control, indicating destruction of 85 percent of the dopamine nerve terminals. However, in the presence of pargyline uptake was reduced to only 58 percent of the untreated control (or 63 percent of the corresponding pargyline control). At the same time, catecholamine histofluorescence showed a healthy outgrowth of fluorescing fibers around the explants in cultures exposed to pargyline plus MPTP (Fig. 1I), while cultures treated with MPTP alone had few fluorescing fibers (Fig. 1H). No significant difference in uptake was seen between control cultures and cultures treated with pargyline alone (Table 1). The protective action seen with pargyline is consistent with the notion that the metabolism of dopamine by monoamine oxidase plays a significant role in the cytotoxic mechanism, but further studies are required to verify such an interpretation.

A direct comparison between the concentrations of MPTP effective in the monkey model in vivo and those used in our studies in vitro is difficult to make. Such factors as tissue distribution and rate of metabolism in vivo and stability in the feeding medium in vitro remain to be examined. As an approximation, however, the amounts of MPTP injected intravenously into rhesus monkeys (*3*) were in the range 0.15 to 0.69 mg per kilogram of body weight, which corresponds to average whole-body concentrations of 1 to 4 μM, for each daily injection over the 5- to 9-day injection schedule. In another study (*4*), squirrel monkeys received repeated intraperitoneal injections in the range 0.5 to 3.0 mg per kilogram of body weight, which corresponds to average whole-body concentrations of 3 to 17 μM. The concentration we selected for investigation in vitro was 10 μM with exposure for 4 to 7 days, which is in a reasonably comparable range.

Our results show that the relative insensitivity of rat brain in vivo to MPTP administered parenterally cannot be ascribed to failure of the tissue to produce a toxic metabolite. Embryonic rat mesencephalon in organotypic culture showed a marked loss of dopamine cell bodies and outgrowths, comparable to changes observed in vivo with monkeys. Organotypic cultures provide a good experimental model for the study of MPTP and its toxic properties on nigrostriatal neurons.

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6. The feeding medium for the cultures consisted of 33 percent human placental serum, 10 percent chick embryo extract, and 57 percent Earl's minimum essential medium (Gibco) supplemented with 3.4 mM glucose. MPTP (Aldrich) was dissolved in 0.1M HCl and subsequently diluted to one-thousandth of its original volume in the feeding medium. Equivalent volumes of 0.1M HCl were added to the control culture medium (without MPTP). The *p*H of the feeding medium was unchanged by this dilution of HCl.
7. Rat dopamine neurons in explant culture exhibit neurochemical characteristics similar to those of the dog [see C. Mytilineou, G. Cohen, D. Dembiec-Cohen, M. Van Woert, E. Hwang, *J. Neural Transm. Suppl.* **19**, 37 (1983)].
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Brain Factor Control of Sex Pheromone Production in the Female Corn Earworm Moth

Abstract. *Sex pheromone production in the female corn earworm moth* Heliothis zea *is controlled by a hormonal substance produced in the female's brain. It is present in the brain in scotophase as well as photophase, but it is released into the hemolymph to stimulate pheromone production only in the scotophase. The stimulatory activity was also detected in the brains of male corn earworm moths and of other moths.*

The female corn earworm moth *Heliothis zea* (Boddie) produces a sex pheromone during scotophase from a glandular area at its abdominal tip to attract and sexually activate a mate (*1*). We report the discovery of a hormonal substance in the brain of *H. zea* that controls production of the sex pheromone in the female. When females were ligated between head and thorax, causing isolation of the brain from the rest of the body, they were incapable of pheromone production. However, ligated females could be stimulated to produce pheromone by injection of a saline extract of the adult insect's brain. The stimulatory substance was found in brains of females in both the scotophase and the photophase, but it was detected in hemolymph only during the scotophase. Results indicate that the hormonal factor is a peptide produced in the female's brain and released under photoperiodic control into the hemolymph, thereby stimulating the pheromone gland to produce sex pheromone. This stimulatory activity is not restricted to extracts of the female brain; extracts of the male corn earworm brain and those of other moth species also triggered pheromone production in the female gland.

Table 1. Effect of ligation and subsequent injection of nervous tissue homogenates on the extractable sex pheromone in female *Heliothis zea*. Females were ligated with cotton thread between the head and thorax 1 hour after emergence. Means followed by the same letter are not statistically different from one another according to Duncan's new multiple range test ($P = 0.05$). BR, brain; CA, corpora allata; CC, corpora cardiaca; TG, thoracic ganglion; *Z*-11-HDAL, (*Z*)-11-hexadecenal.

Treatment	*Z*-11-HDAL ($\bar{x}$ ng per female ± S.E.M.)
Unligated*	122.93 ± 14.17 b
Ligated*	0.57 ± 0.15 e
+ 30 μl of saline*	0.93 ± 0.09 e
+ BR*	131.55 ± 26.55 b
+ BR and CC*	139.27 ± 19.73 b
+ BR (tissue-free)†	189.74 ± 23.13 a
+ CC†	13.56 ± 1.87 c
+ TG†	3.43 ± 0.52 d
+ CA†	0.52 ± 0.06 e

*$n = 10$. †$n = 5$.

Earlier efforts to elucidate physiologic factors that control female sex pheromone production in insects indicated that, in cockroaches, the corpora allata (CA) played some role (*2*), and studies of two species of moths were inconclusive (*3*). In a study of the gypsy moth *Lymantria dispar* (L.) removal of the brain in the pupal stage was reported to prevent pheromone release in 100 percent of the resulting females, but 79 percent of the brain-deficient females were observed to "call" (that is, to extend the terminal

Table 2. Evidence of pheromone gland–activating hormone in brains of female *Heliothis phloxiphaga* and both sexes of *Heliothis zea* moths in scotophase and photophase and its release into hemolymph during scotophase. The (*Z*)-11-hexadecenal (*Z*-11-HDAL) values are means of five replicates. Means followed by the same letter are not statistically different from one another according to Duncan's new multiple range test ($P = 0.05$). F, female; M, male.

Tissue	Photoperiodic phase		Pheromone titer ($\bar{x}$ ng of *Z*-11-HDAL per female ± S.E.M.)
	Donor (unligated)	Recipient (ligated)	
	Heliothis zea		
Brain (F)	Photophase	Scotophase	145.05 ± 7.81 a
Hemolymph	Scotophase	Scotophase	21.50 ± 4.76 c
Hemolymph	Photophase	Scotophase	1.44 ± 0.17 d
Brain (M)	Scotophase	Scotophase	177.56 ± 33.61 a
	Heliothis phloxiphaga		
Brain (F)	Scotophase	Scotophase	105.21 ± 12.61 b

abdominal segments and display the pheromone gland) (*4*). However, whether the calling females produced sex pheromone was not established. In contrast, our findings with *H. zea* indicate that the CA do not act directly in controlling sex pheromone production and show conclusively that female sex pheromone production in this species is regulated by a substance produced in the insect's brain.

The sex pheromone in *H. zea* females is secreted from glandular cells in the intersegmental membrane between the eighth and ninth abdominal segments (*5*). (*Z*)-11-Hexadecenal is the major constituent (~92 percent) of the sex pheromone of this species (*6*). The (*Z*)-11-hexadecenal titer of the pheromone glands of individual females was determined by open-tubular capillary chromatography with the internal standard method of quantitative analysis (*7*).

Maximum pheromone titer in the females was observed in the third scotophase (54 hours after emergence), and glands were extracted at this time. Ligation of a female immediately after emergence or within 2 hours of the extraction caused approximately 98 percent reduction in the extractable pheromone (*8*) relative to the pheromone titer of unligated females of the same age (Table 1). Injection of a brain homogenate (*9*) from a 50-hour-old virgin female restored the pheromone titer of ligated females to the normal amount. A tissue-free brain extract (*9*) injected into ligated females caused an increase in the pheromone titer that was significantly higher than that obtained by unfiltered brain homogenates. This higher titer may be attributed to the absence of catabolic activity that may reside with brain tissue fragments. Injection of homogenates of brain and corpora cardiaca (CC) also increased the pheromone titer in the pheromone glands of ligated females, but the amount was not significantly different from that in the glands of females injected with unfiltered brain homogenate alone. Only a small increase in the pheromone titer was observed after the injection of CC homogenate. Injection of thoracic ganglion homogenate caused an even smaller increase, and the CA homogenate did not cause any increase. A hormonal factor produced by the brain was therefore responsible for the control of sex pheromone production in female *H. zea*, and continuous presence of this stimulatory factor was essential to sustain pheromone production.

Because *H. zea* females normally produce the pheromone during the scotophase, we tested brain homogenates from females in photophase and hemolymph from females obtained either at the third scotophase or third photophase after eclosion (*10*). The results (Table 2) indicated that the hormonal activity was present in the brains of females during photophase and was significantly greater than that of the brains from females of comparable age during scotophase. This suggests that the hormonal substance is stored in the brain during the photophase and is released only during the scotophase. This interpretation was supported by results showing that the stimulatory activity was absent in hemolymph from a female during photophase but was present in hemolymph from a female during scotophase. The low response obtained from the injection of hemolymph compared to brain homogenate from females in scotophase can be attributed to the hormone's being diluted by the total volume of hemolymph circulating in the insect's body.

When ligated females were injected with homogenates of brains from *H. zea* males, a high pheromone titer was generated (Table 2), and significant increases were also obtained when brain homogenates from female *H. phloxiphaga* and moths of five other species (*11*) were injected into ligated *H. zea* females. Whether the stimulatory activity in these brain extracts is due to the same substance that is recoverable from the female corn earworm remains to be determined. We know, however, that the hormonal factor in the female *H. zea* brain is a peptide (*12*) and that the regulation of female sex pheromone production is linked directly to this product of the insect brain.

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7. Photoperiod for insect rearing was 16 hours light and 8 hours dark at 26° and 20°C during the photophase and scotophase, respectively. The ovipositors of individual females were excised at the eighth abdominal segment and extracted for 3 minutes in 5 μl of heptane containing 19 ng of tetradecan-1-ol acetate (internal standard). The extract was injected onto a fused silica chromatographic column (60 by 0.25 mm) that was coated internally with Carbowax 20 M (J and W Scientific). The column was installed in a Hewlett-Packard model 5880A gas chromatograph equipped with a flame ionization detector. Chromatographic conditions were as follows. Hydrogen carrier at 45 cm^3/sec; splitless injection (no collection of fractions) at 225°C; column temperature program: 90°C initial injection temperature with heating (30°C per minute) 0.4 minute after injection to 185°C final column temperature.
8. Females were ligated at 0.25, 1, 2, 8, 16, 24, 32, 40, 48, 52, and 53 hours after emergence. Pheromone extractions were performed 54 hours after emergence. Ligation up to 40 hours caused more than 99 percent reduction in the extractable pheromone, whereas at 48, 52, and 53 hours, the reductions were 98, 97.5, and 78 percent, respectively.
9. Tissues were dissected from 50-hour-old virgin females and homogenized in 30 μl of physiological saline [J. Meyers and T. Miller, *Ann. Entomol. Soc. Am.* **62**, 725 (1969)], and the saline extract was injected into the abdomen of 51-hour-old ligated females with a 50-μl Hamilton syringe. One brain or a pair of CC and CA (or both) was used for injection into a ligated female. Pheromone glands were extracted 3 hours later. A cell-free extract was obtained by filtration of the homogenate through a glass-wool plug in a capillary tube. The extract was examined under a microscope to ensure that no tissue fragments or cells were present.
10. Hemolymph (10 μl) was drawn from the fourth or fifth dorsal intersegmental membrane of the abdomen of a female with a 50-μl Hamilton syringe, mixed with 30 μl of cold (4°C) physiological saline, and injected into the ventral side of the abdomen (fifth or sixth intersegmental membrane) of a ligated female.
11. Brain extracts of the females of navel

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

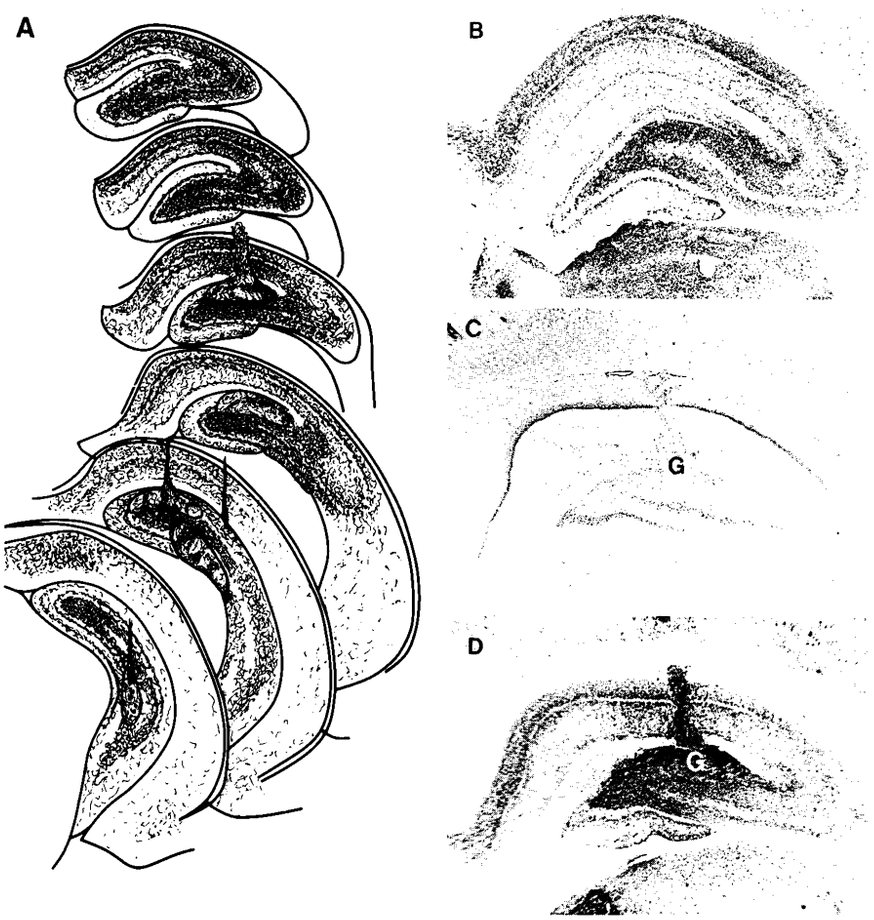

Fig. 1. Graft survival and AChE-positive fiber outgrowth into the host hippocampal formation of an old impaired rat with grafts whose improvement in escape latency represented the median of the whole treated group. (A) Composite schematic drawing of AChE-stained sections of transplant survival and transplant-derived 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*).