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Messengers to the Brain: [Dr. Bruce McEwen]

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Messengers to the Brain

Bruce McEwen

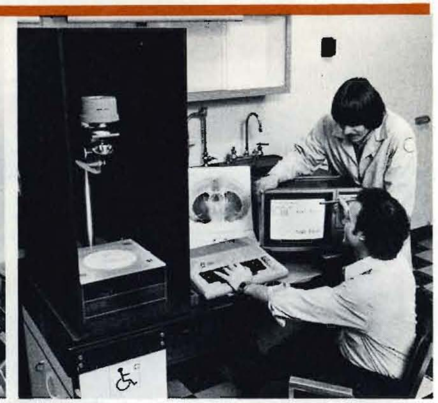
Under the magnifying glass through which neuroendocrinologist Bruce McEwen peers is a section of tissue sliced from the hippocampal region of a rat's brain. With a fine hollow tube he makes a "punch" and puts it into a test tube. The cells in the punch belong to a tiny population of cells, within the billions that make up the walnut-sized labyrinth of the animal's brain, with the particular capacity to bind corticosterone, a hormone secreted by the adrenal glands in response to stress. Before the rat was sacrificed it had been shaken up in a cage like a commuter on a subway train.

In another part of Dr. McEwen's laboratory—a jumble of rooms that to visitors resembles a giant maze—Victoria Luine, using a microscope and a rat-brain "atlas," punches into a frozen slice of hypothalamus containing cells that bind estradiol, a hormone secreted by the gonads, the sex glands.

Corticosterone and estradiol are steroid hormones, so called because they contain sterols (as in cholesterol). Like other hormones, they are biological messengers dispatched by endocrine glands through the bloodstream to different target organs in the body. Hormones affect every life function in higher animals. Adrenal hormones are critical in energy production as well as in the stress response. Gonadal hormones control reproductive function.

Steroid hormones in the brain profoundly influence behavior, as scientists have known from circumstantial evidence for over a century; but it is only in recent years that researchers,

Victoria Luine



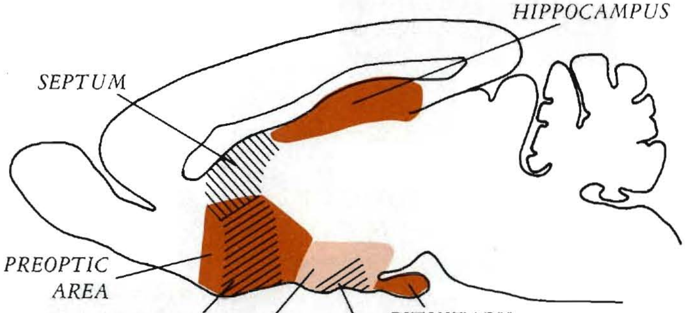
With an instrument called a cryostat microtome, Dr. Lynn O'Connor slices sections from a frozen rat brain.

CENTER PHOTO—*Allan Harrelson, left, a Ph.D. student, and Postdoctoral Fellow Jeffrey Stuckey section fresh hippocampal tissue.*

FAR RIGHT PHOTO—*Dr. Bruce Nock, seated, works a densitometer, which measures the concentration of neurotransmitter receptors in tiny brain regions. The tissue is mounted under a microscope attached to a computer which translates the darkening of the film into quantities of receptors, as displayed on the screen. (See A and B.) Observing is Allan Johnson, a graduate student from Rutgers University.*

with techniques like those illustrated and described in this profile, have been able to ask where in the brain hormones go, what they do, and how they do it. Among the functions of gonadal hormones, as Dr. McEwen and others have confirmed in exquisite detail, are the control of sexual differentiation in the brain as an animal develops and the regulation, in varying degrees in different species, of sexual activity in the adult animal.

Each hormone is taken up by cells equipped with receptor molecules that recognize and bind specific hormones. Neuroendocrinologists have established that nerve cells are targets for steroid hormones; that the receptors are located inside the cells; and that the receptors concentrate the hormone molecules among the genes in the cell nucleus where they deliver an order for the manufacture by the cell of a specific product. The instructions for such production are coded within the genes on segments of deoxyribonucleic acid: DNA. The DNA unfolds, allowing RNA, another nucleic acid, to copy the instructions and convey them outside the



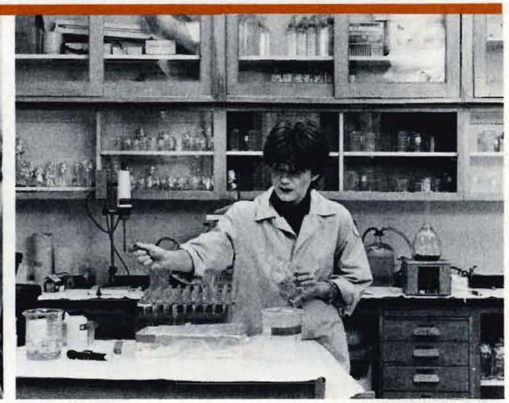
nucleus to the protein factories in the cytoplasm, which surrounds the nucleus. In nerve cells, the instructions transmitted via RNA include the manufacture of enzymes—catalyst proteins—involved in making neurotransmitters. Neurotransmitters are chemicals expelled from nerve endings by electrical signals which transmit these signals to other neurons. Work in Dr. McEwen's laboratory has established that hormones influence the process of neurotransmission, and thereby brain function and behavior, by altering the manufacture of enzyme proteins. The punches Dr. McEwen is shown preparing in the photograph on page one were taken

A

B

from fresh tissue, as opposed to frozen tissue used in most of the work. They were still churning out RNA, protein, and neurotransmitters. They will be incubated and examined to identify their products by means of a new process the laboratory is just putting on line.

Neuroendocrinology is one of several approaches by which scientists are penetrating the dense forest of neural networks within the brain. For Dr. McEwen such research presents an immensely exciting intellectual challenge. Beyond that, it offers, as he states, "the satisfaction of finding new connections to medically relevant problems; of seeing barriers break down between clinical areas, especially psychiatry, and the rest of biology." Recently, members of Dr. McEwen's group



Robert Sapolsky, seated, discovered that the hippocampus loses adrenal steroid receptors with age. Here he examines autoradiograms under the microscope using a technique refined by research assistant John Gerlach, standing. The autoradiograms show the uptake of tritium-labeled adrenal steroid by hippocampal nerve cells.

CENTER PHOTO—*Doctoral candidate Shelley Halpain works a filtration device for trapping and washing cell membranes labeled by neurotransmitter drugs. These drugs label specific receptor sites in membranes which are presumed to be involved in converting chemical information in the form of neurotransmitters into electrical signals. Her work is directed toward understanding how hormones may influence neurotransmission by regulating the levels of membrance receptor sites.*

FAR RIGHT PHOTO—*The samples here being loaded into columns contain hormone molecules that have bound to receptors. The columns will separate the unbound hormone from the hormone-receptor complexes. Preparing the assay is Ljubica Bogic of the University of Belgrade, who spent seven months at Rockefeller learning Dr. McEwen's techniques.*

have found interesting connections between steroid hormone actions in the brain and problems in aging and in depression and other mental disorders. Lewis Krey's studies of the interactions of the gonads, pituitary gland, and central nervous system bear upon problems associated with infertility, menopause, and sex differences in drug response. Dr. Luine is investigating links between estrogen's effects in the brain and Alzheimer's disease.

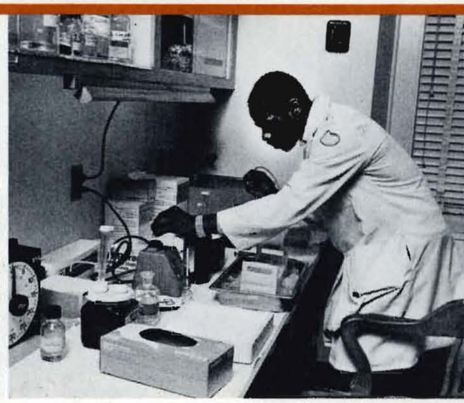
A FORTUITOUS BLIZZARD

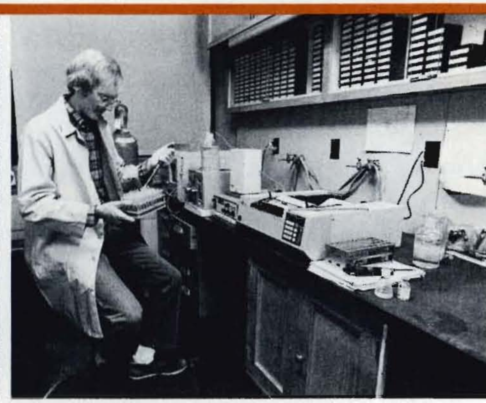
Dr. McEwen, a Rockefeller graduate, has been a member of the University's faculty since 1966. A summa cum laude graduate in chemistry from Oberlin College, he earned his Ph.D. in the cell biology laboratory of Vincent Allfrey and the late Alfred Mirsky for a thesis that, he says, "had nothing to do with the brain. It was a study of the mechanisms of cellular energy production. But I was always interested in psychology, which also interested Dr. Mirsky, who encouraged me to go into neurobiology. He believed it would be a 'hot' area, which was pretty prophetic considering the state of technology for such studies then. He helped me get a postdoctoral appointment in the laboratory of Holger Hydén, in Sweden, one of the places brain research was being done at that time. My wife and I were delighted because it gave us our first chance to travel in Europe." The young Midwesterner returned two years later with a well-developed wine palate (he has since been made a Commander of Honor of a wine-tasting society in Bordeaux), one daughter, another on the way, and a pressing need for employment. Not expecting to find work in neuroscience, he accepted a post in the Department of Zoology of the University of Minnesota.

"In Sweden," he says, "I studied protein metabolism in brain cells. In Minnesota, I began similar nerve-tissue research, working with cockroaches. I remember I kept them in a can on my desk in the lab. But there was very little equipment for what I wanted to do and really no one there to do it with. I was feeling rather discouraged when Alfred Mirsky came out a few months later to give a seminar. He arrived in a blizzard, his seminar was cancelled, and we spent the day snowbound in my apartment talking. Shortly after his visit, I got a call for an interview with Neal Miller, who was just then moving his physiological psychology laboratory from Yale to Rockefeller. Alfred had found out he needed a biochemist and had suggested me. When Dr. Miller offered me the job, I didn't stop to think whether we could afford to live in New York with two kids. I left my cockroaches and we came."

THE SEARCH BEGINS

Before scientists could study the effects of hormones in the brain they had to find them there. In 1962 researchers at the University of Chicago located receptor sites for gonadal hormones in the uterus by injecting rats with estradiol labeled with tritium, the radioactive isotope of hydrogen. With this and related techniques, other investigators began to look for receptors in other tissues and found them in the pituitary gland, whose secretions are controlled by the brain. Among its secretions are the gonadotropins, hormones that activate gonadal development during puberty and regulate gonadal function during adulthood. Gonadotropins stimulate the gonad to secrete steroids—estrogens, androgens, and progestins—which in turn feed back on the brain and pituitary to modify gonadotropin release.





FAR LEFT AND CENTER PHOTOS—*Dr. Gary Dohanich, left, and research assistant Joseph Rhodes analyze enzymes in punches from frozen tissue. Tests like these have led to the discovery of a number of enzymes induced by steroid hormones. The high resolution of the punch procedure and the sensitivity of such assays make it possible to study hormone effects in brain tissue which might otherwise have gone undetected.*

RIGHT PHOTO—*The high-performance liquid chromatography technique being used by Dr. Kenneth Renner electrochemically detects and measures different neurotransmitters in brain tissue.*

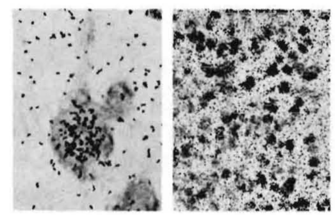
When Dr. McEwen came to Rockefeller, he met Donald Pfaff, a young researcher in the physiological psychology laboratory of Carl Pfaffmann. Dr. Pfaff had shortly before completed the first autoradiographic analysis of estrogen receptors in the hypothalamic area of the brain (see illustration), an achievement that was the major stimulus in directing Dr. McEwen to hormones. "Most people were then interested in the feedback action on pituitary function," he says. "Don and I were interested in behavioral effects." The two men, now both full professors and laboratory heads, have frequently collaborated since, Dr. McEwen's biochemical methods complementing Dr. Pfaff's physiological and neuroanatomical approaches.

The search for hormones in the brain was on and it soon began attracting other young investigators. Victoria Luine, a neurochemist and pharmacologist, joined Dr. McEwen in 1972 to study estrogen effects on enzymes in the brain. Dr. Krey has been a member of the group since 1975. His studies of cellular mechanisms underlying steroid control of pituitary function form a natural complement to the laboratory's primary interest in neural mechanisms of behavior. Of others whose contributions are noted in these pages, many of them students or "postdocs" when they worked with Dr. McEwen, Richard Zigmond is now on the faculty of Harvard Medical School, Carl Denef is a professor in Belgium, Ivan Lieberberg is on the staff of H.C. Moffitt Hospital in San Francisco, Edward Roy is at the University of Illinois, Neil MacLusky is at Yale, Marilyn McGinnis is at Mt. Sinai Medical Center in New York, Bruce Parsons and Thomas Rainbow are at the University of Pennsylvania, Paula Davis is a medical journalist, Christine Fischette is at Hoffmann-La Roche, and Anat Biegon is at the Weizmann Institute in Israel.

MALE AND FEMALE

"Beginning about 1959 work was done that led people to believe the brain undergoes sexual differentiation through the action of steroid hormones," Dr. McEwen says. "The idea that these substances could have a permanent, organizing effect on how an animal will act through its entire life fascinated me, especially since this differentiation process is limited to a specific period in early development; in rats, during the first few days after birth. Later on, the same hormones come along and turn sexual behavior on and off. We also know now, or we think we know, that the same kinds of receptor sites in the same areas in the brain of the developing and the adult animal mediate both these processes. What changes is the state of the nerve cell. In other words, the cell makes use of the hormone and receptor in different ways at different times of life. Shortly after Don Pfaff and I started measuring brain uptake of estradiol, Richard Zigmond refined a procedure for isolating highly purified cell nuclei in the hypothalamus and in the preoptic region, another area where estradiol is concentrated, and it was he who identified cell nuclear estrogen receptors in the brain. His work, done when he was a student, was one of the first important steps in analyzing the biochemical effects of a steroid hormone on the brain."

Estrogens are female hormones and androgens, such as testosterone, are male hormones; but both sexes have both estrogens and androgens. Dr. Pfaff's group and Dr. McEwen's were among the first to ascertain that the male brain contains estrogen receptors and the female brain androgen receptors. Carl Denef, who then began looking at androgens, found enzymes in the pituitary and the brain that convert testosterone to more potent androgens, while others in the lab con-



Autoradiograms show the localization of radioactive corticosterone in a nerve cell nucleus of the hippocampus (left) and of radioactive estradiol in nerve cells of the ventromedial hypothalamus (right).

firmed that the brain, like the gonads, has enzymes that can convert testosterone to estradiol.

"Thus," explains Dr. McEwen, "testosterone is not only a supplier of androgens for androgen receptors, it is also a precursor for estrogens. The conversion of male hormone to female hormone occurs in specific areas of the brain, which were pinpointed, in adult and developing rats, by Ivan Lieberberg. Ed Roy refined an existing technique called an exchange assay so that we could measure how many estrogen receptors in the brain are normally occupied. We found exactly what we had anticipated: that in both adult and developing male brains there were significant amounts of estradiol on estrogen receptor sites in cell nuclei. Paula Davis and Ivan Lieberberg then discovered that this conversion is critical to sexual differentiation, the process by which male and female brains become structurally and functionally different. Block the production of estradiol from testosterone in newborn males and sexual differentiation doesn't take place.

"Well, now we had a problem. If the male and female brain both have androgen and estrogen receptors and the capability of converting testosterone to estradiol, how do males and females differ in response to signals from these hormones? In another series of studies, Neil MacLusky discovered receptors in the brain for progesterone that are induced by estradiol, and Bruce Parsons and Marilyn McGinnis found that this induction is crucial for the appearance of sexual behavior

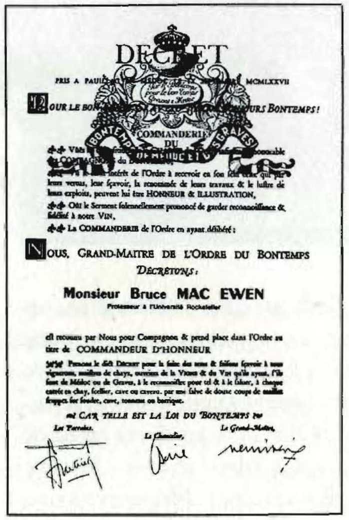
Lewis Krey, left, and Bruce McEwen.

in the female rat. After that, Bruce and Tom Rainbow found that one of the differences between the male and female brain is that males do not produce as many progesterone receptors in a very tiny area of the hypothalamus called the ventromedial nucleus, an area, as Don Pfaff's group and ours had shown, that is essential for female sexual behavior. That was an important observation because it gave us the encouragement we needed to look for other highly localized differences between male and female brains."

STRESS AND THE BRAIN—FROM RATS TO PEOPLE

The physical and behavioral components of stress were important topics under study in Neal Miller's laboratory when Dr. McEwen arrived there in 1966, particularly for Jay Weiss (a recent recipient of a MacArthur Foundation Award). "About a year after I joined the lab," Dr. McEwen relates, "Jay and I decided to see if we could find out where adrenal hormones go. We expected that corticosterone, like estrogen, would localize in the hypothalamus, since at the time it was believed that everything having to do with steroids happened there. I had a very rudimentary knowledge of neuroanatomy but I knew that there was something called the limbic system deep down in the brain, the group of structures that includes the hippocampus and is supposed to be involved in controlling emotions. With only this vague connection, I decided to include the hippocampus in our samples. Lo and behold, it turned out to be the place where corticosterone concentrations were highest. I remember I got my first positive indications on a Saturday in November 1967, when the University of Michigan was playing Ohio State. Ann Arbor is my home town so naturally that was something I followed. Michigan was winning and I was getting these unexpected results. That day was definitely a high point in my career."

The hippocampus has continued to fascinate the McEwen lab over the years, even though the reasons for its hormone sensitivity have remained obscure. Recently, however, Robert Sapolsky, just finishing his thesis, has obtained evidence linking this structure and its hormone sensitivity to the shut-off of the outpouring of adrenal steroid in response to stress.


DECRET

NOUS, GRAND-MAITRE DE L'ORDRE DU BONTEMPS

DÉCRETONS:

Monsieur Bruce MAC EWEN

COMMANDEUR D'HONNEUR

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He has found that aging rats have elevated levels of adrenal steroids, and that the continuous bombardment by these steroids on the brain appears to kill off some of the receptor-containing cells in the hippocampus. As a result, the hippocampus's ability to shut off the system weakens. Through a feedback circuit, the system is gradually destroying itself. What are the factors that precipitate cell loss beyond a normal aging rate? And does what happens inside a rat's brain have any bearing on what happens in a human brain?

"While it's not possible to perform the kinds of experiments on humans beings that can be done with rats," says Dr. McEwen, "a number of studies, including our studies with rhesus monkeys, indicate a high degree of similarity in neural hormone action across species. Coincidentally, considerable clinical data has accrued in recent years showing that in patients with Alzheimer's disease there is a loss of nerve cells comparable to the pattern observed in the aged rats. In Alzheimer's victims, the loss occurs in the basal forebrain, an area that connects with the frontal and temporal cortex where cognition takes place. This similarity induced Vicky Luine to take a closer look at Alzheimer's disease to try to identify the influence of estrogens on the cholinergic nerve cells in the basal forebrain, which produce the neurotransmitter acetylcholine. These cells are especially affected in Alzheimer's disease."

Whereas stress hormone levels rise with age, estrogen levels decrease. A large population of Alzheimer's patients are postmenopausal women. Dr. Luine's findings suggest that cholinergic neurons might begin to degenerate as a result of abnormally reduced levels of estrogen. After she described her research at one of the University's Friday afternoon seminars, Howard Fillit, a Rockefeller immunologist working on Alzheimer's disease, suggested a collaboration. They are now beginning trials at the University's research hospital to see if estrogen therapy will help Alzheimer's patients while the search for primary causes continues.

"Our involvement with clinical problems has suddenly mushroomed," Dr. McEwen states. "This winter, I spoke at the American College of Neuropharmacology at workshops on depressive illness and on sex differences in drug response. Some of us from the lab meet regularly with clinical scientists from a number of other hospitals and research centers. We try to spell out to them the story for the rat and they try to see what relevance it has for people. We're educating one another."

THE SEARCH CONTINUES

The medical applications that may derive from Dr. McEwen's findings have resulted from a search for answers to the question of how cells and tissues work. "Hormones themselves don't cause behavior," Dr. McEwen points out. "They're primers. Their job is to regulate aspects of cell function; in the brain, this involves neurotransmission. In the last few years we've focused a lot of attention on neurotransmitters, on the enzymes that make them or break them down, and on their receptor sites, because neurotransmitters have receptors just as most other biological molecules do. Tom Rainbow, Christine Fischette, and Anat Biegon set up studies using quantitative autoradiography, a powerful methodology with which you can take a section of brain tissue, put it on a glass slide, add a small radioactive molecule that will bind to the receptor, expose the section on film, and read the density of the binding. The marvelous thing about this method is that you don't lose anatomical details so you can go back to the section many times and ask different questions. You can measure neurotransmitter receptor densities, map them in the brain, and study effects of hormones on them."

Ultimately, what cells and tissues do is determined by genes. Dr. McEwen recalls that when he was a student at Rockefeller, the Mirsky-Allfrey laboratory was the setting for "a lot of highly speculative conversations about genetic and environmental influences on behavior." When he was at the University of Minnesota, he applied for and received a research grant with the visionary title "Gene Expression in Nervous Tissue." "I still have the grant," he says, "and it's still my goal. We've spent most of the past seventeen years refining the problem, finding the relevant cells in the brain and finding out what they do. With that done, and with the concepts and techniques of molecular biology and genetic engineering now available to us, we can begin to work on how they do it. We have the underpinnings." □