocampus.

MATERIAL AND METHODS

Subjects

The study used 59 Long-Evans female rats obtained from R. Janvier (France) and which survived until the end of the experiment (initial sample: $n = 62$). They were aged of 3 months at the time of lesion surgery and were housed five or six per Makrolon cage (59 × 38 × 20 cm) except during surgery and behavioral testing periods, for which they were isolated in smaller cages (42 × 26 × 15 cm). Food and water were available *ad libitum* except during radial maze testing, for which the rats were placed under restricted feeding conditions (see below). The colony and experiment rooms were maintained on a 12:12-h light–dark cycle (lights on at 0700) under controlled temperature (21° ± 1°C). All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (council directive no. 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; permission no. 2108 to C.K., no. 6714-bis to H.J. and no. 6212 to J-C.C.; National Institutes of Health publication no. 86-23, revised 1985).

Surgery

All surgery was performed under aseptic conditions, using equithesin anaesthesia (3 ml/kg, i.p.).

Lesion surgery. Aspiration lesions of the fimbria-fornix pathways and overlying structures were performed as usual (e.g., [8,14]). A large hole was drilled in the skull, extending from about 1.5 to 2.5 mm posterior to Bregma [40], and 3.0 mm from the midline on each side. At about 2.1 mm posterior to Bregma, a part of the cortex and the corpus callosum, including the cingular bundle, as well as all the underlying fimbria-fornix pathways were aspirated with a curved Pasteur pipette ending in a 7.0 mm-long straight microcap (Drummond) with an external diameter of 0.7 mm. Laterally, the aspiration extended over about 2.0 mm from the midline. These operations were performed with the rat fixed in a stereotaxic frame, the incisor bar being placed at 3.0 mm below the interaural line.

The control group (sham-operated rats, $n = 11$) consisted of rats which received scalp incision and removal of the bone overlying the region aspirated in the lesion groups.

Transplant surgery. Cells to be grafted were prepared from the brains of Long-Evans fetuses aged 15 days (embryonic day [ED] 15, average crown-rump length [CRL] = 15 mm) for septal grafts and of 13 days (ED 13, average CRL = 12.5 mm) for raphe grafts. About 2 weeks after lesion surgery, a first subgroup of lesioned rats (Group Spt.G, $n = 12$) received bilateral intrahippocampal grafts of a cell suspension prepared from the region including the septum and the diagonal band of Broca, a region rich in cholinergic neurons. A second subgroup of lesioned rats (Group Rph.G, $n = 12$) received grafts of a cell suspension prepared from the region including the mesencephalic raphe, a region rich in serotonergic neurons. In a third subgroup (Group Rph + Spt.G, $n = 11$), the lesioned rats received co-grafts of a cell suspension in which septal and raphe tissue had been mixed prior to the dissociation. The last subgroup (lesion-only, $n = 13$) was a control group consisting of lesioned rats which did not receive grafts.

Tissue fragments were collected into 0.6% glucose saline, incubated for 30 min at 37°C in the same solution containing 0.1% trypsin (Sigma Chemical Co., St. Louis, MO,USA; Grade II), washed three times with 5 ml of glucose saline and brought to a final volume of approximately 10μl per septal tissue piece and 20μl per raphe tissue piece. The tissue pieces were dissociated using a fire-polished Pasteur pipette until a milky suspension was obtained. Injections (2μl/site, 1μl/min) of the resulting suspensions were performed stereotaxically, through a Hamilton syringe, into each dorsal hippocampus at the following coordinates: A 4.0 mm, L ± 1.6 mm, V −3.1 mm; A 2.4 mm, L ± 3.2 mm, V −3.3 mm (from Lambda; [40]). The syringe was left *in situ* for 2 min after each injection. The number of cells injected was counted in a haemocytometer (Thoma chamber) and non-viable cells were identified with 0.05% Trypan Blue. The suspensions contained 81250-117500 cells/μl and 86–93% cells were viable. In our hands, the number of viable cells remains relatively stable for about 3–4 h after preparation of the suspension (our own unpublished observations). Thus, cell suspensions were used within a maximum of 3.5 h after preparation.

Behavioral Tests

Home-cage activity. The rats were not tested before the operations. The spontaneous activity of the rats was recorded for 23 h in the home cages, starting at 11:00 h. This test was performed 1 week, 3 and 5 months after the lesion surgery. A first period of observation (habituation to experimental conditions) lasting for 3 h was immediately followed by a second period that began at 14:00 and lasted for 20 h (nocturnal period: 19:00–07:00). Each cage was traversed by two infrared light beams targeted on two photocells, 4.5 cm above floor level and 28 cm apart. The number of crossings of the home cage (successive interruptions of the beam) was monitored continuously by a microcomputer.

Radial arm maze (RAM). Starting during the fifth post-surgical month and lasting for about 4 months, RAM training and testing were run using two identical grey polyvinylchlorid RAMs placed in an experimental room with several different visual cues around the mazes. The octagonal central platform was 40 cm in diameter. Arms radiating from the platform were 56 cm long and 10 cm wide, with a concave food well located 3 cm from the end of each arm. A 3-cm-high border was fixed to the arms and 30 × 20 cm walls were fixed to each arm entrance. Each maze was elevated 68 cm above floor level. In each maze, 16 infrared photocells (two per arm, one at 12 cm from the entrance and the other 10 cm from the end, with the infrared beam 4 cm above floor level) enabled the entries and movements of rats to be followed. The sequence of photocell beam interruptions were monitored with a microcomputer and errors were defined as re-entries into already visited arms within a given trial.

The body weight of all rats was reduced progressively (over 10 days) 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, Campden, UK) in the maze on 5 consecutive days according to a training schedule described in detail by Jeltsch et al. [31]. Following training, all rats were tested for a series of 48 trials (one trial per day, 5 or 6 days per week) without prior drug treatments and for another series of 15 trials (one trial par day, 5 days per week, one drug condition per trial) with prior drug treatments. The drugs injected 20–25 min before testing were the cholinesterase inhibitor physostigmine hemisulfate (0.05 mg/kg, i.p., Sigma), the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, 0.1 mg/kg, i.p., Sigma), the 5-HT_{1B} receptor agonist 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolol[1,2a]quinoxaline maleate (CGS 12066A, 0.1 mg/kg, i.p., RBI) and the 5-HT_{1A} receptor antagonist n-[2-[4-(2-Methoxyphenyl)-1-piperazinyl-]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate (WAY 100635, 0.2 mg/kg, i.p., RBI). The drugs were dissolved in saline and the injection volume was 2 ml/kg. Saline injections were used as control. Each rat was given three trials under each drug condition, including saline, and the drugs/saline were given on a counterbalanced order from day to day.

Morris water tank (MWT). This test, run during the 9th post-surgical month, was performed with two procedures, one sensitive to disruption of reference memory, the other to disruption of working memory. The Morris water tank consisted of a circular pool (diameter 160 cm, height 60 cm) filled with water to half the height. The water (22°C) was made opaque with powdered milk. The pool was located in an experimental room with many extramaze cues (e.g., chair, computer, desk, cages, lights, pictures on the wall, fan, etc.) and was virtually divided into four equal quadrants with four starting points identified as north, east, south and west. A circular platform, 11 cm in diameter, was placed in the pool, 1 cm underneath the water surface. For each trial, the rat was placed in the pool, facing the wall at a randomly designed starting point from where it was released and given a maximum of 60 s to reach the submerged platform. When the rat had climbed onto the platform, it was allowed to remain there for 10 s before being removed and placed on the next (in the reference memory test) or the same (in the working memory test) starting point. If the rat failed to find the platform within 60 s, it was placed on it for 10 s by the experimenter. Using a video-tracking system (Noldus, The Netherlands), the latency to reach the platform and the distance swam by the rat were recorded for each trial.

Working memory procedure. During 5 consecutive days, the platform was placed in a new location each day, and the rats were released from a single starting point for four consecutive trials separated by 10 s. This testing procedure is assumed to measure primarily working memory.

Reference memory procedure. During 5 other days, the platform was placed in the NW quadrant. Each day, the rats were given four trials for which they where released from each starting point in a randomized order. When the last trial of the last day was completed, the platform was removed and all rats were given a probe trial for 60 s. The testing procedure used before the probe trial is generally considered to provide a measure of learning reflecting spatial reference memory, while the probe trial is considered to measure the strength of spatial learning.

Neurochemical Assessments

Within 8 days after completion of MWT testing, the rats were sacrificed by microwave irradiation (1.8 s, 4.5 kW; Sairem, Villeurbanne, France) in order to rapidly inactivate brain enzymes such as acetylcholinesterase (e.g., [46]). Brains were removed and dissected on a cold plate in order to extract both hippocampi, which were separated into a dorsal (septal pole) and a ventral (temporal pole) portion. The left and right hippocampal portions from each rat were pooled according to their dorsal/ventral origin, weighed and kept at −80°C until neurochemical determinations.

Concentrations of acetylcholine (ACh), noradrenaline (NA), and serotonin (5-HT) were measured using high performance liquid chromatography (HPLC) with electrochemical detection. The tissue samples were prepared for HPLC by homogenisation in 0.05 N formic acid/aceton (15:85, vol./vol.), and the formic extracts were used for ACh and monoamines determinations. The monoamine concentrations were measured without further purification. The HPLC system consisted of an ESA liquid chromatography pump (ESA INC., Bedford, USA) coupled to an ESA Coulochem II detector (Eurosep Instruments) equipped with a 5014 high performance analytic cell (ESA Inc.). The detector potential at the analytic cell was set at +0.4 V. HPLC analysis was performed on a C18 Spherisorb ODS2 reverse phase column (5μm pore size, 4.6 mm in diameter, 25 cm long). The mobile phase consisted of 0.1 M NaH_2PO_4 pH3, containing 0.1 mM/l of EDTA, 1.7 mM/l 1-octane sulfonic acid sodium salt and 10% acetonitril. The flow rate was 1 ml/min.

ACh concentrations were measured after an additional purification of the formic acid extracts. This consisted in tetraphenylboron exchange of the amines in 3-heptanone, followed by 0.1 N HCl extraction [1]. HPLC analysis was performed on a C18 Spherisorb ODS2 reverse phase column (3 μm pore size, 7 mm in diameter, 10 cm long). The mobile phase consisted in 0.05 KH_2PO_4 pH 6.5 containing 600 mg/l tetramethylammonium chloride and 154 mg/l sodium octane sulfate. The flow rate was 0.8 ml/min. ACh was converted into betaine in a post-column reactor with covalently bound acetylcholinesterase (EC3.1.1.7.) and choline oxidase (EC .1.3.17.). The resulting hydrogen peroxide was detected electrochemically using a 5040 ESA cell working electrode at 0.3 V.

Concentrations of ACh, NA, and 5-HT were determined with a data analysis software (Baseline 810, Waters, Div. Millipore, Milford, MA, USA) and were expressed as ng/mg microwaved tissue. Due to technical problems, concentration of ACh could not be measured in two rats, that of NA in eight and that of 5-HT in seven rats.

Statistical Analysis

All data were analysed by analysis of variance (ANOVA) followed, where appropriate, by 2 × 2 comparisons based on the Newman–Keuls test [53]. For analysis of all behavioral data, we had the following group size: Sham, $n = 11$; Les, $n = 13$; Rph.G, $n = 12$; Spt.G, $n = 12$; Rph+Spt.G, $n = 11$. There was some variance in the group size, depending on the neurochemical marker considered; the number of rats per group for each marker is indicated in Table 3. As variances in the concentration of NA and 5-HT were not homogeneous, a square root transformation of individual data was made prior to running the ANOVA [50].

RESULTS

Behavioral Data

Home-cage activity. Data are shown in Figs. 1A,B. ANOVA of the average hourly activity scores recorded during the habituation phase (3 first hours; Fig. 1A) 1 week, 3 and 5 months after the lesion surgeries, showed a significant Group effect [$F(4,54) = 6.31$, $p < 0.001$]. There was neither a significant effect of the Delay, nor a significant interaction between the Group and Delay factors. As indicated by the 2 × 2 comparisons using the test of Newman–Keuls, the Group effect was due to overall activity scores, which were significantly increased in all lesioned rats, whether grafted or not, as compared to their sham-operated counterparts ($p < 0.01$). ANOVA of the average hourly activity scores recorded during the remaining time (Fig. 1B) showed significant Group and Delay effects [$F(4,54)$ and $F(2,108) = 3.60$ and 7.37, respectively, $p < 0.01$ in each case], but no significant Group × Delay interaction. The Group effect was due to overall activity scores, which were increased significantly in all lesioned rats, whether grafted or not, as compared to sham-operated rats ($p < 0.05$). The significant Delay effect was due to overall activity scores, which were significantly lower at the second and third delay as compared to the first one ($p < 0.05$). When the diurnal and nocturnal periods were analyzed separately (data not shown), we found similar overall pictures for both periods, with the difference, however, that the nocturnal activity was two to three times higher than the diurnal one in all groups, and also that the intergroup difference was more pronounced during the night period.

Radial maze performance. Analyses were run on maze performances (errors) averaged over four-trial blocks. Data are shown in Fig. 2. The ANOVA of the number of errors showed significant Group [$F(4,54) = 4.01$, $p < 0.001$] and Block [$F(11,594) = 11.03$, $p < 0.001$] effects, as well as a significant interaction between these two factors [$F(44,594) = 1.46$, $p < 0.05$]. The Group effect was due to an overall number of errors, which was significantly larger in all lesion groups, whether grafted or not, as compared to sham-operated rats ($p < 0.001$ in all cases). Amongst the four groups of lesioned rats, there was no significant difference suggesting that neither graft provided a significant effect on the lesion-induced impairment. The Block effect was mainly due to a significant reduction of the overall number of errors during the three first trial-blocks ($p < 0.05$, at least). Finally, the interaction can be interpreted as resulting from a rapid reduction of the number of errors in the sham-operated rats (over the four first blocks) as opposed to a weaker decrease in the four groups of lesioned rats.

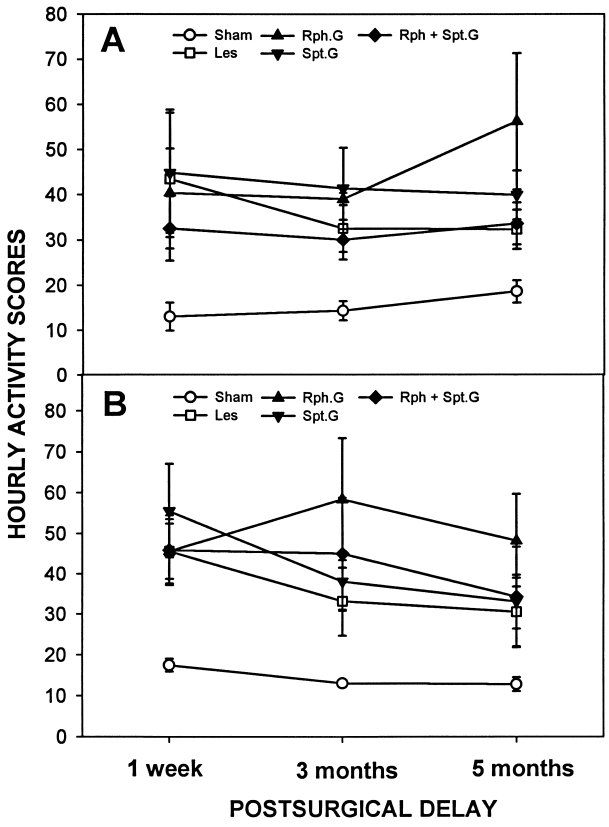

FIG. 1. Average (±SEM) hourly activity scores recorded during 3 h of habituation (A) and the subsequent 20 h (B) in sham-operated (Sham) and lesion-only (Les) rats, and rats with raphe (Rph.G), septal (Spt.G), or raphe + septal (Rph + Spt.G) grafts.

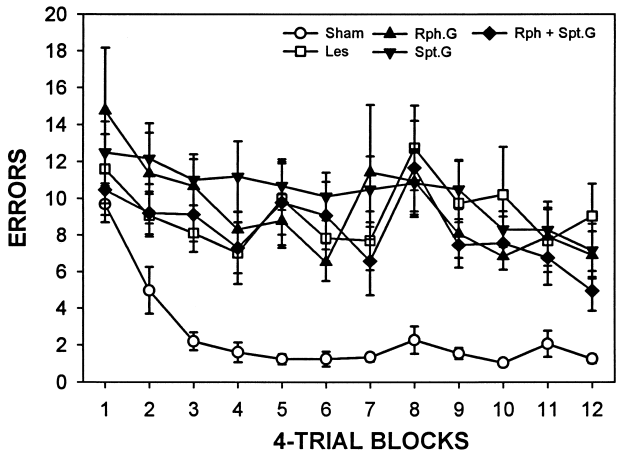

FIG. 2. Average (±SEM) number of errors in the radial maze task. Abbreviations: Sham, sham-operated rats; Les, lesion-only rats; Rphe.G, rats with raphe grafts; Spt.G, rats with septal grafts; Rph + Spt.G, rats with raphe and septal grafts.

TABLE 1

MEAN (± SEM) NUMBER OF ERRORS IN THE RADIAL-MAZE TEST

Drug Condition	Experimental Group				
	Sham	Les	Rph.G	Spt.G	Rph + Spt.G
Saline, 2 ml/kg, i.p.	1.24 ± 0.39	9.79 ± 2.22*	7.00* ± 1.44*	6.44* ± 1.42*	6.27* ± 1.32*
Physostigmine, 0.05 mg/kg, i.p.	1.63 ± 0.46	8.46 ± 2.09*	6.33 ± 1.64*	8.55 ± 1.67*	6.06 ± 1.62*
8-OH-DPAT 0.1 mg/kg, i.p.	2.48 ± 0.63	11.61 ± 2.49*	8.47 ± 1.36*	8.67 ± 1.50*	7.00 ± 1.22*
WAY 100635 0.2 mg/kg, i.p.	3.09 ± 1.03	9.31 ± 1.88*	7.08 ± 2.03*	5.67 ± 1.20*	6.63 ± 2.17*
CGS 12066A 1 mg/kg, i.p.	1.72 ± 0.5	10.12 ± 3.39*	7.78 ± 1.35*	8.36 ± 1.77*	5.69 ± 1.24*

Various drugs were injected 20–25 min before each trial. Each rat was tested at three occasions under each drug condition.

Values given are the mean number per trial computed from the three trials performed under each drug condition (one trial per day).

Sham, sham-operated rats; Les, lesion-only rats; Rph.G, rats with raphe grafts; Spt.G, rats with septal grafts; Rph + Spt.G, rats with raphe and septal grafts; i.p., intraperitoneally.

* Significantly different from sham-operated rats, $p < 0.05$.

Data recorded during the drug trials are shown in Table 1. ANOVA of the number of errors made under CGS 12066A treatment as compared with saline showed a significant Group effect [$F(4,54) = 3.43$, $p < 0.01$] that was due to a significant lesion-induced increase of the number of errors on which neither graft had produced a significant effect. There was no significant effect of the drug and no significant Group × Drug interaction [$F(1,54)$ and $F(4,54) < 1.0$, respectively]. When the number of errors made under 8-OH-DPAT as compared with saline was analyzed, there was a significant Group effect [$F(4,54) = 4.41$, $p < 0.01$] and a significant Drug effect [$F(1,54) = 9.13$, $p < 0.01$], but no significant interaction between both factors. Whilst the overall Group effect could be interpreted as above, the Drug effect reflected an overall level of performance which was significantly impaired by 8-OH-DPAT. ANOVA of the number of errors made under WAY 100635 as compared with saline only showed a significant Group effect [$F(4,54) = 3.26$, $p < 0.05$], which was due to the lesion-induced effect. When the number of errors made after physostigmine or saline injections was analyzed, there was also a significant Group effect [$F(4,54) = 3.83$, $p < 0.01$]; the Drug effect and the interaction between the Group and Drug factors were not significant. The Group effect was due to average performances, which were significantly impaired in the four lesion groups as compared to the sham-operated rats ($p < 0.05$).

Morris water tank performance. Data are shown in Figs. 3A,B. ANOVA of the latencies to reach the platform in the reference memory version of the test (Fig. 3A) showed significant effects of factors Group [$F(4,54) = 11.16$, $p < 0.001$] and Day [$F(4,216) = 74.65$, $p < 0.001$], and of the interaction between both [$F(16,216) = 2.06$, $p < 0.05$]. The Group effect was due to overall latencies, which were significantly higher in all lesion groups, whether grafted or not, as compared to the group of sham-operated rats ($p < 0.001$, in each case). There was no significant difference amongst the four lesion groups. The Day effect was due to latencies which were significantly lower on days 2, 3, and 4 as compared to day 1 or 2 ($p < 0.001$, in each case). Finally, the interaction between both factors can be interpreted as reflecting a marked decrease of the latencies in the group of sham-operated rats as compared to a weaker decrease in the four lesion groups. This interpretation was confirmed by a linear trend analysis showing a significant linear decrease of the latencies in all groups ($p < 0.001$, at least); this decrease was significantly more pronounced in the group of sham-operated rats than in the four other ones ($p < 0.05$, at least). A comparison of the linear trend amongst the four

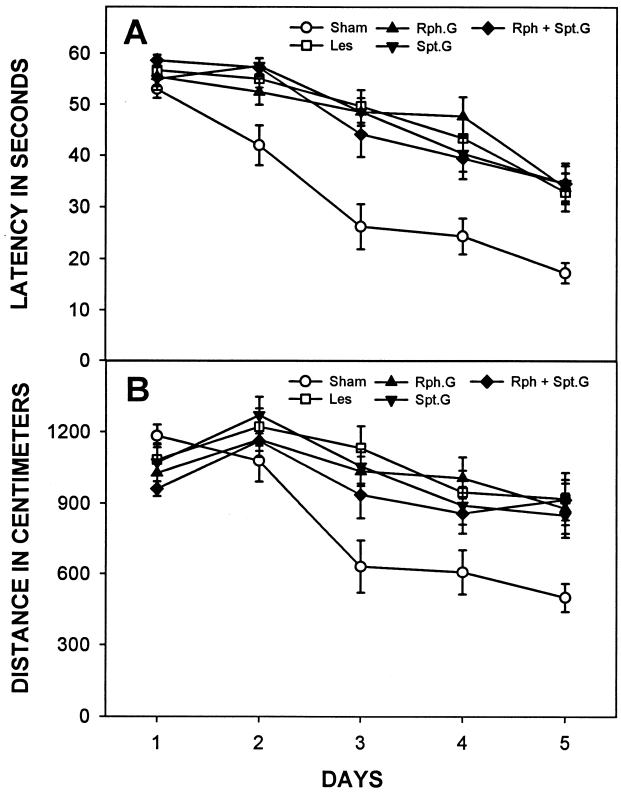

FIG. 3. Average (±SEM) latencies (A) and distances (B) to reach the platform in the water-tank test made according to a protocol sensitive to disruption of reference memory. Data of the probe trial are shown in Table 2.

TABLE 2

ONE-MINUTE PROBE-TRIAL IN THE MORRIS WATER TANK TEST[a]

Behavioral Variable	Experimental Group				
	Sham	Les	Rph.G	Spt.G	Rph + Spt.G
Time (s) in Q2 during the 30 first seconds	10.54 ± 0.78	7.24 ± 1.03*	6.11 ± 0.73*	6.50 ± 0.80*	9.80 ± 1.53
Time (s) in Q2 during the 30 last seconds	10.93 ± 0.73	7.41 ± 1.03	6.19 ± 1.06*	8.01 ± 0.88	8.50 ± 1.65
Number of swims over the former location of the platform	3.81 ± 0.56	1.23 ± 0.28*	1.17 ± 0.34*	2.00 ± 0.41*	1.72 ± 0.27*

All data are means ± SEM.

Sham, sham-operated rats; Les, lesion-only rats; Rph.G, rats with raphe grafts; Spt.G, rats with septal grafts; Rph + Spt.G, rats with raphe and septal grafts; Q2, the quadrant where platform was located during the acquisition trial.

[a]Data of the 30 first seconds are also shown in Fig. 4.

* Significantly different from sham-operated rats, $p < 0.05$.

groups of lesioned rats showed no significant difference. ANOVA of the distances showed significant effects of factors Group [$F(4,54) = 4.08$, $p < 0.01$], Day [$F(4,216) = 21.55$, $p < 0.001$], and of the interaction between both [$F(16,216) = 2.37$, $p < 0.01$]. Overall, these effects can be interpreted as for the latencies, with the exception that all possible differences between days 1, 2, 3 and 4 were significant; average values on days 4 and 5 were not significantly different. A linear trend analysis yielded a picture similar to that described for the latencies with two exceptions: in Rph.G and Rph + Spt.G rats, the distances failed to show a significant decline over trials. The average swimming speed was analyzed as a third variable (data not illustrated). There was no significant Group effect, a significant Day effect [$F(4,216) = 57.6$, $p < 0.001$], and no significant interaction between both factors. The Day effect was due to an overall swimming speed which, at the fifth day, was significantly higher than on all other days. Mean group values were 25.05 ± 0.70 in Sham, 23.15 ± 1.25 in Les, 22.05 ± 0.99 in Rph.G, 22.22 ± 1.12 in Spt.G, and 21.72 ± 1.11 in Rph+Spt.G rats (in cm/s).

The probe trial performances are shown in Table 2. Typical swimpaths are illustrated in Fig. 5. ANOVA of the number of crossings of the former platform location showed a significant Group effect [$F(4,54) = 7.63$, $p < 0.001$], which was due to the rats of all lesion groups swimming significantly less often over this position than the sham-operated rats ($p < 0.01$, in each case). ANOVA of the time spent in the quadrant where the platform was located during the 30 first seconds of the probe trial (Table 2 and Figure 4) also showed a significant Group effect [$F(4,54) = 3.80$, $p < 0.01$]. Performances of lesioned, raphe-grafted or septal-grafted rats did not differ significantly and were all significantly lower than those of sham-operated rats ($p < 0.05$). In rats with co-grafts, the average performance was significantly better than in septal-grafted or raphe-grafted rats ($p < 0.05$); it tended to differ from that of lesion-only rats ($p = 0.08$) and did not differ from that of sham-operated rats. During the last 30 s of the probe trial, ANOVA of the time spent in the "correct" quadrant showed a marginal Group effect [$F(4,54) = 2.48$, $p = 0.055$], which was due to a significant difference between performances of raphe-grafted and sham-operated rats. All other comparisons failed to show significant differences.

ANOVA of the latencies to reach the platform in the working memory version of the test (Figs. 5A,B) showed significant effects of factors Group [$F(4,54) = 5.86$, $p < 0.001$] and Trial [$F(3,162) = 10.73$, $p < 0.001$], but not of the interaction between these two factors. The Group effect was due to overall latencies that were significantly higher in the four groups of lesioned rats, whether grafted or not, as compared to their sham-operated counterparts ($p < 0.01$, in each case). There was no significant difference amongst the four lesion groups. The Trial effect was due to overall latencies which were significantly lower on trials 2, 3 and 4 than on trial 1 ($p < 0.01$, in each case), but also on trial 3 as compared to trial 2 ($p < 0.05$). ANOVA of the distances showed a significant effect of factor Group

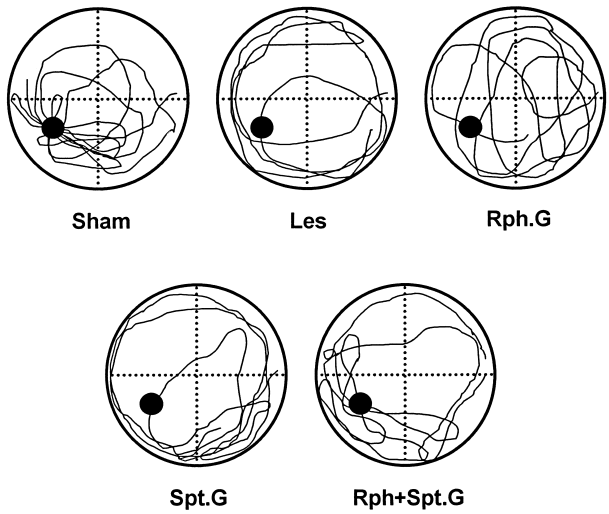

FIG. 4. Representative examples of swimpaths taken by a rat from each group during the 1-min probe trial. The filled circle indicates the location of the platform during the acquisition trials. Abbreviations: Sham, sham-operated rats; Les, lesion-only rats; Rphe.G, rats with raphe grafts; Spt.G, rats with septal grafts; Rph + Spt.G, rats with raphe and septal grafts.

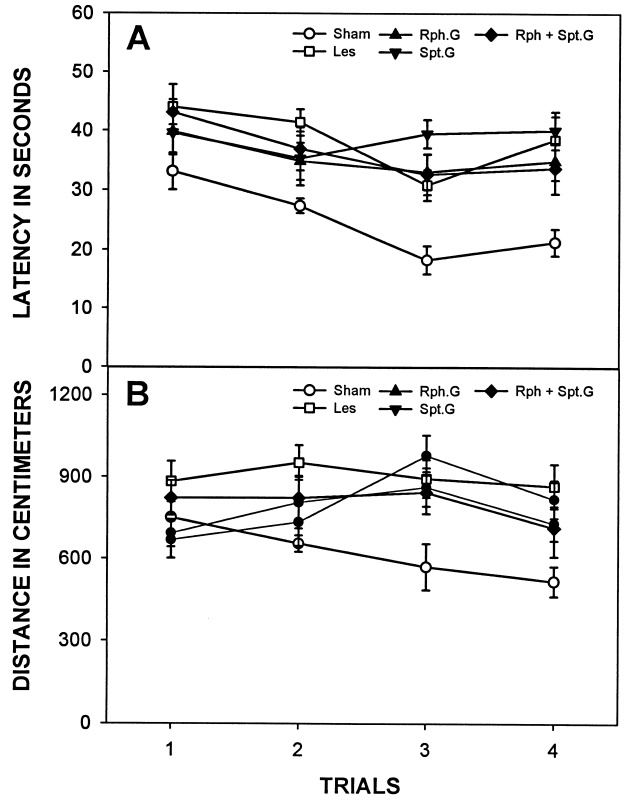

FIG. 5. Average (±SEM) latencies (A) and distances (B) to reach the platform in the water-tank test made according to a protocol sensitive to disruption of working memory.

[$F(4,54) = 4.25$, $p < 0.01$] and only tendencies towards an effect of factor Trial [$F(3,162) = 2.20$, $p < 0.10$] and of the interaction [$F(12,162) = 1.76$, $p < 0.10$]. The Group effect was due to overall distances, which were significantly higher in all rats with lesions, whether grafted or not, as compared to their sham-operated counterparts. As to the average swimming speed (data not shown), ANOVA showed significant Group [$F(4,54) = 2.95$, $p < 0.05$] and Trial [$F(3,162) = 70.1$, $p < 0.001$] effects, but no interaction between both factors. The Group effect was due to a speed which was slightly but significantly reduced in Rph.G rats as compared to Sham rats ($p < 0.05$). All other differences were not significant. The Trial effect was due to an overall speed which was significantly higher on the third trial than on all other ones ($p < 0.001$). Mean group values were: 25.76 ± 0.90 in Sham, 24.20 ± 1.16 in Les, 21.03 ± 1.15 in Rph.G, 22.29 ± 1.04 in Spt.G, and 22.25 ± 1.02 in Rph+Spt.G rats (in cm/s).

Neurochemical Determinations

Concentration of ACh. Data are shown in Table 3. ANOVA showed a significant Group effect in the dorsal [$F(4,52) = 5.85$, $p < 0.001$] and ventral [$F(4,52) = 6.19$, $p < 0.001$] hippocampal pieces. In the dorsal hippocampus, this overall effect was due to a reduced concentration of ACh in lesion-only rats (−57%) or in rats with raphe grafts (−43%) as compared to sham-operated rats ($p < 0.01$ and $p < 0.05$, respectively). A1so, the ACh concentration found in septal-grafted and in co-grafted rats was significantly higher than that found in lesion-only rats ($p < 0.05$, in each case), and did not differ significantly from that measured in sham-operated rats (83% and 99% of normal, respectively). Finally, rats with septal grafts showed values that tended to differ from those of raphe-grafted rats ($p = 0.08$). In the ventral hippocampus, the overall Group effect was due to significantly reduced ACh concentration in lesion-only (−58%) and raphe-grafted (−47%) rats as compared to their sham-operated counterparts ($p < 0.01$, in each case). The difference between co-grafted and lesion-only rats was also significant ($p < 0.05$).

Concentration of NA. Data are shown in Table 3. ANOVA of the square root-transformed data showed a significant Group effect in the dorsal [$F(4,46) = 6.94$, $p < 0.001$] and ventral [$F(4,46) = 3.21$, $p < 0.05$] hippocampal pieces. In the dorsal hippocampus, the overall Group effect was due to NA concentrations, which were significantly higher in lesion-only and raphe-grafted rats as compared to all other groups ($p < 0.02$, at least). The average concentrations found in sham-operated, septal grafted, and co-grafted rats did not differ significantly from each other. In the

TABLE 3

AVERAGE LEVELS (± SEM) OF NEUROCHEMICAL MARKERS OBSERVED IN THE DORSAL AND VENTRAL PORTIONS OF THE HIPPOCAMPUS

Marker	Hippocampal Region	Experimental Group				
		Sham	Les	Rph.G	Spt.G	Rph + Spt.G
[ACh]	Dorsal	1.32 ± 0.11	0.58 ± 0.10*	0.76 ± 0.14*	1.10 ± 0.12	1.31 ± 0.19
	Ventral	1.54 ± 0.11	0.66 ± 0.14*	0.82 ± 0.13*	1.12 ± 0.09	1.20 ± 0.20
		$n = 11$	$n = 12$	$n = 11$	$n = 12$	$n = 11$
[NA]	Dorsal	1.35 ± 0.15	2.43 ± 0.22*	2.68 ± 0.49*	0.98 ± 0.11	1.33 ± 0.22
	Ventral	2.34 ± 0.34	3.79 ± 0.55	4.74 ± 0.70*	2.68 ± 0.32	3.05 ± 0.40
		$n = 9$	$n = 11$	$n = 10$	$n = 11$	$n = 10$
[5-HT]	Dorsal	1.78 ± 0.14	0.79 ± 0.19*	2.60 ± 0.50	0.74 ± 0.19*	2.19 ± 0.45
	Ventral	3.05 ± 0.29	0.93 ± 0.26*	2.99 ± 0.41	1.49 ± 0.32*	2.02 ± 0.36
		$n = 9$	$n = 12$	$n = 10$	$n = 11$	$n = 10$

Data are in ng/mg microwave-irradiated tissue.

Sham, sham-operated rats; Les, lesion-only rats; Rph.G, rats with raphe grafts; Spt.G, rats with septal grafts; Rph + Spt.G, rats with raphe and septal grafts.

* Significantly different from sham-operated rats, $p < 0.05$.

ventral hippocampus, the only significant difference was between the group of raphe-grafted and sham-operated rats ($p < 0.05$).

Concentration of 5-HT. Data are shown in Table 3. ANOVA of the square root-transformed data showed a significant Group effect in the dorsal [$F(4,47) = 7.81$, $p < 0.001$) and ventral [$F(1,47) = 9.0$, $p < 0.001$] hippocampal pieces. In the dorsal hippocampus, this overall effect was due to a reduced concentration of 5-HT in lesion-only rats (−56%) and in rats with septal grafts (−48%) as compared to sham-operated rats ($p < 0.01$ and $p < 0.05$, respectively). There was no significant difference between the raphe-grafted or co-grafted rats and the sham-operated controls (146% and 123% of control, respectively). However, the 5-HT concentrations found in both groups of rats with grafts containing serotonergic neurons were significantly higher than in septal-grafted or lesion-only rats ($p < 0.01$, in any case). In the ventral hippocampus, all differences described in the dorsal hippocampus were also significant, except that the 5-HT concentration of rats with co-grafts did not differ from that of rats with septal grafts.

DISCUSSION

To summarize our results, we observed that lesions of the dorsal septohippocampal pathways decreased the concentration of ACh and 5-HT in the hippocampus, increased locomotor activity in the home cage, impaired spatial working memory in a radial-maze task and altered working and reference memory performances in a water-tank task. Grafts of septal cells almost normalized the concentration of ACh in the dorsal hippocampus but failed to produce any significant behavioral effect. Grafts of raphe cells normalized the concentration of 5-HT in the dorsal hippocampus but had no significant behavioral incidence. Co-grafts of raphe and septal cells simultaneously normalized the concentration of ACh and 5-HT in the dorsal hippocampus and, in the probe trial of the water-tank task, restored some performance to near-normal levels. Finally, whilst an increased NA concentration was found in the dorsal hippocampus of lesion-only and raphe-grafted rats, it was not observed in rats with septal grafts and was not significant in those with co-grafts.

The neurochemical data of the present experiment confirm previous findings showing that (a) extensive lesions of the dorsal septohippocampal pathways not only induce cholinergic and serotonergic denervation of the hippocampus (e.g., [19,22,48]), but also elicit sympathetic sprouting (e.g., [17,28]); (b) intrahippocampal grafts of fetal cell suspensions rich in cholinergic neurons increase markers of cholinergic function to near-normal levels, as do grafts rich in serotonergic neurons with serotonergic markers [14,15,31,35]; (c) the co-grafting technique allows to combine the neurochemical properties of single grafts [14,15,31,35]; (d) grafts providing the hippocampus with a new cholinergic innervation attenuate the lesion-induced increase of NA concentration in the hippocampus [12,14]. Regarding previous studies which showed spared fibers from the ventral hippocampus to sprout and reinnervate part of the hippocampal parenchyma (e.g., [21]), it may seem somewhat puzzling that the 5-HT concentration in the ventral hippocampus remained at a low level even after several postsurgical months. This observation, however, is not at major variance with our previous findings at a comparable delay after aspirative lesions of the fimbria, the fornix, and overlying structures (e.g., [14,28,31]).

The behavioral data of this experiment confirm previous results showing that lesions of the dorsal septohippocampal pathways induce locomotor hyperactivity [3,13,19,30,31] and severely alter cognitive functions related to spatial memory [16,18,39].

Our results with the single grafts rich in cholinergic neurons are not in line with part of the literature. Previous studies had shown that grafts rich in cholinergic neurons may ameliorate performances in a water-tank task [36] and in a radial maze, but only under some conditions, (e.g., appropriate age of the donor [13], additional drug treatment [34], other types of cholinergic lesions [20,26]). In the literature, a view on the functional effects of graft-derived cholinergic reinnervation of the hippocampus considers this reinnervation as necessary, but in no case sufficient, to produce significant and lasting cognitive benefits [18]. This view is in agreement not only with experiments suggesting that co-grafts yield better behavioral results than single grafts [31,35], but also with data suggesting that several types of factors other than the reinnervation of the host structure may influence the functional outcome of a grafting experiment (for a review, see [11]). Regarding previous studies suggesting that the recovery induced by septal grafts alone might reflect functional interactions between the grafted cholinergic neurons and the spared serotonergic afferents that also undergo sprouting [35], it can be speculated that the failure of septal grafts to produce any recovery in the present experiment might result from the rather low levels of serotonergic markers, even after a long postsurgical survival time. Why serotonergic markers remained at levels that were lower in the present study than in other ones remains to be elucidated, though it might be due to variations in the nature or the extent of the lesions.

The grafts rich in serotonergic neurons failed to induce any significant effect on locomotion or cognitive function. This observation is in line with results of previous experiments showing that intrahippocampal grafts rich in serotonergic neurons have no effect on locomotor hyperactivity and cognitive deficits induced by cholinergic and serotonergic denervation of the hippocampus [15,31,35]. Nevertheless, using a lesion technique combining 5,7-DHT lesions of serotonergic neurons and colchicine lesions of cholinergic neurons in the septal region, Richter-Levin et al. [41] found intrahippocampal grafts of raphe neurons to attenuate the lesion-induced deficits in a water-tank test. In this experiment, however, the lesions where quite different from those used in the present experiment.

Finally, as to the co-grafts rich in cholinergic and serotonergic neurons, we confirm and extend our previous findings with lesions restricted to the fimbria and the dorsal fornix [31]. As in our previous study [31], we found that co-grafts rich in cholinergic and serotonergic neurons attenuate the lesion-induced impairment of performance during the probe trial of a water-tank test, but it is noteworthy that, in the present experiment, this beneficial effect was obtained after extensive lesions of the dorsal septohippocampal pathways. The fact that only co-grafts produced beneficial effects is consistent with a viewpoint considering cholinergic-serotonergic interactions to have cognitive implications as substantiated by studies using combined cholinergic and serotonergic lesions (e.g., [37,43]), drug treatments (e.g., [42,45]), or a mixture of both approaches (e.g., [44,45]). The latter aspects have been reviewed by, e.g., Cassel and Jeltsch [9] or Steckler and Sahgal [47]. It must also be stated that not all data collected during the probe trial illustrate a beneficial effect of the co-grafts. Indeed, (a) a significantly increased time spent at searching for the platform in the appropriate quadrant was observed only during the first 30 s of the probe trial; and (b) when the number of swims over the former location of the platform was considered, there was a significant effect of the lesion, but no effect of either graft. While the first observation might be interpreted in terms of different motivations between sham-operated and co-grafted rats (the latter "giving up" earlier in the trial), the second might indicate that whilst the co-grafted rats know in which quadrant they have to look for the plaform according to ambient cues, they remain impaired in reaching the precise location of the platform within this quadrant.

As stated in our Introduction, a major goal of the present experiment was to check whether the behavioral improvement

produced by the co-grafts was specific to a type of task (radial maze vs. water tank) or to a type of memory (working vs. reference). As working and reference memory have been evaluated in the water tank, the finding that the time spent at searching the platform in the appropriate quadrant during the first 30 s of the probe trial did not differ between sham-operated and co-grafted rats suggests that cholinergic and serotonergic reinnervation of the hippocampus preferentially ameliorates spatial reference memory. For consolidation, this suggestion would need to be tested in the radial maze using a protocol allowing to distinguish working memory from reference memory errors (e.g., [29]). In any case, these results provide some support to the hypothesis proposed by Jeltsch et al. [31], namely that co-grafts of cholinergic and serotonergic neurons preferentially improve reference memory capabilities in rats with fimbria-fornix lesions. An alternative but not necessarily exclusive hypothesis may be based on a recent article by Leanza et al. [33,] who showed that complete reinnervation of cortical and hippocampal territories selectively deprived of their cholinergic innervation do contribute to normalize or improve only some aspects of cognitive deficits, particularly those related to reference memory. Conversely, short-term memory capabilities are only inconsistently affected. The authors attribute this limited capacity of the grafts to insufficient integration in the host circuitry because of their ectopic placement (septal cells in the hippocampus). Although we used grafts containing both cholinergic and serotonergic neurons in the present experiment and another lesion model, it cannot be ruled out that a comparable explanation applies to the fact that our grafts affected reference but not working, and thus short term, memory.

The final remarks concern our drug trials in the radial maze. Previous studies had shown that the hippocampal release of ACh is modulated by a serotonergic control involving presynaptic 5-HT_{1B} receptors (e.g., [9,10,47]) and extrahippocampal 5-HT_{1A} receptors (e.g., [27,52]). It is also known that 5-HT_{1A} receptors are located on the soma and the dendrites of serotonergic neurons in the raphe where they are inhibitory (e.g., [25]). Recently 5-HT_{1A} receptors have been found on a subpopulation of cholinergic neurons in the septal region [32]. There are also psychopharmacological data suggesting that systemic activation of 5-HT_{1A} receptors impairs, whereas blockade improves, cognitive performances in rats with cholinergic dysfunctions (e.g., [4,6,7,38]). Even activation of 5-HT_{1A} receptors alone was found to impair cognitive processes [23]. Finally, from previous experiments, it is known that physostigmine can improve radial maze performance in rats with fimbria-fornix lesions and intrahippocampal septal cell suspension grafts (e.g., [34]). For all these reasons and as a preliminary approach, the rats of the present experiment were also tested in the radial maze after injections of physostigmine (inhibitor of acetylcholinesterase), 8-OH-DPAT (5-HT_{1A} receptor agonist), CGS 12066A (5-HT_{1B} receptor antagonist) and WAY 100635 (5-HT_{1A} receptor antagonist). Unfortunately, we found only 8-OH-DPAT to produce a significant effect on radial maze performance. Regardless of the group, when compared to performance after saline injections, this drug produced a weak but significant overall increase of the number of errors. That systemic injections of 8-OH-DPAT could impair cognitive function, in particular working memory, is in line with previous reports [5,7,23,54]. The other drugs had no effect, an observation that could be interpreted as indicating that rats virtually intact, only lesioned, or with septal-, raphe- or co-grafts are not sensitive to these drugs at the doses used. Although doses for these different drugs have been chosen according to the literature, such an interpretation would remain questionable until dose–response relationships for each drug have been evaluated in the radial-maze task. Indeed, most studies on the effects of 8-OH-DPAT or WAY 100635 have used a water-tank task [4,6,7,38] or other tests (e.g., [51]). Furthermore, the effects on spatial memory of systemic treatment with drugs acting at 5-HT_{1B} sites have not been studied so often. Buhot et al. [2] have reported that activation of 5-HT_{1B} receptors produced spatial memory deficits in a radial maze, but they used another 5-HT_{1B} agonist (CP 93,129), and it was microinjected directly into the hippocampus. Future experiments should address these issues more carefully.

In conclusion, the results of this experiment confirm many findings concerning the effects of extensive lesions of the septohippocampal pathways and of intrahippocampal grafts rich in cholinergic and/or serotonergic neurons. It also suggests that reinnervation of the denervated hippocampus by grafted cholinergic and serotonergic neurons induces partial recovery of spatial reference memory in the water-tank task, but fails to improve spatial working memory in the water-tank or radial maze.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. S. Cassel, Mr. O. Bildstein, and Mr. R. Paul for excellent technical assistance. Research funds provided by the Deutsche Forschungsgemeinschaft are also acknowledged (JA 244/4-1).

REFERENCES

1. Beley, A.; Zekhini, A.; Lartillot, S.; Fage, D.; Bralet, J. Improved method for determination of acetylcholine, choline, and other biogenic amines in a single brain tissue sample using high performance liquid chromatography and electrochemical detection. J. Liquid Chromatogr. 10(13):2977–2992; 1987.
2. Buhot, M. C.; Patra, S. K.; Naili, S. Spatial memory deficits following stimulation of hippocampal 5-HT_{1B} receptors in the rat. Eur. J. Pharmacol. 285:221–228; 1995.
3. Capobianco, S.; Hamilton, L. W. Effects of interruption of limbic system pathways on different measures of activity. Physiol. Behav. 17:65–72; 1976.
4. Carli, M.; Bonalimi, P.; Samanin, R. WAY 100635, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial-learning caused by intrahippocampal administration of scopolamine or 7-chlorokynurenic acid. Brain Res. 774:167–174; 1997.
5. Carli, M.; Luschi, R.; Garofalo, P.; Samanin, R. 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT_{1A} receptors in the hippocampus. Behav. Brain Res. 67:67–74; 1995.
6. Carli, M.; Luschi, R.; Samanin, R. (S)-WAY 100135, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial-learning caused by intrahippocampal scopolamine. Eur. J. Pharmacol. 283:133–139; 1995.
7. Carli, M.; Samanin, R. 8-Hydroxy-2-(di-normal-propilamino)tetralin impairs spatial learning in a water maze–Role of postsynaptic 5-HT_{1A} receptors. Br. J. Pharmacol. 105:720–726; 1992.
8. Cassel, J. C.; Duconseille, E.; Jeltsch, H.; Will, B. The fimbria-fornix/cingular bundle pathways: A review of neurochemical and behavioural approaches using lesions and transplantation techniques. Prog. Neurobiol. 51:663–716; 1997.
9. Cassel, J. C.; Jeltsch, H. Serotonergic modulation of cholinergic function in the central nervous system: Cognitive implications. Neuroscience 69:1–41; 1995.
10. Cassel, J. C.; Jeltsch, H.; Neufang, B.; Lauth, D.; Szabo, B.; Jackisch, R. Downregulation of muscarinic- and 5-HT_{1B}- mediated modulation of [^{3}H]acetylcholine release in hippocampal slices of rats with fimbria-fornix lesion and intrahippocampal grafts of septal origin. Brain Res. 704:153–166; 1995.
11. Cassel, J. C.; Kelche, C.; Majchrzak, M.; Will, B. E. Factors influencing structure and function of intracerebral grafts on hippocampal in the mammalian brain: A review. Restor. Neurol. Neurosci. 4:65–96; 1992.
12. Cassel, J. C.; Kelche, C.; Neufang, B.; Will, B. E.; Hertting, G.; Jackisch, R. Graft-derived cholinergic reinnervation of the hippocampus prevents a lasting increase of hippocampal noradrenaline concentration induced by septohippocampal damage in rats. Neurosci. Lett. 138:32–36; 1992.

13. Cassel, J. C.; Kelche, C.; Peterson, G.; Ballough, G. P.; Goepp, I.; Will, B. E. Graft-induced behavioral recovery from subcallosal septohippocampal damage in rats depends on maturity stage of donor tissue. Neuroscience 45:571–586; 1991.
14. Cassel, J. C.; Neufang, B.; Kelche, C.; Jeltsch, H.; Will, B. E.; Hertting, G.; Jackisch, R. Effects of graft containing cholinergic and/or serotoninergic neurons on cholinergic, serotonergic and noradrenergic markers in the denervated rat hippocampus. Brain Res. 604:53–63; 1993.
15. Cassel, J. C.; Neufang, B.; Kelche, C.; Will, B. E.; Hertting, G.; Jackisch, R. Effects of septal and/or raphe cell suspension grafts on hippocampal choline acetyltransferase activity, high affinity synaptosomal uptake of choline and serotonin, and behavior in rats with extensive septohippocampal lesions. Brain Res. 585:243–254; 1992.
16. Collerton, D. Cholinergic function and intellectual decline in Alzheimer's disease. Neuroscience 19(1):1–28; 1986.
17. Crutcher, K. A. Sympathetic sprouting in the central nervous system: A model for studies of axonal growth in the mature mammalian brain. Brain Res. Rev. 12:203–233; 1987.
18. Dunnett, S. B. Neural transplantation in animal models of dementia. Eur. J. Neurosci. 2:567–587; 1990.
19. Dunnett, S. B.; Low, W. C.; Iversen, S. D.; Stenevi, U.; Björklund, A. Septal transplants restaure maze learning in rats with fimbria-fornix lesions. Brain Res. 251:335–348; 1982.
20. Emerich, D. F.; Black, B. A.; Kesslack, J. P.; Cotman, C. W.; Walsh, T. J. Transplantation of fetal cholinergic neurons into the hippocampus attenuates the cognitive and neurochemical deficits induced by AF64A. Brain Res. Bull. 28:219–226; 1992.
21. Gage, F. H.; Björklund, A. Compensatory collateral sprouting of aminergic systems in the hippocampal formation following partial deafferentation. In: Isaacson, R. L.; Pribram, K. H., eds. The hippocampus, vol 3. New York: Plenum Press; 1986:33–64.
22. Gasser, U. E.; Dravid, R. Noradrenergic, serotonergic, and cholinergic sprouting in the hippocampus that follows partial or complete transection of the septohippocampal pathways: Contributions of spared inputs. Exp. Neurol. 96:352–364; 1987.
23. Herremans, A. H. J.; Hijzen, T. H.; Olivier, B.; Slangen, J. L. Serotonergic drug effects on a delayed conditional discrimination task in the rat: Involvement of the 5-HT_{1A} receptor in working memory. J. Psychopharmacol. 9(3):242–250; 1995.
24. Hodges, H. Maze procedures: The radial-arm and water maze compared. Cognitive Brain Res. 3(3/4):167–181; 1996.
25. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Muylecharane, E. J.; Saxena, P.R.; Humphrey, P. P. A. VII. International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin). Pharmacol. Rev. 46:157–203; 1994.
26. Ikegami, S.; Nihonmatsu, I.; Kawamura, H. Transplantation of ventral forebrain cholinergic neurons to the hippocampus ameliorates impairment of radial-arm maze learning in rats with AF64A treatment. Brain Res. 548:187–195; 1991.
27. Izumi, J.; Washizuka, M.; Miura, N.; Hiraga, Y.; Ikeda, Y. Hippocampal serotonin 5-HT_{1A} receptor enhances acetylcholine release in conscious rats. J. Neurochem. 62:1804–1808; 1994.
28. Jackisch, R.; Neufang, B.; Hertting, G.; Jeltsch, H.; Kelche, C.; Will, B. E.; Cassel, J. C. Sympathetic sprouting: Time course of changes of noradrenergic, cholinergic, and serotonergic markers in the denervated rat hippocampus. J. Neurochem. 65:329–337; 1995.
29. Jarrard, L. E. On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. 60:9–26; 1993.
30. Jeltsch, H.; Cassel, J. C.; Jackisch, R.; Neufang, B.; Greene, P. L.; Kelche, C.; Hertting, G.; Will, B. Lesions of supracallosal or infracallosal hippocampal pathways in the rat: Behavioral, neurochemical, and histochemical effects. Behav. Neural Biol. 62:121–133; 1994.
31. Jeltsch, H.; Cassel, J. C.; Neufang, B.; Kelche, C.; Hertting, G.; Jackisch, R.; Will, B. The effects of intrahippocampal raphe and/or septal grafts with fimbria-fornix lesions depend on the origin of the grafted tissue and the behavioural task used. Neuroscience 63(1):19–39; 1994.
32. Kia, H. K.; Brisorgueil, M. J.; Daval, G.; Langlois, X.; Hamon, M.; Verge, D. Serotonin (1A) receptors are expressed by a subpopulation of cholinergic neurons in the rat medial septum and diagonal band of Broca—A double immunocytochemical study. Neuroscience 74(1): 143–154; 1996.
33. Leanza, G.; Martinez-Serrano, A.; Björklund, A. Amelioration of spatial navigation and short-term memory by grafts of foetal basal forebrain tissue placed into the hippocampus and cortex of rats with selective cholinergic lesions. Eur. J. Neurosci. 10:2353–2370; 1998.
34. Low, W. C.; Lewis, P. R.; Bunch, S. T.; Dunnett, S. B.; Thomas, S. R.; Iversen, S. D.; Björklund, A.; Stenevi, U. Function recovery following neural transplantation of embryonic septal nuclei in adult rats septohippocampal lesion. Nature 300(5889):260–262; 1982.
35. Nilsson, O. G.; Brundin, P.; Björklund, A. Amelioration of spatial memory impairment by intrahippocampal graft of mixed septal and raphe tissue in rat with combined cholinergic and serotonergic denervation of the forebrain. Brain Res. 515:193–206; 1990.
36. Nilsson, O. G.; Shapiro, M. L.; Gage, F. H.; Olton, D. S.; Björklund, A. Spatial learning and memory following fimbria-fornix transection and grafting of foetal septal neurons to the hippocampus. Exp. Brain Res. 67:195–215; 1987.
37. Nilsson, O. G.; Streckler, R. E.; 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. 453:235–246; 1988.
38. Ohno, M.; Watanabe, S. Blockade of 5-HT_{1A} receptors compensates loss of hippocampal cholinergic neurotransmission involved in working memory of rats. Brain Res. 736:180–188; 1996.
39. Olton, D. S.; Becker, J. T.; Handelmann, G. E. Hippocampus, space and memory. Behav. Brain Sci. 2:313–365; 1979.
40. Paxinos, G.; Watson, P. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1986.
41. Richter-Levin, G.; Greenberger, V.; Segal, M. Regional specificity of raphe graft-induced recovery of behavioral functions impaired by combined serotonergic/cholinergic lesions. Exp. Neurol. 121:256–260; 1993.
42. Riekkinen, M.; Riekkinen, P.; Sirviö, J.; Riekkinen, P., Jr. Effects of combined methysergide and mecamylamine/scopolamine treatment on spatial navigation. Brain Res. 585:322–326; 1992.
43. Riekkinen, P., Jr.; Sirviö, J.; Riekkinen, P. Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. Brain Res. 527:342–345; 1990.
44. Riekkinen, P., Jr.; Sirviö, J.; Vajakka, A.; Miettinen, R.; Riekkinen, P. Pharmacological consequences of cholinergic plus serotonergic manipulations. Brain Res. 552:23–26; 1991.
45. Riekkinen, M.; Tolonen, R.; Riekkinen, P., Jr. Interaction between 5-HT (1A) and nicotinic cholinergic receptors in the regulation of water maze navigation behavior. Brain Res. 649:174–180; 1994.
46. Stavinhoa, W. B.; Weintraub, S. T.; Modak, A. T. Regional concentrations of choline and acetylcholine in the rat brain. J. Neurochem. 23:885–886; 1973.
47. Steckler, T.; Sahgal, A. The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. Behav. Brain Res. 67:165–199; 1995.
48. Storm-Mathisen, J.; Guldberg, H. C. 5-hydroxytryptamine and noradrenaline in the hippocampal region: Effect of transection of afferent pathways on endogenous levels, high affinity uptake and some transmitter-related enzymes. J. Neurochem. 22:793–803; 1974.
49. Tarricone, B. J.; Simon, J. R.; Li, Y. J.; Low, W. C. Neural grafting of cholinergic neurons in the hippocampal formation. Behav. Brain Res. 74:25–44; 1996.
50. Wallenstein, S.; Zucker, C. L.; Fleiss, J. L. Some statistical methods useful in circulation research. Circ. Res. 47(1):1–9; 1980.
51. Warburton, E. C.; Harrison, A. A.; Robbins, T. W.; Everitt, B. J. Contrasting effects of systemic and intracerebral infusions of the 5-HT_{1A} receptor agonist 8-OH-DPAT on spatial short-term working-memory in rats. Behav. Brain Res. 84:247–258; 1997.
52. Wilkinson, L. O.; Middlemiss, D. N.; Hutson, P. H. 5-HT_{1A} receptor activation increases hippocampal acetylcholine efflux and motor activity in the guinea pig: Agonist efficacy influences functional activity *in vivo*. J. Pharmacol. Exp. Ther. 270:656–664; 1994.
53. Winer, B. J. Statistical principles in experimental design. New York: McGraw Hill; 1971.
54. Winter, J. C. ; Petti, D. T. The effects of 8-hydroxy-2-(di-n-propylamino)tetralin and other serotonergic agonists on performance in a radial maze: A possible role for 5-HT1A receptors in memory. Pharma. Biochem. Behav. 27:625–628; 1987.