A Brain For All Seasons

The metamorphosis of moths between the two major stages of their lives has allowed scientists to discover a surprising flexibility in brain cell development

By JULIE ANN MILLER

The earth-bound early stages built enormous digestive tracts and hauled them around on caterpillar treads. Later in the life-history these assets could be liquidated and reinvested in the construction of an essentially new organism —a flying-machine devoted to sex.
Carroll M. Williams, 1958
Entymologist

While biology cannot argue that a cat has nine lives, it can present a good case for a moth having two. Metamorphosis is, in a sense, the birth of the adult insect. In a pupa, as in an embryo, a distinct form of animal develops. Underlying the obvious anatomical changes, in both instances, cells are born, migrate, remodel and form new connections.

Scientists studying the basics of brain development are taking advantage of this second birth. Developing brain cells are more accessible for study during the caterpillar-to-adult moth transition than during earlier insect embryonic growth, and they are far more accessible than developing brain cells in a mammalian embryo in the womb.

A remarkable flexibility in the development of specific brain cells has been demonstrated by research on metamorphosing hawk moths. In the species *Manduca sexta,* only the male moths sense and react to the attractant scent of the females. On their antennae, the males have special sensory cells for this important task. These cells make connections in the moth brain with characteristic cells in a structure found only in the male.

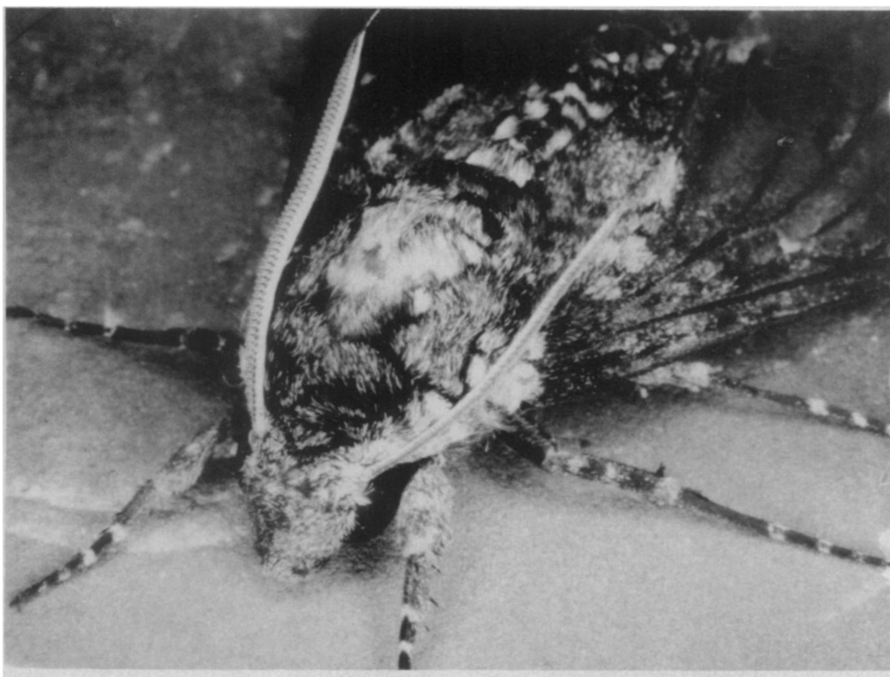
John G. Hildebrand and colleagues of Columbia University now report that it is contact with the appropriate sensory cells that drives the brain cells to develop into the male-specific type. In New York at the recent Society for Neuroscience Science Writers' Seminar, Hildebrand reported that a female moth that has been endowed through surgery with male antennae will develop apparently functional male structures in her brain, even though the brain cells are, of course, genetically female.

From their work on *Manduca* metamorphosis, the scientists expect to learn rules that can be applied to the development of other cells in the moth and also in higher animals, including man. "We hope that a careful experimental analysis of this dramatic case of developmental plasticity will reveal at least one way nerve cells can influence each other's development, and we expect that this mechanism will be found in widely different animal groups," Hildebrand says.

During metamorphosis, the brain of a moth must undergo extensive redevelopment in order to serve bodies of quite different shape and function. The two distinct lifestyles — the caterpillar adapted for growth and the adult for reproduction — are often credited with the great success of moths, and other highly adapted insects. There are more than 100,000 species of moths and butterflies, and they are found in almost all regions of the earth.

"At the pinnacle of the invertebrate evolutionary line, in the most successful strategy of development, the animal takes its most essential life structures and separates them into two bodies: one structure perfectly suited to eating in its own ecological niche and the second structure perfectly suited for finding a mate," Hildebrand says.

The structures for these different activities tend to be incompatible. "The larva with its big jaws and large gut doesn't have



Hildebrand

Mismatched antennae: Transplanting female larval tissue into a male caterpillar resulted in a moth with a male antenna on its right side and a female antenna, which appears narrower, on its left. The larval form (below left) is called a tobacco hornworm. The pupa (below right) has a contoured cuticle with sheaths and cases for adult structures yet to be developed, such as tongue, wing, eye and antenna.

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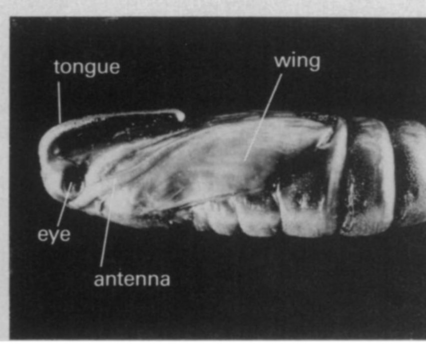

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to go anywhere. It just sits and has an epicurean life," Hildebrand says. These jaws and gut would be excess baggage on the adult, which must travel light as it flies to find a mate. So the adult carries primarily sensory and reproductive organs along with the structures required for flight.

In Hildebrand's laboratory at Columbia, pale green eggs, 1 millimeter in diameter, hatch into tiny green larvae. Even the slight new caterpillars have the black decorative horn attached to their hind end that gives them the name hornworm. The larva eats almost continuously and grows at an amazing rate. In their natural settings, from South America to southern Canada, the larvae, although called tobacco hornworms, devour leaves of tomato, eggplant, potato, pepper, Jerusalem cherry and jimson weed as well as tobacco. In the laboratory they eat an artificial nutrient medium. In a period of about 19 days a *Manduca* grows from a thread-like larva a few millimeters in length into a fat caterpillar, 4 inches long and an inch in diameter.

When the caterpillar reaches the appropriate size, it prepares for transition. The larva stops eating, empties its gut and shrinks in size. It burrows into the ground, where it develops into a pupa. In the laboratory, the scientists store the pupae individually in corked holes bored into a wooden plank, to mimic the natural underground pupation. The pupa is inactive, but within it extensive anatomical renovation is underway. After about 18 days the dart-shaped adult emerges. It can have a body length of more than 3 inches and a wing span of more than 4 inches. Its appearance and behavior have little in common with those of its former self.

The moth brain reflects the two lifestyles and the transition between them. "The brain is very different in the caterpillar and in the adult," Hildebrand says. During metamorphosis, new nerve cells are born, differentiate and establish working connections. Other cells migrate, change functions or even die. In the end, the adult moth brain is at least 10 times as large as the caterpillar brain.

A sensory system for detection of odors important to the adult moth develops during metamorphosis. Odor-sensing cells arise in the developing antennae and their processes grow into the brain where they contact other newly developing nerve cells. Many of the antennal sensory cells are finely tuned to detect the sex-attractant scent of a female moth. These cells, reasonably enough, are found only in the antennae of the males. They give the male antennae a key-hole, instead of an egg, shape in cross-section and a fuzzy coating of long, scent-detecting hairs. These characteristics make the male antennae appear thicker than those of the female.

When the processes of the male-only antennal cells contact cells in the brain, they influence brain development in a strikingly clear-cut manner, Hildebrand says. They trigger their target brain cells to mature into a specific form. These adult cells have a bush of short processes carrying signals in from the sensory nerve cells.

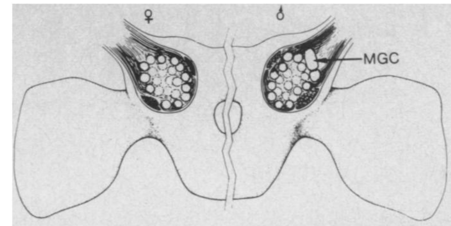
The contact between the male-specific pheromone-detecting sensory neurons and the male-specific brain cells takes place in a structure called the macroglomerular complex. The structure has never been found in the brain of a normal female moth. In contrast, parts of the brain that process odors of plants used as food, for example, are not different in male and female moths. The development of moth antennae and the corresponding brain structures was described by Josh Sanes and Leslie Tolbert working with Hildebrand.

Moth antennae can be mixed and matched by a surgical procedure, devised by Hildebrand and colleagues Anne M. Schneiderman and Steven G. Matsumoto. The scientists graft from the head of one larva into the head of another the tissue that contains precursor cells for an adult antenna. The graft develops into an apparently normal structure, appropriate to the sex of the donor, and it makes connections in the recipient moth's brain.

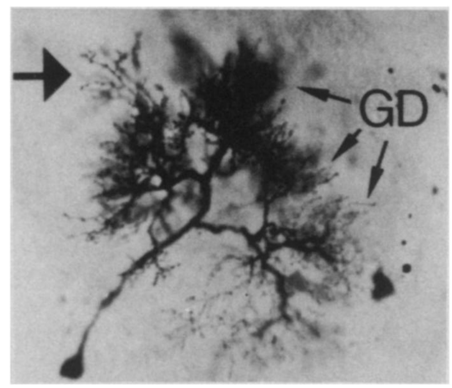
When the sex of the recipient and donor differ, the sensory cell contact alters the course of the brain's development, Hildebrand says. If male sensory cells contact female target cells in the brain, the target cells become male-like in appearance and function. The scientists can even record an electrical response in these cells when the antennae are stimulated with female scent. "It's not just that they have the male sensory cells but that they are wired into the brain in a male-like way," Hildebrand says. On the other hand, the macroglomerular complex and the male-specific brain cells that make connections there are missing from a male brain receiving input from female antennae.

The question remains whether a female with a masculine antenna acts like a male moth in response to female pheromone. At the first whiff of pheromone, will she stop whatever she's doing and follow the scent?

Hildebrand and colleagues are now exploring that question, but it is a difficult task. They are using a wind tunnel at the



In nature, only the brains of male moths (on right in diagram) contain a structure called the macroglomerular complex (MGC). But surgically modified females with male antennae develop a similar structure, and males with female antennae lack the complex.



Female moths surgically endowed with male antennae develop bush-shaped brain cells, like the one above, which are normally only found in male moth brains. It has dendrites (GD) that make contacts within a structure resembling the macroglomerular complex (arrow).

U.S. Department of Agriculture laboratory in Gainesville, Fla., to examine the moth's flight behavior. In work with James H. Tumlinson of USDA, Hildebrand says, they have "some indication that these females move in oriented male-like flight in the presence of pheromone." Thus this aspect of behavior may be fundamentally determined by the sex of a moth's sense organs, rather than of its brain.

Underlying brain development is not a single rule, but a variety of strategies cells can draw on. While these cells of the scent detection system are very flexible in their development, others that scientists have examined are quite set in their ways. Some cells depend on making the correct connection to survive; others survive regardless. Some, like those described now by Hildebrand, depend on the correct connections to differentiate normally. Within a single species, cells only a few microns apart in the brain can have very different strategies, Hildebrand says.

The scientists expect their studies to have wide-ranging applications, from human nutrition to insect control. Hildebrand says, "We hope and expect to gain insights about principles that apply to all nervous systems, including that of mankind." □