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THE EFFECTS OF INTRAHIPPOCAMPAL RAPHE AND/OR SEPTAL GRAFTS IN RATS WITH FIMBRIA–FORNIX LESIONS DEPEND ON THE ORIGIN OF THE GRAFTED TISSUE AND THE BEHAVIOURAL TASK USED

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Abstract—Long–Evans female rats sustained electrolytic lesions of the fimbria and the dorsal fornix and, two weeks later, received intrahippocampal suspension grafts of fetal tissue. The grafts were prepared from regions including either the medial septum and the diagonal band of Broca (septal grafts), or the mesencephalic raphe (raphe grafts), or from both these regions together (co-grafts). All rats were submitted to a series of behavioural tests (home cage and open-field locomotion, spontaneous alternation, radial-arm maze and Morris water maze performance) run over two periods after grafting (one to nine weeks and 20–35 weeks). Two weeks after completion of behavioural testing, histological (acetylcholinesterase and Cresyl Violet staining) and/or neurochemical (choline acetyltransferase activity, high-affinity synaptosomal uptake of choline and serotonin, noradrenaline, serotonin and 5-hydroxyindolacetic acid concentrations) verifications were performed on the hippocampus.

Compared to sham-operated rats, lesion-only rats exhibited hyperactivity which was transient in a familiar environment (home cage) and lasting in an unfamiliar one (open field), decreased rates of spontaneous T-maze alternation, and impaired memory performance in both the radial-arm maze and the Morris water maze. These rats also showed decreased cholinergic and serotonergic markers with a maximal depletion in the septal two-thirds of the hippocampus. Noradrenaline concentration tended to be increased in the dorsal third of the hippocampus, but was not modified in the other two-thirds.

While septal grafts specifically increased the cholinergic markers and raphe grafts the serotonergic ones, neither of these grafts produced a lasting effect on any behavioural variable. Conversely, the co-grafts, which increased both the cholinergic and serotonergic markers in the septal two-thirds of the hippocampus, completely normalized the Morris water maze probe trial performance, but failed to affect any of the other behavioural variables.

Our present results confirm that grafts of fetal neurons injected into the denervated hippocampus may induce a neurochemical recovery that depends on the anatomical origin of the grafted cells, and that co-grafting two fetal brain regions allows the combination of their individual neurochemical properties. Furthermore, our results show that these neurochemical effects of the co-grafts may be involved in the recovery of behavioural function observed in the water maze. However, somewhat paradoxically, those effects appear inefficient for inducing any recovery in other behavioural tasks, even in the radial-arm maze, which is assumed to measure similar spatial functions. Finally, it is suggested that the co-grafts might be more efficient in attenuating the spatial reference memory (Morris water maze) rather than the spatial working memory (radial maze) deficits subsequent to fimbria–fornix lesions.

One major neurochemical feature of Alzheimer's disease (AD) consists of a dramatic degeneration of the cholinergic cerebral neurons.[1,11,32] Although the extent of this cholinergic neuropathology is correlated with the degree of cognitive impairments presented by AD patients,[1,11,58] neurochemical systems other than cholinergic ones are also altered in these patients[45,55] (e.g. serotonergic, noradrenergic, GABAergic).

To test some potential treatments of the cognitive deficits related to AD, several pharmacological models or surgical paradigms of this disease have been developed in animals, particularly in rats.[11,17] One of these surgical paradigms is based on depriving the hippocampus of its cholinergic afferents by extensive lesions of the so-called septohippocampal pathways (i.e. cingular bundle, dorsal fornix and fimbria). Using this lesion paradigm, one experimental approach consists of performing intrahippocampal

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; ChAT, choline acetyltransferase; CRL, crown–rump length; E, embryonic day; EDTA, ethylenediaminetetra-acetate; EGTA, ethyleneglycol-bis(aminoethylether)tetra-acetate; HACU, high-affinity choline uptake; HASU, high-affinity serotonin uptake; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulphonic acid; 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, serotonin; KHB, Krebs–Henseleit buffer; MWM, Morris water maze; NA, noradrenaline; RAM, radial-arm maze.

grafts rich in cholinergic neurons in order to replace the missing hippocampal cholinergic afferents. Thus, studies have demonstrated that such grafts are able to attenuate (or even to fully compensate for) some of the electrophysiological,[43,63,65] neurochemical,[9,10,14,39] histochemical[48,50] and behavioural[6,18,19,43,48,50] deficits subsequent to the disruption of the septohippocampal pathways.

However, in addition to the cholinergic hippocampal afferents, septohippocampal lesions also disrupt serotonergic fibres arising from the medial raphe, noradrenergic fibres arising from the locus coeruleus and GABAergic fibres originating in the septal region.[24,25,71] In agreement with Nilsson *et al.*,[48] we have recently reported that, by using a co-grafting approach, it was possible to compensate for more than one of the neurochemical deficits observed in the hippocampus after septohippocampal lesions; specifically, grafts rich in cholinergic neurons fostered cholinergic recovery, grafts rich in serotonergic neurons fostered serotonergic recovery, whereas co-grafts of both these preparations produced cholinergic and serotonergic recovery;[9] in addition, this recovery was closer to 100% than the recovery observed following single graft implantation. Unfortunately, in our experiment, neither of these grafts was able to attenuate the lesion-induced deficits found in a radial-arm maze (RAM) learning task.

Most studies assessing the structural and/or functional effects of intrahippocampal grafts rich in cholinergic neurons were carried out in rats given extensive (aspiration or knife-cut) damage to the septohippocampal pathways. As a potential animal model of AD, such extensive lesions present at least one major deviation from the overall neurochemical alterations found in AD patients. Apart from their effects on other neurotransmitter systems (see above), these lesions induce an almost complete cholinergic depletion in the hippocampus, whereas the cholinergic degeneration found in the brain of AD patients remains incomplete.[45,47,59] Furthermore, aspiration of the fimbria–fornix, which cannot be carried out without producing severe damage to the overlying cortical structures and the corpus callosum, can also produce sensorimotor disturbances (Cassel *et al.*, unpublished observations). Therefore, partial lesion paradigms consisting, for instance, of damaging more specifically the fimbria and the dorsal fornix, but without altering the overlying structures, might be more appropriate both for modelling AD in animals and assessing the functional effects of various putative therapeutic treatments (e.g. grafts, drugs, neurotrophic factors). However, as regards the utilization of intracerebral grafts, such partial deafferentation paradigms might be helpful only if they induce functional deficits which are not subject to spontaneous recovery over long post-operative periods.

Recently, we found that bilateral lesions of the infracallosal component of the septohippocampal pathways (fimbria and dorsal fornix) induce behavioural and neurochemical deficits which, even after several months, do not result in recovery.[38] Rats given such lesions showed a clear-cut decrease in hippocampal markers of cholinergic and serotonergic function, and exhibited increased locomotion activity in an open field, reduced spontaneous alternation, as well as impaired RAM learning performance.

Given the above, in the present study, we used infracallosal septohippocampal lesions to assess the functional effects of intrahippocampal cell suspension grafts rich in either cholinergic or serotonergic neurons as compared to those of co-grafts of both these preparations. The different types of cell suspensions were injected into the dorsal hippocampus of three-month-old Long–Evans female rats which, two weeks prior to grafting, were submitted to an electrolytic lesion of the fimbria and the dorsal fornix. The grafts rich in cholinergic neurons were prepared from the ventral forebrain region, which includes the septum and the diagonal band of Broca. The grafts rich in serotonergic neurons were prepared from the region of the fetal brain including the medial raphe. The behavioural effects of both the lesions and the grafts were assessed over two periods (one to nine and 20–35 weeks after grafting). We measured home cage activity, open field locomotion, spontaneous alternation in a T-maze, RAM performance and Morris water maze (MWM) learning. All these tasks were chosen because of their classically described sensitivity to hippocampal lesions or denervations.[52,53] Two weeks after completion of behavioural testing, most rats were killed (at least seven per group) and their hippocampi were processed for the following neurochemical determinations: high-affinity synaptosomal uptake of [^{3}H]choline (HACU) and [^{3}H]serotonin (HASU), choline acetyltransferase activity (ChAT), as well as hippocampal serotonin (5-HT), 5-hydroxyindolacetic acid (5-HIAA) and noradrenaline (NA) concentrations. The remaining rats were processed for morphological and histochemical determinations using Cresyl Violet and acetylcholinesterase (AChE) staining, respectively.

EXPERIMENTAL PROCEDURES

Subjects

Long–Evans female rats ($n = 58$), obtained from R. Janvier (France), were used in this study. They were housed in Makrolon cages (59 × 38 × 20 cm) in groups of five or six, except during behavioural testing during which they were isolated in smaller cages (42 × 26 × 15 cm). Food and water were available *ad libitum*, except during RAM testing. The colony room and the testing rooms were maintained on a 12.00 light/12.00 dark cycle (lights on at 07.00) under controlled temperature (23 ± 1°C).

Surgery

All surgery was performed under aseptic conditions, using pentobarbital anaesthesia (40 mg/kg, i.p.) 10 min after an atropine sulphate injection (4 mg/kg, i.p.).

Lesion surgery. At 91 (± 1) days of age, 47 rats received a bilateral electrolytic lesion of the infracallosal septohippocampal pathways (fimbria–fornix), which was made

by passing a rectified current of 1 mA for 40 s through an epoxylite-coated stainless steel electrode (0.15 mm in diameter) which was uninsulated at the tip (*c.* 0.5 mm). The electrode was lowered into the brain at five sites according to the following coordinates: from lambda,[56] A 5.2 mm, L ± 0.8 mm, V − 3.3 mm; A 5.5 mm, L 0.0 mm, V − 3.5 mm; A 5.8 mm, L ± 1.8 mm, V − 3.8 mm. The incisor bar was placed 3.0 mm below the interaural line. The control group (Group Sham; $n = 11$) consisted of rats which received scalp incision and removal of the bone overlying the dorsal parietal cortex.

Transplant surgery. Cells to be grafted were prepared from the brains of Long–Evans fetuses. Two weeks after lesion surgery, a first subgroup of lesioned rats (Group Septum; $n = 10$) received bilateral intrahippocampal grafts of a cell suspension prepared from the region including the septal–diagonal band of Broca, a region which is rich in cholinergic neurons (crown–rump length, CRL: 14 mm; embryonic day, E15). A second subgroup of lesioned rats (Group Raphe; $n = 12$) received grafts of a cell suspension prepared from the region including the medial raphe, which is rich in serotonergic neurons (CRL: 12 mm; E13). In a third subgroup, the lesioned rats (Group Raphe + Septum; $n = 13$) received co-grafts of a cell suspension in which septal and raphe tissue had been mixed prior to the dissociation (CRL: 12 and 14 mm; E13 and E15). The fourth subgroup (Group Lesion; $n = 11$) consisted of lesioned rats which did not receive grafts.

The grafts were performed as described in detail by Cassel *et al.*[9] Briefly, after extraction of the fetal brains, the ventral forebrain region containing the septal area or the region including the mesencephalic raphe was dissected out under a stereoscopic magnifier using sterile instruments and glassware. The dissection procedure of the fetal ventral forebrain was similar to that described by Björklund *et al.*,[3] excision of the region including the fetal mesencephalic raphe was done as described by Seiger[64] and Steinbusch *et al.*[69] Respectively to their anatomical origin, tissue fragments were collected in 0.6% glucose–saline, incubated for 20 min at 37°C in the same solution with 0.1% trypsin (Sigma, Grade II), washed three times with 5 ml of fresh glucose–saline and brought to a final volume of about 10 µl per septal tissue piece and 20 µl per raphe tissue piece. These blocks were then dissociated using a fire-polished Pasteur pipette until a milky suspension was obtained. Injections (2 µl/site, 1 µl/min) of the resulting suspensions were performed stereotaxically, through a Hamilton syringe, into each dorsal hippocampus at the following coordinates: from lambda,[56] A 4.0 mm, L ± 1.6 mm, V 3.1 mm; A 2.4 mm, L ± 3.2 mm, V 3.3 mm. The incisor bar was placed 3.0 mm below the interaural line. The syringe was left *in situ* for 2 min after each injection. Cell suspensions were used within a maximum of 3 h after preparation. The number of cells injected was counted in a haemocytometer (Thoma chamber; for details see Ref. 8) and non-viable cells were identified with 0.05% Trypan Blue. Counts of viable cells per µl were 47,000 for the ventral forebrain suspension, 38,750 for the mesencephalic raphe suspension and 70,000 for the suspension used for the co-grafts. In all cases, the suspensions contained about 10% damaged, non-viable cells.

Behavioural tests

For the first post-surgical period of testing, behavioural testing began one week after transplant surgery and continued for eight weeks. The tests were run in the following order: home cage activity (over three days), open-field activity (over three days), spontaneous alternation (over five days) and RAM (over a period of four weeks: pre-training + 40 uninterrupted trials). After a 12-week interruption of testing, the rats were tested again (second period) according to the same protocol, except for RAM testing, which was modified as follows: (i) only 16 trials were run under conditions identical to those of the first period of testing; (ii) after these 16 trials, the rats were tested with the introduction of a 1 min delay between their fourth and fifth choices (24 trials). When RAM testing was completed, all rats were tested for their learning and retention performances in the MWM task.

Home cage activity. The spontaneous activity of the rats was recorded for 22 h in the home cages, starting at 11.00. A first period of observation lasted for 3 h (habituation to the experimental conditions). A second period of observation, which began at 14.00, lasted for 19 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 was monitored continuously by a microcomputer. Rats were tested one week before lesion surgery, one week after lesion surgery and one, 12 and 20 weeks after transplant surgery.

Open-field activity. The open field consisted of an unpainted wooden square enclosure with 43.5 cm high walls and a 65 × 65 cm floor divided into 25 equal squares. Each rat was placed in a corner square, facing the corner, and observed for 10 min. In the testing room, the only illumination was provided by a 40 W white light placed centrally, 1.80 m above the field. The experimenter was unaware of the surgical treatments of the rats and recorded the number of squares crossed. The rats were tested for two weeks and again during the 21st week after transplant surgery.

Spontaneous alternation. The apparatus was a grey Perspex T-maze (10 cm high × 10 cm wide), with a transparent Perspex roof and a 40 W white lamp located 105 cm above the choice point. The 45-cm-long stem and 21-cm-long side arms ended in 20-cm-long interchangeable start box/goal boxes. Guillotine doors could be placed adjacent to each start/goal box to confine the rat in the start box or at the extremity of the chosen arm. During testing, the rat was placed in the start box for 10 s before the guillotine door was opened; once the rat had reached the end of the chosen arm, it was retained in the goal box for 30 s before another trial was run; two trials were run each day. The goal box, with the rat inside, was interchanged with the start box. The rats were tested during the second week and again during the 21st week after transplant surgery.

Radial-arm maze. Apparatus. RAM training and testing were run using two identical grey wooden RAMs placed in an experimental room with several different visual cues (pictures on the walls, a chair, computer, desk, etc.). The octogonal 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 sentence. Access to each arm was controlled by transparent Perspex guillotine doors. Each maze, elevated 68 cm above floor level, was illuminated by a 40 W white light located about 180 cm above the centre of each maze. In each maze, 16 infrared photocells recorded entries and movements of the rat (two photocells per arm, one at 12 cm from the arm entrance and the other 10 cm from the end of the arm, with the infrared beam 4 cm above floor level). Sequences of photocell beam interruptions were monitored with a microcomputer. Errors were defined as re-entries into already visited arms within a given trial.

Training procedure. The body weight of all rats was reduced (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 food pellets in the maze on five consecutive days prior to testing. On the first day, only one arm was accessible and its food well was filled with eight calibrated food pellets (45 mg, Campden, U.K.). On the second day, two adjacent arms were accessible with four pellets placed in each food well. For the three following days, three adjacent arms were accessible with two pellets placed in each food well. On each day of training, the rat

was returned into its home cage either when all food pellets had been eaten or after 15 min regardless of how many pellets had been consumed. By the fifth day, all rats ate the six pellets in less than 15 min.

Testing procedure. Following training, all rats were tested twice a day (five days per week) for 40 trials during the first period of testing (from the sixth to the ninth weeks after surgery). The daily trials were separated by at least 120 min. At the start of a trial, a rat was placed on the central platform with all guillotine doors open; the trial was finished when all arms had been visited.

For the second period of testing, training began 26 weeks after surgery, starting with three arms open. It lasted for three days. After an initial series of 16 trials (two per day), which were run according to the same procedure as for the first period, testing was continued for 24 days according to an interrupted procedure (analogous to the delayed-non-match-to-sample task). For both the uninterrupted and the interrupted testing procedures, errors were also defined as re-entries into already visited arms in a given trial.

The interrupted procedure (which increases the temporal constraints of the task) was used to prevent some rats from using efficient egocentric choice strategies (e.g. always clockwise or always counter-clockwise repetition of choice directions such as 45° or 135°). Such strategies closely resemble sequential routines. They do not require the use of extramaze cues in performing the task and minimize the mnemonic load of the task. This procedure allowed us to investigate whether rats from the different treatment groups which had developed egocentric choice strategies were also able to use extramaze cues to complete the task. Under this testing condition, the rats were tested only once a day. A rat was placed on the central platform with four of the eight guillotine doors lowered (according to one of four pre-determined patterns which were used alternately in randomized order; i.e. open arms: 2, 4, 6, 8; 4, 5, 6, 7; 2, 3, 7, 8; 1, 4, 5, 6). When the four open arms had been visited, the rat was taken from the apparatus to its home cage, where it remained for 1 min before being reintroduced into the centre of the maze (all doors now open) to complete the trial. Under such testing conditions, the persistent use of egocentric choice strategies results in an increased number of errors. In utilizing this procedure, our goal was not primarily to check whether lesioned rats used egocentric strategies more frequently than the sham-operated ones, but was to impose a testing protocol in which the rats which preferentially use egocentric strategies have to switch to an allocentric strategy in order to keep a good level of performance. However, considering the last eight uninterrupted trials of the second testing period, we have also checked whether there was a difference among our five experimental groups in the number of rats which consistently used efficient egocentric strategies. A rat was considered to preferentially perform the task according to an egocentric strategy when (i) it repeated an angle of 45° or 135° at least six times out of the first seven angular choices of a given trial, (ii) it always did so in the same direction (either clockwise or counter-clockwise) and (iii) such a choice pattern was observed in at least six trials out of the last eight trials of the interrupted testing procedure of the second testing period.

Morris water maze. Apparatus. The MWM consisted of a circular pool (diameter 160 cm; height 60 cm) filled with water to a height of 30 cm. The water (25°C) was made opaque with powdered milk. The pool was located in an experimental room with many extramaze cues (e.g. chair, computer, desk, animal cages, lights, etc.). The pool was divided into four quadrants of equal surface and four starting points were defined as north, south, east and west. A circular platform (diameter 11 cm) was placed at a constant position in the middle of one quadrant of the pool. All movements of the rats were recorded by a camera and analysed by a computer which calculated the distance swum (cm) and the latency (s) to reach the platform.

Pre-training procedure. Pre-training lasted over four days. For the first three days, the platform was 1 cm above the water surface. On days 1 and 2, each rat was placed on the platform for 30 s. On day 3, each rat was given four trials which were made with four different starting positions, 90° apart, according to a randomized order (e.g. E, S, W, N; W, E, S, N; etc.). For each trial, the rat was released in the water, facing the wall, at the designated starting point. It was given a maximum of 120 s to reach the platform. When it had climbed onto the platform, the rat was allowed to remain there for 20 s before being removed and placed at the next pre-determined starting point. If the rat did not find the platform, it was picked up and placed on the platform for 20 s by the experimenter. On day 4 of pre-training, the top of the platform was lowered to the level of the water surface.

Testing procedure. On day 5 and thereafter, the platform was submerged 1 cm under the surface of the water. Each rat was tested for four days with four consecutive trials per day. Immediately after the third trial of the fourth day, the platform was removed and each rat was given a probe trial in which it was allowed to swim for 60 s. The computer calculated the distance as well as the time spent in the quadrant where the platform had been located. Testing began 32 weeks after transplant surgery.

Neurochemical determinations

Tissue preparation. Approximately two weeks after completion of behavioural testing, some of the rats from each group (seven from each control group and eight from each group of grafted rats) were chosen randomly and used for neurochemical determinations. All remaining rats were used for histological and histochemical verifications (see below).

The rats were decapitated and their brains were quickly removed. First, the brain was sectioned transversally at the level of the septal pole of the hippocampus, thereby separating a rostral and a caudal portion of the brain. The rostral portion was collected in 0.1 M phosphate-buffered 1.6% paraformaldehyde and kept at 4°C until it was sectioned for determination of the lesion extent. From the caudal portion, both hippocampi were dissected free and cut into three pieces of approximately equal size, thereby separating a dorsal (septal pole), a "middle" (intermediate) and a ventral (temporal pole) region. According to their septotemporal level of origin, the left and the right regions were collected together into 3 ml of 0.32 M sucrose (in 2.5 mM HEPES, pH 7.4) where they were homogenized in a Potter/Elvehjem glass/Teflon homogenizer (eight strokes at 500 rpm). From this crude homogenate, a 20 μl sample was used for determination of protein content, another 100 μl sample was used for measurements of ChAT activity and a further 100 μl aliquot was mixed with 100 μl 0.2 N $HClO_4$ (containing 250 mg Na_2SO_3 and 200 mg disodium EDTA per litre) and stored at −20°C for high-performance liquid chromatography determinations. The remaining homogenate was centrifuged for 10 min at 1000 *g*; the supernatant was carefully removed by pipetting and centrifuged for 10 min at 17,000 *g*.

Determination of synaptosomal uptake. The pellet of this second centrifugation was carefully resuspended in 700 μl of Krebs–Henseleit buffer (KHB) containing 60 μM pargyline. From this suspension, 375 μl were further diluted into 1125 μl KHB. The resulting suspension (aliquots of 250 μl) was incubated for 5 min at 30°C in the presence of 50 nM of either [^{3}H]choline or [^{3}H]5-HT (samples in triplicates). In a series of preliminary experiments we found that, under these conditions, the synaptosomal uptake was linear between 2 and 8 min for [^{3}H]choline and between 2 and 5 min for [^{3}H]5-HT. In order to correct for unspecific binding or uptake, parallel samples (duplicates) were run in the presence of 1 μM (each) of hemicholinium-3 ([^{3}H]choline uptake) or fluvoxamine ([^{3}H]5-HT uptake). The incubation was stopped by addition of 4 ml of ice-cold KHB (+pargyline), followed by rapid filtration through cellulose nitrate filters (0.65 μm pore size; Sartorius) and further

washing of the filters [three times with 4 ml KHB (+pargyline)]. The filters were dissolved in 2 ml of ethyleneglycol monoethylether and radioactivity was assessed by liquid scintillation counting after addition of 10 ml of toluene scintillator.

Determination of choline acetyltransferase activity. ChAT activity was determined as described by Fonnum[23] with several modifications. In brief, 100 μl of the crude homogenate of hippocampal slices (see above) were diluted with 100 μl of a medium containing 269 mM NaCl, 90 mM NaH_2PO_4, 0.45% Triton X-100, 0.9 mM EDTA, 0.9 mM Na_2EDTA and 179 μM physostigmine. Ten microlitres of this mixture (all samples in triplicates) were added with 5 μl of choline bromide (32 mM). The incubation was started by the addition of 5 μl of [^{14}C]acetyl-coenzyme-A (50 nCi/assay; 0.242 mM final concentration). After 20 min at 37°C, the tip of the incubation tube was cut with a razor blade and put into a mixture of 5 ml sodium phosphate buffer (10 mM, pH 7.4) with 2 ml of sodium tetraphenylborate in acetonitrile (5 mg/ml). From this mixture, the newly formed [^{14}C]acetylcholine was extracted by careful shaking with 10 ml of toluene scintillator. Following separation of the aqueous phase from the organic phase, the samples were directly counted by liquid scintillation counting. In order to correct for non-specific effects, for each hippocampal region, two samples were run at 0°C.

Determination of serotonin, 5-hydroxyindolacetic acid and noradrenaline concentrations. The concentration of these products was evaluated by high-performance liquid chromatography with electrochemical detection (detector model M20, pump model 300c, Gynkotek). Each sample (see above) was centrifuged and the supernatant (aproximately 90 μl) filtrated through 0.22 μm filters (Millipore). Twenty microlitres of the filtrated supernatant were injected into a C18 reversed-phase column (ODSII, 5 μm). The separation of the different compounds was obtained using a citrate–acetate buffer (pH 4.1, 39.8 mM sodium acetate, 17.3 mM citric acid monohydrate, 86.7 μM disodium EDTA) containing 80 ml/l methanol, 6.5 ml/l ethanol and 518 mg/l sodium octane sulphonate. Electrochemical detection (Gynkotek) was performed at 0.6 V using a calomel reference electrode. Sensitivity of the detection was 2.5 pg for NA and 5-HIAA, and 7.4 pg for 5-HT.

Protein content. Protein content was assessed in both the crude homogenate (used for determination of ChAT activity, 5-HT, 5-HIAA and NA concentrations) and in the suspension of synaptosomes, according to the method described by Lowry *et al.*[44]

Histological/histochemical verifications

All rats which were not used for neurochemical determinations were anaesthetized with an overdose of pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 50 ml of 0.9% saline followed by 60 ml of 0.1 M phosphate-buffered 4% paraformaldehyde (4°C). After extraction, the brains were post-fixed for about 4 h and transferred into a 0.1 M phosphate-buffered 20% sucrose solution for 36–40 h. The brains were then quickly frozen and cut into 30-μm-thick coronal sections using a cryostat (−20°C). From the posterior septum to the posterior region of the hippocampus, each fifth section was collected onto gelatine-coated slides. The sections were dried at room temperature for 36 h and stained either with Cresyl Violet[68] or for AChE according to a method similar to that of Koelle:[40] ethopropazine (0.3 mM) was used to block non-specific cholinesterases and acetylthiocholine iodide (4 mM) was used as the substrate.

Statistical analysis

All data were analysed by an analysis of variance[78] (ANOVA) followed, where appropriate, by 2 × 2 comparisons based on Duncan's multiple range test.[72] Neurochemical data were analysed separately for each hippocampal region (dorsal, "middle" and ventral). Since variances in home cage activity, open-field scores and RAM performances did not show homogeneity, a square root transformation of these data was made prior to running the ANOVA.[77]

For comparing the proportion of rats which, in the five experimental groups, showed a persistent use of egocentric strategies in the radial maze, we used a Chi-Square test.[72] The computation of the correlation coefficients between behavioural and neurochemical variables was performed according to the method described by Tallarida and Murray.[72]

RESULTS

Behavioural assessment

Home cage activity. Day period (Fig. 1A). Average pre-operative scores did not differ significantly among the five groups. Following the lesion and transplantation surgeries (one week, and one, 12 and 20 weeks, respectively), there was no significant overall Group effect, $F(4/52) = 2.3$, $P = 0.06$ (5 × 5 "Group" Factor × "Test Period" Factor ANOVA), but a significant Test Period effect, $F(4/208) = 31.3$, $P < 0.001$, as well as a significant Group × Test Period interaction, $F(16/208) = 1.9$, $P = 0.02$. The

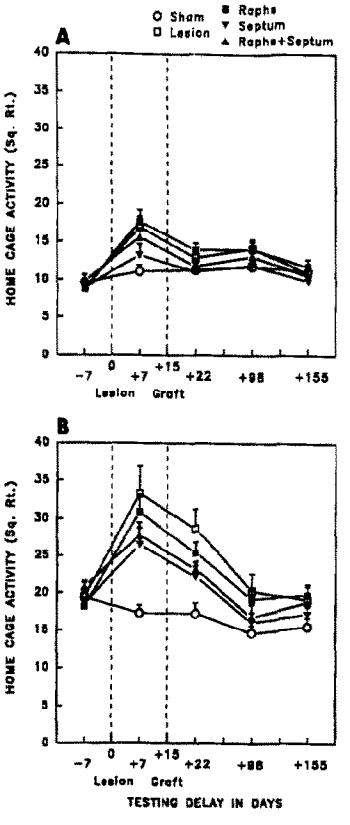

Fig. 1. Average home cage diurnal (A) and nocturnal (B) activity scores (number of cage crossings; square roots) found before lesion surgery (−7) and at four post-surgical delays in sham-operated (Sham), lesion-only (Lesion) and lesioned rats with raphe (Raphe) or septal (Septum) grafts, or with co-grafts of both raphe and septal tissues (Raphe + Septum).

Test Period effect, as well as the interaction between factors "Group" and "Test Period", were due to both an increase in activity shortly after lesion surgery ($P < 0.05$ in all cases) and a subsequent decrease in all lesioned rats, as opposed to the more stable activity noted in sham-operated rats. Neither type of graft provided any significant effect on the lesion-induced hyperactivity.

Night period (Fig. 1B). Average pre-operative scores did not differ significantly among the five groups. A 5 × 5 (Factor "Group" × Factor "Test Period") ANOVA revealed significant Group, $F(4/52) = 5.0$, $P = 0.001$, and Test Period effects, $F(4/208) = 51.7$, $P < 0.001$, as well as a significant Group × Test Period interaction, $F(16/208) = 3.8$, $P < 0.001$. The Group effect consisted of increased activity in all lesioned rats, whether grafted or not ($P < 0.05$ in all cases). No significant difference was observed between grafted and lesion-only rats, whatever type of graft was considered. The significant Test Period effect, as well as the significant Group × Test Period interaction, reflect a decline, over time, of the lesion-induced hyperactivity.

Open-field locomotion. For the first post-surgical period of testing (two weeks after transplantation surgery; Fig. 2A), the ANOVA of the number of squares crossed (Factor "Group" × Factor "Block") showed a significant overall Group effect, $F(4/52) = 6.8$, $P < 0.001$, a significant Block effect, $F(4/208) = 25.4$, $P < 0.001$, but no significant Group × Block interaction. The Group effect was due to increased locomotion in all rats with lesions, as compared to sham-operated rats ($P < 0.05$ in all cases). Neither type of graft produced any significant effect on the lesion-induced hyperlocomotion. Regardless of the surgical treatment, the Block effect was due to a decline in activity during the 10 min of observation. The lack of interaction indicates that the activity decline between the first and last blocks did not differ significantly among the groups.

For the second post-surgical period of testing (21 weeks after transplantation surgery; Fig. 2B), there was again a significant overall Group effect, $F(4/52) = 3.2$, $P = 0.019$, which was due to a significantly increased locomotion in rats with raphe grafts, as compared to either sham-operated, lesion-only or rats with septal grafts ($P < 0.05$ in all cases). There was no other significant difference among the groups. There was also a significant Block effect, $F(4/208) = 11.1$, $P < 0.001$, but no significant Group × Block interaction. We propose the same interpretation for both this Block effect and the lack of interaction as for the first period of testing.

Spontaneous alternation. For the first post-surgical period of testing (two weeks after transplantation surgery; Table 1), the ANOVA showed a significant Group effect, $F(4/52) = 4.5$, $P = 0.003$: the mean alternation scores were significantly lower in all lesioned rats, whether grafted or not, as compared to Sham rats ($P < 0.05$ in all cases).

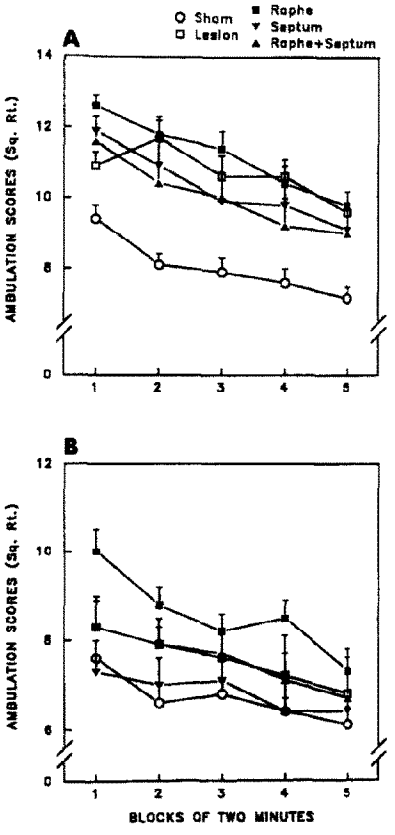

Fig. 2. Average open-field ambulation scores (square roots) found two weeks (A) and 20 weeks (B) after transplant surgery. Group abbreviations as in Fig. 1.

For the second post-surgical period of testing (21 weeks after transplantation surgery; Table 1), the ANOVA showed a significant Group effect, $F(4/52) = 3.5$, $P = 0.012$. The mean alternation scores were significantly lower in lesion-only rats and in rats with only septal grafts or co-grafts, as compared to Sham rats ($P < 0.05$ in all cases). Conversely, rats with raphe grafts showed an average alternation rate which did not differ significantly either from that of Sham rats ($P < 0.05$) or from that of lesion-only rats.

Radial-arm maze performance. Procedure with no interruption. Analyses were run on maze performances averaged over four-trial blocks.

For the first period of testing (six to nine weeks after transplantation surgery; Fig. 3A), the ANOVA of the number of errors (Factor "Group" × Factor "Block") showed significant Group, $F(4/52) = 10.8$, $P < 0.001$, and Block, $F(9/468) = 40.9$, $P < 0.001$, effects, as well as a significant Group × Block interaction, $F(36/468) = 1.7$, $P = 0.01$. The Group effect was due to a number of errors which was significantly increased in all rats with lesions, whether grafted or not, as compared to sham-operated rats ($P < 0.05$ in all cases). In addition, the overall number of errors of rats with septal grafts was significantly lower than that of lesion-only rats ($P < 0.05$).

Table 1. Average (S.E.M.) spontaneous alternation rates (%) found during both post-grafting testing periods in sham-operated, lesion-only rats and lesioned rats with a raphe, a septal or a mixture of raphe and septal grafts

	Surgical treatment				
Period of testing	Sham	Lesion	Raphe	Septum	Raphe + Septum
2 weeks post-grafting	65.5 (5.7)	41.8* (5.0)	42.5* (4.3)	35.0* (4.5)	46.9* (6.5)
21 weeks post-grafting	78.2 (3.5)	53.6* (6.0)	67.5 (6.5)	51.0* (6.4)	57.7* (6.2)

*Significant difference compared to Sham rats (Duncan's test; $P < 0.05$).

For the second period of testing (26–32 weeks after transplantation surgery; Fig. 3B), there were still significant overall Group, $F(4/52) = 5.7$, $P < 0.001$, and Block, $F(3/156) = 6.5$, $P < 0.001$, effects, but no significant interaction between these two factors. Compared to that found in Sham rats, the overall number of errors was significantly increased in all groups of rats with lesions, whether grafted or not ($P < 0.05$ in all cases). Neither type of graft provided any significant effect on the lesion-induced impairment. In the last eight trials of this test, we found that two sham-operated rats (out of 11), three lesion-only rats (out of 11), three rats with raphe grafts (out of 12), five rats with septal grafts (out of 10) and four rats with co-grafts (out of 13) showed a preference for performing the maze with egocentric strategies. Statistical analysis of these data did not reveal a significant difference among the five groups ($\chi^2 = 2.83$, 4 d.f., ns).

Procedure with interruption. Data are shown in Fig. 3C. The ANOVA of the number of errors (Factor "Group" × Factor "Block") showed significant Group, $F(4/52) = 13.0$, $P < 0.001$, and Block, $F(5/260) = 29.5$, $P < 0.001$, effects, but no significant Group × Block interaction. Compared to that found in sham-operated rats, the number of errors was significantly increased in all rats with lesions, whether grafted or not ($P < 0.05$ in all cases), and neither type of graft provided any significant effect on the lesion-induced impairment. The Block effect was due to an improvement of the scores, over time, which was significant in all groups of rats.

Morris water maze. Acquisition (Fig. 4A and B). The ANOVA of the escape mean distances as well as that of the escape mean latencies (Factor "Group" × Factor "Day") revealed significant overall Group [Distance: $F(4/52) = 3.5$, $P = 0.01$; Latency: $F(4/52) = 4.8$, $P = 0.002$] and Day [Distance: $F(3/156) = 10.0$, $P < 0.001$; Latency: $F(3/156) = 11.2$, $P < 0.001$] effects, but no significant Group × Day interaction. The Group effect was due to the poor performances of the rats with single grafts (group S and group R) which showed increased distances and latencies to find the platform, as compared to the sham-operated rats ($P < 0.05$ in all cases). The lesion-only and the co-grafted rats were not as impaired as single-grafted rats, and performed at a level which did not differ from that found in Sham rats. The Day effect was due to an overall improvement of the performances over time. This was observed in the Sham group but also in all other groups, an observation which may also explain the lack of a significant Group × Day interaction.

Probe trial (Table 2). During the probe trial, the rats showed average swimming speeds which did not differ significantly across the five groups (Table 2). Concerning the distance and the time spent in the quadrant where the platform was located during the acquisition phase of the test, the ANOVA revealed a significant Group effect [Distance: $F(4/52) = 8.3$, $P < 0.001$; Time: $F(4/52) = 7.6$, $P < 0.001$]. Performances of rats with only lesions, with raphe grafts or with septal grafts did not differ significantly and were all significantly lower than those of sham-operated rats ($P < 0.05$). In rats with co-grafts, the performances were significantly better than in lesion-only rats ($P < 0.05$) and did not differ significantly from those of sham-operated rats. An illustration of a swimpath from one typical rat in each of the five groups is shown in Fig. 5. A comparison of the number of crossings of the former platform position showed that both Sham and Septum + Raphe rats swam more often over this position than the rats in the three other groups, but this difference was not significant, $F(4/52) = 1.7$. Unfortunately, this finding does not allow us to make any clear conclusion concerning a between-group difference in the ability of the rats to precisely localize the platform position.

Summary of the behavioural data. Rats with infracallosal lesions showed increased locomotor activity in both a familiar (home cage) and an unfamiliar (open field; only for the first testing period) environment, impaired performance in spontaneous alternation and RAM tasks, as well as in the probe trial of the MWM task. The use of an interrupted RAM procedure further impaired performances of all rats with lesions without significantly affecting those of sham-operated control rats. Raphe grafts were found to improve recovery of only spontaneous alternation, but also to hamper recovery of normal ambulation scores in the open-field test. Septal grafts were found to improve only RAM performance, but only during

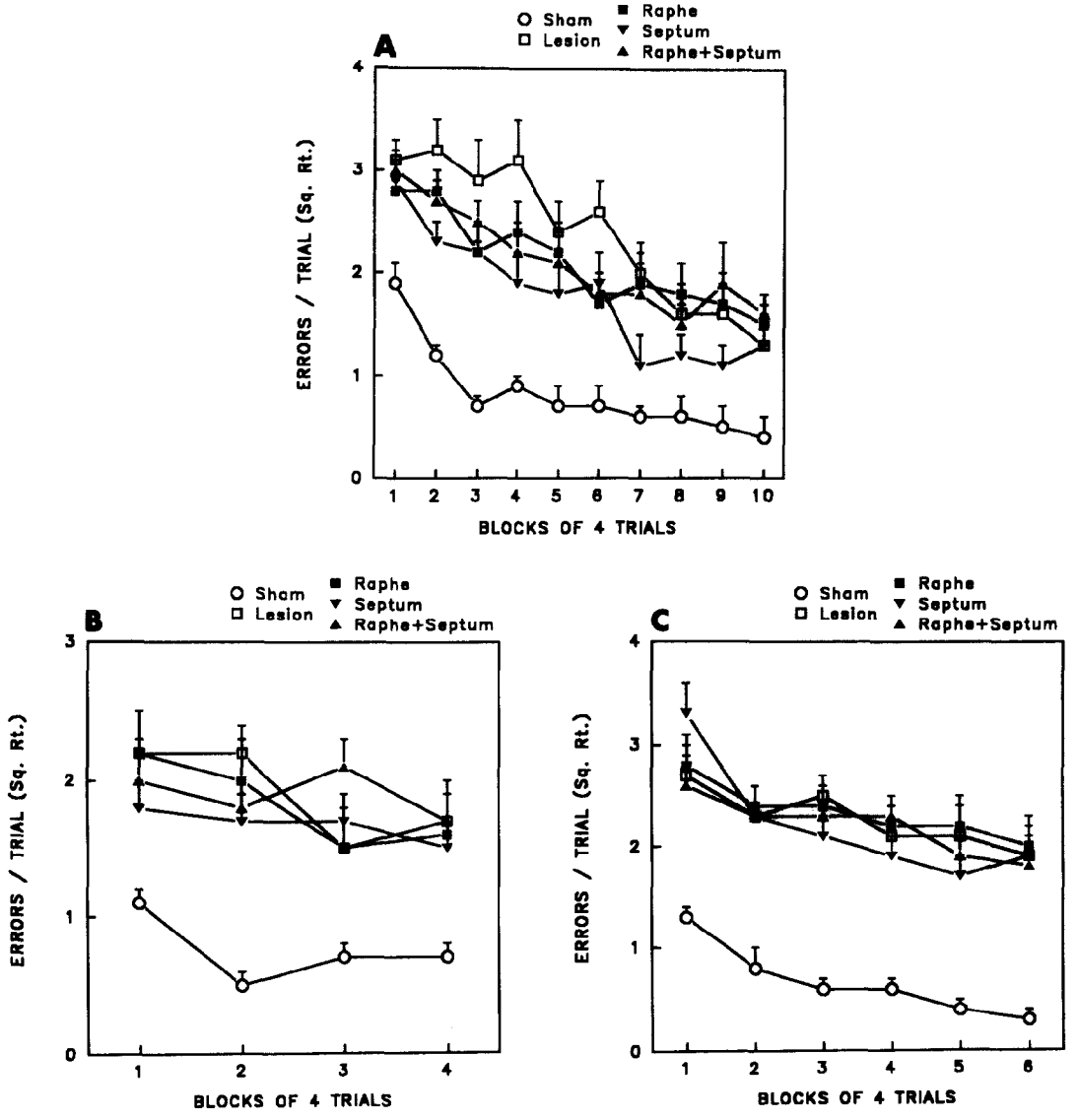

Fig. 3. Number of errors per trial averaged over four-trial blocks found in the RAM test. (A) Uninterrupted testing performed between six and nine weeks after transplant surgery. (B) Uninterrupted testing performed during the 26th and 27th weeks after transplant surgery. (C) Interrupted testing performed between 27 and 30 weeks after transplant surgery. Group abbreviations as in Fig. 1.

the first period of testing. Rats with co-grafts of raphe and septal origin were the only ones to show normalized performance in the MWM probe trial, although they showed no normalization of performance in any other test.

Histological verification

Extent of the lesions. Infracallosal lesions were comparable to the lesions that we performed in a previous experiment.[38] A typical example of such a lesion is presented in Fig. 6A. Briefly, these lesions damaged the dorsal fornix and the major part of the fimbria. In some rats, however, a thin ventral part of the latter was almost intact. These lesions also slightly encroached onto the most dorsal portion of the lateral septum, the medial part of the corpus callosum and, in about half of the rats, onto the most anterior pole of the hippocampus.

Hippocampal distribution of acetylcholinesterase reaction products and Cresyl Violet-stained material (Fig. 6). Compared to that found in Sham rats (Fig. 6B), there was a substantial reduction of the hippocampal AChE-staining in lesion-only rats (Fig. 6C). Some residual AChE staining could, however, be observed in the dentate gyrus and in both the subiculum and the medial part of region CA1. As found in previous experiments,[6,8,9] all grafts (raphe, septal and co-grafts) consisted of rather well delineated small cell aggregates which developed partly outside the hippocampus and partly within the dentate gyrus

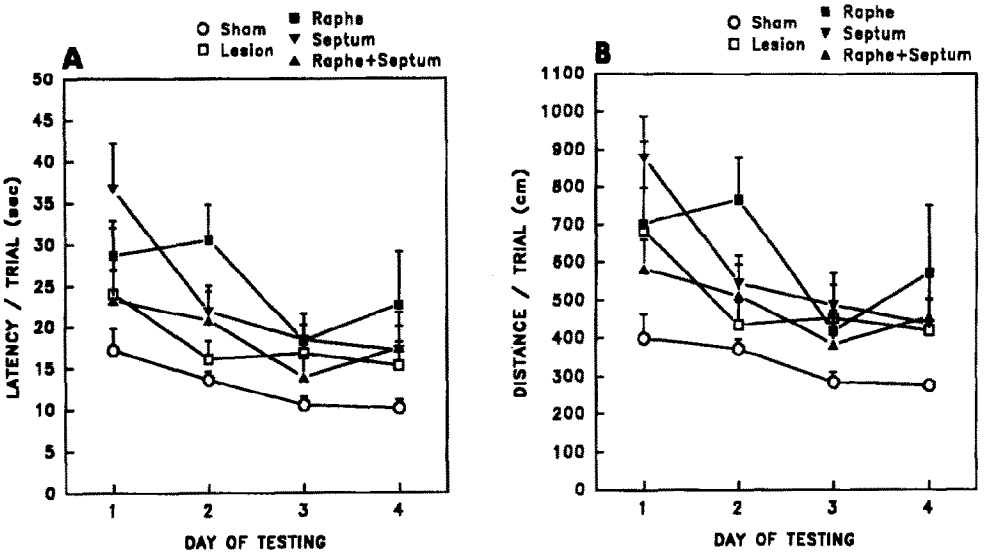

Fig. 4. Average scores recorded during the acquisition phase of the MWM test. Group abbreviations as in Fig. 1. The test was begun 32 weeks after transplant surgery. (A) Average latency to find the platform during the acquisition trials. Each point represents the mean of four consecutive trials. (B) Average distance swum to find the platform during the acquisition trials. Each point represents the mean of four consecutive trials.

and/or Ammon's horn (through the cannula track). When located in the dentate gyrus, the grafts often produced topographically limited granule cell degeneration (for detailed discussion of this morphological aspect, see Ref. 10). Neither the variability in the location of the grafts within the host structure nor the injection-induced granule cell degeneration showed any obvious relationship with the level of behavioural performance. Concerning the neurochemical data, it was not possible to address the question of a relationship between either the graft location or the graft-induced morphological effects in the host structure (e.g. granule cell degeneration) and the various neurochemical markers, since the brains of the rats used for neurochemical determinations could not be used for morphological verifications. Septal, raphe and co-grafts were all found to be densely AChE-positive, but only grafts of septal

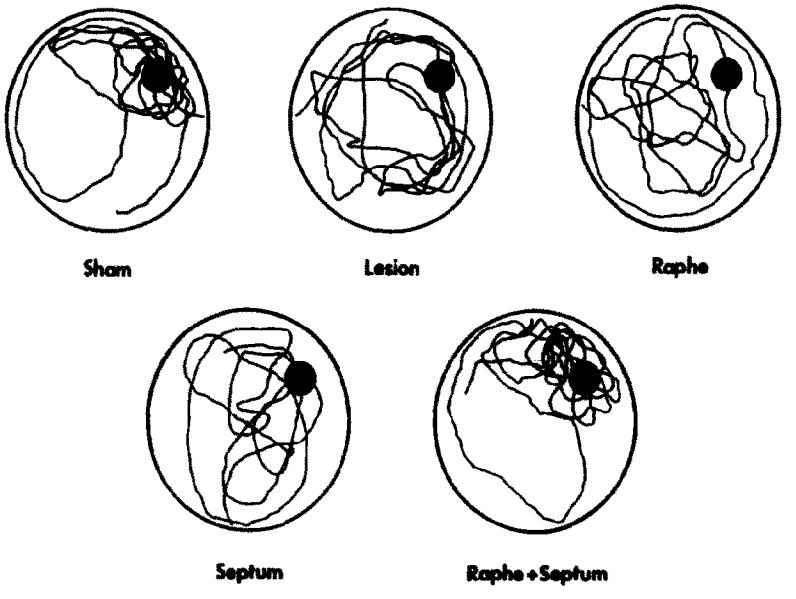

Fig. 5. Representative examples of swimpaths taken by a rat from each of the five experimental groups during the 1 min probe trial. The filled circles indicate the locus where the platform was located during the acquisition trials. Only sham-operated and co-grafted rats exhibited a search pattern which was focused on the former location of the platform.

Table 2. One-minute probe trial in the Morris water maze test: swimming speed computed over the whole duration of the probe trial, distance swum and time spent in the quadrant (Q2) where the platform was located during the acquisition trials and number of swimmings across the platform position

Behavioural variable	Surgical treatment				
	Sham	Lesion	Raphe	Septum	Raphe + Septum
Swimming speed (m/min)	15.4	14.7	14.3	14.8	14.1
	(0.4)	(0.6)	(0.5)	(0.6)	(0.7)
Distance in Q2 (cm)	913.9	567.6*	588.3*	630.4*	856.9
	(86)	(30)	(46)	(56)	(47)
Time in Q2 (s)	36.0	22.0*	23.7*	25.2*	35.3
	(3.5)	(1.1)	(1.9)	(2.6)	(2.9)
Platform crossings (n)	3.8	2.6	3.2	3.5	4.4
	(0.5)	(0.4)	(0.6)	(0.5)	(0.4)

Values are means; S.E.M. indicated in parentheses.
*Significant difference compared to Sham rats (Duncan's test; $P < 0.05$).

origin or co-grafts provided the denervated hippocampus with a dense organotypic AChE-positive reinnervation (Fig. 6E and F, respectively). Although the hippocampal AChE positivity found in rats with raphe grafts was increased as compared to that observed in lesion-only rats (Fig. 6D), it was neither as dense nor as widely distributed as that found in the hippocampus of sham-operated rats or of rats with either septal or co-grafts.

Neurochemical determinations

Data for choline acetyltransferase activity. Data are shown in Table 3. ANOVA showed a significant Group effect in the three hippocampal regions [$F(4/33) = 15.0$, 16.2, 10.2 for the dorsal, "middle" and ventral regions, respectively; $P < 0.001$ in all cases]. In lesion-only rats, the average ChAT activity was significantly reduced (dorsal −68%; "middle" −56%; ventral −38%) as compared to that observed in sham-operated rats ($P < 0.05$ in all cases). In raphe-grafted rats, whatever hippocampal region was considered, the ChAT activity was significantly lower than in sham-operated rats ($P < 0.05$ in all cases) and did not differ significantly from that of lesion-only rats. In septal and co-grafted rats, the ChAT activity found in both the dorsal and "middle" hippocampal regions was significantly higher than that found in either lesion-only and raphe-grafted rats ($P < 0.05$ in all cases). Additionally, in the "middle" hippocampal region, rats with septal grafts showed a ChAT activity which was significantly lower than in sham-operated rats ($P < 0.05$). Also, in the ventral hippocampal region, the ChAT activity observed in septal and co-grafted rats was significantly lower than in sham-operated rats.

Data for high-affinity choline uptake. Data are shown in Table 3. ANOVA showed a significant Group effect in the three hippocampal regions [$F(4/33) = 11.7$, 10.5, 2.6 for the dorsal, "middle" and ventral regions, respectively; $P < 0.05$]. In lesion-only rats, the average HACU was significantly reduced (dorsal −77%; "middle" −65%; ventral −36%) as compared to that observed in sham-operated rats. In raphe-grafted rats, the HACU observed in both the dorsal and the "middle" hippocampal regions was significantly lower than that of sham-operated rats ($P < 0.05$ in all cases) and, whatever hippocampal region was considered, it did not differ significantly from that of lesion-only rats. Rats with septal grafts showed, in the dorsal and the "middle" regions, a HACU value which was significantly higher than that found in lesion-only and raphe-grafted rats ($P < 0.05$, in all cases). Additionally, we noted that this value differed significantly from that of sham-operated rats in the "middle" hippocampal region. Concerning co-grafted rats, their average HACU value was significantly higher than that found in both lesion-only and raphe-grafted rats in the dorsal hippocampal region ($P < 0.05$ in all cases). In both the "middle" and the ventral regions, co-grafted rats showed a HACU which differed significantly from that of sham-operated rats ($P < 0.05$ in all cases).

Data for high-affinity serotonin uptake. Data are shown in Table 3, ANOVA showed a significant Group effect in the three hippocampal regions [$F(4/33) = 10.8$, 5.7, 6.6 for the dorsal, "middle" and ventral regions, respectively; $P < 0.001$]. In lesion-only rats, the average HASU was significantly reduced (dorsal −65%; "middle" −52%; ventral −34%) as compared to that observed in sham-operated rats ($P < 0.05$ in all cases). In raphe-grafted rats, in the dorsal region of the hippocampus, the average HASU was significantly higher than that found in lesion-only rats and did not differ significantly from that of sham-operated rats ($P < 0.05$ in all cases). However, this effect was not observed in the other two hippocampal regions. In co-grafted rats, in both the dorsal and the "middle" hippocampal regions, the average HASU was significantly higher than in either lesion-only, raphe-grafted or septal-grafted rats ($P < 0.05$, in all cases). In the dorsal hippocampus, it also significantly exceeded that found in sham-operated rats ($P < 0.05$). This was not the case in the ventral hippocampus, where no significant difference was observed among the values of the four lesion

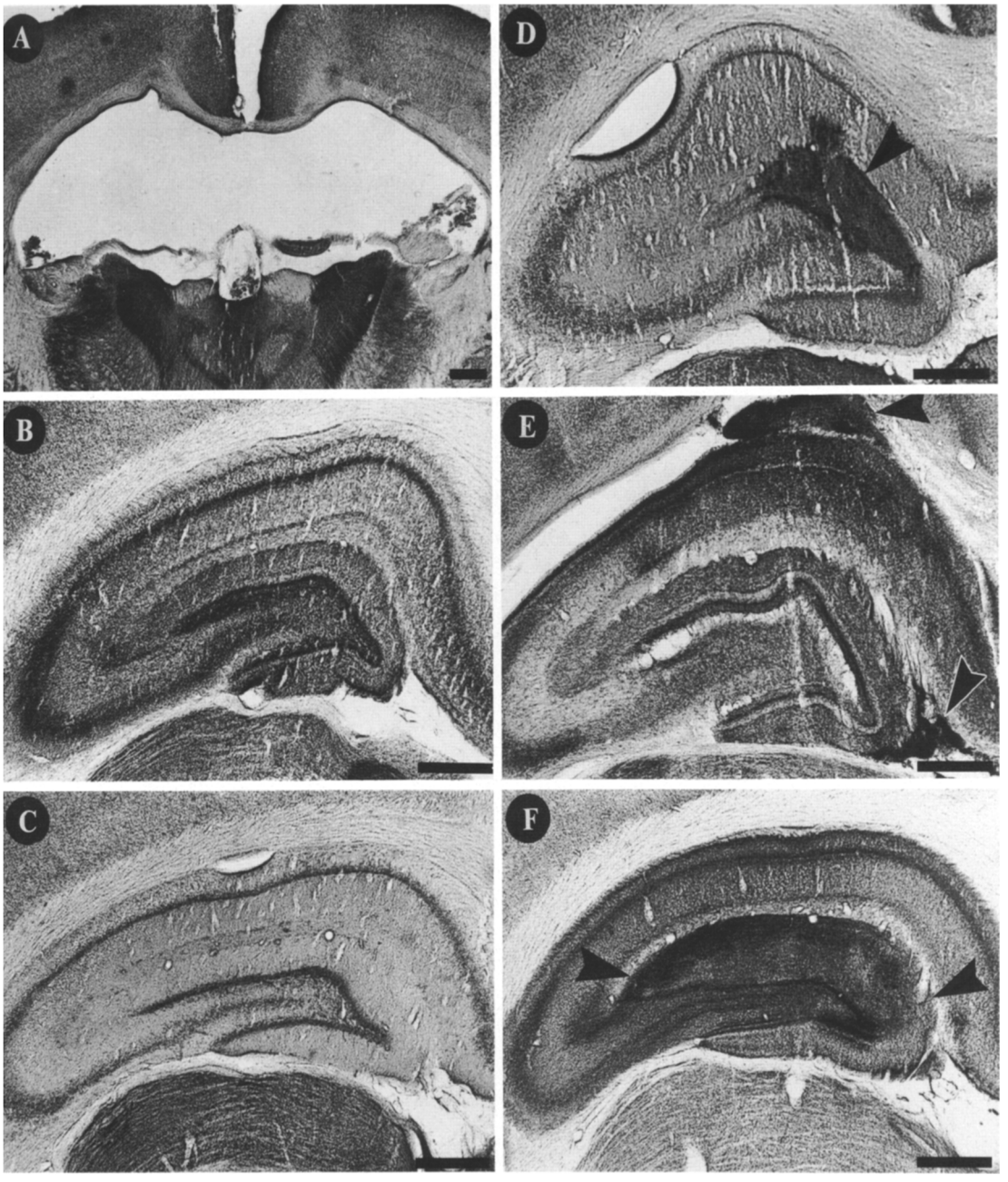

Fig. 6. (A) Photograph showing the extent of a representative fimbria–fornix lesion on a coronal section (stained for AChE) located about 1.3 mm posterior to bregma. (B–F) Photographs showing examples of AChE positivity found in coronal sections through the dorsal hippocampus of rats given a sham operation (B), a fimbria–fornix lesion (C), a raphe graft (D), a septal graft (E) or a co-graft of both raphe and septal tissue (F). Arrowheads indicate graft location. Scale bars = 500 μm.

groups, whether grafted or not. Whichever hippocampal region was considered, there was no significant difference between the average values found in rats with septal grafts and those found in sham-operated rats.

Data for serotonin concentration. Data are shown in Table 3. ANOVA of the [5-HT] values showed a significant Group effect in both the dorsal and the "middle" hippocampal regions [$F(4/33) = 8.5$, $P < 0.001$; $F(4/33) = 2.9$, $P = 0.03$, respectively], but not in the ventral one. The lesions had significantly decreased [5-HT] in both the dorsal and the "middle" hippocampal regions ($P < 0.05$ in all cases). In those two regions, raphe grafts normalized the [5-HT]. In the dorsal hippocampus, co-grafts resulted in a significantly increased [5-HT] as compared to that found

Table 3. Average levels (S.E.M.) of neurochemical markers found in the three hippocampal regions of control rats, lesion-only rats and lesioned rats with a raphe, a septal or a mixture of raphe and septal grafts

		Surgical treatment				
Neurochemical marker		Sham	Lesion	Raphe	Septum	Raphe + Septum
ChAT	Dorsal	0.7	0.2*	0.25*	0.85*†‡	0.9†‡
(nM/min/mg prot.)		(0.03)	(0.06)	(0.03)	(0.07)	(0.15)
	"Middle"	0.8	0.3*	0.3*	0.6*†‡	0.65†‡
		(0.03)	(0.04)	(0.03)	(0.05)	(0.07)
	Ventral	0.9	0.6*	0.5*	0.7*‡	0.7*
		(0.04)	(0.04)	(0.03)	(0.04)	(0.08)
HACU	Dorsal	0.7	0.2*	0.25*	0.8†‡	0.7†‡
(pM/min/mg prot.)		(0.1)	(0.04)	(0.05)	(0.1)	(0.09)
	"Middle"	0.8	0.3*	0.35*	0.5*†‡	0.4*
		(0.09)	(0.04)	(0.05)	(0.07)	(0.04)
	Ventral	0.9	0.6*	0.7	0.7	0.6*
		(0.09)	(0.09)	(0.08)	(0.08)	(0.06)
HASU	Dorsal	0.8	0.3*	0.8†	0.3*	1.9*†‡§
(pM/min/mg prot.)		(0.08)	(0.02)	(0.2)	(0.07)	(0.4)
	"Middle"	1.0	0.4*	0.5*	0.45*	1.1†‡§
		(0.05)	(0.03)	(0.1)	(0.04)	(0.3)
	Ventral	1.35	0.9*	0.9*	0.9*	0.9*
		(0.07)	(0.04)	(0.07)	(0.1)	(0.07)
[5-HT]	Dorsal	2.4	0.8*	2.1†	1.1	6.5*†‡§
(ng/mg prot.)		(0.4)	(0.5)	(0.6)	(0.4)	(1.6)
	"Middle"	2.1	1.15*	2.0	1.8	3.4†
		(0.2)	(0.4)	(0.3)	(0.2)	(0.9)
	Ventral	3.3	1.9	3.0	2.9	2.7
		(0.3)	(0.5)	(0.4)	(0.35)	(0.4)
[5-HIAA]	Dorsal	3.9	3.2	4.4	3.3	6.7*†‡§
(ng/mg prot.)		(0.4)	(0.2)	(0.5)	(0.4)	(1.3)
	"Middle"	3.6	2.9*	3.7	3.4	5.0†
		(0.2)	(0.2)	(0.3)	(0.5)	(1.0)
	Ventral	4.3	3.7	4.2	1.4	2.2
		(0.3)	(0.2)	(0.4)	(0.5)	(0.75)
[NA]	Dorsal	2.4	3.9	3.2	1.5†‡	2.4
(ng/mg prot.)		(0.15)	(0.9)	(0.5)	(0.2)	(0.3)
	"Middle"	2.7	3.0	3.1	2.1	2.9
		(0.2)	(0.4)	(0.55)	(0.2)	(0.2)
	Ventral	3.8	3.9	4.0	3.6	4.0
		(0.2)	(0.7)	(0.3)	(0.3)	(0.55)

*Significant difference compared to Sham rats (Duncan's test; $P < 0.05$); †significant difference compared to Lesion-only rats ($P < 0.05$); ‡significant difference compared to Raphe-grafted rats ($P < 0.05$); §significant difference compared to Septum-grafted rats ($P < 0.05$).

in the four other groups ($P < 0.05$ in all cases). In the "middle" region, the [5-HT] of co-grafted rats was still higher than in the other four groups, but only the difference with lesion-only rats was significant ($P < 0.05$). Whichever hippocampal region was considered, there was no significant effect of septal grafts on [5-HT].

Data for 5-hydroxyindolacetic acid. Data are shown in Table 3. ANOVA of the [5-HIAA] values showed a significant Group effect in both the dorsal and the ventral hippocampal regions [$F(4/33) = 3.9$, $P = 0.01$; $F(4/33) = 3.1$, $P = 0.02$, respectively], but not in the "middle" one. In the dorsal hippocampus, this effect was due to significantly increased [5-HIAA] in co-grafted rats as compared to the values found in the other four groups ($P < 0.05$ in all cases). In the ventral hippocampus, the [5-HIAA] was found to be significantly higher in co-grafted rats as compared to either lesion-only or sham-operated rats ($P < 0.05$ in all cases).

Data for noradrenaline concentration. Data are shown in Table 3. ANOVA of the [NA] values showed a significant Group effect in the dorsal hippocampal region [$F(4/33) = 3.7$, $P = 0.013$], but not in the two other ones. This effect was due to significantly higher [NA] in both lesion-only and raphe-grafted rats as compared to septal-grafted rats ($P < 0.05$).

Summary of the neurochemical data. These data are summarized in Table 4. Fimbria–fornix lesions induced a cholinergic and a serotonergic denervation of essentially the septal two-thirds of the hippocampus (i.e. dorsal and "middle" regions). Raphe grafts attenuated the serotonergic deficit, septal grafts attenuated the cholinergic one, while co-grafts combined the neurochemical properties of both single grafts. Concerning the hippocampal [NA], neither the

Table 4. Lesion- and graft-induced neurochemical effects observed in the three hippocampal regions of lesion-only rats and lesioned rats with a raphe, a septal or a mixture of raphe and septal grafts

Neurochemical marker		Surgical treatment			
		Lesion	Raphe	Septal	Raphe + Septal
HACU	Dorsal	23	34^{ns}	104*†	97*†
	"Middle"	35	45^{ns}	69*	54^{ns}
	Ventral	64	72^{ns}	80^{ns}	67^{ns}
ChAT	Dorsal	32	35^{ns}	120*†	132*†
	"Middle"	44	44^{ns}	77*	81*†
	Ventral	62	54^{ns}	71^{ns}	70^{ns}
HASU	Dorsal	35	97*†	42^{ns}	245*‡
	"Middle"	37	54^{ns}	46^{ns}	117*†
	Ventral	66	64^{ns}	68^{ns}	67^{ns}
[5-HT]	Dorsal	36	86*†	55^{ns}	160‡
	"Middle"	62	97^{ns}	91^{ns}	119*†
	Ventral	65	94^{ns}	93^{ns}	89^{ns}
[5-HIAA]	Dorsal	83	112^{ns}	85^{ns}	171*‡
	"Middle"	82	103^{ns}	97^{ns}	140^{ns}
	Ventral	86	97^{ns}	77^{ns}	131*
[NA]	Dorsal	161	134^{ns}	62*	99^{ns}
	"Middle"	113	116^{ns}	79^{ns}	108^{ns}
	Ventral	104	106^{ns}	96^{ns}	105^{ns}

*Significant graft-induced effect (compared to lesion-only rats); †graft-induced normalization (no significant difference as compared to values found in sham-operated rats and significant effect as compared to lesion-only rats); ‡significant graft-induced overcompensation (compared to sham-operated rats); ^{ns}no significant effect of the graft (compared to lesion-only rats).

lesions nor the three types of grafts were found to induce noteworthy effects in the hippocampus, although an increased [NA] was observed where cholinergic denervation was most pronounced.

Neurochemical and behavioural correlations

To run these analyses, the neurochemical and behavioural data from Sham rats were not included, since consideration of these data would have resulted in an irrelevant inflation of the correlation coefficients. Among the different meaningful correlations that have been computed between all aforementioned variables (neurochemical–neurochemical, neurochemical–behavioural, behavioural–behavioural), we found a significant correlation between MWM probe trial performance with (i) HACU in the dorsal hippocampus ($r = 0.38$, $P < 0.05$, 29 d.f.), (ii) ChAT activity in all hippocampal regions ($r = 0.40$, 0.50 and 0.48, in the dorsal, "middle" and ventral regions, respectively, $P < 0.05$, 29 d.f.) and (iii) HASU ($r = 0.58$ and 0.61, in the dorsal and "middle" regions, $P < 0.05$, 29 d.f.). Whichever hippocampal region was considered, none of the neurochemical variables was significantly correlated with any of the other behavioural variables considered, namely home-cage or open-field activity, spontaneous alternation rates or RAM performance (uninterrupted and interrupted procedures). Among the different behavioural variables, it is particularly interesting that there was no significant correlation between the uninterrupted RAM performance (errors in the last four-trial block of the second test period) and either the distance or the latency in the MWM probe trial ($r = 0.21$ and 0.24, respectively, d.f. = 29, ns). Also, the correlation between the interrupted RAM performance and the MWM variables (latency, distance) was not significant ($r = 0.21$ and 0.19, respectively, d.f. = 29).

DISCUSSION

In a previous experiment, we grafted septal or raphe tissue into the hippocampus of rats which had sustained aspiration lesions of the septohippocampal pathways and we found such grafts to foster a neurochemical recovery that depended upon the neuroanatomical origin of the grafted tissues.[10] Also, the technique of co-grafting both these tissues allowed the combination of neurochemical properties of each single graft. More recently,[38] we observed that electrolytic lesions restricted to the infracallosal (i.e. fimbria and dorsal fornix) component of the septohippocampal pathways not only resulted in lasting cholinergic and serotonergic hippocampal depletions, but also induced long-lasting behavioural impairments similar to those reported after more extensive septohippocampal damage (knife-cut or aspiration). The rats given such lesions displayed increased locomotor activity, reduced spontaneous alternation rates in a T-maze and dramatically impaired spatial working memory performance in an RAM test.

Our present findings in lesion-only rats completely confirm those reported by Jeltsch *et al.*[38] In addition, they show that an attenuation of only the serotoner-

gic deficit by raphe grafts or of the cholinergic one by septal grafts does not permit behavioural recovery in the test battery that we have used, with the exception of raphe grafts enhancing recovery of spontaneous alternation in a T-maze, and reducing recovery of normal ambulation scores in an open-field test. However, and this is particularly noteworthy, when both the cholinergic and serotonergic deficits are compensated for or attenuated simultaneously by co-grafts of septal and raphe cells, a complete recovery is observed in the MWM probe trial. On all other behavioural variables assessed so far, the co-grafts remained ineffective.

Neurochemical and behavioural effects of the lesions

In a previous experiment,[38] we reported that electrolytic lesions of only the fimbria and the dorsal fornix induced cholinergic and serotonergic hippocampal denervations, as well as behavioural deficits which lasted over six months. In our present experiment, we found all these deficits to still be detectable after a 10-month post-operative period. This finding suggests that neither the neurochemical nor the behavioural deficits subsequent to lesions restricted to the fimbria and the dorsal fornix are subject to complete spontaneous recovery. Unfortunately, the experimental design used in the present study does not allow us to determine whether the neurochemical deficits may have been somewhat attenuated over time, as was reported previously after more partial lesions of the septohippocampal pathways or of the septum.[6,25,27,28,54]

Since the lesion-induced neurochemical effects were found to be most dramatic in the septal pole of the hippocampus, one may assume that the fibres coursing through the fimbria and the dorsal fornix, which were lesioned, preferentially project onto the dorsal half of the hippocampus. Interestingly, after aspirative lesions of the septohippocampal pathways (i.e. fimbria, dorsal fornix, overlying corpus callosum, cingular bundle), it appears that both the cholinergic and serotonergic depletions are much more pronounced in the "middle" and ventral hippocampal regions than in the case of lesions restricted to only the fimbria and the dorsal fornix. However, in the dorsal region of the hippocampus, the neurochemical consequences of both these lesion types are of similar magnitude (compare our present results with those reported by Cassel *et al.*[10]). Thus, whereas electrolytic fimbria–fornix lesions induce cholinergic and serotonergic hippocampal denervations with a decreasing severity along the septotemporal axis of the hippocampus (see Table 3), aspirative lesions of these pathways produce neurochemical depletions which are of comparable magnitude along this axis (HACU, ChAT, HASU, [5-HT]: between about −70% and −80%, as reported by Cassel *et al.*[10]).

Despite this clear difference in the amplitude of the neurochemical effects found after electrolytic lesions as compared to aspirative ones, it is noteworthy that the behavioural consequences of both lesion paradigms are quite similar. This suggests that denervating mainly the dorsal half of the hippocampus might be sufficient to account for the vast majority of behavioural deficits which are classically reported after more extensive hippocampal denervations. These deficits include, among other perturbations, locomotor hyperactivity, reduced alternation rates and impaired mnesic performance assessed in either the RAM or the MWM.[11,17,50,63] In that regard, one may recall a recent study by Moser *et al.*,[46] who showed that the dorsal hippocampus plays a more important role for spatial learning than the ventral one, a functional distinction which had already been raised more than 20 years ago, also for aspects other than only spatial learning.[33,47,70] Therefore, our present set of neurochemical and behavioural data might also be regarded as providing further evidence for the view that the hippocampal region more critically involved in (at least) spatial learning processes is the dorsal region. If so, it can be speculated that the septohippocampal fibres projecting onto the dorsal hippocampus constitute one component of the dorsal hippocampal network involved in the neurobiological processes which may underlie spatial learning.

That such "locally" denervating lesions result in lasting neurochemical and behavioural impairments is also an interesting observation in relation to experiments aimed at assessing the functional effects of intrahippocampal cell suspension grafts of fetal neural tissues. Indeed, over the last 20 years, in most studies which used such grafting approaches, the grafts had been implanted into only the dorsal hippocampus of rats which sustained an extensive aspirative lesion of the septohippocampal pathways. Whether rich in cholinergic, serotonergic or other types of neurons, such dorsally implanted grafts fostered neurochemical effects which were maximal in the vicinity of the grafted neurons and declined progressively as the distance from the graft increased. Accordingly, in the most ventral part of the hippocampus, the graft-induced neurochemical effects were generally weak or even non-existent.[2,10,76] Thus, with electrolytic fimbria–fornix lesions, one has at least this advantage of having a lesion paradigm inducing lasting behavioural effects with a maximal denervation precisely restricted to the region in which the grafts are classically implanted.

A last point deserving discussion in relation to the lesion-induced neurochemical effects is the finding that in lesion-only rats (but also in the raphe-grafted ones), [NA] tended to be increased as compared to that measured in sham-operated rats, but only in the dorsal region of the hippocampus. In both other regions, [NA] was similar to that found in virtually intact rats. Since we found that, two to three weeks post-surgery, infracallosal lesions reduced [NA] by about 60% in the dorsal third of the hippocampus (unpublished observations), we may interpret this slightly about normal increase of [NA] as resulting

mainly from sympathetic fibre ingrowth, a well known phenomenon which is elicited by cholinergic denervation of the hippocampus.[12] In both other hippocampal regions, infracallosal lesions only weakly reduced [NA], when assessed shortly after surgery (also two to three weeks). In that concern, the near-normal levels of [NA] found in our present experiment in the "middle" and ventral hippocampal regions may probably be regarded as indicating that, in these regions, the lesions had no or only a weak effect on this noradrenergic marker.

Neurochemical effects of the grafts

The grafts that we performed were rich in either serotonergic or cholinergic neurons, or in both of these neurochemical categories of cells. In a previous experiment in which the same three types of intrahippocampal grafts were used, but in rats given an aspirative and thus an extensive lesion of the septohippocampal pathways, we found the grafts to induce neurochemical effects which depended on the neuroanatomical origin of the grafted tissue.[10] As compared to the study by Cassel *et al.*,[10] our present series of neurochemical data confirms the points stressed hereafter. First, while mesencephalic raphe grafts produced serotonergic effects and septal grafts cholinergic effects, co-grafts of cells from both these nuclei were observed to produce serotonergic as well as cholinergic effects. Second, the single graft-induced effects can be considered as rather specific, since raphe grafts did not alter the cholinergic markers and, reciprocally, septal grafts did not alter the serotonergic ones. Further evidence for the neurochemical specificity of the single grafts has been obtained more recently by determining the hippocampal concentration of GABA, glutamate, glutamine, aspartate, taurine, serine, alanine and glycine in the rats of the present experiment. Neither of these amino acid concentrations (which are only weakly or not affected by septohippocampal lesions; see Ref. 41), was modified by either raphe, septal or co-grafts (unpublished observations). Third, whichever type of graft is considered, the graft-induced neurochemical effects are expressed with a decreasing gradient along the septotemporal axis of the hippocampus, these effects being maximal in the dorsal region of the hippocampus.

There are, however, three differences between our present study and that of Cassel *et al.*[10] The first difference concerns the serotonergic markers. In the dorsal region of the hippocampus, Cassel *et al.*[10] have reported that, six months after transplant surgery, single raphe grafts increased the levels of serotonergic markers to about 250% and co-grafts to about 350% of normal. In our present experiment in which the post-grafting delay was longer, the serotonergic markers were overcompensated only in the presence of co-grafts and only to 160% for [5-HT] and 245% for HASU. This discrepancy might be explained by a different yield in the preparation of the cell suspensions, which was actually lower in the present study (i.e. raphe: 38,750 viable cells/μl; co-grafts: 70,000 cells/μl) than in the former one (i.e. raphe: 53,000 viable cells/μl; co-grafts: 80,000 viable cells/μl).

The second difference, which concerns mainly the cholinergic markers, concerns the extent of the graft-induced effects along the septotemporal axis of the hippocampus. Cassel *et al.*[10] have found septal and co-grafts to induce significant cholinergic effects (ChAT activity and HACU) down to the ventral region of the hippocampus, whereas in the present study these types of grafts exerted significant cholinergic effects only in the "middle" region of the hippocampus. This discrepancy in the extent of the graft-induced effects along the septotemporal axis of the hippocampus might be explained by the aforementioned difference in the yield of the respective cell preparations. Indeed, it is possible that the potential for grafts to grow fibres over the whole hippocampus closely depends upon the amount of viable cells originally present in the cell suspension. Another explanation would consider the degree of hippocampal denervation along the septotemporal axis as a factor which may influence the reinnervation potential of the grafted cells. Actually, previous studies have shown that, in the hippocampus, both the survival and the development of a graft, as well as the graft-derived reinnervation, are optimal when the denervation of this structure is maximal.[26] In our present study, the electrolytic fimbria–fornix lesions produced a denervation of the ventral hippocampal region which was far less pronounced than that reported after aspirative lesions by Cassel *et al.*[10] Therefore, one may speculate that the lack of graft-induced cholinergic effects in the ventral hippocampal region is due to the lower degree of denervation of this region. In other words, the spared fibres may have hindered the graft-derived cholinergic fibre ingrowth in both the "middle" and ventral hippocampal regions.

The third difference concerns the noradrenergic marker (i.e. hippocampal [NA]). In our previous study, we found aspirative lesions of the septohippocampal pathways to result in [NA] which was dramatically increased over the whole hippocampus (dorsal 183%; "middle" 213%; ventral 228% of normal). This phenomenon, which was interpreted as reflecting sympathetic sprouting, was inhibited in both the dorsal and "middle" hippocampal regions by grafts rich in cholinergic neurons (septal and co-grafts), but not by single raphe grafts. In the present study, in which the lesions alone or without cholinergic-rich grafts tended to increase [NA] in the dorsal region of the hippocampus (see first part of the Discussion), such a graft-induced inhibition was observed only with septal grafts; although the [NA] found in the dorsal region of co-grafted rats was lower than that found in lesion-only rats, this difference failed to reach significance. All these observations, however, should not be regarded as a

contradiction of the findings by Cassel *et al.*[10] The first reason might be of statistical origin. Indeed, the variability in [NA] found within lesion-only rats from the present study was higher than in the other four groups, but also than in lesion-only rats from our previous work.[9,10] The second reason might be more related to the respective lesion paradigms. The study by Cassel *et al.*[10] used rats with more pronounced (i) lesion-induced cholinergic denervation of the hippocampus, (ii) reactional sympathetic sprouting and (iii) septal and co-graft derived effects on cholinergic markers in the denervated hippocampus.

Nevertheless, altogether, the neurochemical results of our present experiment clearly confirm that the technique of co-grafting different categories of cerebral tissue allows the promotion of recovery from more than only one lesion-induced neurochemical deficit in a given cerebral structure. These results also demonstrate that most of the graft-induced neurochemical effects found in our previous experiment[10] can also be obtained after lesions of only the infracallosal component of the septohippocampal pathways.

Graft-induced behavioural effects

There are several data suggesting that the septohippocampal cholinergic system plays an important role in the expression of cognitive functions.[52,53] However, the relationship between the cholinergic innervation of the hippocampus and cognitive capabilities is not exclusive. As demonstrated recently, the pharmacological interaction which may occur between the cholinergic and other neurotransmitter systems (e.g. noradrenergic, serotonergic, etc.) may also have some cognitive implications.[15,16,29,60,74] More precisely, there are some data demonstrating that a pharmacological interaction between the cholinergic and serotonergic systems may be involved in cognitive processes.[49,51,60,61,74,75] For instance, when a reduction of the cholinergic transmission is combined with a reduction of the serotonergic one, the impairment observed in some spatial tasks is larger than the sum of the impairments observed after depleting either of these systems alone.[49,51] Also, when rats given 5,7-dihydroxytryptamine lesions of the septohippocampal pathways are tested under atropine treatment in an MWM task, they show a clear-cut deficit which is prevented by intrahippocampal grafts rich in serotonergic neurons.[60]

Part of the behavioural data obtained in our present experiment is in line with the aforementioned view on the implication of a cholinergic by serotonergic interaction in cognitive function. Actually, neither the cholinergic reinnervation (in the presence of septal grafts) nor the serotonergic one (in the presence of raphe grafts) was sufficient to promote lasting behavioural recovery. This statement is valid for all of the behavioural variables assessed. Conversely, with concomitant cholinergic and serotonergic reinnervations of the denervated hippocampus (in the presence of co-grafts), there was a complete recovery in the MWM probe trial: lesioned rats with co-grafts were able to remember the location of the platform as accurately as sham-operated rats.

Concerning the fact that the MWM probe trial performance was the only behavioural variable to be enhanced by intrahippocampal co-grafts, several comments can be made. First, if there is general agreement on the fact that lesions of the septohippocampal pathways result, among other behavioural perturbations, in locomotor hyperactivity and reduced spontaneous alternation rates, one must remember that after intrahippocampal grafts rich in cholinergic neurons, both these variables were never found to be consistently affected by grafts providing the denervated hippocampus with a cholinergic reinnervation.[4,6,9,18, but see 6,19] Our data suggest that this might also be true, but only for the lesion-induced hyperactivity, for grafts which provide the hippocampus with a new serotonergic innervation, all the more because rats with raphe grafts were found to be rather hyperactive during the second testing period as compared to the lesioned ones. Therefore, it may be speculated that the partial disruption of the cholinergic and/or the serotonergic hippocampal afferents probably does not account for the hyperactivity. However, concerning the impaired alternation performance observed in lesioned rats, one cannot completely exclude a participation of the serotonergic denervation of the hippocampus. Indeed, during the second period of testing, rats with raphe grafts showed alternation performance which did not differ significantly from those of sham-operated rats. However, since these performances did not differ from those of lesion-only rats it is difficult to establish a clear-cut relationship between serotonergic denervation of the hippocampus and impaired spontaneous alternation performance. The latter qualifying statement is in line with another study in which[38] we found that, following fimbria–fornix lesions similar to the ones used here, there was no significant correlation between spontaneous alternation rates (but also the locomotor hyperactivity) and the degree of either cholinergic or serotonergic hippocampal denervations. It is therefore tempting to speculate that a disruption of other septohippocampal neurotransmitter systems may be involved in these deficits. According to Sidel *et al.*,[66] it seems that the central GABAergic system may interact with the locomotor hyperactivity which can be induced by systemic pharmacological disruption of cholinergic transmission. Otherwise, according to Isaacson *et al.*,[36] it seems that the central noradrenergic system might also be involved in both the lesion-induced hyperactivity and the impaired spontaneous alternation performance. Finally, one might also consider whether the disruption of the hippocampal efferents which course through the fimbria–fornix would not also have some functional significance in both aforementioned deficits. Further studies based on pharmacological

manipulations or on more neurochemically-specific lesion paradigms should contribute to clarify this point.

The second comment concerns the RAM performances of lesioned rats which received grafts rich in cholinergic neurons (septal or co-grafts). Only during the first post-surgical period of testing did these rats show RAM performances which were slightly better than in lesion-only rats (see Fig. 3A), but this difference did not reach significance. One must point out here that some lasting improvement of RAM performance may have been expected in these rats. Actually, previous studies have reported improvements in spatial learning abilities after intrahippocampal septal grafts in rats which sustained cholinergic denervation of the hippocampus.[7,13,14,18,21,50,54,62] Several factors may account for these contradictory results. Most studies which reported beneficial effects on spatial learning after graft-derived cholinergic reinnervation of the hippocampus used a reinforced alternation task[13,18] or a MWM task.[42,48,50,73] Only in a few studies was the RAM task used to assess the graft-induced effects on spatial learning.

In one of these studies in which the septo-hippocampal pathways were aspirated, significant beneficial effects of the grafts could only be obtained when the rats were tested under physostigmine treatment.[43] In another study,[7] which also used aspiration of the septohippocampal pathways, a beneficial graft-induced effect in RAM performance was found to depend upon the time which had elapsed between grafting and testing: observed at about five months post-surgery, this effect could not be found at an earlier (one month) or a later delay (10 months). Finally, four other studies which reported beneficial effects of septal grafts on RAM learning used different treatments for inducing cholinergic damage; the lesions were made either with AF64A[21,34,35] or with chronic ethanol treatment, which actually induced more partial lesions.[31] Here it should be noted that several factors related to the experimental conditions may influence the survival, development, integration and functional expression of intracerebral grafts.[10] Therefore, one may speculate that the lack of a significant septal graft-induced behavioural effect in our present study, particularly as concerns RAM learning, might be explained by differences (as compared to the aforementioned studies) in the lesion paradigms used, the age of the donor tissue,[6] the delay between grafting and testing, the type of tissue implanted into the denervated structure, or even the type of test(s) used to evaluate the lesion- and graft-induced effects. That the post-surgical delay may have been one of these factors is indirectly suggested by the observation that rats with septal grafts showed slightly improved RAM performance only during the first testing period. Finally, it must also be pointed out that a recent view on the functional effects of graft-derived cholinergic innervation of the hippocampus considers this innervation as necessary, but not as sufficient, to produce significant and lasting cognitive effects.[17] Our data in rats which received grafts rich in cholinergic neurons, particularly in rats with single septal grafts, might be considered as further support to that view. Furthermore, one cannot rule out the fact that even with a partial fimbria–fornix lesion paradigm, the damage to the corresponding hippocampal afferents may have been too extensive and the engraftment too limited to sustain functional recovery. However, in co-grafted rats, such a statement would need some qualification since performance in the MWM probe trial, another variable accounting for spatial learning abilities, was completely normalized.

The latter point introduces our third comment, namely that the graft-induced effects may be task-specific and that several tasks measuring spatial learning performance (e.g. RAM or MWM tasks) are not necessarily equipotent in their ability to measure graft-induced effects in lesioned animals. Such a statement is consistent with a report by Sinden *et al.*,[57] who showed that basal forebrain grafts implanted into the cortex of rats given ibotenic acid-induced lesions of the nucleus basalis failed to ameliorate performance in a complex operant conditioning task, but improved passive avoidance performance. In parallel, Emerich *et al.*[21] have clearly shown that the behavioural effects of intrahippocampal grafts rich in cholinergic neurons after AF64A-induced lesions of the cholinergic hippocampal afferents may closely depend upon the difficulty of a RAM learning task. Actually, whereas grafted rats showed improved performance in a standard version of the task (similar to our uninterrupted testing protocol), these rats remained impaired on the more difficult version, which imposed a delay of 1 h between the selection of the fourth and the fifth arms. It is also noteworthy that these authors found a similar task-specific recovery of RAM performance in rats which had sustained AF64A-induced lesions and were subsequently treated with the ganglioside AGF2.[22] Therefore, we may suggest that, for studies assessing the behavioural effects, whether cognitive or not, of intrahippocampal grafts, the investigation and the interpretation of the graft-induced effects should be based not only on one single behavioural test, but on a battery of tests, all able to detect the lesion-induced effects that the grafts should counteract. The task specificity of our behavioural findings may also be interpreted as showing that the co-grafts ameliorated spatial memory performances involving reference memory processes rather than those involving working memory ones. Due to our respective testing protocols, the RAM test requested an intact working memory capability, whereas the MWM requested an intact reference memory one. In future experiments, it might be relevant to use an RAM testing protocol allowing a parallel assessment of working memory and reference memory errors (i.e. in baiting only a

subset of arms, see Refs 30 and 37) and to compare these RAM performances to those found in an MWM task. Furthermore, the differences between the RAM and the MWM tasks may not only be considered in terms of mnemonic processes required to solve the problem (reference versus working memory); one may also wonder whether these tasks actually measure the same spatial functions. This question is of particular relevance regarding the absence of a significant correlation between the uninterrupted RAM and the MWM performances in all lesioned rats (i.e. whether grafted or not). The RAM and the MWM tasks may be solved by using completely different strategies. In the RAM task, rats may use allocentric or egocentric (guidance) strategies (see Experimental Procedures), whereas in the MWM, only an allocentric strategy would be appropriate to find the platform. Further differences may be, for instance, in terms of complexity of the tasks (e.g. eight versus one important locations), motivational aspects (appetitive versus aversive), or even of required capacities to deal with spatial information in a flexible manner[20] (egocentric strategies do not suppose any flexibility). Further experiments are necessary to address these possibilities more accurately.

CONCLUSION

In conclusion, our neurochemical data show that a raphe or a septal cell suspension graft, when implanted into the partially denervated hippocampus, may foster a neurotransmitter-specific recovery depending upon the anatomical origin of the grafted cells. These data also demonstrate that co-grafting a mixture of both these cell suspensions is an appropriate technique to combine, in one preparation, the neurochemical properties of each single graft. From a behavioural point of view, these results show that, whereas single grafts exerted only restricted or short-term effects on the behavioural outcome of fimbria–fornix lesions, only the co-grafts, which provide the partially denervated hippocampus with both a new serotonergic and a new cholinergic hippocampal reinnervation, may induce beneficial effects on spatial memory and orientation. However, since these effects were found to be task-specific, we may also assume that the RAM and the MWM, two tasks which both measure spatial learning and memory abilities, do not share the same degree of sensitivity to graft-induced effects, and/or do not measure exactly the same spatial learning and memory capacities. For instance, it is possible that co-grafts producing the types of neurochemical effects found in our present experiment after fimbria–fornix lesions are more efficient in attenuating spatial reference memory deficits (e.g. in the MWM test) than spatial working memory ones (e.g. in the RAM test).

In any case, the neurochemical effects of co-grafts which may be involved in the virtually complete behavioural recovery observed in the MWM probe trial may be insufficient to trigger recovery in another spatial learning and memory task. This experiment, therefore, suggests that the neurochemical specificities of the donor tissue as well as the behavioural task used to assess the functional consequences of the grafts may constitute additional factors to be considered among the factors which may influence the functional outcome of intracerebral grafting experiments.[5]

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REFERENCES

1. Bartus R. T., Dean R. L., Beer B. and Lippa A. S. (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217,** 408–414.
2. Björklund A., Gage F. H., Schmidt R. H., Stenevi U. and Dunnett S. (1983) Intracerebral grafting of neuronal cell suspension. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. *Acta physiol. scand.* **522,** Suppl., 49–58.
3. Björklund A., Stenevi U., Schmidt R. H., Dunnett S. B. and Gage F. H. (1983) Intracerebral grafting of neuronal cell suspensions. I. Introduction and general methods of preparation. *Acta physiol. scand.* **522,** Suppl., 1–7.
4. Cassel J. C., Kelche C., Hornsperger J. M., Jackisch R., Hertting G. and Will B. E. (1990) Graft-induced learning impairment despite graft-enhanced cholinergic functions in the hippocampus of rats with septohippocampal lesions. *Brain Res.* **534,** 295–298.
5. Cassel J. C., Kelche C., Majchrzak M. and Will B. E. (1992) Factors influencing structure and function of intracerebral grafts in the mammalian brain: a review. *Restor. Neurol. Neurosci.* **4,** 65–96.
6. Cassel J. C., Kelche C., Peterson G. M., Ballough G. P., Goepp I. and Will B. (1991) Graft-induced behavioural recovery from subcallosal septo-hippocampal damage in rats depends on maturity of donor tissue. *Neuroscience* **45,** 571–586.
7. Cassel J. C., Kelche C. and Will B. (1990) Time-dependent effects of intrahippocampal grafts in rats with fimbria–fornix lesions. *Expl Brain Res.* **81,** 179–190.
8. Cassel J. C., Kelche C. and Will B. E. (1991) Susceptibility to pentylenetetrazol-induced and audiogenic seizures in rats given aspirative lesions of the fimbria–fornix pathways followed by intrahippocampal grafts: a time-course approach. *Restor. Neurol. Neurosci.* **3,** 55 64.

9. Cassel J. C., Neufang B., Kelche C., Aiple F., Will B. E., Hertting G. and Jackisch R. (1992) Effects of septal and/or raphe cell suspension grafts on hippocampal choline acetyltransferase activity, high affinity synaptosomal uptake of choline and serotonin, and behaviour in rats with extensive septohippocampal lesions. *Brain Res.* **585,** 243–254.
10. Cassel J. C., Neufang B., Kelche C., Jeltsch H., Will B. E., Hertting G. and Jackisch R. (1993) Effects of grafts containing cholinergic and/or serotonergic neurons on cholinergic, serotonergic and noradrenergic markers in the denervated rat hippocampus. *Brain Res.* **604,** 53–63.
11. Collerton D. (1986) Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience* **19,** 1–28.
12. Crutcher K. A. (1987) 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.
13. Daniloff J. K., Low W. C., Bodony R. P. and Wells J. (1985) Cross-species transplants of embryonic septal nuclei to the hippocampal formation of adult rats. *Expl Brain Res.* **59,** 73–82.
14. Daniloff J. K., Wells J. and Ellis J. (1984) Cross-species septal transplants: recovery of choline acetyltransferase activity. *Brain Res.* **324,** 151–154.
15. Decker M. W., Gill T. M. and McGaugh J. L. (1990) Concurrent muscarinic and beta-adrenergic blockade in rats impairs place-learning in a water maze and retention of inhibitory avoidance. *Brain Res.* **513,** 81–85.
16. Decker M. W. and McGaugh J. L. (1991) The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* **7,** 151–168.
17. Dunnett S. B. (1990) Neural transplantation in animal models of dementia. *Eur. J. Neurosci.* **2,** 567–587.
18. Dunnett S. B., Low W. C., Iversen S. D., Stenevi U. and Björklund A. (1982) Septal transplants restore maze learning in rats with fornix–fimbria lesions. *Brain Res.* **251,** 335–348.
19. Dunnett S. B., Martel F. L., Rogers D. C. and Finger S. (1989) Factors affecting septal graft amelioration of differential reinforcement of low rates (DRL) and activity deficits after fimbria–fornix lesions. *Restor. Neurol. Neurosci.* **1,** 83–92.
20. Eichenbaum H., Stewart C. and Morris R. G. M. (1990) Hippocampal representation in place learning. *J. Neurosci.* **10,** 3531–3542.
21. Emerich D. F., Black B. A., Kesslak J. P., Cotman C. W. and Walsh T. J. (1992) Transplantation of fetal cholinergic neurons into the hippocampus attenuates the cognitive and neurochemical deficits induced by AF64A. *Brain Res. Bull.* **28,** 219–226.
22. Emerich D. F. and Walsh T. J. (1990) Ganglioside AGF2 promotes task-dependent recovery and attenuates the cholinergic hypofunction induced by AF64A. *Brain Res.* **527,** 299–307.
23. Fonnum F. (1975) A rapid chemical method for the determination of choline acetyltransferase. *J. Neurochem.* **24,** 407–409.
24. Freund T. F. and Antal M. (1988) GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* **336,** 170–173.
25. Gage F. H. and Björklund A. (1986) Compensatory collateral sprouting of aminergic systems in the hippocampal formation following partial deafferentation. In *The Hippocampus*, Vol. 3 (eds Isaacson R. L. and Pribram K. H.), pp. 33–64. Plenum Press, New York.
26. Gage F. H. and Björklund A. (1986) Enhanced graft survival in the hippocampus following selective denervation. *Neuroscience* **17,** 89–98.
27. Gage F. H., Björklund A., Stenevi U. and Dunnett S. B. (1983) Functional correlates of compensatory collateral sprouting by aminergic and cholinergic afferents in the hippocampal formation. *Brain Res.* **268,** 39–47.
28. Gasser U. E., Van Deusen E. B. and Dravid A. R. (1986) Homologous cholinergic efferents spared by partial fimbrial lesions contribute to the recovery of hippocampal cholinergic enzymes in adult rats. *Brain Res.* **367,** 368–373.
29. Haroutunian V., Santucci A. C. and Davis K. L. (1990) Implications of multiple transmitter system lesions for cholinomimetic therapy in Alzheimer's disease. In *Progress in Brain Research*, Vol. 84 (eds Aquilonius S. M. and Gillberg P. G.), pp. 333–346. Elsevier, Amsterdam.
30. Hodges H., Allen Y., Kershaw T., Lantos P. L., Gray J. A. and Sinden J. (1991) Effects of cholinergic-rich neural grafts on radial maze performance of rats after excitotoxic lesions of the forebrain cholinergic projection system—I. Amelioration of cognitive deficits by transplants into cortex and hippocampus but not into basal forebrain. *Neuroscience* **45,** 587–607.
31. Hodges H., Allen Y., Sinden J., Mitchell S. N., Arendt T., Lantos P. L. and Gray J. A. (1991) The effects of cholinergic drugs and cholinergic-rich foetal neural transplants on alcohol-induced deficits in radial maze performance in rats. *Behav. Brain Res.* **43,** 7–28.
32. Holttum J. R. and Gershon S. (1992) The cholinergic model of dementia, Alzheimer type: progression from the unitary transmitter concept. *Dementia* **3,** 174–185.
33. Hughes K. R. (1965) Dorsal and ventral hippocampus lesions and maze learning: influence of preoperative environment. *Can. J. Psychol.* **19,** 325–332.
34. Ikegami S., Nihonmatsu I., Hatanaka H., Takei N. and Kawamura H. (1989) Transplantation of septal cholinergic neurons to the hippocampus improves memory impairments of spatial learning in rats treated with AF64A. *Brain Res.* **496,** 321–326.
35. Ikegami S., Nihonmatsu I. and Kawamura H. (1991) 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.
36. Isaacson R. L., Springer J. E. and Ryan J. P. (1986) Cholinergic and catecholaminergic modification of the hippocampal lesion syndrome. In *The Hippocampus*, Vol. 4 (eds Isaacson R. L. and Pribram K. H.), pp. 128–158. Plenum Press, New York.
37. Jarrard L. E. (1986) Selective hippocampal lesions and behaviour. In *The Hippocampus*, Vol. 4 (eds Isaacson R. L. and Pribram K. H.), pp. 93–126. Plenum Press, New York.
38. Jeltsch H., Cassel J. C., Jackisch R., Neufang B., Greene P. L., Kelche C., Hertting G. and Will B. (1994) Lesions of supracallosal or infracallosal hippocampal pathways in the rat: behavioural, neurochemical and histochemical effects. *Behav. neural Biol.* (in press).
39. Kaseda Y., Simon J. R. and Low W. C. (1989) Restoration of high affinity choline uptake in the hippocampal formation following septal cell suspension transplants in rats with fimbria–fornix lesions. *J. Neurochem.* **53,** 482–488.

40. Koelle G. B. (1954) The histochemical localization of cholinesterases in the central nervous system of the rat. *J. comp. Neurol.* **100,** 211–235.
41. Lahtinen H., Miettinen R., Ylinen A., Halonen T. and Riekkinen S. R. (1993) Biochemical and morphological changes in the rat hippocampus following transection of the fimbria–fornix. *Brain Res. Bull.* **31,** 311–318.
42. Li Y. J., Simon J. R. and Low W. C. (1992) Intrahippocampal grafts of cholinergic-rich striatal tissue ameliorate spatial memory deficits in rats with fornix lesions. *Brain Res. Bull.* **29,** 147–155.
43. Low W. C., Lewis P. R., Bunch S. T., Dunnett S. B., Thomas S. R., Iversen S. D., Björklund A. and Stenevi U. (1982) Functional recovery following neural transplantation of embryonic septal nuclei in adult rats with septo-hippocampal lesions. *Nature* **300,** 260–262.
44. Lowry O. H., Rosebrough N. J., Farr A. C. and Randall R. S. (1951) Protein measurement with the folin reagent. *J. biol. Chem.* **193,** 265–275.
45. Mann D. M. A. and Yates P. O. (1986) Neurotransmitter deficits in Alzheimer's disease and in other dementing disorders. *Human Neurobiol.* **5,** 147–158.
46. Moser E., Moser M. B. and Andersen P. (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J. Neurosci.* **13,** 3916–3925.
47. Nadel L. (1968) Dorsal and ventral hippocampal lesions and behaviour. *Physiol. Behav.* **3,** 891–900.
48. Nilsson O. G., Brundin P. and Björklund A. (1990) Amelioration of spatial memory impairment by intrahippocampal grafts of mixed septal and raphe tissue in rats with combined cholinergic and serotonergic denervation of the forebrain. *Brain Res.* **515,** 193–206.
49. Nilsson O. G., Clarke D. J., Brundin P. and Björklund A. (1988) Comparison of growth and reinnervation properties of cholinergic neurons from different brain regions grafted to the hippocampus. *J. comp. Neurol.* **268,** 204–222.
50. Nilsson O. G., Shapiro M. L., Gage F. H., Olton D. S. and Björklund A. (1987) Spatial learning and memory following fimbria–fornix transection and grafting of fetal septal neurons to the hippocampus. *Expl Brain Res.* **67,** 195–215.
51. Ogren S. O., Stone W. S. and Altman H. J. (1985) Evidence for a functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. *Soc. Neurosci. Abstr.* **11,** 256.11.
52. O'Keefe J. and Nadel L. (1978) *The Hippocampus as a Cognitive Map.* Oxford University Press, Oxford.
53. Olton D. S., Becker J. T. and Handelmann G. E. (1979) Hippocampus, space and memory. *Behav. Brain Sci.* **2,** 313–365.
54. Pallage V., Toniolo G., Will B. E. and Hefti F. (1986) Long-term effects of nerve growth factor and neural transplants on behaviour of rats with medial septal lesions. *Brain Res.* **386,** 197–208.
55. Palmer A. M., Wilcock G. K., Esiri M. M., Francis P. T. and Bowen D. M. (1987) Monoaminergic innervation of the frontal and temporal lobes in Alzheimer's disease. *Brain Res.* **401,** 231–238.
56. Paxinos G. and Watson P. (1986) *The Rat Brain in Stereotaxic Coordinates.* Academic Press, Sydney.
57. Perry E. K. (1986) The cholinergic hypothesis—ten years on. *Br. med. Bull.* **42,** 63–69.
58. Perry E. K., Curtis M., Dick D. J., Candy J. M., Atack J. R., Bloxham C. A., Blessed G., Fairbairn A., Tomlinson B. E. and Perry R. H. (1985) Cholinergic correlates of cognitive impairment in Parkinson's disease: comparison with Alzheimer's disease. *J. Neurol. Neurosurg. Psychiat.* **48,** 413 421.
59. Perry E. K., Tomlinson B. E., Blessed G., Bergmann K., Gibson P. H. and Perry R. H. (1978) Correlation of cholinergic abnormalities with senile plaques and mental scores in senile dementia. *Br. med. J.* **2,** 1457–1459.
60. Richter-Levin G. and Segal M. (1989) Raphe cells grafted into the hippocampus can ameliorate spatial memory deficits in rats with combined serotonergic/cholinergic deficiencies. *Brain Res.* **478,** 184–186.
61. Riekkinen P. Jr., Sirviö J. and Riekkinen P. J. (1990) Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. *Brain Res.* **527,** 342–345.
62. Segal M., Greenberger V. and Milgram N. W. (1987) A functional analysis of connections between grafted septal neurons and a host hippocampus. In *Progress in Brain Research* (eds Seil F. J., Herbert E. and Carlsson B. M.), pp. 247–251. Elsevier, Amsterdam.
63. Segal M., Greenberger V. and Pearl E. (1989) Septal transplants ameliorate spatial deficits and restore cholinergic functions in rats with a damaged septo-hippocampal connection. *Brain Res.* **500,** 139–148.
64. Seiger A. (1985) Preparation of immature central nervous system regions for transplantations. In *Neural Grafting in the Mammalian Brain* (eds Björklund A. and Stenevi U.), pp. 71–77. Elsevier, Amsterdam.
65. Shapiro M. L., Simon D. K., Olton D. S., Gage F. H., Nilsson O. and Björklund A. (1989) Intrahippocampal grafts of fetal basal forebrain tissue alter place fields in the hippocampus of rats with fimbria–fornix lesions. *Neuroscience* **32,** 1–18.
66. Sidel E. S., Tilson H. A., McLamb R. L., Wilson W. A. and Swartzwelder H. S. (1988) Potential interactions between GABAb and cholinergic systems: baclofen augments scopolamine-induced performance deficits in the eight-arm radial maze. *Psychopharmacologia* **96,** 116–120.
67. Sinden J. D., Allen Y. S., Rawlins J. N. P. and Gray J. A. (1990) The effects of ibotenic acid lesions of the nucleus basalis and cholinergic-rich neural transplants on win-stay/lose-shift and win-shift/lose-stay performance in the rat. *Behav. Brain Res.* **36,** 229–249.
68. Sirkin D. W. (1983) Critical defatting of frozen brain sections for optimal differentiation with the cresyl violet stain. *Stain Technol.* **58,** 121–122.
69. Steinbusch H. W. M., Beek A., Frankhuyzen A. L., Tonnaer J. A. D. M., Gage F. H. and Björklund A. (1987) Functional activity of raphe neurons transplanted to the hippocampus and the caudate–putamen. In *Cell and Tissue Transplantation into the Adult Brain, Annals of the New York Academy of Sciences,* Vol. 495 (eds Azmitia E. C. and Björklund A.), pp. 169–184.
70. Stevens R. and Cowey A. (1973) Effects of dorsal and ventral hippocampal lesions on spontaneous alternation, learned alternation and probability learning in rats. *Brain Res.* **52,** 203–224.
71. Storm-Mathisen J. and Guldberg H. C. (1974) 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.
72. Tallarida R. J. and Murray R. B. (1987) *Manual of Pharmacologic Calculations with Computer Programs.* Springer, New York.
73. Tarricone B. J., Klein S. R., Simon J. R. and Low W. C. (1991) Intrahippocampal transplants of septal cholinergic neurons: high-affinity choline uptake and spatial memory function. *Brain Res.* **548,** 55–62.

74. Vanderwolf C. H. (1987) Near-total loss of "learning" and "memory" as a result of combined cholinergic and serotonergic blockade in the rat. *Behav. Brain Res.* **23,** 43–57.
75. Vanderwolf C. H., Leung L. W. S., Baker G. B. and Stewart D. J. (1989) A general role for serotonin in the control of behaviour: studies with intracerebral 5,7-dihydroxytryptamine. *Brain Res.* **504,** 192–198.
76. Van Luijtelaar M. G. P. A., Tonnaer J. A. D. M., Frankhuijzen A. L., Dijkstra H., Hagan J. J. and Steinbusch H. W. M. (1991) Morphological, neurochemical, and behavioural studies on serotonergic denervation and graft-induced reinnervation of the rat hippocampus. *Neuroscience* **42,** 365–377.
77. Wallenstein S., Zucker C. L. and Fleiss J. L. (1980) Some statistical methods useful in circulation research. *Circulation Res.* **47,** 1–9.
78. Winer B. J. (1971) *Statistical Principles in Experimental Design.* McGraw-Hill, New York.

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