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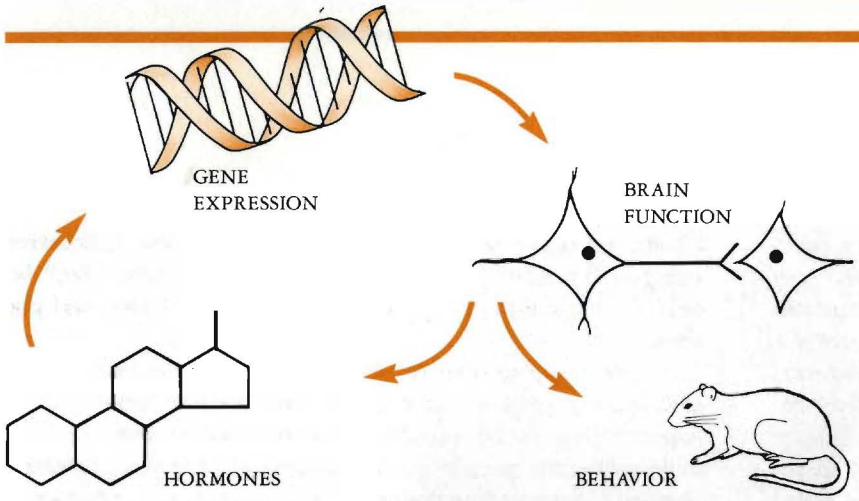
Brainwork: [Dr. Donald W. Pfaff]

Fulvio Bardossi



THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

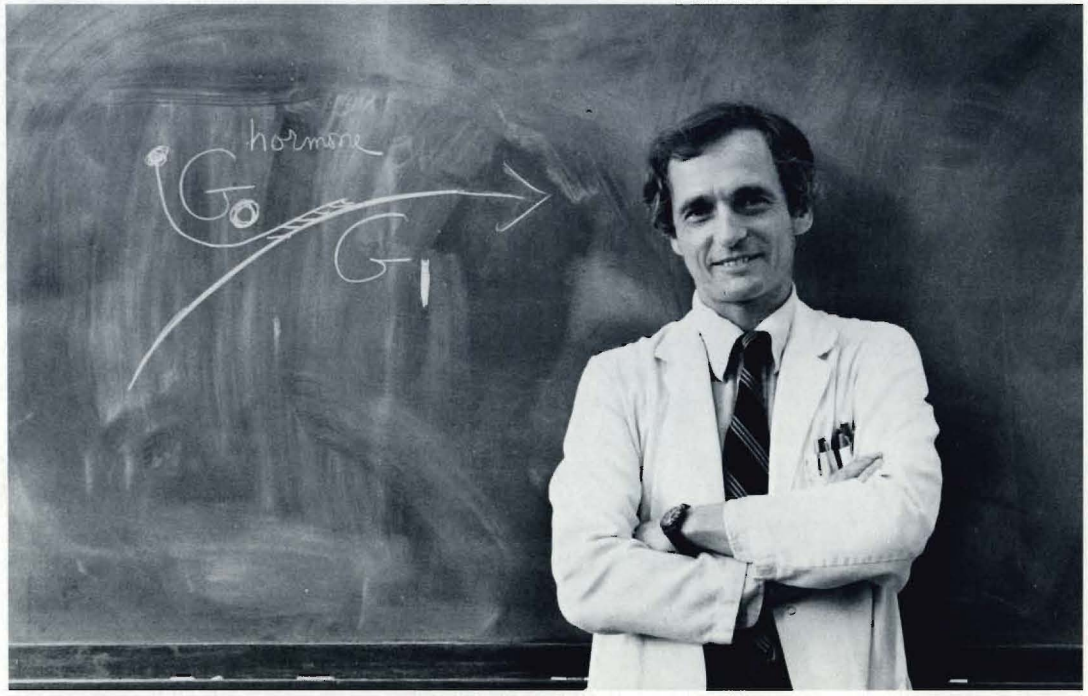
SPRING 1989

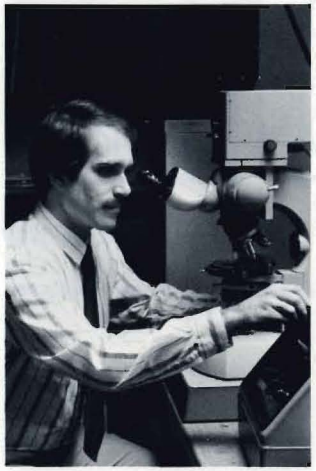
Brainwork

It has taken science some two thousand years to recognize the brain as the master organ of the body—the locus of thought, the seat of the emotions, the governor of behavior. The gradual discovery of the brain's architecture and its billions of nerve cells has inspired such analogies as electrical switchboards and supercomputers. Only in the past thirty years have scientists begun to evolve a more subtle concept of the brain. In this view, electrical signals are transmitted from one nerve cell to another by chemical molecules released at the junction—or synapse—between two cells. Scientists have also begun to learn how nerve cells react to and are altered in crucial ways by other chemical messengers called hormones.

The word hormone means "to set in motion," although, in fact, hormones can also inhibit the response of cells they affect. Among the most potent and specific hormones are the steroids, the subjects of study in Donald Pfaff's laboratory of neurobiology and behavior at The Rockefeller University. Steroid hormones include those that control the response to stress and the so-called sex steroids. Sex steroids include the androgens (controllers of sperm production), the estrogens (regulators of the menstrual cycle), and progesterone (which helps to prepare the uterus for ovulation).

Donald W. Pfaff





Postdoctoral Fellow Robert Gibbs is studying genes involved in the growth and maintenance of nerve cells. He recently employed new DNA probe techniques to analyze the receptors in the brain for Nerve Growth Factor. Now he is contrasting patterns of expression, between uterus and brain, for a cancer-related gene, c-fos.

Some hormones are secreted into the bloodstream by endocrine glands; some originate in the brain itself. Acting on nerve cells in the portion of the brain called the hypothalamus and in the spinal cord, these chemical messengers bring about sex-related physical changes in developing animals and humans, affect such behaviors as feeding, mating, and aggressiveness, and influence moods and other mental states.

Donald Pfaff began thinking about brain hormones in his graduate school days in the 1960s, when not very much was known about them. Today, Dr. Pfaff and his team of researchers, using a wide variety of research techniques, are manipulating hormones and other chemical molecules to uncover how the brain works.

FROM BRAIN TO BEHAVIOR

After graduating from Harvard College in 1961, Don Pfaff decided to forego a family tradition in surgery to enter a new brain research program at the Massachusetts Institute of Technology. In late 1961, when he was exploring ideas for his Ph.D., his thesis advisor, neurobiologist Joseph Altman, mentioned in passing that he would "like to do something with hormones." His student was intrigued and began reading up on them. He learned that some behaviors are controlled by the brain and are specifically dependent on hormonal chemicals carried by the blood to target tissues all over the body.

Today, it is known that for a hormone to work it must fit and bind to a specifically shaped receptor molecule jutting out from the membrane of a target cell or embedded in its nucleus. But in the early '60s nothing was known about the existence of receptor sites on cells to which hormone molecules could bind and subsequently activate.

Dr. Pfaff's initial efforts to understand hormone activity in the brain had two fortuitous boosts. In 1962 researchers at the University of Chicago reported the first proof of steroid receptors in other cells in the body. Just about the same time, a drug company in Boston began manufacturing the first radioactively labeled estrogens. Dr. Pfaff decided to use these radioactive estrogens to locate hormone receptors in the brain. "So," he recalls, "I got into my car, crossed the Charles River, and got some."

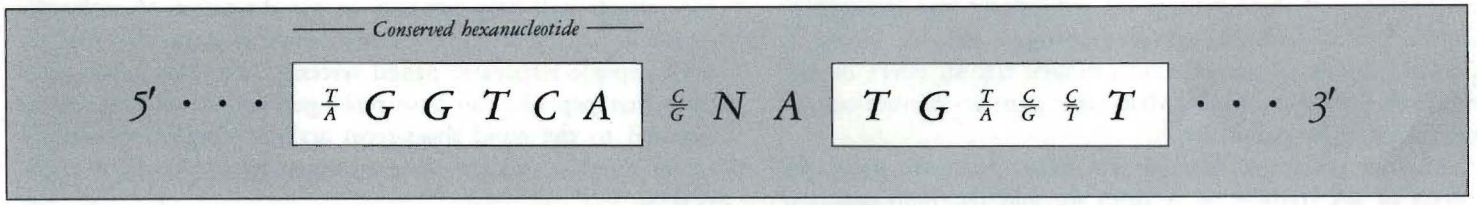
By preparing sections of nerve-cell tissue treated with these radioactive estrogens and a special photographic emulsion, he hoped it would be possible to detect where the hormone molecules attached on the cell. Sure enough, the new procedure revealed clusters of tiny grains—due to the radioactive estrogens—wherever the hormone molecules had collected.

As it turned out, he and his colleagues at MIT and later at the Rockefeller, where he began his postdoctoral studies in 1966, were able to prove that steroid hormone binding occurs in all vertebrate brains from fish and amphibians to reptiles, birds, and mammals. The patterns and mechanisms of binding by estrogens, androgens, progesterone, and the stress hormones (glucocorticoids and mineralcorticoids) appear to be universal. That hormones and their receptors are similar in many different organisms is a major theme in the lab's work because, as Dr. Pfaff says, "we are interested in those things that you can discover in an animal brain that would also be true in a human brain."

"As we were reinforced by the positive findings," he adds, "we began to say, 'Aha. We have hit an island of knowledge here. How large is it?' It's like the Dutch discovering Australia: they didn't realize it was a continent, otherwise they would have stayed." Dr. Pfaff stayed, and he and his fellow neurobiologists are still exploring their continent: mechanisms of hormone action.

The mapping by Dr. Pfaff and others of steroid-binding sites in the hypothalamus and related areas of the brain made it possible to work out, for the first time, the neural circuit for a mammalian behavior. The model was the hormone-dependent mating behavior of the female rat.

When prompted by the sex hormone estradiol, a form of estrogen that acts on nerve cells in the brain, the female rat responds to physical stimuli from the male on her flanks and rear by assuming a posture—the lordosis reflex—that allows



The distinct sequence of nucleotide bases to the left shows a classical reverse symmetry (compare boxed bases) which comprises the consensus Estrogen Response Element. A very similar sequence appears to account for Gary Romano's demonstration that estradiol raises preproenkephalin messenger RNA levels in specific hypothalamic neurons. Lab members now focus on steroid receptors as transcription factors, capable of altering expression in particular subsets of nerve cells

mating. Estradiol must be present in the female brain for the stimulation to elicit the mating response. Dr. Pfaff and his colleagues have correlated their hormonal receptor data with measures of electrical activity in steroid-binding neurons and analyses of sensory and motor mechanisms. "The power of the hormone effect," he notes, "is like a key that allows us to open the gateway to an entire circuit, from brain to behavior, and to analyze it."

Currently, the lab studies gene expression for the hormone receptors themselves. By showing that estrogen turns on the gene for the progesterone receptor, the researchers related, for the first time, a gene transcription factor to a behavior.

GENE AND PROTEIN EXPRESSION

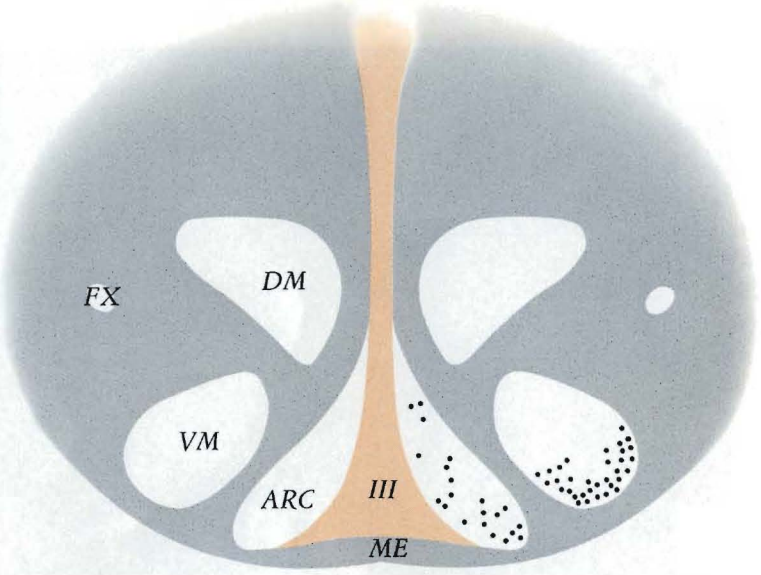
Hormonal control of neurons has proved to be a powerful model for other kinds of experiments. Charles Mobbs is using it to explore the biochemical mechanisms by which steroids regulate the functioning of nerve cell connections in the brain. Very little is known about these mechanisms, though they are believed to be relevant not only to basic neurobiological processes but also to many clinical impairments. One question under investigation is whether, during reproductive senescence, the hormone could cause selective loss of neural synaptic hookups that it once facilitated.

Scientists have found that steroids can trigger receptors in the cell nucleus that, in turn, switch on certain genes controlling production of specific polypeptides or proteins. On the basis of this and other data, Drs. Pfaff and Mobbs theorize that estradiol induces the synthesis of proteins in the hypothalamus, some of which are then transported to the midbrain. From there these proteins may activate working connections between sensory and motor neurons governing contractions of the muscles involved in specific behaviors. The identification of such proteins could provide tools for investigating those biochemical mechanisms related to the transfer of messages across the synaptic gaps between nerve cells. Until recently, no protein with the right credentials was known. Dr. Mobbs now thinks he has a potential candidate.

Genes controlling the manufacture of certain hypothalamic chemicals, neuropeptides, are of special interest to Assistant Professor Joseph T. McCabe. Pioneering a labeling method for identifying RNA in brain tissue, he is able to correlate synthesis of vasopressin and oxytocin to levels of other hormones and to hypertension. Genetically different rat and mouse strains can usefully be compared, since some have high blood pressure and others will not manufacture vasopressin.

In his search he has been working with pairs of female rats from which the ovaries have been removed. Both rats in a pair are injected in the hypothalamus with radioactively labeled protein precursors; one of them also receives an estradiol implant. Dr. Mobbs then analyzes tissue from several brain regions of each rat, using an intricate technique by which proteins are separated according to molecular weight and electrical charge, and imaged as spots on specially treated photographic film.

He has found a protein that almost always appears in the hypothalamus and midbrain of rats given estradiol, but rarely in rats not given an infusion of the hormone. All the evidence suggests that estradiol causes the protein to appear in the hypothalamus; from there it is transported to the midbrain. "It is the only protein out of the hundreds examined to meet this test," he says. Now he is investigating the unanswered questions: What is the function of this protein? Can we clone it?



In this cross-section through the rat hypothalamus, each dot shows a nerve cell expressing the gene for the progesterone receptor. With a tritiated single-stranded DNA probe, Gary Romano and Postdoctoral Fellow Andrea Lauber used in situ *hybridization to find expression. Pretreatment with estradiol turns this gene on, whereas it reduces messenger RNA levels for the estrogen receptor itself.*

ABBREVIATIONS:

ARC — arcuate nucleus
DM — dorsomedial nucleus
FX — fornix
ME — median eminence
III — third ventricle
VM — ventromedial nucleus of hypothalamus

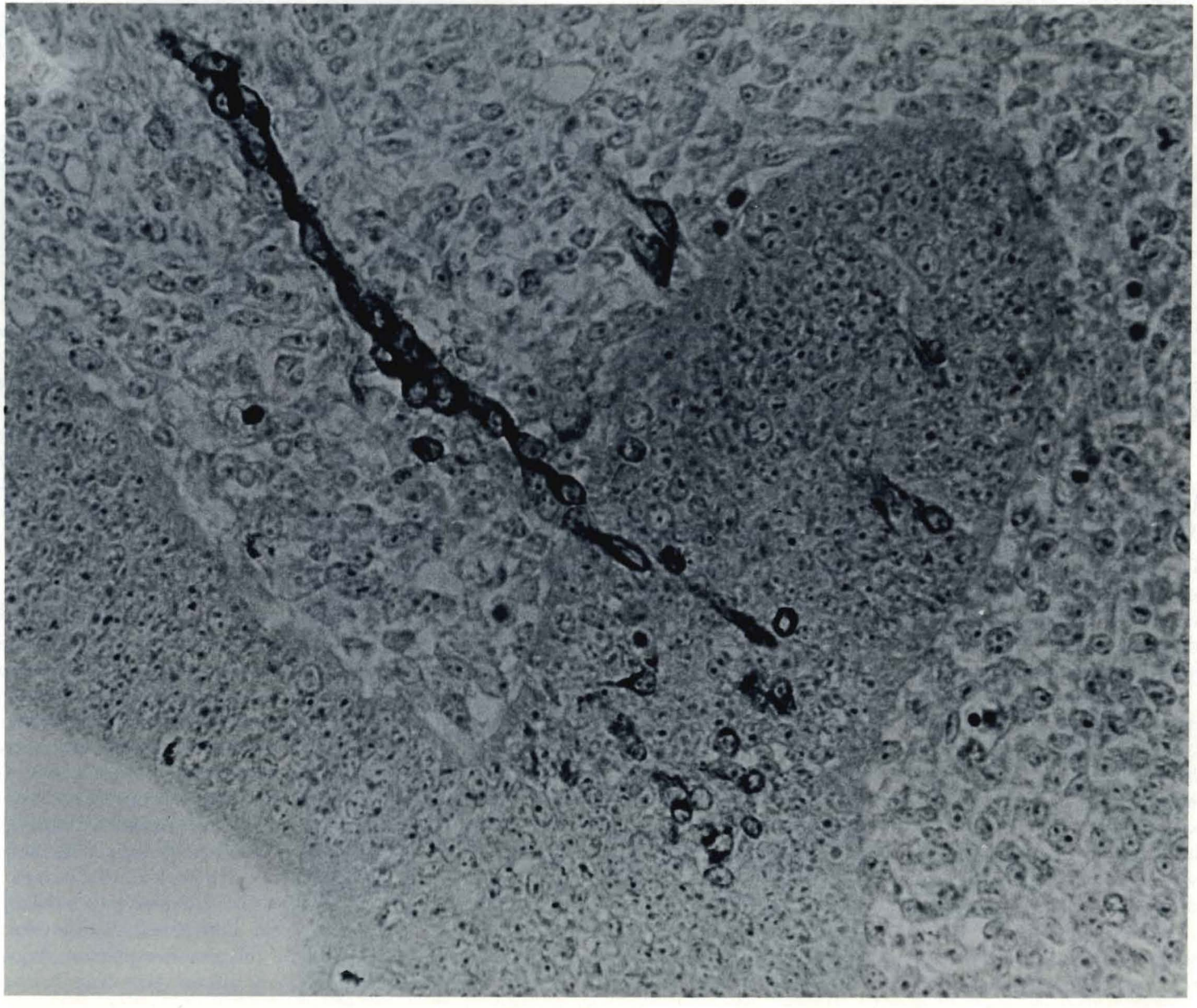
UBIQUITOUS GABA AND VERSATILE PEPTIDES

Drs. Susan Schwartz-Giblin and Lee Ming Kow are two veteran members of the laboratory whose work in neuroendocrinology is opening windows on such problems as epilepsy, pain, and drug side effects. Both are electrophysiologists who use recordings of electrical activity in neurons to study hormonal effects.

Dr. Schwartz-Giblin is particularly interested in steroid hormone effects on the spinal cord. Even though most estrogen-collecting cells in the nervous system are located in the hypothalamus, a few are found in the spinal cord, in a region where primary sensory nerve fibers come in and their signals are processed. Dr. Schwartz-Giblin says she "couldn't help but be struck by the evidence that these cells in the spinal cord seemed to be strategically located for interaction with a class of neurons that bind a chemical known as GABA."

In earlier research she had shown that GABA-binding cells are found in almost every area of the brain and that GABA can act as an inhibitory transmitter. One component of the GABA receptor is sensitive to the chemical benzodiazepine, which enhances the power of GABA to inhibit or dampen incoming sensory signals. Working on reproductive behavior in the female rat, Dr. Schwartz-Giblin demonstrated that progesterone increases benzodiazepine binding, which in turn strengthens GABA power, but that the presence of estradiol weakens it. Since the receptivity of the female to male stimulation peaks at a point in the estrus cycle when estrogen levels dip but progesterone levels remain high, the data indicated that progesterone acted via benzodiazepine receptors to regulate the intensity of the signals entering the spinal cord.

Dr. Schwartz-Giblin is also exploring the possibility that estrogen plays a role in reducing pain and discomfort during pregnancy. Other researchers have shown that pain and discomfort thresholds rise just before rats give birth. A similar increase occurs in humans. Graduate fellow Gary Romano, who received his Rockefeller Ph.D. in 1988, discovered that in the hypothalamus, estrogen regulates the genetic expression of enkephalin, one of the body's natural opiates. Knowing that in



LHRH hormone-expressing cells migrating from their origin in the tip of the nose, across the nasal septum, and toward the brain (top of photo) where they will find their final destinations from which they will function. This finding by Marlene Schwanzel-Fukuda is the first example of nerve cells expressing a specific neuropeptide and moving into the brain during its development.

the spinal cord the receptors for enkephalin and dynorphin, another opioid, are found parallel to estrogen cells, Dr. Schwartz-Giblin intends to see whether estrogen has an effect on the messenger RNA molecules that carry genetic instructions for producing these painkillers.

Another project of interest is a follow-up study about the effects of sex steroids on seizures she and Dr. Pfaff reported recently. The two colleagues found that estrogen protects rats against seizures induced by picrotoxin, a chemoconvulsant that blocks GABA synaptic transmission. "What we know is that all drugs given to control seizures, for example the barbiturates, act through the GABA system," Dr. Schwartz-Giblin points out. "So here we are back again to having to figure out an interaction between steroid hormones and GABA."

Dr. Kow, who has made important contributions to the technique of recording electrical signals in vitro, is using his expertise to study neuropeptides. These include opioids, such as enkephalin, hormones such as oxytocin, which speeds the emergence of the fetus during labor, and the antidiuretic vasopressin.

Before peptides were studied thoroughly in the nervous system, they were thought to be just another category of neurotransmitter. Now, scientists like Dr. Kow are demonstrating that a neuropeptide can be involved in a variety of biological actions beyond simple transmission. By a much slower process called modulation, neuropeptides markedly alter responses of neurons to a classical transmitter such as norepinephrine.

In a study investigating feeding behavior in rats, Dr. Kow recorded the electrical activity of hypothalamic neurons treated with TRH, a hormone involved in the regulation of food intake. His findings showed that the peptide can reduce feeding by modulating responses to the neurotransmitters norepinephrine and serotonin.

"It's a whole new ball game," says Dr. Pfaff in describing the change in viewpoint that Dr. Kow and other researchers' work has brought about. Because modulation in the brain is widely involved in the peptide regulation of neural and endocrine function, Drs. Kow and Pfaff maintain that "analyzing modulatory effects will help not only in the dissection of molecular mechanisms, but also in the development of drugs that interact with peptide-responsive neural systems." Dr. Kow's data illustrate that peptides can have prolonged actions on neurons, as opposed to the rapid short-term actions of neurotransmitter-active drugs which are often accompanied by unwelcome side effects.

CELLS INVADING THE BRAIN

Surprise, be it frustrating or exhilarating, is an inevitable ingredient of research. One of the most surprising recent discoveries of the Pfaff laboratory was made by Marlene Schwanzel-Fukuda. It concerned neurons that produce the peptide LHRH. LHRH interacts with the pituitary gland, which in turn controls testosterone production in developing vertebrates. Testosterone plays a key role in the growth of male sex characteristics and the production of sperm.

"As little as a year ago," Dr. Pfaff says, "everybody would have assumed that LHRH neurons are born in the brain." But Dr. Schwanzel-Fukuda discovered that LHRH cells are formed in an area at the tip of the nose called the olfactory placode and actually travel from there through the nose into the bottom of the brain, eventually to reach the hypothalamus.

Dr. Schwanzel-Fukuda believes her discovery may help explain Kallmann's syndrome, a relatively rare genetic disease in humans that inhibits sexual development in males and females by arresting normal development of testes or ovaries.

Chatting in his office or in introducing his colleagues, Dr. Pfaff communicates his excitement about the group's work and the scientific and practical promises of neurobiology. "Recently, it seems findings have been tumbling into our laps," he says. "We've been lucky in that we've been able to incorporate many techniques of molecular endocrinology into our studies. But these days, for discoveries in molecular brain research, it's just a matter of being there." □

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