orangeworm (*Amyelois transitella*), gypsy moth (*Lymantria dispar*), granulate cutworm (*Feltia subterranea*), fall cankerworm (*Alsophila pometaria*), and the bagworm (*Thyridopteryx ephemeraeformis*) stimulated pheromone production in ligated corn earworm females. These insects represent five families in the order Lepidoptera (Pyralidae, Lymantriidae, Noctuidae, Geometridae, and Psychidae, respectively), and the chemistry of their pheromones is different from that of *H. zea*.

12. The peptide has been isolated from *H. zea* brain extracts by reversed-phase high-performance liquid chromatography. Details of its isolation and characterization will be reported elsewhere.
13. Supported in part by U.S. Department of Agriculture Cooperative Agreement 58-32U4-1-299. Scientific article A3757. Contribution number 6734 of the Maryland Agricultural Experiment Station.

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Intrahippocampal Septal Grafts Ameliorate Learning Impairments in Aged Rats

Abstract. *Grafts of fetal septal tissue rich in cholinergic neurons were implanted as a dissociated cell suspension into the depth of the hippocampal formation in aged rats with severe impairments in spatial learning abilities. After 2½ to 3 months, the rats with grafts, but not the controls, had improved their performance in a spatial learning test. Their improvement was due, at least in part, to an increased ability to use spatial cues in the task. In all animals the grafts had produced an extensive acetylcholinesterase-positive terminal network in the surrounding host hippocampal formation. Thus, the action of cholinergic neurons in the graft onto elements in the host hippocampal circuitry may be a necessary, but perhaps not sufficient, prerequisite for the observed functional recovery.*

Brain aging is associated with both behavioral impairments and structural and biochemical alterations in discrete neuronal subsystems. Both aged rats and aged humans can exhibit severe deficits in cognitive abilities (*1*, *2*). Regional analyses of neuronal cell losses (*3*) and decrements in glucose utilization (*4*, *5*) have suggested that the age-related decline in cognitive performance may be related to dysfunction or degenerative changes in specific limbic or cortical neuronal circuitries. In particular, recent studies (*2*, *6*) have shown that the degree of cognitive impairments in humans is highly correlated with degeneration or atrophy of the basal forebrain cholinergic projection system, which provides major cholinergic afferent inputs to both the hippocampal formation and the neocortical mantle. Decline in cerebral cholinergic function is seen also with aging in rodents (*2*, *7*), and since the age-related decline in learning and memory is reminiscent of the deficits seen after lesions to the septo-hippocampal system in rats, decrements or degenerative changes in the septo-hippocampal cholinergic projection system may play a role in the development of such deficits.

In young rats with surgical lesions of the septo-hippocampal connections, we have previously shown that grafts of fetal septal tissue, rich in cholinergic neurons, can partly compensate for impairments in spatial learning in several maze tasks (*8*, *9*). We now report that grafts of neuronal cell suspensions, obtained from the septal–diagonal band area of rat fetuses and injected into the depth of the hippocampal formation in aged rats, can improve severe age-dependent deficits in spatial learning in a place navigation test.

Female Sprague-Dawley rats (Anticimex, Stockholm, Sweden) were used. Aged rats were obtained as retired breeders at 9 to 11 months of age. They were housed in groups of four to six rats in a clean, controlled environment for an additional year before the experiment started. Young control rats were bought at 2 months of age and were allowed 3 weeks to adapt in the new environment.

Behavioral testing was conducted in the Morris's water maze task (*10*) 1 week before and 2½ to 3 months after transplantation surgery. The experiment was carried out on two batches of rats. Batch 1 consisted of 53 aged rats (21 to 23 months old) and 10 young controls (3 months old), and batch 2 of an additional

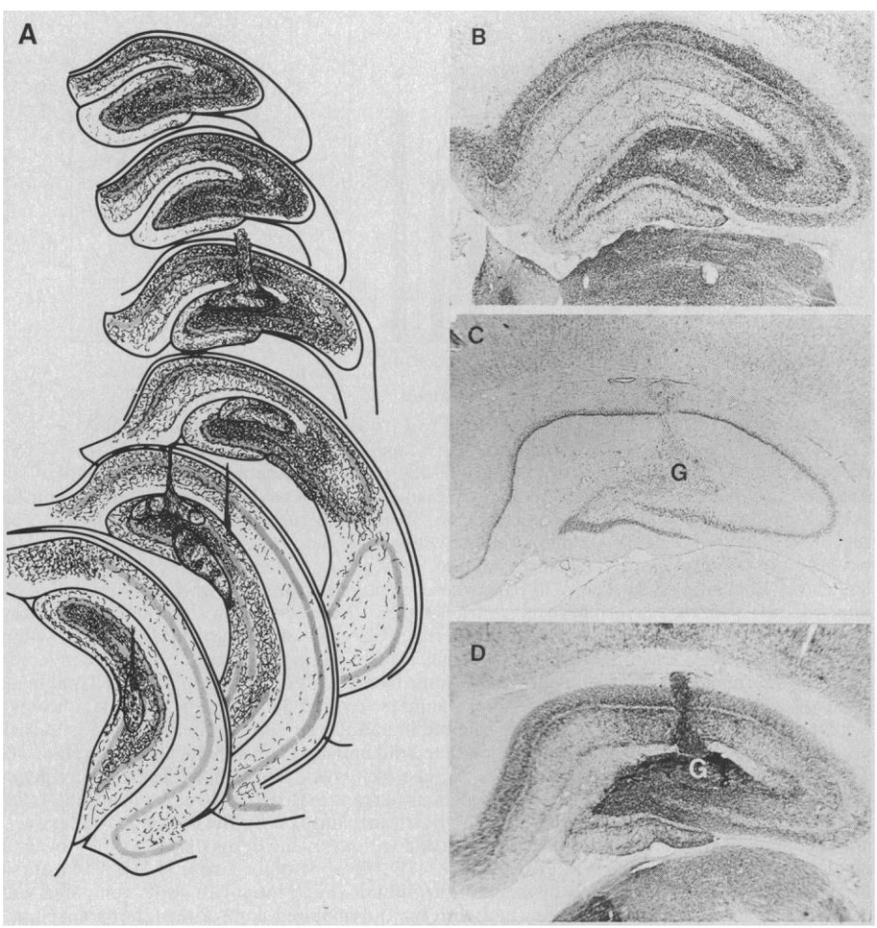

Fig. 1. Graft survival and AChE-positive fiber outgrowth into the host hippocampal formation of an old impaired rat with grafts whose improvement in escape latency represented the median of the whole treated group. (A) Composite schematic drawing of AChE-stained sections of transplant survival and transplant-derived fiber outgrowth throughout the entire host hippocampal formation, represented in six equally spaced coronal planes. (B) Photomicrograph of the AChE-positive, graft-derived fiber plexus in the host dorsal hippocampus rostral to the implantation sites. (C) Photomicrograph of hippocampus stained with cresyl violet at the site of the rostral graft (*G*) placement. (D) Photomicrograph of an AChE-stained section adjacent to the one in (C), showing the rostral graft placement and the extent of AChE-positive fiber outgrowth into the host hippocampus from the graft (*G*).

40 aged rats. Since our previous studies (*5, 11*) showed that only about a quarter to a third of our aged rat population is markedly impaired in spatial learning in the water maze task, the pretransplant test identified those impaired individuals in the aged rat group. Based on the performance of the young control rats in batch 1, we set the criterion for impaired performance in the aged rats such that the mean escape latency (swim time to find the platform) should be above the 99 percent confidence limit of the young control group. The young controls showed a mean swim time over all trials of 17.9 seconds with an upper 99 percent confidence limit of 38.5 seconds. Twelve old rats in batch 1 and 17 old rats in batch 2 showed mean swim times greater than 38.5 seconds and were thus allocated to the "old-impaired" group used for transplantation. The remaining aged rats constituted the "old-nonimpaired" group, 12 of which (from batch one) were included, together with the young control group, during the second test as reference groups.

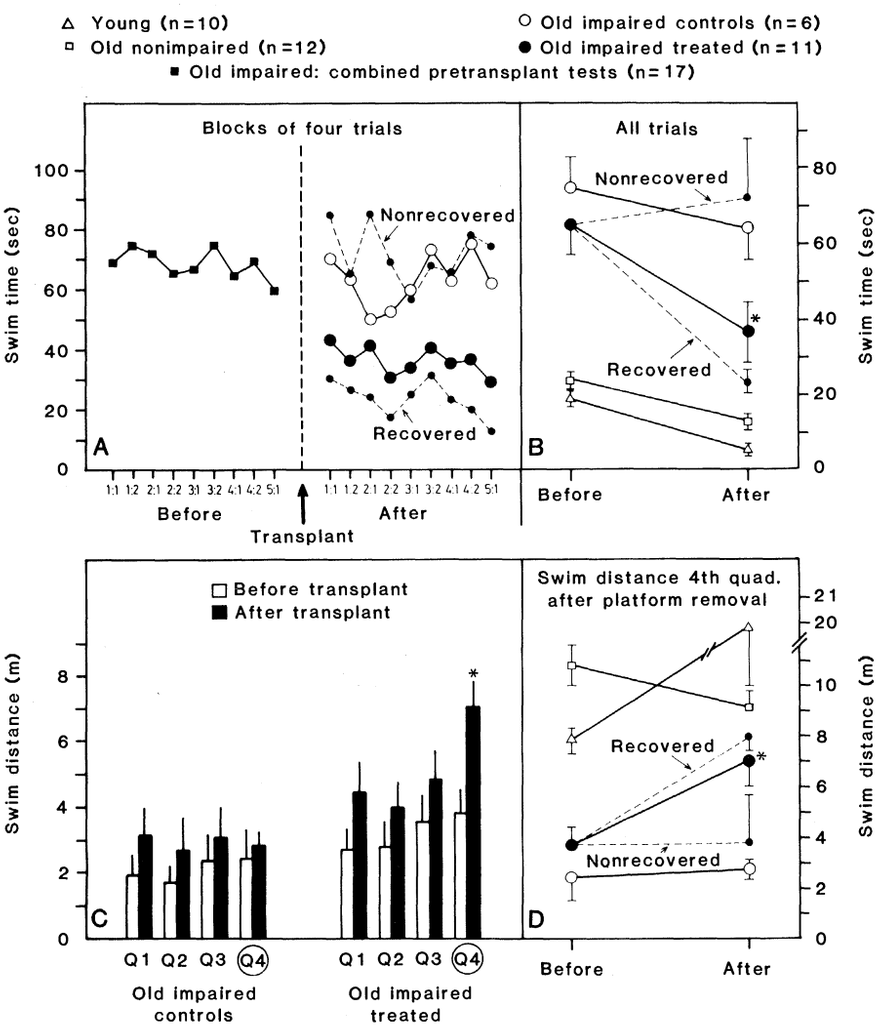

Fig. 2. Morris water maze performance. (A) Escape latency is plotted as nine blocks of four trials, with eight trials presented each day. The left panel represents the performance of all old impaired rats before transplant. The statistical analysis of these data was conducted with the pretransplant performance of the aged-impaired group subdivided into their respective categories of old impaired controls and old impaired with grafts. Since no statistical difference existed between the two groups before transplantation, they were combined graphically for simplicity and clarity of presentation. The right panel represents the performance of the two groups of old impaired rats after the one group received transplants. "Recovered" (in A, B, and D) refers to 8 of the 11 old impaired rats with grafts that on the posttransplant trials had recovered to within the 99 percent confidence interval of the young control group performance. "Nonrecovered" refers to the remaining three that showed no improvement after the transplant. (B) Total mean (± standard error of the mean) escape latency summed over all 36 trials. The asterisk indicates a significant decrease in escape latency by the old impaired rats with grafts compared with their pretransplant performance and compared with the old impaired control rats after transplant. (C) Total distance swum in each quadrant on the last trial when the platform had been removed from the pool. The asterisk indicates a significant increase in distance swum in quadrant 4 (Q4) (where the platform had been located) in the posttransplant test by the old impaired rats with grafts compared with their earlier performance and with the mean of the distances they swam in quadrants 1 to 3 (Q1, Q2, Q3) after transplants. (D) Total distance swum in Q4. The asterisk indicates a significant increase in distance of the old impaired group with grafts compared with its performance before the transplant and with the old impaired control rats after transplant.

Twelve of the 29 rats in the old-impaired group received bilateral suspension grafts prepared from the septal–diagonal band area obtained from 14- to 16-day-old embryos of the same rat strain (*12–14*). Three implant deposits were made stereotaxically into the hippocampal formation on each side (Fig. 1A). The remaining 17 aged rats were left untreated and served as the old-impaired control group.

By the time of the second testing (2½ to 3 months after transplantation), all 12 old impaired rats with grafts remained alive, but only 6 of the 17 old impaired controls survived. One of the treated rats had to be excluded from the test since it swam in a corkscrew-like pattern below the surface of the water as soon as it was placed in the water maze.

Water maze performance is summarized in Fig. 2. Acquisition was measured as the mean escape latency over all trials to find the hidden platform in the pool (Fig. 2B). Both groups of old impaired rats had longer escape latencies than both the young rats and the old nonimpaired rats before grafting. On the second test, 2½ to 3 months after grafting, the group without grafts remained as impaired, whereas the treated animals as a group improved significantly [−42 percent; related groups comparison, $t(20) = 3.3$, $P < 0.01$]. In the posttransplant test, the performance of the treated rats was significantly better than that of the untreated old-impaired controls [unrelated groups comparison, $t(15) = 5.3$, $P < 0.01$].

The old impaired rats did not significantly reduce their escape latencies over the 36 trials in the initial test session (plotted as nine blocks of four trials each, Fig. 2A, left), and they did not reduce their escape latencies to a statistically significant extent even over the second test session (open circles in Fig. 2A, right). The animals with implants showed a reduced escape latency already within the first four trials after transplant (block 1:1), and the reduction over the subsequent trials (filled circles in Fig. 2A, right) was not statistically significant [two-way analysis of variance, main group effects, $F(3, 28) = 5.9$, $P < 0.01$; group-by-trial interactions, $F(8, 16) = 0.51$, $P > 0.05$]. The reduced escape latencies in the treated group were not due simply to faster swim speeds since the distance to reach the platform was also significantly reduced

in this group but not in the controls without grafts [7.6 ± 0.5 m in the old impaired rats before transplantation versus 5.4 ± 0.7 m after transplantation; related group comparison, $t(20) = 3.6$, $P < 0.01$].

Within the treated group 8 of the 11 animals reached escape latencies in the posttransplant test within the 99 percent confidence limit of the young rats (as defined above in the pretransplant test). Both the range (11 to 33 seconds) and the mean (23.8 seconds) of the escape latencies of these recovered animals were close to those of the nonimpaired aged rats in the pretransplant test (mean, 24.3 seconds; range, 12 to 27 seconds). Three of the rats with grafts remained impaired (nonrecovered); their performance in the posttransplant test was similar to that of the rats without grafts in the old impaired control group.

The ability of the rats to use spatial cues for the location of the platform in the pool was assessed by analyzing their search behavior after removal of the platform on day 5 of testing. Whereas the young rats and the rats in the old nonimpaired group focused their search on the fourth quadrant, where the platform had previously been placed, the old impaired rats failed to do so in the pretransplant test; their swim was equally distributed over all four quadrants (Fig. 2C) and the distance swum in the fourth quadrant was considerably less than in the young and the old nonimpaired groups (Fig. 2D). In the posttransplant test the rats with grafts, but not those without, showed significantly improved performance; their swim distance in the fourth quadrant increased by 83 percent [related groups comparison, $t(20) = 3.6$, $P < 0.01$] (asterisks in Fig. 2, C and D), and they swam significantly more in the fourth quadrant (where the platform had been located) than in the other quadrants of the pool [quadrant 4 compared with the mean distance in quadrants 1 to 3; related groups comparison, $t(20) = 3.3$, $P < 0.01$) (Fig. 2C). By contrast, the untreated controls showed no significant change over their earlier performance (Fig. 2C). Again, the performance of the eight recovered rats in the group with grafts (Fig. 2D) was within the range of the nonimpaired aged rats, and it resembled that of the young rats in the initial test.

At the completion of behavioral testing, the brains from the rats with grafts were processed for acetylcholinesterase (AChE) histochemistry (*15*). In all rats, the intrinsic septo-hippocampal cholinergic pathway was transected unilaterally by a fimbria-fornix lesion 7 days before they were killed to remove the intrinsic AChE-positive innervation of the hippocampal formation, thus allowing observations on the AChE-positive fiber outgrowth from the septal grafts. Surviving AChE-rich grafts were found in two or three implantation sites on both sides in all animals; on the side with the lesion, the grafts produced an extensive AChE-positive terminal network in all major subdivisions of the hippocampal formation (dentate gyrus, CA3, CA1, and subiculum) (Fig. 1). No clear-cut differences in graft survival or AChE-positive fiber outgrowth were noted between the three rats that remained impaired and the eight that had recovered in the behavioral test.

These results demonstrate the ability of intracerebral neural grafts to ameliorate age-related impairments in complex, cognitive behavior. The mechanism of action of the septal grafts is an intriguing issue for further investigation. It is of interest that intrahippocampal septal grafts—identical to the ones used here—in our previous study had no effect on another aspect of age-related behavioral impairment (motor coordination disabilities), whereas grafts of embryonic substantia nigra implanted into the striatum were effective (*16*). This suggests that the behavioral effects of the grafts are regionally specific and may be mediated through a direct action on elements in the surrounding region of the host brain. Our previous studies in young rats with behavioral deficits induced by discrete surgical brain lesions provide evidence that intracerebral neural grafts can act by substituting for a lost afferent input to a denervated target area (*8, 9, 17*). As a working hypothesis, therefore, we propose that the behavioral effect of the septal grafts in the present study is due to their ability to extend axons into the host hippocampal formation that can substitute for age-dependent impairments in synaptic transmission in the hippocampal neural circuitries of the host. The conspicuous difference in survival between the two groups of old impaired rats over the 3-month experimental period could perhaps also reflect some (primary or secondary) effects of the grafts.

The AChE histochemistry showed that the septal grafts were rich in AChE-positive neurons and had formed an extensive AChE-positive (probably cholinergic) terminal network in the host hippocampal formation, suggesting that the cholinergic neurons in the graft may play a role in the recovery of spatial learning in the water-maze task. In young rats, interference with cholinergic transmission as well as lesions of the septo-hippocampal connections disrupt performance in the water-maze task (*18*). Moreover, measures of cholinergic neurotransmission in the hippocampal formation decline in aged rats (*2, 7*), and in other types of learning tests the performance of aged rats can be improved by administering cholinergic precursors. However, since the recovery in water-maze performance in the aged rats with grafts did not show any obvious correlation with the extent of AChE-positive fiber ingrowth, such ingrowth seems not to be the sole critical factor in the present graft effect. Our previous study (*8*) on the effects of intrahippocampal septal grafts on T-maze learning in young rats with lesions indicated that cholinergic reinnervation was a necessary but not sufficient prerequisite for recovery. This may also be the case for the graft-induced recovery of spatial learning in aged animals without lesions. In any event, the intracerebral neural grafting technique should be a useful tool to gain a deeper understanding of the neurological deficits underlying the age-dependent cognitive decline in animal models of aging and dementia.

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Interaction Between Perceived Self-Motion and Object-Motion Impairs Vehicle Guidance

Abstract. *When one is riding in a vehicle, perceptual thresholds for motion of objects are significantly elevated above those determined under corresponding but simulated conditions in the laboratory without concurrent self-motion perception. Authorities on road traffic accidents should thus consider an additional perceptual time of at least 300 milliseconds for detecting critical changes in headway beyond the usual reaction time. Detection times thus corrected consequently lead to an alteration of our conception of safe intervehicle distances in a convoy. This elevation of thresholds for object-motion during self-motion, with its consequences for visual control of vehicle guidance, can be seen as a disadvantageous side effect of an otherwise beneficial space-constancy mechanism, which provides us with a stable world during locomotion.*

Under laboratory conditions thresholds for detecting object-motion are traditionally determined with the subject's head fixed by a biteboard. Under natural environmental conditions, however, a person moves freely with the twofold perceptual task of controlling self-motion and perceiving object-motion simultaneously. The incidental observation that one has considerable difficulties seeing the treetops moving in the wind while driving a vehicle led us to a systematic study of egocentric object-motion perception during concurrent self-motion. In a series of laboratory experiments we demonstrated significantly increased thresholds of object-motion perception during simultaneous self-motion perception under various stimulus conditions.

Active head oscillations about the vertical z-axis (amplitude, ±20°) raised the detection thresholds for object-motion (with a 1° target, $\dot{\varphi}$ = 5 deg/sec) with increasing frequency of the sinusoidal head movements (0, 0.5, 1.0, and 1.5 Hz) up to a factor of 3 (1.5 Hz) above that measured when the head was stationary (0 Hz). These elevated thresholds are not due to a retinal slip of the fixated target, because stabilization of the retinal image was complete for ±20° rotatory head movements up to a frequency of 1.5 Hz (*1*).

Passive lateral triangular head movements (amplitude, ±60°) with the vestibulo-ocular reflex suppressed by fixation of a head-coupled target result in a doubling of the thresholds for object-motion detection with head movements at 60° per second angular velocity (*2, 3*).

That real self-motion is not the essential stimulus for suppressed object-motion perception was demonstrated with objectively stationary subjects for whom apparent self-motion was visually induced by full-field optokinetic stimulation. Elevated object-motion thresholds were linked to the sensation of self-motion, as evidenced by comparison with small-field pattern stimulation, which did not induce apparent self-motion (*4, 5*).

On the basis of these laboratory data we hypothesized that while a person is riding in a vehicle, thresholds for the detection of changes in headway should be elevated because object-motion thresholds are elevated during self-motion. We tested our hypothesis with a field study (vehicle guidance under natural conditions) and a corresponding simulation in the laboratory, in which a

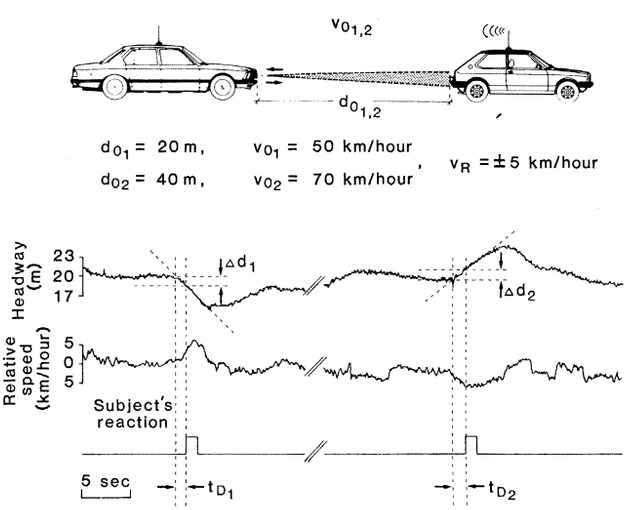

Fig. 1. Perception of changes in headway determined under real road conditions, at different absolute speeds ($v_{O_{1,2}}$) and distances ($d_{O_{1,2}}$). The intercar distance (headway) as well as relative speed (v_R) and subject's reaction latency (t_D) were recorded simultaneously.