Exp Brain Res (1987) 67: 195–215

Experimental Brain Research

Spatial learning and memory following fimbria-fornix transection and grafting of fetal septal neurons to the hippocampus

O.G. Nilsson[1], M.L. Shapiro[2], F.H. Gage[3], D.S. Olton[2], and A. Björklund[1]

[1] Department of Histology, University of Lund, Biskopsgatan 5, S–223 62 Lund, Sweden
[2] Department of Psychology, Johns Hopkins University, Baltimore, MD 21218, USA
[3] Department of Neurosciences, UCSD, La Jolla, CA 92093, USA

Summary. The ability of intrahippocampal grafts of fetal septal-diagonal band tissue, rich in developing cholinergic neurons, to ameliorate cognitive impairments induced by bilateral fimbria-fornix transections in rats was examined in three experiments using the Morris water-maze to test different aspects of spatial memory. *Experiment 1.* Rats with fimbria-fornix lesions received either septal cell suspension grafts or solid septal grafts; normal rats and rats with lesions alone were used as controls. Sixteen weeks after surgery, the rats' spatial learning and memory were tested in the water-maze using a place test, designed to investigate place navigation performance, in which rats learned to escape from the water by swimming to a platform hidden beneath the water's surface. After 5 days of training, the rats were given a spatial probe test in which the platform was removed from the tank to test spatial reference memory. *Experiment 2.* The same rats used in Exp. 1 were tested in a delayed-match-to-sample, working memory version of the water-maze task. The platform was located in one of two possible locations during each trial, which was composed of 2 swims. If the rat remembered the location of the platform on the 2nd swim of a trial, it should find the platform more quickly on that swim, and thereby demonstrate working memory. *Experiment 3.* Prior to receiving fimbria-fornix lesions, normal rats were trained in a modification of the water-maze task using alternating cue navigation and place navigation trials (i.e., with visible or non-visible escape platforms). The retention and reacquisition of the place task and the spatial probe test were examined in repeated tests up to 6 months after the lesion and intrahippocampal grafting of septal cell suspensions. The effects of central muscarinic cholinergic receptor blockade with atropine were also tested. Normal rats performed well in both the place and spatial probe tests. In contrast, rats with fimbria-fornix lesions only were unable to acquire or retain spatial information in any test. Instead, these rats adopted a random, non-spatial search strategy, whereby their latencies to find the platform decreased in the place navigation tasks. Sixty to 80% of the rats with septal suspension or solid grafts had recovered place navigation, i.e., the ability to locate the platform site in the tank, in Exp. 1 and 3, and they showed a significantly improved performance in the working memory test in Exp. 2. Atropine abolished the recovered place navigation in the grafted rats, whereas normal rats were impaired to a lesser extent. In contrast, atropine had no effect on the non-spatial strategy adopted by rats with fimbria-fornix lesions only. The results show that: (1) fimbria-fornix lesions disrupt spatial learning and memory in both naive and pretrained rats; (2) with extended training the fimbria-fornix lesioned rats develop an efficient non-spatial strategy, which enables them to reduce their escape latency to levels close to those of intact controls; (3) intrahippocampal septal grafts can restore the ability of the lesioned rats to use spatial cues in the localization of the platform site; and (4) the behavioural recovery produced by grafts is dependent upon an atropine sensitive mechanism.

Key words: Neural transplantation – Acetylcholine – Hippocampus – Spatial memory – Atropine

Offprint requests to: O.G. Nilsson (address see above)

Introduction

Lesions of the septo-hippocampal system in rats, including transection of the fimbria-fornix (FF) pathways which carry afferents to and efferents from the hippocampal formation, produce permanent behavioural impairments in many tasks, particularly

those requiring spatial memory (Morris 1983; O'Keefe and Nadel 1978; Olton et al. 1979; Rawlins 1985).

Previous studies have demonstrated that intrahippocampal grafts of fetal septal-diagonal band tissue, rich in developing cholinergic neurons, can partly restore learning and memory in rats with medial septal or FF lesions (Low et al. 1982; Dunnett et al. 1982; Daniloff et al. 1985; Pallage et al. 1986). These studies used T-maze and 8-arm radial maze tasks that are sensitive to septo-hippocampal damage (Olton 1983).

In the present study we investigated in three experiments the behavioural effects of intrahippocampal septal grafts in further detail using reference and working memory versions of the Morris water-maze task (Morris 1981, 1984). The Morris water-maze has been widely used to assess the functional effects of hippocampal system lesions. This task requires navigation, and is particularly useful for analyzing the role of the hippocampus in spatial learning and memory (see Morris 1983 for a review). The standard reference memory procedure of this task (used in two of the present experiments) tests the ability of a rat to swim to a platform hidden just beneath the surface of the water in a swimming pool; the rat can then climb onto and rest upon the platform. Normal rats use a *spatial strategy* to find the platform in the pool. During a *place test*, when the platform is present in the tank, they swim directly from the starting point to the platform. During a *spatial probe test*, when the platform is removed from the pool, they spend more time swimming in the *training quadrant*, the part of the pool that had contained the platform during the place test, than in the other quadrants (Morris 1981, 1984). Rats with hippocampal system lesions are severely impaired in locating the platform site, and use a *non-spatial strategy* to find the platform. Thus, during the place test, they swim indirectly to the platform, and during the spatial probe test, they spend equivalent amounts of time in all quadrants, rather than swimming mostly in the training quadrant (Morris et al. 1982; Sutherland et al. 1982a; Morris 1983). Performance in this task is also sensitive to central cholinergic receptor blockade (Sutherland et al. 1982b; Whishaw 1985; Whishaw et al. 1985). In addition to this reference memory task, a modified delayed-match-to-sample version of the water-maze task was designed to test spatial working memory in the second experiment of the present study.

Two types of grafts were studied: septal cell suspensions injected directly into the hippocampus, and solid pieces of septal tissue placed into the cavity made by the FF lesion. Because suspension grafts, unlike solid grafts, cannot act as a bridge to allow fibers to grow across the FF lesion into the denervated hippocampus from the host septum (cf. Buzsaki et al. 1986), any recovery should be due to the effects of the graft on the hippocampus, rather than to reinnervation from the host septum.

Several lines of evidence suggest that cholinergic activity is required for the recovery produced by the septal grafts. Such grafts, rich in developing cholinergic neurons, when implanted in or adjacent to the hippocampal formation in adult recipient rats can restore several of the morphological and functional deficits produced by FF lesions (for a review, see Gage et al. 1987). The septal grafts can re-establish a fairly normal cholinergic innervation of the initially denervated hippocampus (Björklund and Stenevi 1977; Björklund et al. 1983a) and can form abundant functional cholinergic synapses with dentate granule cells and hippocampal pyramidal neurons of the host (Segal et al. 1985, 1987; Clarke et al. 1986; Anderson et al. 1986). Moreover, biochemical measures of acetylcholine synthesis in vitro have indicated that the grafted cholinergic neurons are spontaneously active at a near normal rate (Björklund et al. 1983c). Together, these data suggest that fetal septal neurons can innervate the hippocampus of rats with FF lesions, and form new, functional cholinergic synapses with neurons in the host.

To study the role of the cholinergic system in septal graft function, Experiment 3 also examined the effects of atropine, a cholinergic muscarinic receptor antagonist, on the graft-induced behavioural recovery in the water-maze. If this recovery was dependent on a cholinergic mechanism, then atropine should abolish the graft effect. If, however, the behavioural recovery induced by septal grafts was independent of cholinergic mechanisms, then atropine should have no effect.

Four questions were thus addressed in the present study. (1) Can septal grafts restore place navigation in the water-maze in rats with FF lesions? (2) Is the restoration due to the recovery of normal spatial learning and memory mechanisms, including working memory? (3) Does pre-training of the rats influence the amnesic effect of subsequent FF lesions or the recovery produced by grafts? (4) Is the graft-induced behavioural recovery affected by cholinergic receptor blockade?

General methods

Subjects

Young adult female Sprague-Dawley rats (ALAB, Stockholm, Sweden) weighing about 200 g at the time of surgery served as

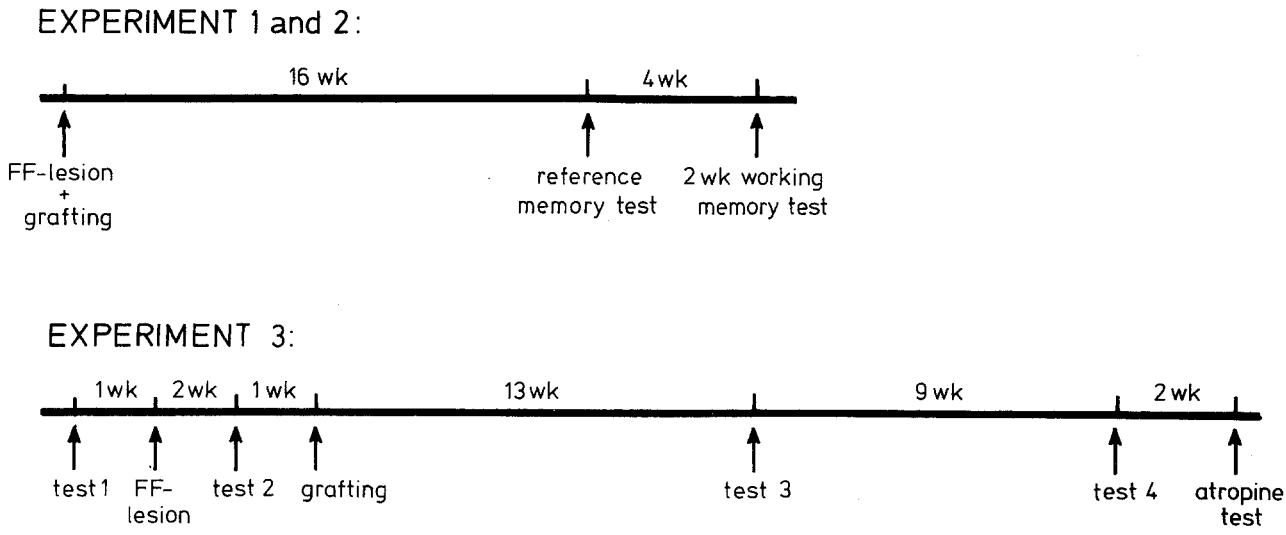


Fig. 1. General design of the three experiments showing the order of surgery and tests and the intervals between them

subjects. They were housed in groups with ad lib access to food and water throughout the experiments.

Surgery

All surgery was conducted in a Kopf stereotaxic apparatus. Bilateral aspiration lesions of the FF were made in rats anaesthetized with methyl hexital (Brietal, Lilly; 40 mg/kg i.p.). Some of the rats with FF lesions were given septal grafts within 4 weeks of receiving the lesion. A mixture of ketamine (Parke-Davis, 10 mg/kg) and xylazine (Rompun, Hoechst, 5 mg/kg), given i.p. or i.m., was used as anaesthetic in the transplantation surgery.

Rats serving as normal controls were not treated surgically. Bilateral FF lesions were made by aspiration (Stenevi et al. 1976). A whole was drilled in the skull posterior to bregma on either side of the midline. The cortex, corpus callosum, and underlying fimbria-fornix were removed by aspiration until the dorsal aspect of the thalamus was visible. The lesion transected the septo-hippocampal fibers in the fimbriae, the dorsal fornices, and the supracallosal striae.

The cell suspensions were prepared as described previously (Björklund et al. 1983b). The developing septal-diagonal band area was dissected from the basal forebrain of E14–E16 donor rat fetuses (crown-rump length, CRL = 12–16 mm) of the same strain as the graft recipients. Tissue from 10–15 fetuses was collected in sterile 0.6% glucose-saline at room temperature, incubated in crude trypsin (Sigma type II: 0.1% in the glucose-saline medium) for 20 min at 37° C, washed 4–5 times with fresh glucose-saline, and mechanically dissociated in about 100 µl of this medium to form a milky suspension. Three 2 µl aliquots of the suspension were injected stereotaxically into the hippocampus bilaterally at the following coordinates (in mm): (1) A = 4.5 anterior to the interaural line, L = ±3.3 from midline, V = 2.8 ventral to dura; (2) A = +3.0, L = ±3.5, V = 3.5; (3) A = +3.0, L = ±4.8, V = 5.7. The incisor bar was set at the same dorso-ventral plane as the interaural line. Each injection was performed over 4 min.

The solid septal grafts, 1–2 mm^3, were dissected as described above from E16–E17 donor rat fetuses (CRL = 16–20 mm). They were kept in saline until being implanted into the FF cavities produced by the FF lesion one week earlier. Tissue from one fetus was placed on each side on the dorsal thalamus, in contact with the exposed hippocampal surface. The remainder of the cavity was filled with saline-soaked gelfoam before the wound was closed (Björklund and Stenevi 1977).

Apparatus: the Morris water-maze

A cylindrical tank (120 cm in diameter, 45 cm deep) was filled to a depth of 30 cm with room temperature water made opaque by the addition of powdered milk (Morris 1981, 1984). A transparent plastic platform (10.5 × 10.5 cm), mounted on a plastic column, was placed into the tank so that the platform was 1–2 cm below the water's surface. The platform and column were anchored to the floor of the tank with a lead weight, allowing the platform to be moved by the experimenter. A removable cover for the platform (3 cm thick) was made of wood and sheet metal, and had a vertical rod (5 cm tall) in its middle. When placed on the platform, the cover extended about 1–2 cm above the water. Four starting points, 90° apart, were marked on the edge of the tank. The pool was located in a corner of a room containing many extra-maze cues (i.e., windows, posters, video and computer equipment, etc.). A digitized TV system connected to an ABC 800 microcomputer recorded the rat's position and movements in the tank.

Acetylcholinesterase histochemistry

At the end of the experiment, all surviving rats with grafts or FF lesions were perfused via the ascending aorta with 150 ml of ice cold 4% phosphate buffered paraformaldehyde. The brains were fixed in the same solution for 2 h, and then placed in phosphate buffer at 4° C. Before sectioning, the brains were transferred to a 10% sucrose solution for a minimum of 12 h. Sections 15 µm thick were cut on a cryostat from the rostral end of the lesion cavity to the entorhinal cortex. Every third (in grafted brains) or sixth (in lesioned brains) section was stained for acetylcholinesterase (AChE), using prometazine (10^{-4} M) to inhibit non-specific esterases (Holmstedt 1957) and silver nitrate to intensify the reaction product (Geneser-Jensen and Blackstad 1971).

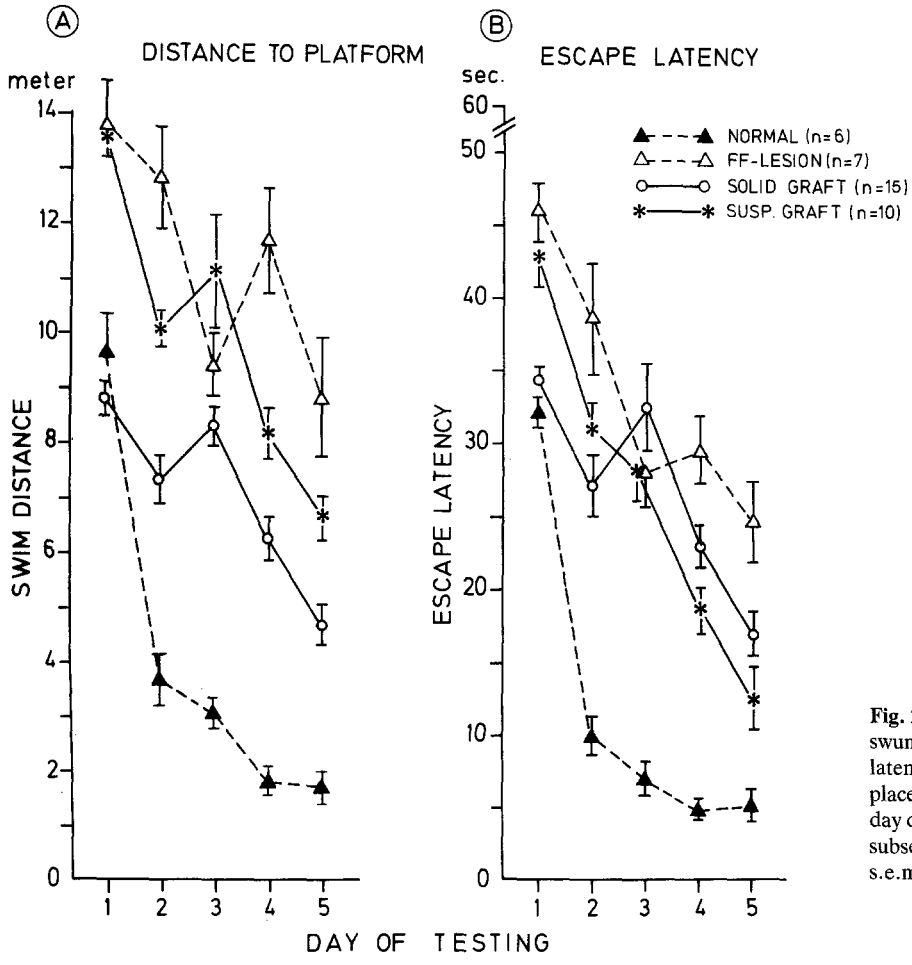


Fig. 2A, B. *Experiment 1.* Distance swum in meters (**A**) and escape latencies in seconds (s) (**B**) in the place test, given as the mean of each day during the test week. In this and subsequent figures, bars represent ± s.e.m.

Experiment 1

This experiment was designed to test whether septal grafts could restore the acquisition of spatial memory in rats with bilateral FF lesions. The behavioural effects of two types of grafts were examined: septal cell suspensions injected directly into the hippocampus, and solid septal tissue pieces placed in the cavity left by the FF lesion. Sixteen weeks following surgery, rats were tested in the Morris water-maze with place and spatial probe tests (Fig. 1).

Procedure

The experiment comprised: Normals (n = 6), FF lesions only (n = 7), FF lesions with suspension grafts (n = 10), and FF lesions with solid grafts (n = 15). The cell suspensions were injected during the same surgical session in which the FF lesions were made; the solid grafts were placed in the cavity one week following the FF lesions.

Place test. The platform was placed in a constant position in the middle of the training quadrant of the tank so that it was 1–2 cm below the surface of the water. Rats were given 2 blocks of 4 trials on each day for 4 consecutive days, and 1 block of 4 trials the fifth day. The four start positions were used in a pseudo-random, counterbalanced fashion so that each location was used once in each block of 4 trials. For each trial, the rat was placed in the water facing the wall at the designated start position. The rat was given 60 s to find the platform and climb onto it, and was then allowed to rest for 10–15 s. The rat was then removed from the platform and placed at the next predetermined starting point. If the rat did not find the platform within 60 s, the experimenter picked up and placed the rat on the platform for 10–15 s. The path from the starting location to the platform was recorded by the computer. Two measurements were obtained: *latency*, the amount of time (in seconds), and *distance*, the length of the path (in meters), from the starting point to the platform.

Spatial probe test. Immediately following the last place test (on the fifth day) described above, the platform was removed. The rat was placed at one of the starting points, allowed to swim for 120 s, and then removed from the tank. The rat's swim path was recorded by the experimenter. In addition, four *annuli* were defined as a circular area in the middle of each of the four quadrants of the tank. The center of the annulus was located where the center of the platform would have been if a platform had been placed in the quadrant. The area of the annulus was twice that of the platform. The number of times the rat passed through each annulus during the trial was counted. The *heading angle* was the angle of the rat's swimming direction relative to the platform's location as the rat left the starting point. The heading angle ranged from 0 (swimming

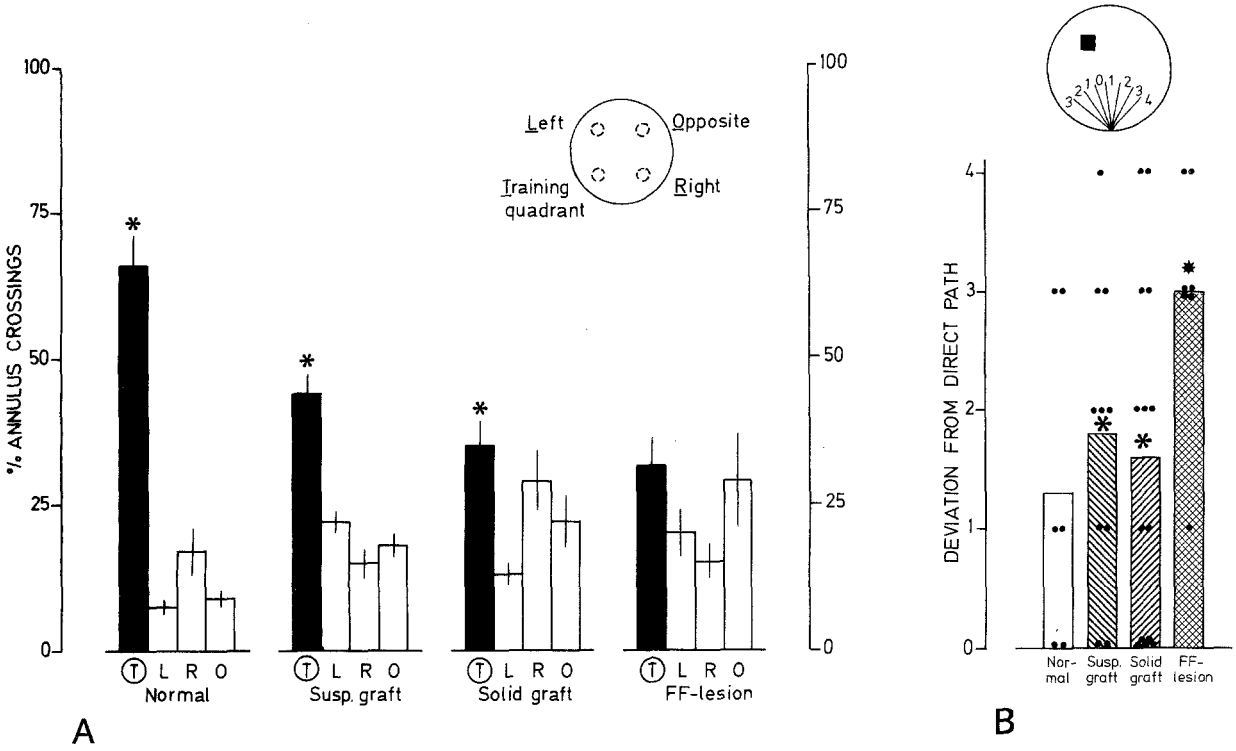


Fig. 3A, B. *Experiment 1.* **A** Relative distribution of annulus crossings in the different quadrants, as indicated in the inset, in the spatial probe trial when the platform was removed from the pool and the rat was allowed to swim for 2 min. Each annulus marks an area twice the actual size of the platform and is located in the middle of each quadrant. Asterisks denote higher number of crossings in the training quadrant compared to all other quadrants, $p < 0.01$. **B** Scores given each rat, as indicated in the inset, based on the heading angle taken from the start position by the rat when placed in the tank in the last spatial probe trial. Star indicates significant difference from the normal group, and asterisks a difference from the FF-lesioned group, one-tailed, $p < 0.05$

directly towards the platform) to 4 (swimming 75–90° away from the direct path to the platform; see inset in Fig. 3B).

Results

Place test. The escape latency and swim distance of all four groups of rats is illustrated in Fig. 2. Normal rats learned the task rapidly, reached asymptotic performance within 4 days of testing, and at the end of testing reached the platform in a mean of 4.7 s. Rats with FF lesions alone showed overall slower acquisition of the task, but the performance of the individual rats differed markedly. Thus, by the end of the test week their escape latencies varied from 7 to 60 s, with a mean of 24.9 s. Rats with grafts performed at a level intermediate between normal rats and those with FF lesions alone. At the end of testing, rats with suspension grafts reached the platform after a mean of 12.9 s, and those with solid grafts after a mean of 17.5 s.

Although each group showed significant acquisition of the task when examined individually (repeated measures ANOVA: distance and escape latency, $p < 0.05$), the groups differed (distance, $F(3, 34) = 6.4$, $p < 0.01$; latency, $F(3, 34) = 4.83$, $p < 0.01$), and the groups acquired the task at different rates (group × day interaction: distance, $F(12, 82) = 2.04$, $p < 0.05$; latency, $F(12, 82) = 2.11$, $p < 0.05$). During the last two days of testing, the FF lesioned rats differed significantly from the normal controls, whereas the two grafted groups did not differ from either the normal controls or the FF lesioned rats (Distance: $F(3, 34) = 4.4$, $p < 0.01$; latency: $F(3, 34) = 3.22$, $p < 0.05$, followed by Tukey's HSD: $p < 0.05$).

Spatial probe test. Figure 3 illustrates the ability of the rats to locate the platform site in the final spatial probe trial. Normal rats had a pronounced bias for the original platform site (T in Fig. 3A), and most of them swam directly towards the platform site (Fig. 3B). As illustrated in Fig. 4, upper panel, normal rats swam primarily in the training quadrant. All rats with FF lesions were markedly impaired on these measures. They did not swim preferentially over the platform site annulus relative to the other annuli (Fig. 3A), and only 1 of the 7 rats with FF lesions swam directly towards the platform in the

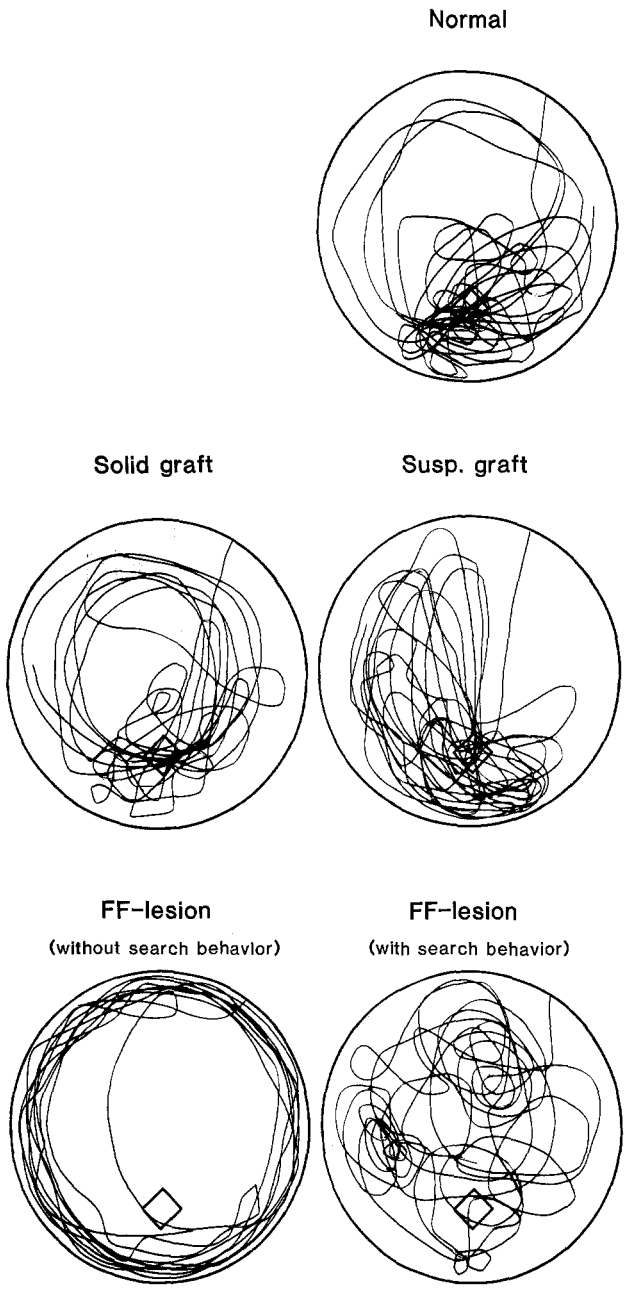


Fig. 4. *Experiment 1.* Actual swim paths taken during the spatial probe trial of a normal rat, of recovered solid and suspension grafted rats, and two different examples of the FF-lesioned rats, illustrating the different kinds of search behaviour

spatial probe trial (Fig. 3B; Mann-Whitney U: $p < 0.05$ one tailed for comparison with normal rats). Three of the rats with FF lesions had developed an active but seemingly random search behaviour, which made them swim at a distance from the edge and cover all four quadrants of the tank. The other four showed a more stereotyped, thigmotaxic behaviour, swimming predominantly along the edge of the pool (compare the two FF lesioned rats illustrated in Fig. 4).

Rats with either suspension or solid septal grafts showed significantly improved performance in the spatial probe test compared to rats given FF lesions alone (Fig. 4). The grafted rats crossed over the platform annulus more often relative to the three non-platform annuli (Fig. 3A) (Suspension grafted group: $F(3, 7) = 9.13$, $p < 0.01$; Solid grafted group: $F(3, 12) = 9.45$, $p < 0.01$), and they performed significantly better than rats with lesions alone in their ability to swim directly towards the platform (Fig. 3B) (one-tailed Mann-Whitney U, $p < 0.05$ for both groups). Eight of the 10 rats in the suspension graft group and 9 of the 15 rats in the solid graft group but none in the FF lesioned group showed a clear place preference in the spatial probe test. Five of the rats with suspension grafts and 3 of the rats with solid grafts had scores within the normal range in the spatial probe test.

In the final microscopic analysis (see below) 3 of the non-recovered rats in the solid graft group had extensive unintended pathology (hydrocephalus and thalamic-septal damage) and 1 recovered rat had some minor sparing of the lateral fimbria on one side. However, even when these 4 rats were excluded from the statistical analysis, the graft-induced effects on the spatial probe measures remained significant in the solid graft group.

Comments

Normal rats learned to find the hidden platform quickly and directly by using a spatial strategy. In the spatial probe test, they swam directly towards the former platform site from one of the arbitrarily chosen starting points at the edge of the tank, and focussed their swim over the former platform site. As shown previously (Morris 1981, 1984) this reflects the ability of normal rats to learn and remember the location of the platform, and use extra-maze cues to navigate to the platform.

The primary deficit induced by the FF lesion was the inability of the lesioned rats to locate the platform site in the spatial probe test: None of the rats with FF lesions alone showed a clear spatial bias in the search for the platform. In the place test, the FF lesioned rats showed as a group impaired acquisition, but the individual rats differed markedly. Thus, some of the lesioned rats adopted a non-spatial, seemingly random search strategy, and the best performing rat had an escape latency by the end of the test week that was within the normal range. As further emphasized by the results obtained in Exp. 3,

below, this shows that the FF lesioned rats can develop an efficient non-spatial strategy which allows them to reduce their escape latency in the water-maze task. Thus, thigmotaxic behaviour was characteristic only of some of the FF lesioned rats, and with extended training most of the lesioned rats will adopt the non-spatial type of search behaviour (see below). Because of this, escape latency is not a reliable or stable measure of the deficit induced by FF lesions in the analysis of place navigation in the water-maze task.

The grafted animals showed a significant recovery of place navigation in the spatial probe test. The recovery induced by the septal grafts appeared to be due to a specific effect on the ability to use extra-maze cues to locate the platform site in the tank, and thus to acquire a spatial strategy in the search for the hidden platform. Many of the grafted rats, like the normal rats but unlike rats with FF lesions only, were able to swim directly towards the platform from the starting point, and the best behaviourally recovered rats focussed their search over the platform site in the spatial probe test to the same extent as the intact animals. The graft effect was thus not due simply to inhibition of the thigmotaxic behaviour (i.e., swimming along the perimeter of the tank) seen in some of the FF lesioned rats.

It might be argued that the grafted rats could perform well in the place test using an abnormal strategy. However, the restoration or place navigation in the spatial probe test provides evidence that spatial memory, an ability associated with normal hippocampal function and not displayed by rats with FF lesions, was at least partly restored by the septal grafts.

Experiment 2

In Experiment 1, the escape platform was always located in one place. Thus, rats could use the same information to solve the task on every trial, a *reference memory* procedure (Honig 1978; Olton et al. 1979). In contrast, *working memory* procedures require the use of information embedded in the temporal context of a single trial. A delayed-match-to-sample (DMTS) task is an example of a working memory procedure. A trial begins when a sample stimulus is presented to a subject and then withdrawn. After a delay, the sample stimulus and another stimulus are presented. The subject's task is to indicate which of these was the sample stimulus at the beginning of that trial. For the next trial, a different sample stimulus may be presented, so that the subject must remember which one was presented during the current trial to perform correctly. Because the information required to solve the task changes from trial to trial, DMTS procedures involve working memory.

Hippocampal lesions are known to impair the ability of rats to perform a DMTS working memory procedure in the water-maze (Morris 1983; Whishaw 1985). Even if grafts restore normal spatial reference memory, i.e., the ability to represent a spatial location, they may not restore working memory, which is a process requiring the rapid storage and retrieval of information in a temporal context. Experiment 2 addressed this point: To what extent do grafts enable normal spatial working memory?

Procedure

The same groups of rats used in Exp. 1 were used as subjects in Exp. 2. Testing started 4 weeks after the end of that experiment (Fig. 1). The critical difference between this and the procedure described in Exp. 1 is that here the submerged platform was placed in one of *two* locations that varied between trials in a DMTS procedure (see inset in Fig. 5). For each trial, the platform was placed in one of these predetermined locations. Each trial was composed of 2 *swims*. The platform remained in the same location during both swims. For the first swim, the rat was placed in the tank facing one of two starting points (determined by a pseudo-random order) and allowed to swim to the hidden platform. For the second swim, the same rat was placed in the tank facing one of the two starting points (again determined by a pseudo-random order) and allowed to search for the platform again. In either swim, if a rat did not find the platform after 60 s, it was placed onto the platform by the experimenter for 15–30 s. After the second swim, the rat was placed in a warm cage. Groups of 4–7 rats were tested at a time. After each rat had been given one trial, the next trial begun. Four trials per day were given 5 days per week for 2 weeks. Working memory was quantified as *difference scores* in swim distance or escape latency between swim 1 and swim 2.

Results

Both groups of rats with septal grafts performed better than rats with lesions only in the working memory test, as did normal rats (1-way ANOVA by groups: mean difference between swims, $F(3, 34) = 4.13$, $p < 0.02$; Planned comparisons: Suspension grafted vs FF lesioned only, $F(1,34) = 6.48$, $p < 0.02$; Solid grafted vs FF lesioned only, $F(1, 34) = 7.47$, $p < 0.01$; Normal vs FF lesioned only, $F(1, 34) = 11.02$, $p < 0.005$).

Normal rats consistently escaped more quickly to the platform, and swam less distance on swim 2 than on swim 1 (repeated measures ANOVA on two factors: escape latency: $F(1, 5) = 22.9$, $p < 0.01$; swim distance: $F(1, 5) = 216.5$, $p < 0.001$). This pattern was evident on each day of testing (Fig. 5).

Rats with FF lesions had impaired performance. They did not escape more quickly, nor did they swim

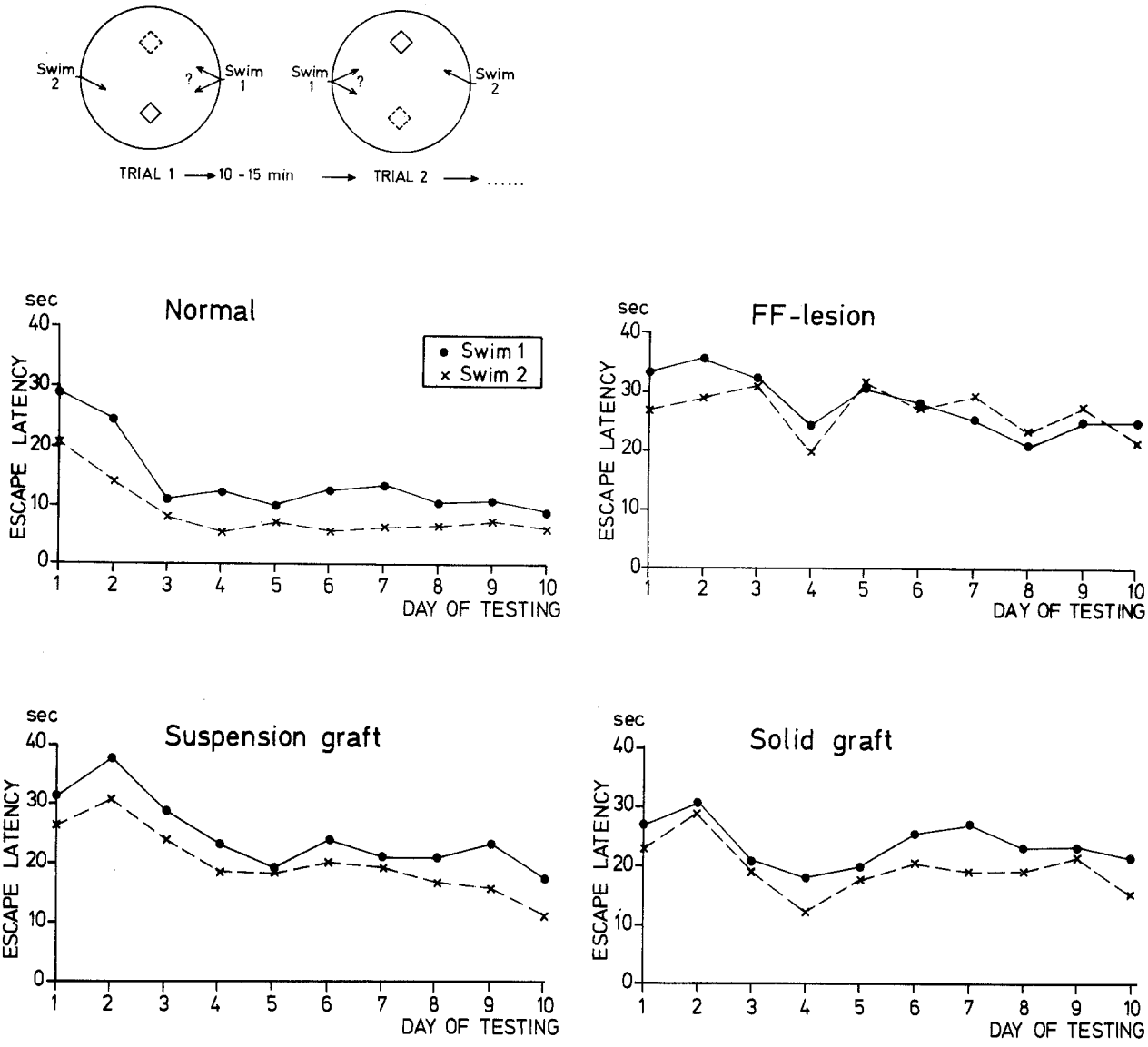


Fig. 5. *Experiment 2.* The inset outlines the working memory procedure diagrammatically. For each trial (composed of two swims), the escape platform was placed in one of the locations shown as diamonds. In swim 1 the rat had to explore the pool to determine the platform's location. In swim 2, if the rat remembered the platform's location from swim 1, it could swim to the platform more directly. After a 10–15 min intertrial interval, another trial was given. The platform's location (the solid diamond) on any given trial could not be predicted from the previous trial. The graphs illustrate escape latencies in seconds (s) during the 10 day working memory test for normal, FF-lesioned, suspension grafted, and solid grafted rats. Normals and rats with either suspension or solid septal grafts showed significantly lower escape latencies in swim 2 compared to swim 1 (i.e., displaying working memory, see text)

less distance on the second swim (escape latency: $F(1,6) = 1.59$, $p = 0.25$; swim distance: $F(1,6) = 1.6$, $p = 0.25$).

Rats with suspension grafts performed like normal rats, consistently escaping more quickly and swimming shorter distances on the second swim of a trial (escape latency: $F(1, 9) = 18.9$, $p < 0.005$; swim distance: $F(1, 9) = 5.7$, $p < 0.05$). This effect was evident from the first day of testing. Rats given solid grafts were similar (escape latency $F(1, 14) = 10.4$, $p < 0.01$; swim distance: $F(1, 14) = 5.7$, $p < 0.05$).

Comments

This experiment used a working memory procedure that required rats to remember information from a single experience to perform correctly. If the rat remembered the location of the platform from the first swim of a trial, it could escape from the water more quickly and directly in the second swim. Because the platform was moved from trial to trial, information from previous trials could not be used to determine the platform's location. Thus, only by

using working memory (and acquiring information quickly) could be rat escape more efficiently in swim 2 than in swim 1.

Normal rats demonstrated working memory by consistently swimming more quickly and directly to the platform on the second swim than on the first. In contrast, rats with FF lesions were severely impaired in working memory. This result is consistent with previous findings that rats with hippocampal damage perform poorly in working memory procedures in the water-maze (Morris 1983) and in other types of tests (Olton et al. 1979).

Rats with septal grafts, like normal rats but unlike those with FF lesions, performed well in the working memory task. Thus septal grafts enabled some rats with FF lesions to use spatial information acquired in only one trial. Taken together, these results provide converging evidence that the septal grafts, can improve spatial learning and memory, including place navigation and working memory.

Experiment 3

Experiments 1 and 2 tested acquisition of spatial memory in experimentally naive rats. Experiment 3 tested: (1) Whether FF lesions impaired the retention of previously acquired spatial memory; (2) Whether septal grafts could enable rats to reacquire spatial memory lost as a result of the FF lesion; (3) Whether the presence of local cues (i.e., a visible platform on alternating trials) could improve the performance of FF lesioned rats with or without septal suspension grafts; and (4) Whether cholinergic receptor blockade by atropine treatment would impair the graft-induced behavioural recovery in the water-maze.

Procedure

As illustrated in Fig. 1, 23 rats were trained before surgery (Test 1). One week later, 17 of the rats were given bilateral FF lesions and tested again 2 weeks later (Test 2). One week after Test 2, 6 of the lesioned rats received bilateral septal suspension grafts to the hippocampus. Thirteen and 23 weeks later the rats were tested again (Tests 3 and 4), followed shortly by an atropine challenge (Test 5). By the end of Test 4, 6 normal, 6 rats with FF lesions only, and 5 rats with both FF lesions and grafts survived. One FF lesioned and one grafted rat died during the atropine test.

The water-maze *place test* was used as described in Exp. 1, with the modification that the rats were now trained using non-visible and visible escape platforms on alternating trials. On each day, the platform was visible on trials 1, 3, 5, and 7 and not visible on trials 2, 4, 6, and 8. The platform was made visible with the platform cover described in the Apparatus section above. A 1 min *spatial probe* test, as described in Exp. 1, was administered at the end of each of the 4 test periods.

The *atropine test* was given to all rats during Test 5, performed 2 weeks after Test 4 (Fig. 1). On each day, each rat was given 2 blocks of 4 trials with the alternating visible and non-visible platform procedure. After these 8 trials, a 9th spatial probe trial was given that lasted 2 min. On the first day the rats were tested without drug treatment. On the second day each rat was injected with atropine sulfate (Sigma; 50 mg/kg, i.p) 20–60 min before the start of the test. On the third day each rat was given equimolar amounts of atropine methylbromide (Sigma; 28.4 mg/kg, i.p.), to control for effects of peripheral cholinergic blockade.

Results

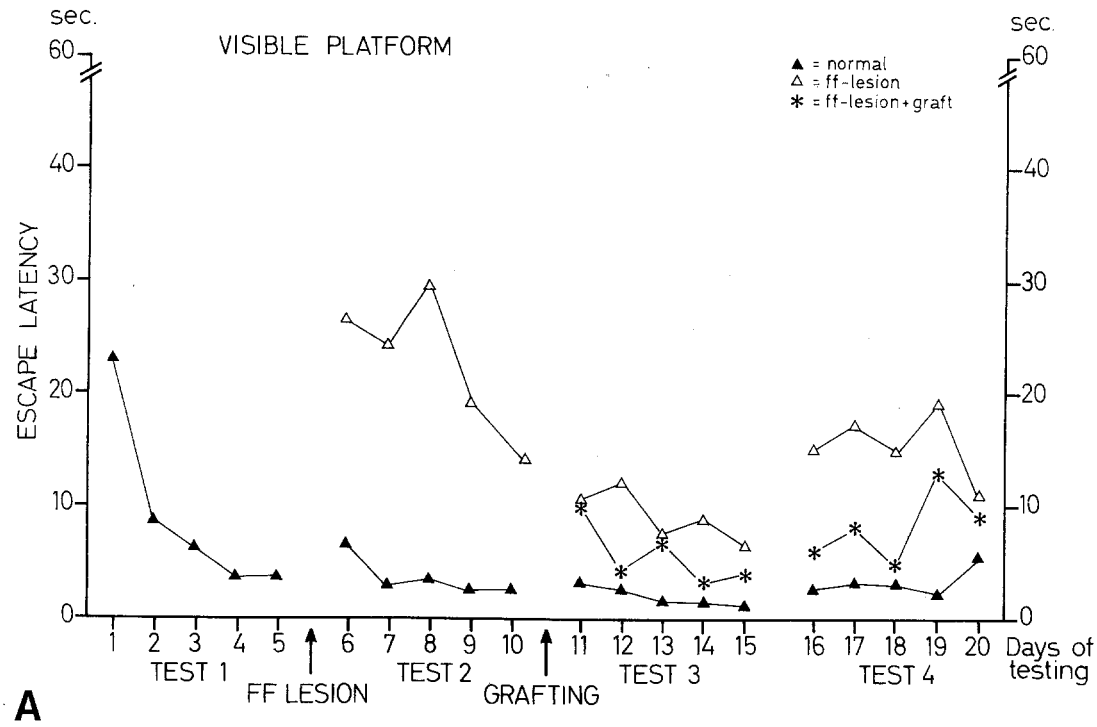
Place test. Before surgery (Test 1) all rats acquired both the visible (V) and the non-visible (NV) platform tasks (repeated measures ANOVA on two factors: $F(1, 22) = 150.9$, $p < 0.001$), reaching asymptotic performance after 4 days of testing (Fig. 6). These results were similar to those obtained in Exp. 1. The rats acquired the V task significantly faster than the NV task ($F(1,22) = 16.69$, $p < 0.01$).

Normal rats performed well on Test 2, showing good retention of the task over the 3 week interval in both V and NV trials. The performances in the V and NV trials were no longer different. Rats with FF lesions performed poorly on both V and NV tasks on Test 2 compared with their pre-lesion performance on Test 1 (paired t-tests on V and NV: days 4 and 5 in Test 1 vs day 1 in Test 2, $p < 0.01$). They performed poorly throughout Test 2, with much longer escape latencies on both V and NV tasks compared to normal rats ($F(1, 16) = 53.14$, $p < 0.001$), although the deficit was more severe on NV than on the V tasks ($F(1, 11) = 65.37$, $p < 0.001$). However, the performance of the FF lesioned rats improved during Test 2 ($F(1, 11) = 11.49$, $p < 0.01$). Thus, in the NV trials, 6 of the 12 lesioned rats had reduced their escape latency to less than 20 s by the end of Test 2.

In Test 3, all groups improved performance on both V and NV tasks ($F(4, 56) = 3.78$; $F(4, 56) = 4.31$, respectively, $p < 0.01$) and rats in all groups escaped more quickly on V than NV trials ($p < 0.05$). Now, however, groups differed only on NV trials ($F(2, 14) = 7.53$, $p < 0.01$) because of the superior performance of normal rats (Fig. 6B).

In Tests 4 and 5, the groups no longer differed significantly, on either the V or the NV trials. As seen from the scatter-plots in Fig. 8A, 4 of the 5 FF lesioned rats and 3 of the 4 grafted rats had reduced their escape latencies to within the normal range in Test 5 (2–8 s), which demonstrates that both the grafted and the non-grafted rats with FF lesions had developed an efficient search behaviour by the end of the experiment.

Spatial probe test. Normal rats performed similarly to those in Exp. 1. Between 50 and 75% of all annulus crossings were located over the former platform site



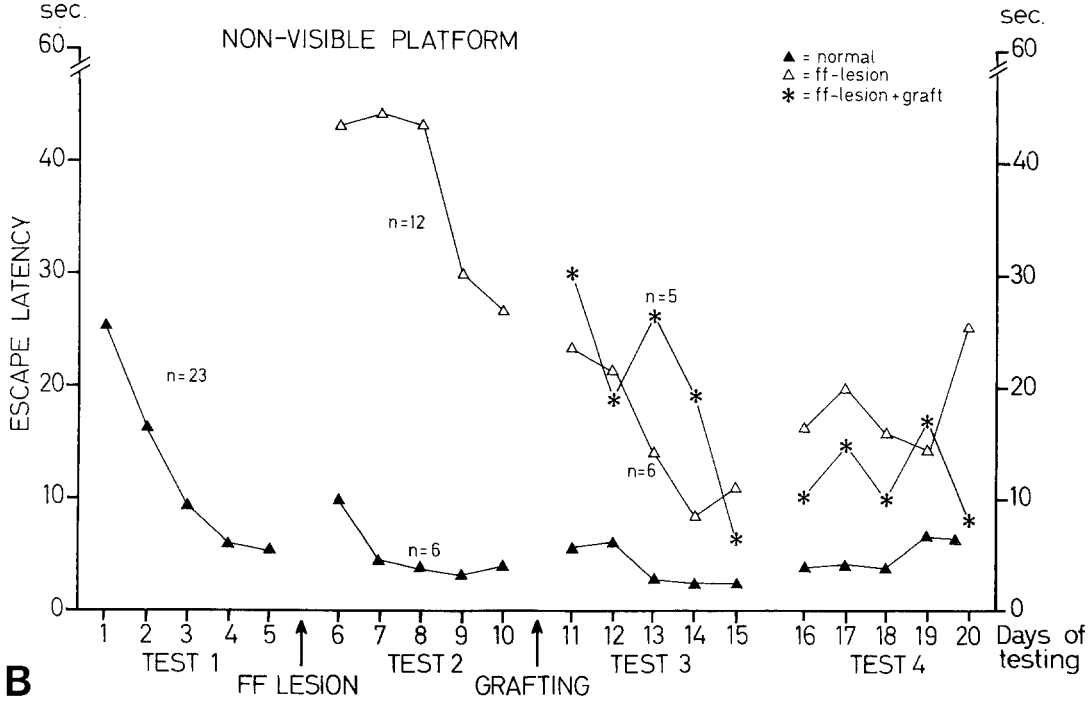


Fig. 6A, B. *Experiment 3.* Mean escape latencies in seconds (s) for each group in the successive tests using visible (cue navigation) (**A**), and non-visible (place navigation) (**B**) platforms on alternating trials. See Fig. 1 for details on surgery and intervals between tests

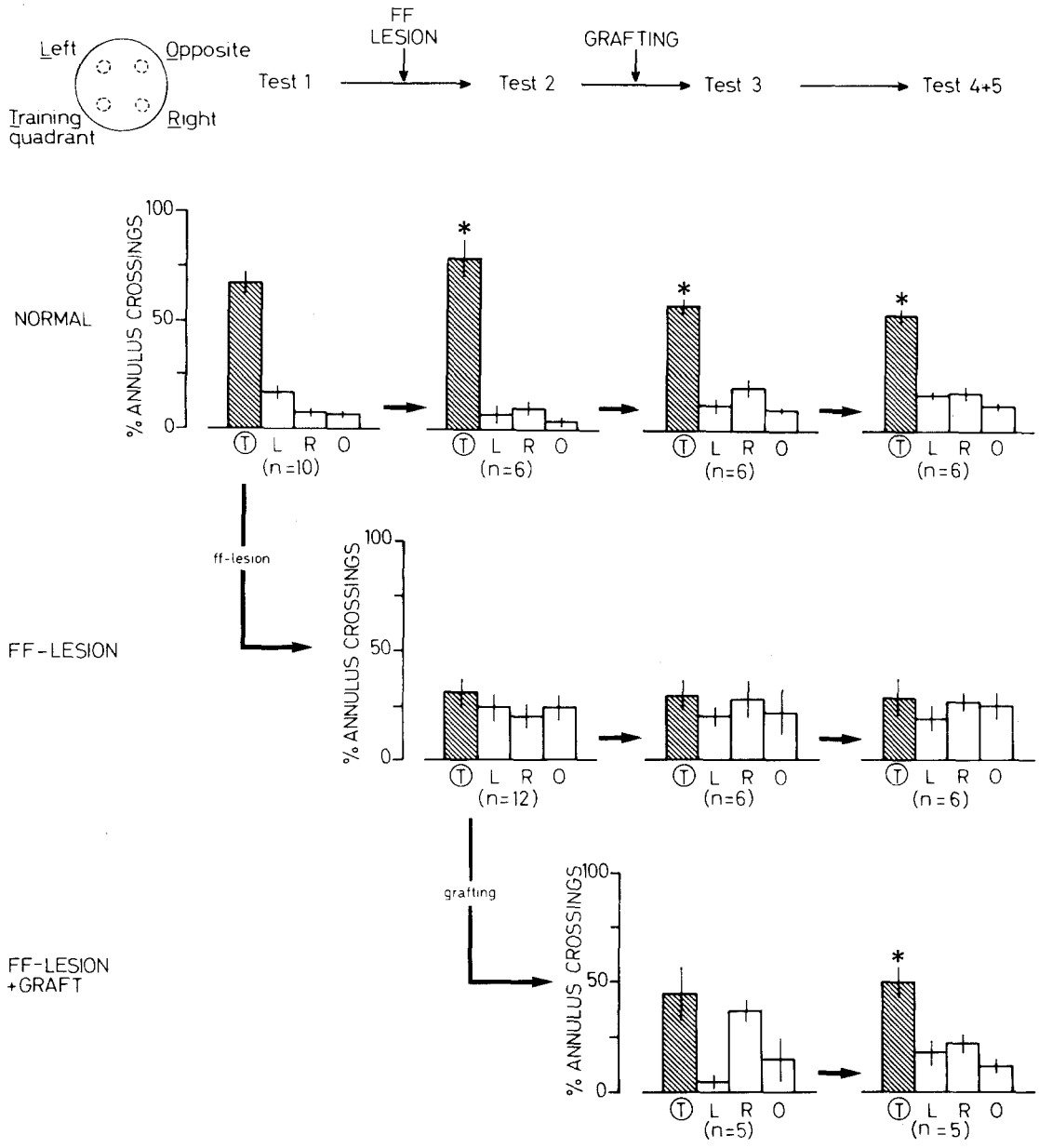
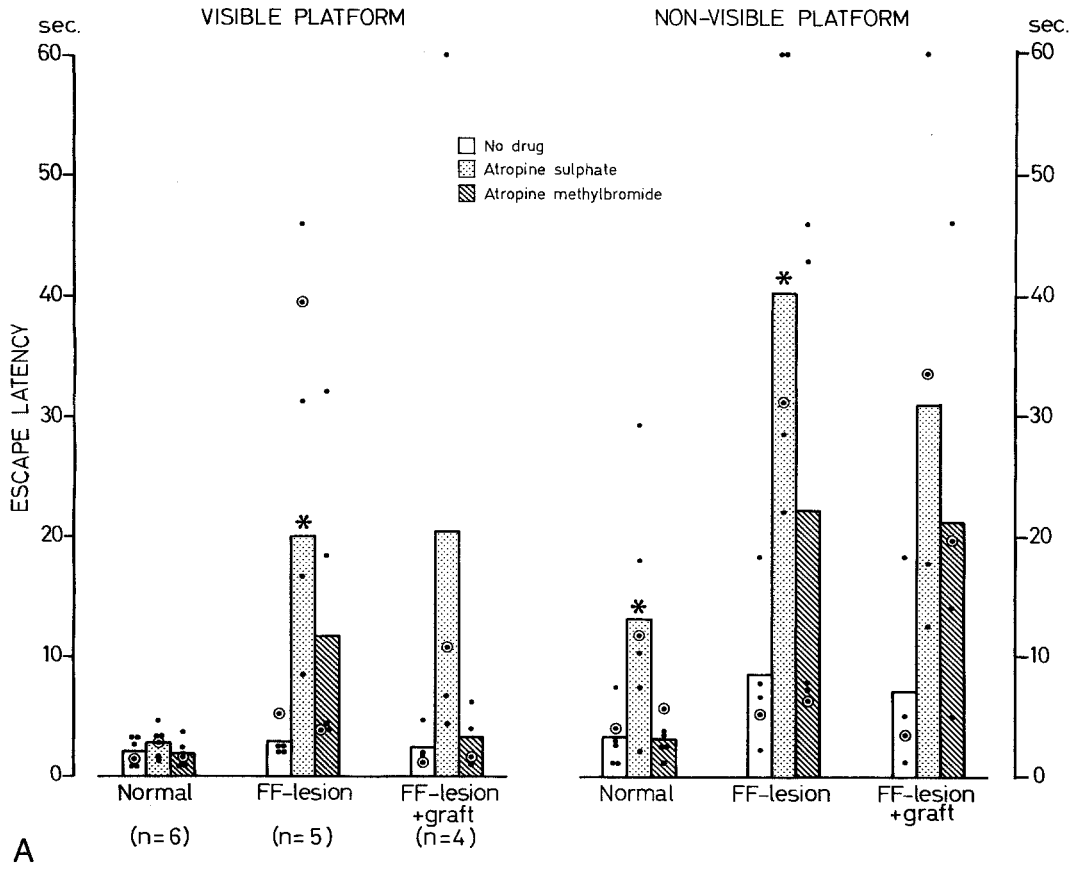


Fig. 7. *Experiment 3.* Analysis of the spatial probe trial in the different tests, where Test 4 and the first test day in the drug test (No drug day, Test 5) have been analyzed together (note spatial probe trial was performed for 1 min in this experiment). Bars give the relative distribution (means ± s.e.m.) of annulus crossings over the four quadrants in the spatial probe trials in the different tests, as indicated in the inset at the top of the figure. Asterisks denote differences from the FF-lesioned group $p < 0.05$. Note that in Test 1, data was obtained from only 10 of the 23 tested rats

(upper panel in Figs. 7 and 9). The FF lesion totally abolished the previously acquired place navigational ability (Test 2), and the lesioned rats remained impaired on the spatial probe measures, despite extended training, also in the subsequent tests. Thus, they distributed their swim equally over all four quadrants of the pool throughout tests 2–5 (middle panel in Fig. 7). An efficient non-spatial search behaviour seemed to develop gradually in the FF lesioned rats, as illustrated in Fig. 9 (middle panel): The majority of the rats displayed the thigmotaxic swimming pattern by the end of Test 2, whereas by the end of Test 3 all but one of the rats displayed the non-spatial more random search behaviour. The efficiency of this search behaviour is illustrated in Fig. 8B by the number of total annulus crossings (i.e., crossings over all four annuli) in the FF-lesioned group in Test 5.



For legend see page 207

Rats with graft crossed over the platform site more often than rats with lesions alone in Tests 4 and 5, and they restricted their swimming more narrowly to the training quadrant (Fig. 7, lower panel; Fig. 9, lower middle panel). In fact, the recovered grafted rats focussed their swim to the platform quadrant to the same extent as the normal controls. This spatial bias seemed to develop gradually, appearing in 2 of the 5 rats in Test 3, and in 4 of the 5 rats in Tests 4 and 5. The rats with grafts never swam in the random pattern that was typical of rats with lesions alone. The apparent blockade of a random swimming pattern by the grafts is further shown in the behaviour of the only grafted rat that never developed a spatial search strategy: it rarely crossed any annulus in Tests 4 and 5.

These observations were verified statistically. Normal rats swam more often over the annulus containing the former platform site than over other sites in Test 1 (Fig. 7: $F(2, 7) = 49.96$, $p < 0.01$). This performance persisted throughout the experiment. Rats with FF lesions did not display this spatial bias, as reflected in an interaction between group and annulus position in Test 2 ($F(3, 48) = 12.23$, $p < 0.01$) and in Tests 4 and 5 ($F(6, 42) = 3.15$, $p < 0.025$). The rats with FF lesions also swam over the platform site fewer times than normal rats (Test 4 and 5: $F(2, 14) = 3.78$, $p < 0.05$); Tukey's HSD, $p < 0.05$). Rats with septal grafts made relatively more crossings over the former platform site than did those with FF lesions only (Test 4 and 5: $F(2, 14) = 5.9$, $p < 0.025$; Tukey's HSD, $p < 0.05$) (Fig. 7). Whereas rats with FF lesions made significantly fewer crossings over the platform site than normal rats in Tests 4 and 5 (see above), rats with grafts did not differ from either group.

Atropine test

Atropine sulphate and atropine methylbromide were given to all rats on the days immediately following the no-drug day in Test 5 (Fig. 8). Because many rats did not find the platform within the maximum 60 s; and because of the large variance in performance, a non-parametric test (Wilcoxon Signed Rank Test) was used in all of the following measures except for swim speed.

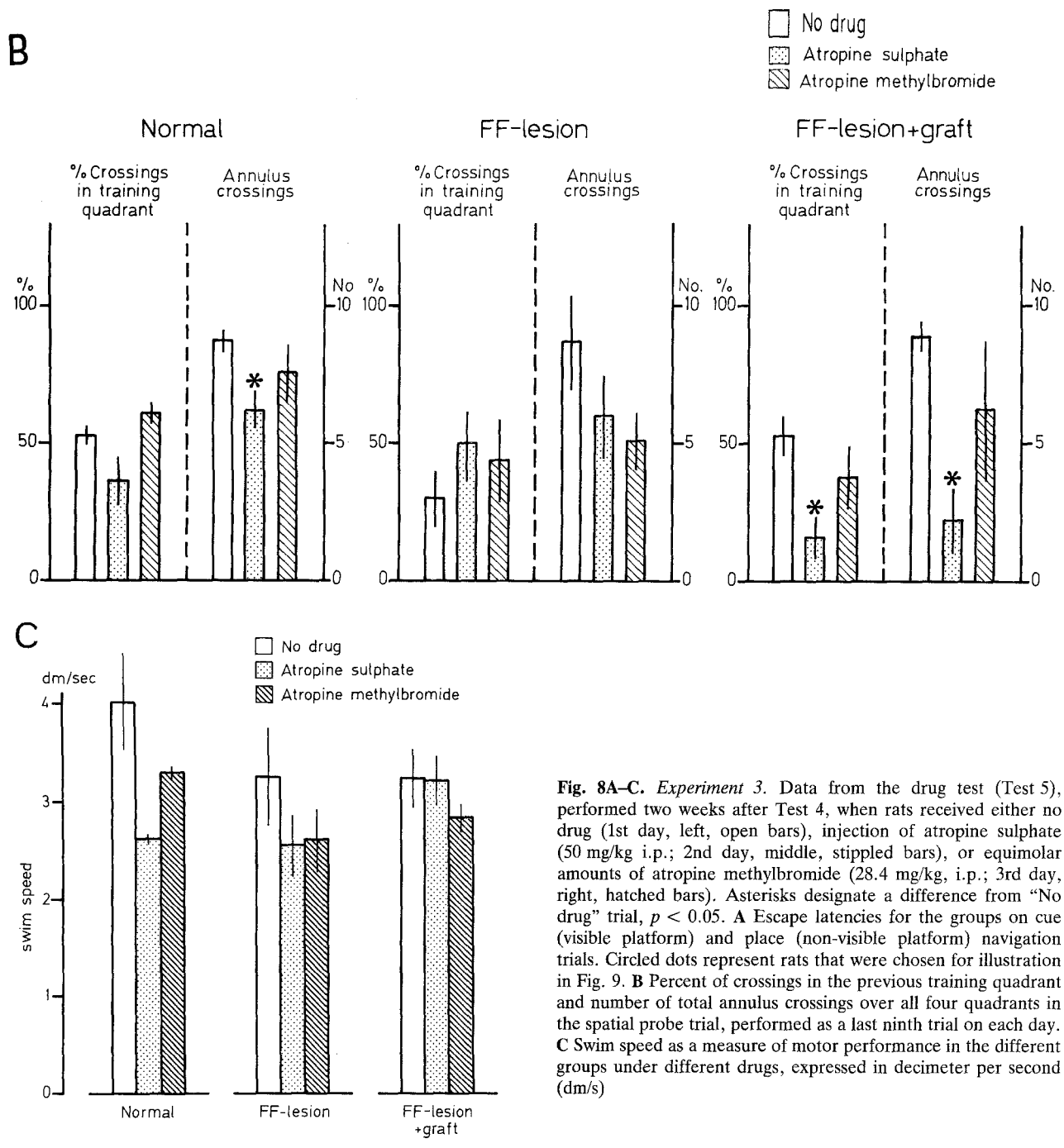


Fig. 8A–C. *Experiment 3.* Data from the drug test (Test 5), performed two weeks after Test 4, when rats received either no drug (1st day, left, open bars), injection of atropine sulphate (50 mg/kg i.p.; 2nd day, middle, stippled bars), or equimolar amounts of atropine methylbromide (28.4 mg/kg, i.p.; 3rd day, right, hatched bars). Asterisks designate a difference from "No drug" trial, $p < 0.05$. **A** Escape latencies for the groups on cue (visible platform) and place (non-visible platform) navigation trials. Circled dots represent rats that were chosen for illustration in Fig. 9. **B** Percent of crossings in the previous training quadrant and number of total annulus crossings over all four quadrants in the spatial probe trial, performed as a last ninth trial on each day. **C** Swim speed as a measure of motor performance in the different groups under different drugs, expressed in decimeter per second (dm/s)

Place test: escape latency (Fig. 8A). Normal rats exhibited increased escape latencies under atropine on NV trials only ($p < 0.05$), whereas rats with FF lesions showed increased escape latencies under atropine on both V and NV trials ($p < 0.05$). Atropine impaired the performance of the grafted rats also, but this effect did not reach significance. None of these effects were seen after peripheral cholinergic blockade induced by atropine methylbromide.

Spatial probe test (Fig. 8B). Normal rats were not significantly impaired by atropine in the spatial probe test as measured by the percent of crossings located over the platform annulus relative to the other three annuli. However, atropine did reduce the total number of annulus crossings (i.e., crossings over all four annuli) in normal rats ($p < 0.05$). Rats with FF lesions alone were unaffected by atropine on either measure. By contrast, rats with grafts were severely impaired in both the percent of crossings over the

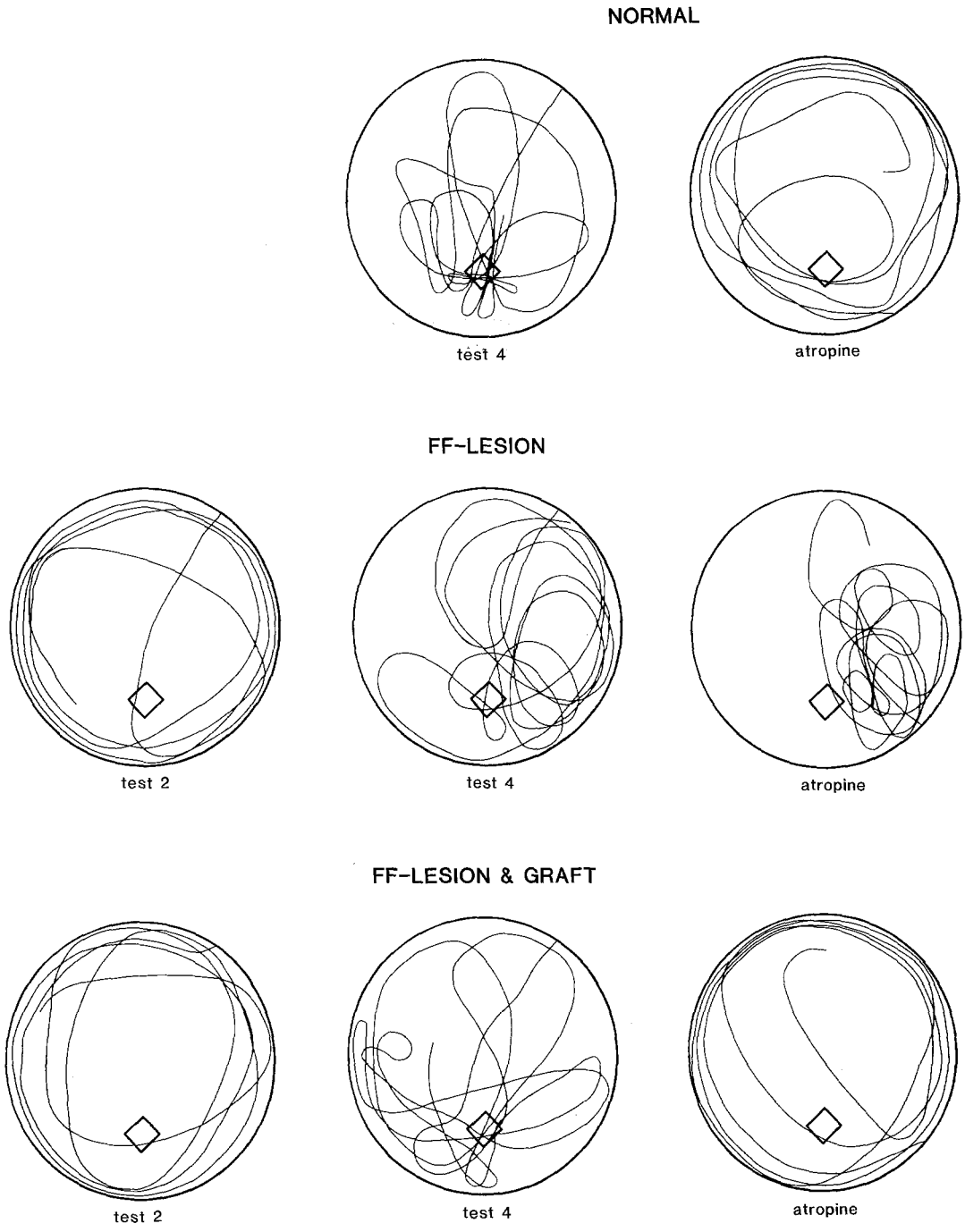


Fig. 9. *Experiment 3.* Actual swim paths taken by one normal rat (top panel), one FF-lesioned rat (middle), and one rat with both FF-lesion and septal graft (bottom) in the spatial probe trials in Test 2, Test 4, and in the atropine test. Rats chosen for illustration are depicted in Fig. 8A with circles around their dots

platform annulus and the total number of annulus crossings ($p < 0.05$). None of these effects was seen after atropine methylbromide.

Inspection of the actual swim paths (Fig. 9, right hand panels) showed that normal rats tended to swim in wider circles under the influence of atropine, thus making less focussed searches for the platform. This effect was even more pronounced in the grafted rats. In contrast, rats with FF lesions swam in a simular random fashion before, during, and after atropine treatment. The increased escape latencies seen under atropine in the FF lesioned rats could be attributed to a less efficient search over all four quadrants of the pool.

Swim speed (Fig. 8C). Swim speeds were monitored as a general measure of the rats' motor performance. These were unaffected by either FF lesions or grafts. Atropine and atropine methylbromide administration tended to reduce swim speeds, but not significantly (repeated measures ANOVA; groups: $F(2, 12) = 1.49$, $p > 0.26$; drugs: $F(2, 11) = 3.31$, $p = 0.075$; group × drug interaction: $F(4, 22) = 1.42$, $p > 0.26$).

Comments

The results show that: (1) FF-lesions produce deficits in place navigation even when rats were given prior training; (2) rats with FF lesions develop with extended training an efficient non-spatial search strategy which allows them to reduce their escape latencies to within the normal range; (3) septal grafts can restore the ability of the FF lesioned rats to use spatial cues to locate the platform site; and (4) this restoration is dependent upon the integrity of cholinergic function.

Although the retention of the already learned water-maze task was severely impaired by the FF lesion, the lesioned rats showed an apparent, gradual improvement after repeated training. Thus, by the end of the experiment (Test 5) (i.e., the fourth test week after the lesion, about 6 months after surgery) all but one of the FF-lesioned rats had escape latencies within the normal range (i.e., less than 8 s) in the NV task. However, as in Exp. 1, analysis of the swim paths in the spatial probe trials showed that none of the FF-lesioned rats used a place navigation strategy to locate the platform site in the pool, not even after extensive training. Instead, they distributed their search over all four quadrants without any place preference. Thus, consistent with the observations in Exp. 1, the present results show that rats with bilateral FF lesions are unable to retain or acquire any spatial memory in the water-maze task.

Previous studies (Morris et al. 1982; Schenk and Morris 1985) have shown that cue-navigation in the water-maze task, using a visible platform, is unimpaired by hippocampal lesions. However, in the present study, the FF-lesioned rats were impaired on both visible and non-visible platform trials. This may be due to the procedure using alternating trials with visible and non-visible platforms. The rats were trained pre-operatively on both types of trials. This probably favored a spatial strategy for locating the platform even when the platform was visible (cf. Olton 1979), and the relevance of the cue may to some extent have been overshadowed by the more consistent spatial location of the platform (cf. Sutherland and Mackintosh 1971; Rescorla and Wagner 1972). For the same reason, the alternation may have prevented the rats with FF lesions from learning to use the visible platform as a cue post-operatively. Thus, if the FF lesioned rats used the same random, non-spatial search strategy to locate the platform on both types of trials, they might be impaired initially in the cued trials as well. However, this account does not explain why the rats persisted in ignoring the visible platform after repeated trials, and future experiments may be needed to determine whether the particular trial sequence used here is unusually disruptive to cued navigation.

The septal suspension grafts reinstated a significant place navigation ability and a spatial memory in the pretrained rats with FF lesions. This appeared as a reacquisition of the ability to locate the platform site in the spatial probe trials, and developed gradually over repeated testing. Thus, by 2.5 months after grafting, only half of the grafted rats were clearly locating the platform site, whereas at 5 months all but one of the grafted rats were doing so. Interestingly, in the 2.5 month test (Test 3), those rats that did not display any significant place navigation had not developed the non-spatial type of search strategy evident in the FF-lesioned rats at that time. This suggests that the grafts had prevented the development of the alternative search strategy in the lesioned rats, which may be interpreted as an early sign of a graft-induced effect in rats which later developed recovery of place navigation. It is unclear, however, whether the graft-induced recovery in spatial memory in the pretrained FF lesioned rats was due to a gradual restoration of previously acquired memory (i.e., a recall of information acquired before the lesion surgery) or to a recovered ability to learn new information.

Microscopic analysis

Experiments 1 and 2

Twelve of the rats with solid grafts and 8 of the rats with suspension grafts survived throughout testing and were processed together with the lesioned controls for AChE histochemistry. In all grafted and non-grafted animals, except one solid grafted animal, the lesion had completely transected the fimbria-fornix and the supracallosal pathways on both sides, resulting in a virtually complete denervation of the dorsal two-thirds of the hippocampal formation, the posterior cingulate cortex, the medial parietal cortex and the retrosplenial cortex. In the incompletely lesioned rat a small portion of the lateral fimbria

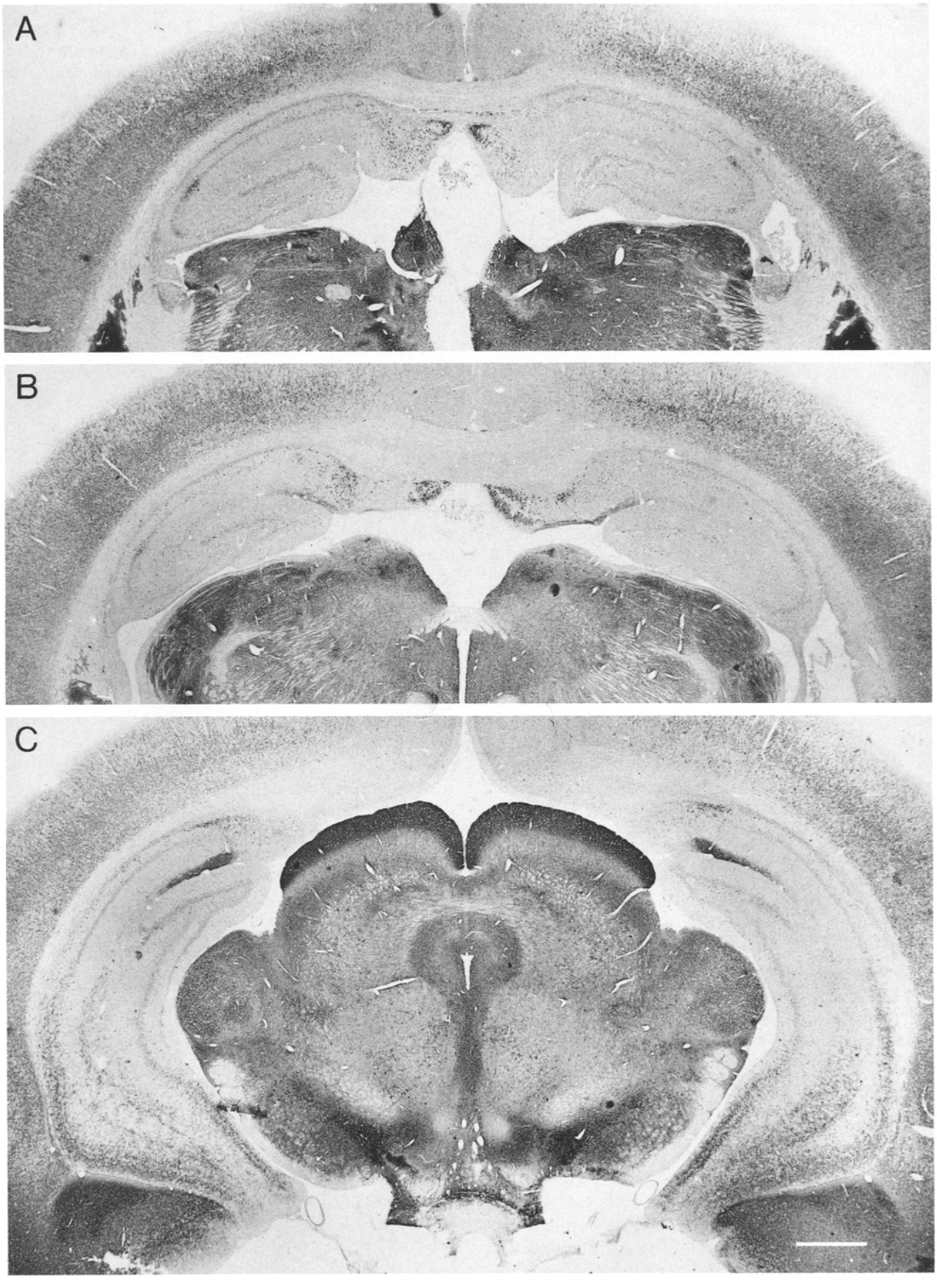


Fig. 10

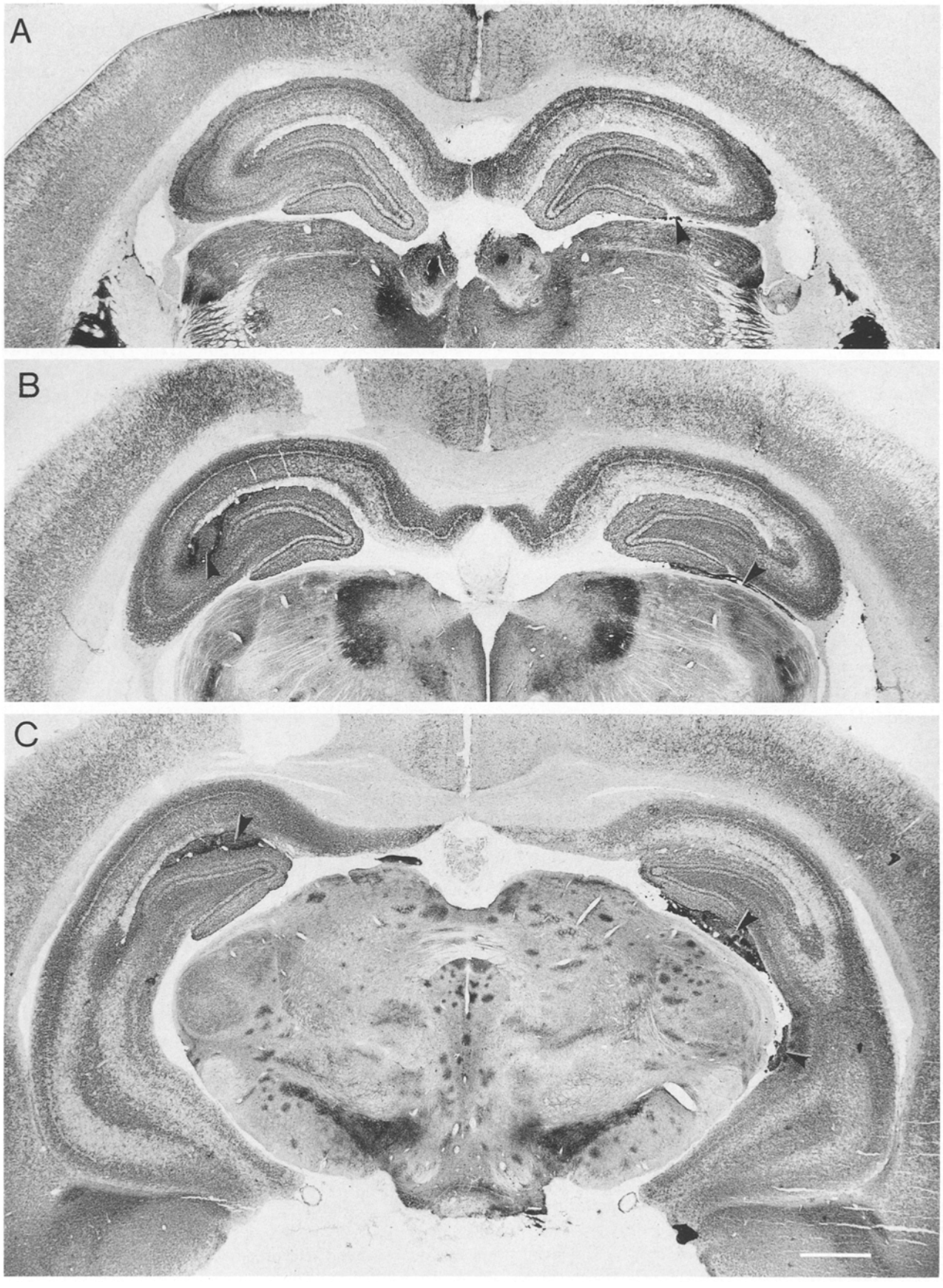


Fig. 11

(innervating the ventral hippocampus) was spared on one side.

All animals had surviving grafts. In the suspension graft group all rats had surviving grafts on both sides, and AChE-positive fibers extended throughout the host hippocampus to provide a partial to complete bilateral reinnervation of all hippocampal subfields, including the subiculum. In most rats, the cingulate, medial parietal and retrosplenial cortices remained denervated. In the rats with solid grafts, all grafts had fused with the septum rostrally and with one or both hippocampi caudally. Five of the rats had AChE-positive fibers throughout the dorsal 3–5 mm of the hippocampus bilaterally, whereas 7 animals had primarily or exclusively unilateral AChE-positive graft-derived reinnervation. Three rats with solid grafts had extensive unintended pathology, including extensive bilateral hydrocephalus and in one case also substantial damage to the septum and the anterodorsal thalamus. These three rats were among the six solid graft rats which showed no clear recovery of place navigation in Exp. 1. The animals in the suspension graft group had consistently more extensive, bilateral graft-induced reinnervation, particularly of the caudal and ventral aspects of the hippocampal formation than the rats with solid grafts.

In both the suspension and solid graft groups, all behaviourally recovered rats had extensive hippocampal AChE-positive reinnervation on at least one side. However, there was no clear-cut difference in graft size or extent and patterning of the AChE-positive fiber outgrowth between the non-recovered rats (without hydrocephalus) and the behaviourally recovered animals. Thus, in the solid graft group, rats with only unilateral AChE-positive reinnervation of the hippocampal formation occurred in both subgroups, and one of the non-recovered rats had extensive bilateral AChE-positive reinnervation of all hippocampal subfields, including the dorsal part of the subiculum.

Experiment 3

AChE histochemistry showed surviving grafts on both sides in all rats in the grafted group that survived to the end of the experiment. All of these rats had extensive AChE-positive fiber ingrowth into the host hippocampal formation as shown in Fig. 11. This occurred on both sides of the brain in all rats except one, where the fiber ingrowth was less on one side due to a small graft. In all FF-lesioned rats the dorsal two thirds of the hippocampal formation, as well as the posterior cingulate, medial parietal and retrosplenial cortices, had a near complete loss of AChE-positive innervation on both sides, as shown in Fig. 10. Inspection of the lesion sites showed complete bilateral transection of the fimbriae, dorsal fornices, and supracallosal striae in all rats in the lesioned and grafted groups, and the size and extent of the lesions were comparable in the two groups. No obvious difference in graft size or extent of AChE-fiber outgrowth was seen between the four behaviourally recovered grafted rats and the single rat that did not show any clear recovery of place navigation in the spatial probe test.

Discussion

The results demonstrate that rats with bilateral FF lesions are unable to acquire place navigation and spatial memory in the water-maze task, whether or not they are pretrained on the task prior to the lesion surgery. The lesioned rats showed an apparent, gradual recovery which was due to the development of an alternative non-spatial search strategy. This suggests that the ability of the rats to acquire *spatial* learning and memory for place navigation in the water-maze is normally dependent on the integrity of the septo-hippocampal connections. In Exp. 1 and 3 this spatial learning and memory deficit was significantly ameliorated by either solid septal grafts, placed within the FF lesion cavity, or by septal suspension grafts implanted into the denervated host hippocampus. In Exp. 2, a significant graft-induced recovery was seen also in a spatial working memory version of the water-maze test. Taken together, these observations suggest that the ability of the septal grafts to improve performance of FF lesioned rats in the water-maze is specifically due to an effect on the ability of the lesioned rats to use extra maze cues in the localization of the platform site.

Figs. 10, 11. Photomicrographs of AChE-stained frontal sections through the hippocampus at three levels (**A–C**)

Fig. 10A–C. Rat with FF lesion only, showing the characteristic lack of AChE-positive fibers throughout the dorsal hippocampus. Note the sparing and some sprouting of AChE-positive fibers in the ventral hippocampus (**C**) coming through a ventral route not affected by the FF lesion

Fig. 11A–C. Rat with both FF lesion and septal suspension grafts. Note the near complete and homotypic reinnervation of the hippocampus by AChE-positive fibers. White arrowheads indicate location of graft tissue. Scale bar = 1 mm

As in normal rats during early stages of training (Sutherland et al. 1982b; Whishaw 1985; Whishaw et al. 1985), the recovered water-maze performance in the grafted rats was sensitive to central cholinergic muscarinic receptor blockade. In Exp. 3, atropine impaired place navigation in the grafted rats and abolished their ability to locate the platform site in the spatial probe trial. This effect was more pronounced in the grafted rats than in the normal controls. In normal rats, Whishaw (1985) has reported that place navigation is sensitive to atropine only during early stages of training, and that the well-trained response is relatively insensitive to cholinergic blockade. Whishaw interpreted this as a transition from atropine-sensitive to atropine-insensitive place learning. The higher sensitivity of the grafted rats to atropine in the present study may signify, therefore, that they had not yet fully acquired the task, and thus that they remained in the atropine-sensitive stage of learning, despite the fact that they had reached asymptotic performance. If this interpretation is correct, the pronounced atropine sensitivity of the grafted rats could mean that they remained impaired with respect to the ability to acquire the well-trained, atropine-resistant type of place learning.

Another interpretation of the difference in atropine sensitivity between the normal controls and the grafted rats is that the grafted cholinergic system might be more vulnerable to muscarinic receptor blockade. This would be the case, for example, if cholinergic transmission in the graft-to-host connections is less efficient, or marginally sufficient to maintain synaptic function. Such a mechanism may be valid also for the interpretation of the effect of atropine on escape latency in the extensively trained FF-lesioned control rats, since extensive damage to the cholinergic projection system could make the remaining parts of the system (e.g. the basalocortical projections) more sensitive to pharmacological interference.

These observations are consistent with the idea that the graft-induced recovery of place navigation in the FF-lesioned animals is dependent on a cholinergic mechanism. Other observations in rats with intrahippocampal septal grafts lend support to this interpretation. (*i*) Immunocytochemical studies, using antibodies to choline acetyltransferase, have shown that the grafted septal neurons re-establish abundant cholinergic synapses onto neurons in the host hippocampus, including dentate granule and pyramidal neurons (Clarke et al. 1986; Anderson et al. 1986). (*ii*) In electrophysiological studies using intracellular recordings in slice preparations which contained the grafts, Segal et al. (1985) have demonstrated normal synaptic responses of host CA1 pyramidal cells to septal graft stimulation. These effects were antagonized by atropine and potentiated by physostigmine, and were thus most probably mediated by cholinergic synapses. (*iii*) In FF lesioned rats with solid septal grafts, Buzsaki et al. (1987) reported the recovery of behaviour-dependent rhythmic slow activity (or theta rhythm) in the parts of the hippocampus that were reinnervated by AChE-positive fibers, but not outside this area. (*iv*) In a neurochemical study, Kelly et al. (1985) reported that solid septal grafts were capable of normalizing the regional glucose utilization rate in the FF denervated hippocampal formation, and that this effect was highly correlated with the degree of reinnervation by AChE-positive fibers from the graft. (*v*) In a study by Dunnett et al. (1982), graft-induced recovery of a forced-choice alternation task in a T-maze was seen with septal solid or suspension grafts, providing a cholinergic reinnervation of the host hippocampal formation, but not with noradrenaline-rich grafts of the locus coeruleus region lacking cholinergic neurons.

These various observations provide substantial support for the idea that grafts of fetal septal-diagonal band neurons can provide a new cholinergic afferent control system to the previously denervated host hippocampal formation, and that this new input is functional not only at the synaptic level, but also in the recovery of hippocampus-dependent spatial learning and memory in the otherwise permanently impaired lesioned rat. Since the septo-hippocampal cholinergic pathway normally seems to act as a modulatory system, which is capable of regulating the excitability of dentate granule cells and hippocampal pyramidal neurons (Krnjević and Ropert 1982; Nicoll 1985), the behavioural effects of the grafted cholinergic neurons could be mediated via the restoration of relatively non-specific, tonic cholinergic neurotransmission at the cholinergic receptor sites.

The observations of Dunnett et al. (1982) on the recovery of forced-choice alternation in rats with FF lesions, and of Gage et al. (1984) on the recovery of place navigation in the water-maze in aged rats, indicate however, that cholinergic reinnervation by the septal grafts may be necessary but not sufficient for behavioural recovery to occur. This is consistent with the findings of the present study, showing that recovery of place navigation was quite variable also in animals with good bilateral, graft-derived, AChE-positive reinnervation of the host hippocampus. Thus, in Exp. 1, 2 of the 10 rats with suspension grafts and 6 of the 15 rats with solid grafts showed no clear place preference in the spatial probe test. In 3 of the rats with solid grafts this failure could be

accounted for by hydrocephalus and unintended septal and thalamic damage associated with the aspiration cavities. In other cases the cause of the variable results is not clear. The grafts would likely require a minimum of integration into the host hippocampal circuitry, including afferent connections from the host brain, for adequate functioning. This factor, which was not controlled for in this study, may vary from animal to animal. Moreover, the aspirative FF lesion used here is extensive, affecting not only the cholinergic afferents to the hippocampus, but also other major afferent and efferent systems of the hippocampal formation, as well as parts of the corpus callosum and the cingulate cortex.

It is obvious that the septal grafts can substitute only for some aspects of the structural damage induced by FF lesions, and that the normal afferent and efferent connections of the hippocampal formation running through the fimbria-fornix and supracallosal pathways were not restored by the grafts. This may explain why the performance in the water-maze task remained impaired also in the best grafted rats. Nevertheless, the performance of the most successful grafted animals in the spatial probe test was within the normal range, which indicates that at least one aspect of hippocampal function, i.e., place navigation in the water-maze, can be substantially improved also in the absence of these connections. Apparently, the hippocampal mechanisms underlying spatial memory can function at least to some degree also in the absence of major connections between the hippocampal system and subcortical structures, perhaps through a direct modulation of the host hippocampal circuitry by the grafted neurons.

In summary, the results of the present study demonstrated the usefulness of the intracerebral transplantation technique as a tool for the analysis of mechanisms underlying behavioural recovery after brain damage and for the study of the role of the septo-hippocampal cholinergic system in hippocampus-dependent learning and memory. FF lesions are extensive, and disconnect many areas in addition to the medial septum and hippocampus, yet septal grafts can restore some aspects of normal memory function. This finding suggests a model of hippocampal function in which the basal forebrain cholinergic system operates in a permissive fashion to modulate information processing or storage in the hippocampus and its associated cortical structures.

The experimental animal work suggests that the intracerebral grafting technique may have some potential in the therapy of cognitive deficits in patients with neurodegenerative diseases. However, a necessary prerequisite for any future efforts in this direction is the detailed analysis of the mechanisms and structural requirements for grafted neural tissue to influence different aspects of impaired cognitive function. Further studies should therefore address questions of the specific actions and the limitations of graft functions: What aspects of memory do the grafts not affect, and can different types of grafts affect different aspects of impaired memory? What is the role of glia and glia-derived trophic mechanisms in the restoration of behaviour after cortical lesions? And why do surviving AChE-rich septal grafts not always work and what are the requirements for grafted septal neurons to become functional in hippocampal learning and memory mechanisms?

Acknowledgements. We thank Gertrude Stridsberg, Carine Jönsson, Birgit Haraldsson, Benedikte Falck, Yvette Jönsson, Alicja Flasch and Siv Carlson for excellent technical assistance, and Ragnar Mårtensson for the photographic work. The studies were supported by grants from the Swedish MRC (04X-3874) and from the National Institutes of Health and Aging (NS 06701 and AG 03766). MLS was supported by a grant from the National Science Foundation.

References

Anderson KJ, Gibbs RB, Salvaterra PM, Cotman CW (1986) Ultrastructural characterization of identified cholinergic neurons transplanted to the hippocampal formation of the rat. J Comp Neurol 249: 279–292

Björklund A, Stenevi U (1977) Reformation of the severed septo-hippocampal cholinergic pathway in the rat by transplanted septal neurons. Cell Tiss Res 185: 289–302

Björklund A, Gage FH, Stenevi U, Dunnett SB (1983a) Intracerebral grafting of neuronal cell suspensions. VI. Survival and growth of intrahippocampal implants of septal cell suspensions. Acta Physiol Scand Suppl 522: 49–58

Björklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH (1983b) Intracerebral grafting of neuronal cell suspensions. I. Introduction and general methods of preparation. Acta Physiol Scand Suppl 522: 1–7

Björklund A, Gage FH, Schmidt RH, Stenevi U, Dunnett SB (1983c) Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. Acta Physiol Scand Suppl 522: 59–66

Buzsaki G, Gage FH, Czopf I, Björklund A (1987) Restoration of rhythmic slow activity (theta) in the subcortically denervated hippocampus by fetal CNS transplants. Brain Res 400: 334–347

Clarke DJ, Gage FH, Björklund A (1986) Formation of synapses by intrahippocampal grafts as revealed by choline acetyltransferase immunocytochemistry. Brain Res 369: 151–162

Daniloff JK, Bodony RP, Low WC, Wells J (1985) Cross-species embryonic septal transplants: restoration of conditioned learning behaviour. Brain Res 346: 176–180

Dunnett SB, Low WC, Iversen SD, Stenevi U, Björklund A (1982) Septal transplants restore maze learning in rats with fornix-fimbria lesions. Brain Res 251: 335–348

Gage FH, Björklund A, Stenevi U, Dunnett SB, Kelly PAT (1984) Intrahippocampal septal grafts ameliorate learning impairments in aged rats. Science 225: 533–535

Gage FH, Buzsaki G, Nilsson OG, Björklund A (1987) Grafts of fetal cholinergic neurons to the deafferented hippocampus. Prog Brain Res (in press)

Geneser-Jensen FA, Blackstad TW (1971) Distribution of acetylcholinesterase in the hippocampal region of the guinea pig. Z Zellforsch 114: 460–481

Holmstedt B (1957) A modification of the thiocholine method for the determination of cholinesterase. Acta Physiol Scand 40: 331–357

Honig WK (1978) Studies of working memory in the pigeon. In: Hulse SH, Fowler H, Honig WK (eds) Cognitive processes in animal behaviour. Hillsdale, Erlbaum, pp 211–248

Kelly PAT, Gage FH, Ingvar M, Lindvall O, Stenevi U, Björklund A (1985) Functional reactivation of the deafferented hippocampus by embryonic septal grafts as assessed by measurements of local glucose utilization. Exp Brain Res 58: 570–579

Krnjević K, Ropert N (1982) Electrophysiological and pharmacological characteristics of facilitation of hippocampal population spikes by stimulation of the medial septum. Neuroscience 7: 2165–2183

Low WC, Lewis PR, Bunch ST, Dunnett SB, Thomas SR, Iversen SD, Björklund A, Stenevi U (1982) Functional recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions. Nature 300: 260–262

Morris RGM (1981) Spatial localization does not require the presence of local cues. Learn Motiv 12: 239–260

Morris RGM (1983) An attempt to dissociate "spatial mapping" and "working memory" theories of hippocampal function. In: Seifert W (ed) The neurobiology of the hippocampus. Academic Press, London, pp 405–432

Morris RGM (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Meth 11: 47–60

Morris RGM, Garrud P, Rawlins JNP, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297: 681–683

Nicoll RA (1985) The septo-hippocampal projection: a model cholinergic pathway. Trends Neurosci 8: 533–536

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Clarendon Press, Oxford

Olton DS (1979) Mazes, maps, and memory. Am Psycholog 34: 583–596

Olton DS (1983) Memory functions of the hippocampus. In: Seifert W (ed) The neurobiology of the hippocampus. Academic Press, London, pp 335–374

Olton DS, Becker JT, Handelman GE (1979) Hippocampus, space and memory. Behav Brain Sci 2: 313–365

Pallage V, Toniolo G, Will B, 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

Rawlins JNP (1985) Associations across time: the hippocampus as a temporary memory store. Behav Brain Sci 8: 479–497

Rescorla RA, Wagner AR (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and non-reinforcement. In: Black AH, Prokasy WF (eds) Classical conditioning II. Appleton-Century-Crofts Inc, New York

Schenk F, Morris RGM (1985) Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. Exp Brain Res 58: 11–28

Segal M, Björklund A, Gage FH (1985) Transplanted septal neurons make viable cholinergic synapses with a host hippocampus. Brain Res 336: 302–307

Segal M, Greenberger V, Milgram NW (1987) A functional analysis of connections between grafted septal nuclei and a host hippocampus. Prog Brain Res (in press)

Stenevi U, Björklund A, Svendgaard N-Aa (1976) Transplantation of central and peripheral mono-amine neurons to the adult rat brain: techniques and conditions for survival. Brain Res 114: 1–20

Sutherland NS, Mackintosh NJ (1971) Mechanisms of animal discrimination learning. Academic Press, New York

Sutherland RJ, Kolb B, Whishaw IQ (1982a) Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neurosci Lett 31: 271–276

Sutherland RJ, Whishaw IQ, Regehr JC (1982b) Cholinergic receptor blockade impairs spatial localization using distal cues. J Comp Physiol Psychol 96: 563–573

Whishaw IQ (1985) Cholinergic receptor blockade impairs locale but not taxon strategies for place navigation in a swimming pool. Behav Neurosci 99: 979–1005

Whishaw IQ, O'Connor WT, Dunnett SB (1985) Disruption of central cholinergic systems in the rat by basal forebrain lesion or atropine: effects on feeding, sensorimotor behaviour, locomotor activity, and spatial navigation. Behav Brain Res 17: 103–115

Received September 9, 1986 / Accepted December 24, 1986