

The History of Neuroscience in Autobiography
Volume 14

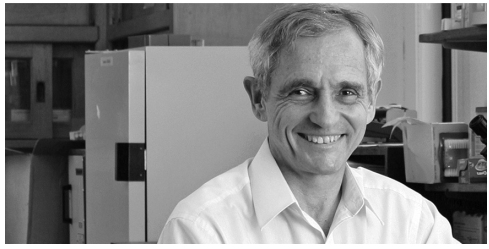
Edited by Thomas D. Albright
and Larry R. Squire
Published by Society for Neuroscience
ISBN: 978-0-916110-13-0

Donald Pfaff

pp. 300–333

https://www.doi.org/10.1523/hon.014007

Donald Pfaff



Donald Pfaff

Born:

Rochester, New York
December 9, 1939

Education:

Harvard College, AB *magna cum laude* (Biological Psychology, 1961)
Massachusetts Institute of Technology (MIT), PhD (Brain and Cognitive Sciences, 1965)

Appointments:

Research Associate, MIT, Department of Psychology (1965–1966)
Postdoctoral Fellow, Rockefeller University (1966–1968)
Assistant to Professor Emeritus, Rockefeller University (1969–present)

Honors and Awards (Selected):

National Merit Scholarship (1957–1961)
Harvard National Scholarship (1957–1961)
MIT President's Award Fellowship (1962–1963)
Elected, American Academy of Arts and Sciences (1992)
Elected, USA National Academy of Sciences (1994)
MERIT NIH grant award (2003–2013)
Elected, The Harvey Society (2004)
Elected Fellow, New York Academy of Sciences (2006)
Elected, Collegium Internationale Neuro-Psychopharmacologicum (2007)
Honorary Doctorate, Pace University (2008)
IPSEN Foundation Prize in Neural Plasticity (2010)
Lehrman Memorial Prize, Soc. Behavioral Neuroendocrinology (2011)
Honorary Professor, Huazhong University, Wuhan, China (2013–2018)
Academician, Guangdong Medical University, China (2017–2022)

Donald Pfaff spent his career on a quest to discover a body-to-brain-to-behavior causal connection and to take on the explanation of mechanisms for a mammalian behavior as a scientific problem to solve. He stated his scientific problem: "Is it possible to discover the brain mechanisms for a mammalian behavior?" Studying hormone-regulated behaviors would give some advantages, so he discovered the neurons expressing the genes for estrogen receptors. Then he settled on lordosis as a simple behavior: a social behavior essential for reproduction. His team worked out the entire circuit for producing lordosis behavior. Then, since the estrogen receptors he had discovered in the brain are ligand-activated transcription factors, he set out to discover some genes whose transcription is estrogen regulated in neurons. Then he manipulated mRNA levels for these genes and discovered predicted changes in lordosis. Collectively these studies confirmed that it is possible to discover the brain mechanisms for a complete mammalian behavior. Along the way he discovered that, during development, GnRH-producing neurons are born in the nose and migrate into the forebrain. As a result, he ended up studying a mechanistic chain from histone protein modifications through transcription through electrophysiology to behavior.

Donald Pfaff

Family

I was lucky. Born into a medical family I came to understand that my father, a grandfather, and a great-grandfather were successful surgeons and my mother had been an operating room nurse. (I think that was where she met my father.) Furthermore, I was born into a big Roman Catholic family—my oldest brother was 26 years older than my youngest brother. My older three siblings were pre–Great Depression babies, with all that meant for the pressures on the family. I was the first post-Depression baby, resented by my older siblings for having things easier.

The following are three important things about Roman Catholicism: First, because of the many theological dogmas the nuns shot at you during early grade school, a relaxed, critical mindset emerged naturally. Second, I was chosen as altar boy in third grade because I could memorize the Latin (without knowing what it meant). Third, discipline. Trudging through knee-height snow in upstate Rochester, New York, to serve at a 6:30 a.m. mass took some discipline. That's been with me ever since. But the Catholicism had to stop.

My older brothers had gone to a Catholic high school and my parents were not happy with it. They said the priest and teachers there "almost ruined my brothers' minds" (even though one went to Harvard and the other went to Massachusetts Institute of Technology [MIT]). Various tests I took, including the conventional IQ test, confirmed my parents' impression of my intelligence. So, my father called his Catholic friend who was the principal of the local, public Monroe High School and asked how her school would treat me. She said, "no problem"—in each grade, they have a small honors group. As a result, I was surrounded by great students and pleased to be among them.

During those long-ago days, even the honors students did not get to be instructed in calculus, but the science classes were excellent. My first great psychological reward as a science student came in Mr. Sam Gold's chemistry class. When the reaction for making potassium permanganate was written on the board, and I carried out the reaction and, indeed, the solution turned purple as predicted, I was on my way. I was hooked on science.

In upstate New York in the 1950s, if you were a good high school student in science and you were a boy, you were expected to go into physics or chemistry, and if you were a girl, you were more likely to go into biology. So, I headed for college thinking about math and physics.

College

I was accepted at the four places I applied, but it boiled down to choosing between MIT and Harvard. I made a big mistake. Because I was uncertain about wanting to become an engineer, I passed up MIT and went to Harvard. Among the Harvard "preppies," there was a surprising anti-intellectual attitude, in addition to the fact that these students had better secondary school preparation, had fake British accents, and dressed expensively. Ugh. Anyway, as a so-called math whiz, I was thrown into advanced math taught by a super-brilliant guy, Andrew Gleason, who had actually solved one of the "big problems for the 20th century," as proclaimed by the famous German mathematician David Hilbert. Gleason did not care about teaching and raced through the material using symbols we had never seen. It took five of us students, all with very high SAT scores, working together for about five hours to do each homework set. This discouraged me from advancing in higher math, except for mathematical statistics, which I loved and still do. Later in graduate school at MIT, the course on finite state automata taught by Michael Dertouzos was a lot of fun. My private ambitions about formal approaches to nervous system theory continue to this day.

With a couple of exceptions, the science professors at Harvard were not interested in teaching. In Physics 12C (wave equations), for example, the entire game was to be able to read and then quickly write out extremely long equations. On the hour-long exam, the highest scoring student got 76 out of 100, and the second highest (me) got 28. The teacher did not care.

Two events turned around the Harvard experience for me. The first was an inspiring course taught by the brilliant assistant professor Paul Rozin, who held a double doctoral degree in biology and psychology. His lectures showed me how I could turn my successful academic work in organic chemistry and certain (electronic) aspects of physics into useful techniques for studying brain and behavior.

The second turn of events occurred when I volunteered for Harvard's Phillips Brooks House mental health committee, for which we visited a state mental hospital. I eventually became chair of that committee, but the experience was miserable. I had "my own" schizophrenic patient to help with but witnessed many things: insulin shock, in which a patient is put into a coma and then receives a strong, convulsive electric shock to the brain; a schizophrenic patient throwing chairs at their television; long rows of catatonic patients sitting motionless and staring at nothing. My private internal reaction: Would my father approve of this low level of medical care? No! This reaction influenced my career plan. Rather than going to medical school headed for neurology or psychiatry, I planned to get a doctorate in neuroscience to concentrate on research that might help address mental problems in the future.

The 1960s and 1970s were a time of great hope for biological psychiatry. Many brilliant people were attracted to this field, including Julius Axelrod (see volume 1), Solomon Snyder (see volume 6), and Floyd Bloom (see volume 7). But what is my opinion about biological psychiatry after more than six decades of witnessing biological psychiatry? Not so great. In my recent MIT Press book *Laws of Human Behavior: Steps Toward Hard Science*, I paraphrase former National Institute of Mental Health (NIMH) director Tom Insel who wrote, essentially, that "we have done great science but have not advanced the quality of patient care very much."

My honors thesis was carried out at the Massachusetts Mental Health Center, part of Harvard Medical School. The research had to do with Brandeis and MIT professor Richard Held's theory that for a human to adapt successfully to an altered perceptual environment (s)he must carry out active movements within that environment. Held's database was in visual perception. My honors thesis added auditory spatial localization data, which provided some support to Held's theory.

Graduate School, MIT

I wanted to go to graduate school in behavioral neuroscience with the long-term vision of adding solid scientific understanding of human behavior, "normal and abnormal." My applications were accepted widely, but I did not want to leave Cambridge because I was in love with a Radcliffe and Harvard undergraduate, a beautiful woman. I did not want to stay at Harvard, but then, luckily, I found out from Richard Held (see volume 6) that MIT was just about to start a brand-new department, which would later become the current Department of Brain and Cognitive Sciences. The new department head, Hans-Lukas Teuber, had made great discoveries at New York University's Veterans Administration hospital about brain damage from headshot wounds to US soldiers during World War II. Teuber accepted three students, including me.

This was great for several reasons. First, the faculty at MIT was terrific, both inside and outside of my new department. Second, I walked into Joe Altman's histochemistry laboratory and knew in about five minutes that I would like to work there. Joe was an immigrant from Hungary who escaped (was he escaping the Nazis or the Russians?) by swimming in the Danube. These were extremely early days for investigation of the histochemistry of the nervous system, and Joe's beautiful work in rat brain combined neurochemical precision with anatomical and spatial precision. Third, after my college mistake, four years later I realized that I was "an MIT guy," devoted to my work no matter how long the days were or how grungy the work. For example, I was devoting my life to basic research on brain and behavior using laboratory animals to which I was allergic.

Fourth, because we three new students were the very first students in the new Department of Brain and Cognitive Sciences, we realized that the faculty did not have the faintest idea of what courses they wanted us to take. Because I narrowly decided not to go to medical school, I realized that, with permission from MIT, I could get on my bike and ride to Harvard Medical School and take all the nervous system courses there. That was a breakthrough because the neurobiology instruction, led by the fantastic neurophysiologist Stephen Kuffler, was first rate. Moreover, while some of the medical students were grumbling "do I need this in my daily medical practice," I was grabbing every syllable in triplicate and got A's in everything. That in turn led to my being accepted into Kuffler's exclusive summer course at the Marine Biological Laboratory at Woods Hole, where I could learn how to turn the histochemical discoveries I had made in my MIT graduate school work into postdoctoral electrophysiological insight.

As an aside, at Harvard Medical School I took courses in endocrine physiology (called "Guts and Glands") and molecular endocrinology to support the important research initiatives I'll discuss.

Parenthetically, I must reflect that my college girlfriend dumped me in favor of her graduate adviser, an accomplished guy five years older than me and more sophisticated. Thus, choosing MIT was an excellent academic decision made for a wrong, romantic reason!

At MIT, my adviser Joe Altman had made a great discovery, overturning the dogma that the mammalian brain at birth has all the neurons it will ever have. That is, he discovered postnatal neurogenesis, now an important and thriving field of research. My first job in Joe's lab was following the migrations of these newly born, thymidine-labeled nerve cells. This work with Joe helped to spring a wonderful discovery 27 years later, which I'll describe in the section "Histochemistry."

On the basis of my disappointment with the backward state of mental hospitals, I hoped to work on neural mechanisms more directly related to behavior. I said, "Joe, for my doctoral thesis work, I'd like to do something more closely related to brain function (i.e., behavior)." Joe's *seven-word sentence* in reply was: "I'd like to do something with hormones." That seven-word sentence led me to a train of discoveries that included the first demonstration of body-to-brain-to-behavior causal connections.

I'd been a good student in undergraduate organic chemistry and knew about steroids. Choosing between the family of stress-related steroids and sex-related steroids, I made the following comparison: Stress hormones fan out across the body, initiating diverse and mechanistically independent processes in the service of preparing the entire body to respond to stress. In dramatic contrast, sex hormones are tightly organized to zoom in on a single type of behavioral process, sex behavior (male-typical and female-typical). And I knew that estrogenic hormones and androgenic hormones are required for female-typical and male-typical mating behaviors. Thinking

of what would make a coherent, focused research problem, in the manner of a physical science problem, I decided that I would have a much better chance of making significant discoveries by concentrating on sex behavior. That turned out to be right.

The work would link molecular chemistry to neuronal cell biology and physiology, and thus to the causation of a social behavior required for reproduction. Causal links proved from body to brain to behavior.

Histochemistry

How do sex hormones act in the brain to foster sex behavior? Thinking about sex hormone actions in the uterus and the prostate gland, there was evidence for some kind of hormone receptors. Could I discover hormone receptors in the brain? The answer is "yes." I did so, in fact, but the path to success was long and laborious (summarized in Pfaff, 2017). To provide a coherent account of that process, the following several paragraphs link my graduate student work to experiments in my lab performed at Rockefeller long after my doctorate.

The organic chemist Elwood Jensen, in 1961, had just produced the first carrier-free tritiated estradiol and licensed it to New England Nuclear. I got one of the first shipments, which enabled me to begin using autoradiography for exact, precise cellular localization of estrogen and androgen receptors in the brain. Tritium is important for spatial resolution of hormone receptors in the brain because the beta particle emitted has such weak kinetic energy that the only way it can reach the nuclear track photographic (NTB)-3 emulsion to render a grain black (when developed) is to go straight down into the emulsion. That meant great spatial resolution for cell-by-cell measurements.

What would be my new histochemical procedure? I made a list of 13 technical approaches, in order of my *a priori* estimate of likelihood of success. The actual work took more than three years. The winning procedure was the ninth, and the results were published in *Science* (1968). At every step, there was a tremendous amount of work in the darkroom: coating microscope slides with the Kodak nuclear emulsion NTB-3, serial sectioning brain tissue at 6 micra, and carefully loading the tissue sections (without mechanical pressure) onto the emulsion-coated slides. Then the microscope slide boxes, with a drying agent, had to be sealed and stored in a lead box (protection against ambient radiation), which remained sealed for about three months. That long a time was required because of the low specific activity of the tritium in those days. Thus, the rhythm became: (1) Try a new method and get the slides into the lead boxes; (2) go to Harvard Medical School to take a course in neuroscience or molecular endocrinology; and (3) return to the lab to develop the autoradiograms. Fail, and move on to the next cycle of three steps using the next method from my list.

Eventually, I succeeded and got good autoradiograms to chart cells that had bound tritiated estradiol carefully and comprehensively (preliminary report; Pfaff, 1965). The biggest populations of labeled cells were in the hypothalamus, the preoptic areas (POAs), the corticomedial amygdala, the lateral septum, and the hippocampus. Thus, a limbic-hypothalamic system, called a *system* because later we documented dense anatomical connections among these cell groups (Cottingham and Pfaff, 1986).

For female-typical mating behavior, the most important estrogen-binding neurons were located in the ventrolateral corner of the ventromedial nucleus of the hypothalamus (vlVMN). We discovered this by eliminating estrogen throughout the body (ovariectomized rats) and inserting a tiny cannula that delivered estradiol to vlVMH, which caused the female to exhibit typical mating behavior: vertebral dorsiflexion of the head, back, and rump to achieve a sway-backed posture, which permitted fertilization by the male (Davis et al., 1979). This was an estrogen-regulated social behavior essential for reproduction, which revealed that the essential molecular estrogenic actions on nerve cells are located on neurons in vlVMH.

Years later, we came back to the receptor localization project with a different histochemical technique and got the same results, describing the same limbic-hypothalamic system (Pfaff and Keiner, 1973). Likewise following up the autoradiography with biochemical measures of cell nuclear estrogen binding, with Richard Zigmond and Bruce McEwen (see volume 8), we confirmed our early histochemical results.

Of course, we wanted to follow up the discovery of hormone receptors in the brain, a discovery made in the rat brain, with work in other species. Over many years, my lab and other labs studied estrogen receptors (ERs) and androgen receptors in all classes of vertebrates: fish, amphibians, reptiles, birds, and mammals (including humans). Our lab focused on paradise fish, frogs, lizards and snakes, chickens, and chaffinches, and among mammals, on mice, hamsters, and rhesus monkeys. Dick Swaab, in the Netherlands, and Jeff Blaustein, at the University of Massachusetts, demonstrated human ER immunoreactivity in the same limbic-hypothalamic groups my results had highlighted. Thus, our limbic-hypothalamic system of neurons, which expressed genes for sex steroid hormone receptors, appeared to be universal among vertebrates.

To extend the same set of discoveries, we later used *in situ* hybridization to demonstrate ER mRNA, for example, in vlVMN, the most important neuronal group for activating lordosis behavior (Lauber et al., 1990). Moreover, postdoc Andrea Lauber's results revealed that estrogen administration downregulated expression of the mRNA for its ER, and that this downregulation was more significant in females than in males (Lauber et al., 1991).

The conclusion? There is a limbic-hypothalamic system of sex hormone receptors in the brain that is universal among vertebrates. What types of hypothalamic neurons bind estrogens? The first example came from the work of graduate student Harker Rhodes working with Joan Morrell in my lab. They showed that oxytocin-expressing and vasopressin-expressing neurons in the paraventricular nucleus of the hypothalamus bind estradiol (Rhodes et al., 1981).

Knowing that estrogen-concentrating neurons in vlVMN are crucial for permitting lordosis behavior by the female in response to mating by the male, we later asked where they send their axons—that is, where might an estrogen-influenced signal be registered? Joan Morrell, combining estrogen autoradiography with neuroanatomy using a retrograde-transported dye, discovered that estrogen-binding neurons in vlVMN send axons to the dorsal portion of the midbrain central gray (MCG; Morrell and Pfaff, 1982), itself an estrogen-binding cell group. This accounts for one example of the tendency for estrogen-receptive cell groups to connect with other estrogen-receptive cell groups (Cottingham and Pfaff, 1986).

Another Lucky Break: Rockefeller University

When I was close to finishing my doctoral work at MIT, I wanted to follow up my histochemical results on hormone receptors with neurophysiological investigations to look for the effects of androgenic and estrogenic hormones on electrical activity in neurons with the appropriate receptors. Two professors at Yale, one a neurophysiologist and the other a behavioral biologist, agreed to be my postdoctoral advisers, and I was successful in getting National Science Foundation funding for my project. But then they both decided to leave Yale, one to Emory and one to Rockefeller. Despite growing up in New York State in a medical family, I had never heard of Rockefeller. I asked my esteemed neuroanatomy teacher at MIT, Walle J. H. Nauta, "was Rockefeller a good place?" A normally polite Dutch-born guy, he was uncharacteristically brusque, letting me know that Rockefeller was a fantastic place to do research. So, there I went.

On the way from Cambridge to New York, I took advantage of having been accepted into Steven Kuffler's summer course in neurophysiological techniques at the Marine Biological Laboratory. There I learned intracellular recording techniques by recording from neurons in the visceral ganglion of Aplysia and replicating Eric Kandel's (see volume 9) discovery of heterosynaptic facilitation.

Electrophysiology

After discovering sex hormone receptors in the brain, I wondered what was next. It seemed clear to me that the next step should be to determine

whether nuclear hormone binding would have any physiological effects. Can sex hormones affect electrophysiological responses to sex-relevant stimulation? Comparing opportunities to follow up my estrogen-binding results to my androgen-binding results, at first the androgen route of neurophysiological recording seemed to be better. This was because the responses of the male to female odors are so important in rodent sex behavior. Indeed, by recoding from individual male rat POA neurons, which regulate male sex behavior, we showed potentiating roles for testosterone (Pfaff, 1969). Administration of testosterone, either directly into the POA or by systemic injection, influenced the electrical activity of preoptic neurons. Direct preoptic administration increased the magnitude of single-cell responses to electrical shock of the olfactory bulb and to odor.

Despite the interesting results with testosterone, I began to reflect, soberly, on how hard it might be to get a complete set of neuronal mechanisms for male sex behavior. After all, my scientific "problem," stated in the manner of the "hard sciences," was to answer the question: "Is it possible to work out the brain mechanisms for producing a mammalian behavior?" The female's vertebral dorsiflexion response (called "lordosis behavior"), which is essential for permitting fertilization, seemed like a much more tractable problem, so my emphasis in a lot of studies henceforth was to analyze estrogen actions in brain tissue, especially in vlVMN. So the problem became: Using combinations of electrophysiological and neuroanatomical techniques, could I work out the complete circuit for a mammalian behavior? The answer was "yes."

What that meant was studying sensory processing underlying tactile stimulation of the male on the flanks and perineum, sending signals into the dorsal horn of the spinal cord, up spinoreticular tracts into the hindbrain. Likewise, that meant working my way "backward" from lordosis-executing muscles, to the relevant motoneurons, back up reticulospinal and vestibulospinal tracts to the hindbrain. And, of course, we had to discover the estrogen-influenced efferents from vlVMN to the midbrain. All of this took a lot of time.

Knowing that estrogen binding in vlVMN hypothalamic neurons is necessary and sufficient for permitting lordosis behavior by the female, could we demonstrate the electrophysiological effects of estrogens on the neuronal activity of those neurons? Yes. Research associate Lee-Ming Kow and I (1985) made comparisons of VMN neuronal electrical activity between estrogen-treated and untreated preparations and showed that chronic, in vivo estrogen treatments, while having no effect on resting firing rate, selectively facilitated the responsiveness of silent neurons to excitatory orthodromic inputs, including responsiveness to the excitatory actions of acetylcholine and norepinephrine. Estrogen administration also potentiated VMN neuronal depolarizing responses to histamine and NMDA (Kow, Pataky, and Pfaff, 2006) replicated by studies of responses to histamine

mediated by H1 receptors (Easton et al., 2004; Dupré et al., 2010). In other patch clamp experiments run by postdoc Anna Lee, we found that estrogen increased VMN neuronal responses to phenylephrine (Lee 2008a, 2008b), with the currents being mediated by N- and L-type calcium channels. So we had several experiments showing that estrogens heighten physiological responsiveness in VMN neurons, a site where estrogens facilitate reproductive behavior.

Toward a Complete Behavioral Circuit

Activation of VMN neurons by estrogen is necessary and sufficient for the production of the female social behavior essential for reproduction, lordosis. Thus, our research aimed at determining a complete neural circuit adequate for producing female reproductive behavior, lordosis, used three complementary approaches: (1) discovering the targets of axons descending from VMN; (2) sensory physiology, following pathways from the behavior-triggering somatosensory receptors; and (3) working our way "backwards" from the muscles used for executing lordosis, back to the behavior-relevant motoneurons, and then to the relevant systems descending from the brainstem. The overall result, summarized in the following three sections, is a coherent behavioral circuit that is organized in modules.

VMN Outputs

Axons descending from VMN form two streams. The first is a periventricular route that yields synapses in the rostral pole of the MCG. This region also contains estrogen-binding neurons and receives ascending sensory input from the lower brainstem (Krieger et al., 1979). The second stream runs laterally and cannot be implicated in the behavioral mechanisms of interest to us. The behaviorally relevant periventricular route just mentioned has no axons that extend all the way to the pons, thus helping to define the midbrain module of the lordosis behavior circuit.

We used electron microscopy to define the synapses from VMN neurons in the MCG (Chung et al., 1990a, 1990b). Following lesions of the VMN we saw a type of degenerative pattern in the central gray known as "watery degeneration": Both in presynaptic and postsynaptic processes, there was a swollen appearance, electron lucent. We confirmed these results using kainic acid lesions of ventromedial hypothalamus (VMH) to rule out the possibility that fibers of passage contributed to the foregoing results. Further confirmation of this crucial VMN/MCG projection came from electrophysiological results (Sakuma and Pfaff, 1981, 1982) in which orthodromic spikes coming from VMH cell bodies were canceled by antidromic spikes coming up from MCG stimulation.

Thus, with the hypothalamic module of the behavior circuit defined neuroendocrinologically by estrogen actions in VMN (ultrastructural and molecular results are given in the next section), and the midbrain module defined by the results just quoted, we were left to define the sensory and motor components of the system.

Sensory Components

Frame-by-frame film analyses showed that the female's behavior is triggered by the male contacting her flanks and tail base (Pfaff and Lewis, 1974), which was revealed, for example, by female skin marked by a dye from the mounting male (Pfaff, Montgomery, and Lewis, 1977). Application of a local anesthetic to those regions of the female virtually eliminated the female's response (Kow and Pfaff, 1976).

Using electrical recordings, we found that single cells in the appropriate lumbar dorsal root ganglia (Kow and Pfaff, 1979) responded to very small pressure (less than 500 millibars), indicating input from a type of skin receptor called Ruffini endings. In the spinal cord, dorsal horn neurons gave very similar responses to behaviorally relevant stimuli (Kow, Zemlan, and Pfaff, 1980). Estrogens are involved as well: Small numbers of estrogen-binding neurons are seen in the lumbar dorsal horn (Morrell et al., 1982), and hormone administration significantly increases the size of receptive fields of primary sensory neurons contributing to the pudendal nerve (Kow and Pfaff, 1973).

Spinal circuitry is not sufficient for lordosis behavior to be performed (Kow, Montgomery, and Pfaff, 1977). Selective small transections of the spinal cord revealed that ascending fibers in the anterolateral columns carry the ascending information adequate for lordosis behavior. The behaviorally relevant ascending spinoreticular fibers terminate among the large neurons of the medullary reticular formation, which make up the nucleus gigantocellularis (NGC; Robbins, Schwartz-Giblin, and Pfaff, 1990).

Multiple experiments led us to the conclusion that MCG neurons, which receive estrogen-dependent hypothalamic output, send descending behaviorally relevant signals both to the lateral vestibular nucleus and to the medullary NGC. Both of these neuronal groups are responsible for launching lordosis-facilitating vestibulospinal and reticulospinal signals. Medical doctor–doctoral student Sandra Cottingham showed that MCG stimulation potentiates the ability of vestibulospinal neurons to activate (through vestibulospinal axons) electrical responses in the deep back muscles that are responsible for lordosis behavior (Cottingham and Pfaff, 1987a). MCG neurons also project to NGC (Robbins et al., 1990). Cottingham et al. (1987b) showed that not only does medullary NGC stimulation activate axial muscles crucial for lordosis behavior, but MCG stimulation vastly amplifies the NGC effect.

Thus, neurons in the MCG relay hormone-dependent excitation from the hypothalamic by descending axons that potentiate both vestibulospinal and reticulospinal actions on the motoneurons and axial muscles for lordosis behavior.

Motor Components

Graduate student Emily Brink (Brink and Pfaff, 1980, 1981) defined the anatomy and physiology of the muscles essential for the performance of lordosis behavior. These are deep back muscles of several groups (e.g., medial longissimus, lateral longissimus, transversospinalis) whose separate contributions to lordosis behavior depend on their sizes, their fiber lengths, and their insertions. Bilateral symmetry is essential.

The motoneurons that control these muscles are extremely large, with cell bodies tucked in the ventromedial corner of the ventral horn of the lumbar spinal cord (Brink, Morrell, and Pfaff, 1979). Emily showed that these motoneurons respond vigorously to stimulation of the behaviorally relevant dorsal roots. Because we knew that spinal mechanisms are not sufficient for lordosis behavior to occur (Kow et al., 1977), it was exciting to discover how descending inputs from the lower brainstem could potentiate the electrical activity of these axial muscle motoneurons. If electrical stimulation of large reticulospinal neurons in the medullary reticular formation, NGC, precedes sensory segmental stimulation by 10 to 100 milliseconds, the motoneuron response to sensory segmental stimulation is roughly tripled (Brink and Pfaff, 1981; Cohen et al., 1987a). Furthermore, selective transections showed that behaviorally relevant descending reticulospinal axons travel in the lateral columns of the spinal cord. Transecting these abolishes the motoneuron response.

As expected, we found these motoneurons responsive to natural cutaneous stimulation in the skin areas contacted by the male and in the sensory field of the pudendal nerve, and responsive to stimulation of the pudendal nerve (Kow and Pfaff, 1982; Cohen, Schwartz-Giblin, and Pfaff, 1987b).

So this series of papers told us how the motor side of the lordosis behavior circuit worked.

Modules of the Neural Circuit for Producing the Behavior

By approximately 1990, it became obvious that we had worked out the first circuit for an entire vertebrate behavior, and that it was a modular network. The spinal module handles business by spinal segment. The lower brainstem module integrates behavioral regulation across segments. The hypothalamic module adds the estrogen dependence of the circuit. And the midbrain module integrates the relatively slow neurophysiology of the hypothalamic output into the rapid neurophysiological kinetics of the rest of the circuit.

Ligand-Activated Transcription Factors

I was lucky that the hormone receptors I had discovered in brain turned out to be ligand-activated transcription factors. Over the years, primarily with *in situ* hybridization, we discovered several transcriptional mechanisms whereby estrogens heightened gene expression for genes whose products would, in turn, facilitate female reproductive behavior.

One strong line of evidence showed that estrogen administration increases transcription from the gene for the progesterone receptor (PR; Parsons et al., 1979, 1982a, 1982b; Ogawa et al., 1994), through which progesterone significantly enhances estrogen's activation of lordosis behavior. Estrogen also enhances gene expression for adrenergic alpha-1 receptors, muscarinic acetylcholine receptors, and enkephalin (Romano et al., 1990), all of which act to increase female reproductive behavior. In much of this work, our results dovetailed nicely with the work of Ann Etgen, at Albert Einstein.

Interestingly, estrogens turn on both the genes for the neuropeptide oxytocin (Chung et al., 1991; Quinones-Jenab et al., 1997) and for the oxytocin receptor (Oxtr). The subsequent effects of these transcriptional events on lordosis behavior have the capacity to multiply each other. Likewise, estrogen increases transcription for both gonadotropin-releasing hormone (GnRH) and the GnRH receptor (Rothfeld et al., 1989; Quinones-Jenab et al., 1996), both of which enhance lordosis behavior. Later, we studied effects on gene expression for nitric oxide synthase (Ceccatelli et al., 1996; Rachman et al., 1998) as it positively affects female reproductive behavior. In vlVMN, estrogens enhance growth-related processes, notably a large increase in ribosomal RNA (Jones et al., 1986, 1990). Through these multiple molecular mechanisms, female reproductive behavior is overdetermined when hormonal support kicks in.

Actually, before all of the studies mentioned thus far, I was looking to test the hypothesis that estrogen administration would influence protein synthesis in estrogen-binding neurons. I knew that new protein synthesis in hypothalamic neurons after estrogen treatment was required for female-typical sex behavior (Parsons et al., 1982). But I was handicapped in that all my experience at that time was in histochemistry, not conventional protein chemistry. I had completed course work in organic chemistry at Harvard and in biochemistry at Massachusetts Institute of Technology (MIT) but did not have corresponding laboratory techniques in hand. So I marshaled my courage to ask the advice of Rockefeller professor Stanford Moore, who had won the Nobel Prize for Chemistry based on the first-ever determination of the amino acid sequence of an enzyme, RNAse. He and his lab showed us how to use liquid chromatography to separate labeled proteins after microinjections of labeled amino acids into VMH (Pfaff, Rosello, and Blackburn, 1984). All estrogen-pretreated rats showed lordosis

behavior; none of the control ovariectomized rats did. In the estrogen-pretreated animals, there were significantly more radioactively labeled proteins in microdissected VMH. Additionally, some of these proteins had been axonal transported to the MCG. One specific class of labeled proteins in the MCG (based on molecular weight) was 10 times more abundant in the estrogen-pretreated animals than in the controls.

A second chemical approach to the same issues was spearheaded by Charles Mobbs in our lab (Mobbs et al., 1988, 1989, 1990). To separate labeled proteins in VMH, he used very large two-dimensional gels. One dimension separated proteins according to molecular weight; the second dimension separated proteins according to isoelectric point. He could discriminate more than 250 labeled proteins but was especially excited to detect a strong estrogen effect on one protein with a molecular weight of 70 kilodaltons and an isoelectric point of 5.9. This labeled protein was indeed transported from VMH to MCG—disrupting axoplasmic flow by colchicine treatment reduced arrival of this protein in MCG by about 85 percent.

These two chemical approaches complemented our subsequent decades of work with *in situ* hybridization summarized earlier.

Ultrastructure

The large increase in ribosomal RNA consequent to estrogen treatment suggested that we do some ultrastructural work with VMN neurons. We expected to see growth-related changes resulting from estrogen treatment.

Estrogen treatment led to growth of a larger, spherical cell nucleus in VMN neurons and obvious nucleolus-associated chromatin (Jones et al., 1985). In many cell types, segregation of nucleolar components is associated with conditions in which the demand for ribosomal RNA (synthesized in the nucleolus) exceeds the rate of synthesis. Furthermore, in the cytoplasm, we found estrogen treatment to be associated with a massive elaboration of the rough endoplasmic reticulum, "protein synthetic machinery" (Cohen and Pfaff, 1981; Meisel and Pfaff, 1985). Knowing of the important, behaviorally relevant projections to the MCG, we did ultrastructural work there as well (Chung et al., 1988, 1990). In MCG synapses, estrogen treatment caused an increase in the number of synaptic terminals with dense-cored vesicles; an increase in the lengths of postsynaptic densities; and an increase in the absolute number of synapses per unit area.

Overall, these changes seemed to indicate a stronger hormone-dependent signal from VMN to the MCG in the lordosis behavior circuit.

Epigenetics

How do the hormone-regulated, behaviorally relevant transcriptional events I discussed previously come about? How are they regulated? To

answer these questions, we needed to apply the so-called epigenetic chemical methodologies, focusing mainly on histone N-terminus modifications. Fortunately for me, one of the scientific fathers of epigenetics, C. David Allis, led a large laboratory at Rockefeller, just two floors away from my lab. Dave and his lab members were incredibly generous with their instructions and reagents.

For background, if and only if ER proteins are liganded by estrogen molecules, they will dimerize and bind to DNA at an estrogen response element (ERE; consensus sequence is AGGTCAnnnTGACCT). But because inactive DNA is covered by chromatin proteins, how do the dimerized ER proteins get to the ERE? To answer this question, it is important to understand the basic proteins called histones. Chemical modifications of histones are the key events, and our work was dedicated to linking them into a molecular understanding of the transcriptional regulation of reproductive behavior. In short, we found histone modifications that create a permissive environment for the transcriptional activity necessary for lordosis behavior.

The molecular biologist Khatuna Gagnidze arrived from Tbilisi, Georgia, and accomplished a mammoth amount of work, as described in the next few paragraphs. Because the application of epigenetic techniques to neuroscience was so new at the time, I'll cover Khatuna's contributions in some detail.

Khatuna and Richard Hunter in the lab (see their work reviewed in Pfaff et al., 2017) found that estradiol (E) induces global changes in histone modifications in the VMH. That is, in the E time course of 1, 3, 6, and 9 hours, different histone marks were altered significantly at different times after E administration (Gagnidze et al., 2013). For example, the double-alteration on histone H3, on amino acids 10 and 14, and on S10K14Ac, with these alterations being transcription-promoting, was elevated by 1 hour after E; however, by 6 hours following E, the levels of this marker returned to baseline. In contrast, total H3 acetylation increased with slower kinetics and reached significantly higher levels only 6 hours after E treatment. Another transcriptional-activating histone modification, H3K4me3, showed significantly higher levels 3 and 6 hours after E treatment. Thus, even global histone modifications provide part of the basis for increased transcriptional rates in VMH following E treatment.

Furthermore, E-induced histone modifications are accompanied by ER target gene upregulation. We would expect mRNA elevations in E-responsive genes to follow on from the types of histone changes summarized earlier. The PR gene is a classic ER target gene, which is regulated by E in VMH as well as in other E-responsive tissues. As expected, protein levels of both PR isoforms, PR-A and PR-B, were significantly increased following E administration for 6 hours. These data reveal that in the VMH of female mice, an "active" chromatin state brought about by E exposure coincides with the transcriptional activation of an ER target gene, PR.

How did this transcriptional activation happen? E treatment induces association of activating histone marks with PR promoter in VMH (Gagnidze et al., 2013). To connect these two findings, we used chromatin immunoprecipitation (ChIP) analysis of VMH following E for 3 and 5 hours with antibodies specific for H3Ac and H3K4me3. Two functional promoters, for PR-A and PR-B isoforms, have been described for rat as well as for human PR genes. Association of H3 histone containing either of two transcription-promoting marks, H3acetyl or H3K4me3, was significantly increased at the promoter of the PR gene in the VMH of female mice treated with E. Moreover, H3 acetylation was rapid and sustained and association of H3K4me3 became evident only after 5 hours of E. Summarizing a large set of results, the timing of the E effects depended on which promoter segment was analyzed, which histone mark was measured, and the time after the beginning of E treatment.

Furthermore, we studied the E effect on the recruitment of the canonical estrogen receptor alpha (ERα) to putative regulatory sequences on the PR gene. The induction of the PR gene, whose expression in VMH is behaviorally relevant for female mice as well as the subsequent expression of lordosis behavior, critically requires expression of ERα (Ogawa et al., 1998; Musatov et al., 2006). On chromatin isolated from VMH, can E treatment of ovariectomized female mice result in the recruitment of ERα to the promoter of the PR gene? Mice were treated with vehicle or E for 5 hours and chromatin isolated from VMH was analyzed using the ChIP assay. We did not observe E-regulated recruitment of ERα to the promoter region of the PR gene. We did, however, detect E-induced binding of ERα at two putative enhancer regions located ~81 kb and 200 kb upstream of the PR transcription start site. Whether this ERα binding is precisely that which supports E-induced transcription in VMH remains to be determined. In addition, in an estradiol-responsive mHypoE-N42 mouse embryonic hypothalamic cell line, PR-B expression was transiently induced by estradiol after 6 hours of treatment in an ERα-dependent manner. This induction was associated with an increase in ERα phosphorylation (serine 118) and its recruitment to one of the PR forms of PR-B's promoter as well as with an increased association of SP1 (a transcription factor involved in ERα tethering to target gene promoters), H3K4me3, and RNA polymerase II occupancy. Thus, the induction of PR-B expression is associated with specific transcription factor interactions and epigenetic changes in its promoter in these embryonic hypothalamic cells.

These results also depended on which E-sensitive brain region was dissected. In the POA, we observed a slight but significant effect of E treatment on H3Ac and a stronger effect on H3K4me3 after 5 hours of E treatment. These and other results demonstrate that the time course and the pattern of E-induced histone modifications at the promoter of the PR gene is brain-region specific.

As you would expect, the pattern of E-dependent histone modifications depends on which gene is studied. Another lordosis behavior-relevant gene is OTR. In VMH, there was no effect of treatment on either of the "activating" histone marks at 3 hours of E, but there was a significant effect of treatment on both histone modifications after 5 hours. This time course is accurately reflected in the kinetics of OTR expression in VMH. In POA, 5-hour E treatment resulted in hyperacetylation of H3 histone at the promoter of OTR gene, but no H3K4me3 was detected.

E also affected the expression of ERα coactivators in the VMH of female mice. Histone modifications are induced by protein complexes, including certain enzymes that are recruited by transcription factors to promote transcription of hormone-sensitive target genes. Thus, we examined the mRNA expression levels of specific transcriptional coregulators. For example, we found that two SRC isoforms with intrinsic histone acetyltransferase activity, SRC1 and SRC2, were highly regulated by E exposure; they were significantly upregulated at the 3-hour time point. However, 5 hours after E treatment SRC1 stays elevated while SRC2 mRNA levels returned to baseline. Thus, in principle, these and similar findings reported in Gagnidze et al. (2013) established the basis for the construction of an ER-organized transcriptional complex in VMH, but the timing of coregulator induction remains to be explained.

Larissa Faustino, from São Paolo, worked with Khatuna Gagnidze (2015) and knew that elevated levels of thyroid hormones (TH) reduce estradiol (E2)-dependent female sexual behavior. E2 stimulates PR and Oxtr within the VMH and POA, critical hypothalamic nuclei for sexual and maternal behavior, respectively. Here, we investigated the impact of TH on E2-dependent transcriptional mechanisms in female mice. First, we observed that triiodothyronine (T3) inhibited the E2 induction of Pgr and Oxtr. We hypothesized that differences in histone modifications and receptor recruitment could explain the influence of TH on E2-responsive Pgr and Oxtr expression. We observed that histone H3 acetylation (H3Ac) and methylation (H3K4me3) was gene and brain-region specific. We then analyzed the recruitment of ERα and TH receptor α (TRα) on the putative regulatory sequences of Pgr and Oxtr. Interestingly, T3 inhibited E2-induced ERα binding to a specific Pgr enhancer site, whereas TRα binding was not affected, corroborating our theory that the competitive binding of TRα to an ERα-binding site can inhibit ERα transactivation and the subsequent E2-responsive gene expression. On the Oxtr promoter, E2 and T3 worked together to modulate ERα and TRα binding. Finally, the E2-dependent induction of cofactors was reduced by hypothyroidism and T3. Thus, we determined that the Pgr and Oxtr promoter regions are responsive to E2 and that T3 interferes with the E2 regulation of Pgr and Oxtr expression by altering the recruitment of receptors to DNA and changing the availability of cofactors. Collectively, our findings provide

insight into molecular mechanisms of response to E2 and TH interactions controlling sex behavior in the hypothalamus.

One of the consequences of neonatal androgen exposure during the neonatal critical period for sexual differentiation is the absence of lordosis behavior in the male. Molecular biologist Rod Scott joined the lab from Glasgow (Scott, Wu-Peng, and Pfaff, 2002) to measure protein binding to the PR EREs: PR-A codes the shorter PR transcript, compared with PR-B, which codes the longer PR transcript. In brief, the greater estrogen effect on PR-B transcription in the female compared with the male likely contributes to the greater effect of progesterone on lordosis behavior in females.

Transcriptional Systems Overdetermining Reproductive Behavior

As previously noted, we have at least four examples of multiplicative transcriptional systems revved up by estradiol: (1) both GnRH and the GnRH receptor; (2) both oxytocin and Oxtr; (3) both tyrosine hydroxylase for the production of norepinephrine and the norepinephrine receptor alpha-1; and (4) both the opioid peptide enkephalin and its receptor the delta opioid receptor.

Furthermore, we were able to identify specific physiological roles for some of the estrogen-amplified transcriptional systems. I have a couple of examples: PR transcription clearly *amplifies* the estrogen effect on behavior. GnRH clearly *synchronizes* female reproductive behavior with ovulation, thus helping to guarantee a biologically productive behavior. Estrogen-stimulated nitric oxide coordinates with glutamatergic neurons to heighten behavioral excitability, allowing the generation of a heightened arousal state necessary for the initiation of mating behavior by the female.

These results and the *in situ* hybridization studies mentioned earlier show that several transcriptional systems participate in overdetermining female reproductive behavior at the time of ovulation. We conducted epigenetic studies to begin to envision how those transcriptional systems are themselves controlled.

Genes Regulating Behavior

The ERα gene offers a starkly clear example of a gene product regulating behavior. Microinjection, into the VMH, of an adeno-associated virus encoding a small interfering RNA to depress ER reduces lordosis behavior virtually to zero and instead causes the female actively to reject the male (Ogawa Eng et al., 1998). Furthermore, when postdoc Sergei Musatov joined the lab to work with the brilliant molecular biologist Michael Kaplitt, they used microinjections of a short-hairpin RNA, interfering RNA, into VMH to abolish lordosis behavior (Musatov et al., 2006). ER gene knockout (ERKO)

females rejected males for at least two reasons (Ogawa et al., 1996): They were less responsive to somatosensory stimuli of the sort that ordinarily would trigger lordosis, and the ERKO females were treated as invading males by the resident stud males who attacked the ERKO females.

Another crucial component of reproduction, maternal behavior, is not controlled by vlVMN neurons but instead by medial POA neurons located farther anterior in the basal forebrain. Postdoc Ana Ribeiro in the lab (Ribeiro et al., 2012) used microinjections of a small-interfering RNA against ER transcription into the medial POA and completely abolished maternal behavior: Treated mothers did not retrieve babies to the nest, did not lick them to keep babies moist and warm, and did not nurse them.

Estrogen Receptor Beta

Neuroscientists and especially neuroendocrinologists were intrigued when the laboratory of Jan-Ake Gustafsson discovered a second ER, beta (ß), of physiological importance throughout the body (Pettersson and Gustafsson, 2001). Sonoko Ogawa (1999) working with Jan-Ake found that females' lordosis behavior is stronger than normal in betaERKO mice, whereas males with both ERα and ERß knocked out did not show any components of sexual behaviors (Ogawa et al., 2000). It was especially interesting that the aggressive behaviors of male mice are increased by ERß gene disruption (Nomura et al., 2002, 2006).

ERß plays a prominent role in the regulation of emotional behaviors. For example, female mice without a functional ERß showed enhanced anxiety, which went along with lower serotonin content in several brain regions (Imwalle et al., 2005). In another experiment, Kazuya Tomihara gonadectomized ERß knockout (betaERKO) female mice, and their wild-type (betaWT) littermates were implanted with several different doses (experiment 1: 2.0 microg/day; experiment 2: 1.0, 0.4, 0.2 or 0.1 microg/day) of an estradiol benzoate (EB) or placebo pellet. Ten days after pellet implant, behavioral tests commenced to measure anxiety levels (experiment 1: light-dark transition test [LDT]; experiment 2: LDT, elevated plus maze test [EPM] and social investigation test [SIT]). We found that, at higher doses, long-term treatment of EB had anxiogenic effects in both betaWT and betaERKO mice, as indicated by a decrease of the time spent in the light side and the number of transitions between two sides during LDT (Tomihara et al., 2009).

BetaERKO neuronal survival later in life is compromised (Koehler et al., 2005), suggesting that the roles for this gene product in cognitive decline among the elderly need further investigation. This is an especially compelling topic because Emilie Rissman, working with Jan-Ake, showed that ERß is required for normal spatial learning (Rissman et al., 2002).

Particularly notable are roles for ERß in brain development (Koehler et al., 2005). One sign of this is the fivefold reduction of ERß deletion on genes involved in neuronal migration and axon guidance and genes involved in growth and differentiation. For example, in betaERKO, newborn neurons cannot migrate to their correct positions in the cerebral cortex. Furthermore, the development of sexual dimorphisms in brain and behavior, particularly defeminization in the male, depends on ERß (Kudwa et al., 2005).

Micronet

Elena Choleris, working with Jan-Ake Gustafsson (2003), did some thinking about how estrogens control many physiological and behavioral processes, some of which are connected to reproduction. These include sexual and other social behaviors. They implicate four gene products in a micronet required for mammalian social recognition, through which an individual learns to recognize other individuals. Female mice whose genes for the neuropeptide oxytocin (OT), ERß, or ERα have been selectively "knocked out" were deficient specifically in social recognition and social anxiety. There was a remarkable parallelism among results from these three separate gene knockouts. The data strongly suggest the involvement in social recognition of the four genes coding for ERα, ERß, OT, and the OT receptor. They thus proposed a four-gene micronet, which links hypothalamic and limbic forebrain neurons in estrogen control over OT regulation of social recognition. In our model, estrogens act on the OT system at two levels: Through ERß, they regulate the production of OT in the hypothalamic paraventricular nucleus, and through ERα, they drive the transcription of the OT receptor in the amygdala. The proper operation of a social recognition mechanism allows for the expression of appropriate social behaviors, aggressive or affiliative.

Gonadotropin Releasing Hormone

We paid a lot of attention to the neuropeptide gonadotropin releasing hormone (GnRH) because of its dual role in priming the anterior pituitary gland to orchestrate the hormonal basis for ovulation, coordinated with GnRH's role in fostering lordosis behavior (Pfaff, 1973).

In 1973 it was easier to do research on brain and behavior than it is now. The bureaucracy, most of it the product of federal regulations, has increased substantially. Thus, I got the idea of direct effects of GnRH on lordosis behavior about 9 p.m. on a Tuesday, and I ordered the animals from Charles River Laboratories about 9 a.m. on Wednesday. The entire experiment was done in the time it takes now to get an animal use protocol

approved. The positive effect of GnRH on lordosis behavior was published in *Science* (1973).

A supremely talented histochemist and neuroanatomist in my lab, Marlene Schwanzel-Fukuda, was doing a standard developmental study of the beginning of GnRH gene expression in the mouse embryo. After she had collected a lot of data, she invited me to review it with her. At embryonic 11–13 days after birth, major GnRH expression was seen in the olfactory placode, outside the brain. Then, 12–16 days after birth, major expression appeared in GnRH cells on the nasal septum. Then, days 13–20 GnRH cells were detected in the arch above the nasal placode and in the space below the developing forebrain. Then, by day 20 after birth, GnRH neurons could be seen in the POA, their functional location throughout the rest of life (Schwanzel-Fukuda, Nature, 1989).

As I looked over Marlene's shoulder, my memory flashed back to my days as a first-year graduate student in Joe Altman's lab at MIT. I was helping Joe chart the migrations of newly born neurons in the hippocampus—Joe had discovered postnatal neurogenesis, thus overturning the dogma that "at the time of birth, vertebrates have all the neurons they will ever have." Anyway, that flashback caused me to blurt out "Marlene, you have discovered a migration of newly born GnRH neurons from the nose into the brain!" These are the only neurons born outside the brain that migrate into the brain.

The data in the embryonic mouse nose and brain were clear, and I wondered if Marlene might have discovered a vertebrate-wide phenomenon. At the time, we had a fish brain expert in the lab, Ishwar Parhar, who came to us from Singapore. Quickly, Ishwar did the same type of developmental series in the brain of a salmonid fish (Parhar et al., 1995) followed by work with another fish, tilapia (Parhar et al., 1996) and obtained evidence that was equivalent to Marlene's for the developmental migration of GnRH neurons from the olfactory apparatus into the forebrain. Over the years, it became clear that GnRH migration turned out to be true for all vertebrates studied.

From this, and from confirming evidence in other labs, two stories emerged, one funny and one serious.

Marlene's histochemistry and the raw data illustrated in her posters and papers were so beautiful—she was a real scientific artist—that her slides made beautiful material for talks to nonscientists. I gave one such late-afternoon talk at Rockefeller to a lot of lawyers considering contributions to Rockefeller. Here was the drill: The lawyers would come to campus, have a couple of drinks, listen to me, and then have dinner. After my talk, which featured Marlene's beautiful histochemical slides on GnRH, many attendees, including me, went downstairs to the restroom before dinner.

As I was going downstairs, a guy coming upstairs, his eyes a bit glazed, said, "You just gave a talk, right?"

I said, "right!"

He followed up: "It was about those sex cells going from the nose into the brain, right?"

I said, "right!"

Then his face lit up and he said, "Now I know why, when my wife had a nose job, she lost her interest in sex!"

A much more serious topic had to do with human disease. Kallmann's Syndrome, inherited hypogonadotropic hypogonadism coupled with anosmia, is associated with an X chromosome deletion at Xp 22.3. Kallmann's Syndrome is a well-recognized disorder that prevents a person from going through puberty, which means no reproduction and no reproductive behavior. In a Kallmann fetus we found an absence of GnRH neurons in the brain despite dense clusters of GnRH cells in the nose (Schwanzel-Fukuda et al., 1989b). It turned out that our neuroanatomical work in New York was complementary to the genetic work of Christene Petit, in Paris. She demonstrated the Kall gene mutation, which gave rise to our results.

NCAM

We got the idea that neural cell adhesion molecules (NCAM) might be involved in the likely complex mechanisms of the GnRH neuron migration. It turned out that NCAM immunoreactive cells set up a scaffolding along the migration route (Schwanzel-Fukuda et al., 1992), and as the GnRH neurons traverse the boundary between the olfactory pit and the forebrain, GnRH neurons are never seen to be independent of this NCAM scaffolding. Later we showed that NCAM was expressed in a population of cells in the olfactory placode of humans and then throughout the GnRH cell migration route (Schwanzel-Fukuda et al., 1996).

The chemistry of GnRH is interesting. The protein chemist Tom Kaiser at the University of Chicago had gotten a lot of attention for showing that certain physiologically important peptide hormones are "amphiphilic": One face of a peptide hormone would be lipophilic (hydrophobic) and the other face would be hydrophilic. That is, alternating sidechains with these properties would project below or above the peptide backbone. To test the physiological importance of the amphiphilic structure, Jenn-Tser Pan came from Taiwan to work with Lee-Ming Kow and me (Pan et al., 1986), which began our collaboration with Tom Kaiser. We used micropipettes to record from hypothalamic and preoptic neurons that responded to GnRH. About 70 percent of GnRH-responsive neurons also responded to an amphiphilic analog. We thus suspected that the amphiphilic property of GnRH accounts for an important part of its mechanism of action as a neuropeptide.

Indirect Genetic Actions on Behavior

The absence of reproductive behavior in Kallmann's patients (noted previously) shows how a genetic change can affect human behavior indirectly, in this case, through a chain of seven clearly defined steps: (1) Males with Kallmann's have no libido, no sexual desire, *because* (2) they have very low testosterone, *because* (3) there are very low concentrations of gonadotropic hormones coming from the pituitary gland, *because* (4) the pituitary is not receiving GnRH from the hypothalamus, *because* (5) the GnRH neurons are not in the hypothalamus, *because* (6) of the failure of the GnRH neuronal migration from the nose to the brain, and *because* (7) of the absence of the cell surface protein normally produced by the Kall gene at Xp22.3.

Principle: Gene/Behavior Causal Relations

Because of the just-mentioned series of seven causal steps and myriad other examples from this autobiography, genetic contributions to the regulation of behavior—lab animals and, importantly, humans—are not to be doubted. Capsulizing some contributions from my lab, the easiest work was using gene knockout mice (e.g., ERKO alpha and beta) to affect behavior, and the hardest work was specific microinjections of AAV vectors and specific microRNAs into discrete neuroanatomical cell groups in the forebrains of lab animals.

These techniques, in a paragraph, demonstrated the regulation of three types of behavior required for reproduction: (1) ERα expression in neurons of the lateral POA is required for female courtship behaviors; (2) expression of that same gene in neurons of vlVMN hypothalamus is required for female mating behavior; and (3) finally, expression of that same gene in medial preoptic neurons is required for maternal behaviors.

Central Nervous System Arousal

Having worked out the motivational mechanisms for one behavior, our lab expanded our focus to ask: Does a form of motivation exist that helps to initiate *all* behaviors? The lab formulated the concept of generalized central nervous system (CNS) arousal (Pfaff, 2006), came up with a physical theory (Pfaff and Banavar, 2007), produced evidence that it exists, and has continued working on its mechanisms. We started by showing that neurons of the reticular formation's NGC foster generalized CNS arousal (Wu, Stavarache, et al., 2007; Martin et al., 2011; Tabansky et al., 2018). Now we are considering the likelihood that vestibular-influenced NGC neurons have especially important arousing influences through projections both to the spinal cord and toward the forebrain. Of course, the concept of and work on generalized arousal does not contradict work on the somewhat

more complex arousing influences of, for example, norepinephrine and dopamine.

Intellectual and Educational Insight

Many years ago, famed MIT professor Nicholas Negroponte quit his job at the MIT Media Lab to start the program called "One Laptop per Child." It was successful in distributing free computers to children in economically developing countries. These instruments did not need line electricity—they could be charged just by winding a handle. Because access was so easy, it was said that some African children learned "Google" as their first word in English.

Inspired by Negroponte, I received permission from Springer to write a neuroscience text similarly sent for free to scholars in countries with low gross domestic products per capita. Nora D. Volkow, a medical doctor, joined me as coeditor of the second volume, and John L. Rubenstein joined us as coeditor of the third volume, which now features 161 chapters in five volumes. The third edition was much expanded by an emphasis on molecular developmental neuroscience, thus becoming the most comprehensive neuroscience text ever (so far) to be made available for free in countries with the poorest economies.

More recently, I've been disturbed by newspaper stories asserting the unreliability of certain scientific papers, especially because research on brain and behavior, always fraught with multiple contingencies, can be potentially susceptible to such problems. In response, I wrote a book for MIT Press (Pfaff and Sherman, 2024) citing major "wins" for behavioral science and associated neuroscience. It has a wide scope in time (research from two centuries ago up to 2023) and in subject matter (from psychophysics to social behavior). Hopefully, it will be received as a proper defense of our field.

Reflection

The work covered included an early demonstration of a body-to-brain-to-behavior causal connection. My lab began by asking whether it is possible to demonstrate the mechanisms causing the production of a vertebrate behavior. The answer is yes. Together with our team, we worked out the mechanisms that produce a simple social behavior essential for reproduction. The work proceeded in four steps: (1) discover hormone receptors in the brain; (2) work out the neural circuitry; (3) show facilitation of behaviorally relevant gene expression by the behaviorally effective steroid hormone (an estrogen); and (4) demonstrate that knocking out the genes for ERs or estrogen-sensitive genes significantly reduces the social behavior. Thus,

working out mechanisms for a vertebrate (here, a mammalian) behavior is proven possible.

Our lucky break came from the fact that the hormone receptors I had discovered in the brain turned out to be ligand-activated transcription factors. This opened an intellectual interstate highway linking molecular biology to neuroscience to behavior.

In this autobiography, which reviews many discoveries, I haven't expressed how much fun it was to work, over 60 years, with about 500 younger scientists who usually were easy to work with and sometimes had great senses of humor. About 30 feet from my office door, for example, two brilliant electrophysiologists, Paola Calderon and Alex Proekt, could be heard chuckling at one joke after another. It was a joy to sit at my computer listening to them. Earlier, Charles Mobbs was running giant two-dimensional gels that took a long time to run. While they ran, Charles, who loved show business, went out into his marble-floored hallway and practiced tap dancing before a large mirror. The Rockefeller scientific environment lends itself to high-level, pleasant scientific involvement, tending to avoid scrapes of the sort I've seen elsewhere.

Looking Forward

We have been working during a boom in neuroscience. Indeed, just reading the exciting biographies of the neuroscientists in these 13 Society for Neuroscience volumes has heightened my understanding of how lucky I've been to work in this field. It is easy to look forward, anticipating a golden age of neuroscience when all the techniques my lab has used will be augmented both by the data crunching and sophisticated modeling made possible by supercomputers, as well as by the evolution of medical practices better prepared to absorb and apply new neuroscientific knowledge. My own work will play out in a greater understanding and therapeutic treatment of emotional disorders, especially those linked to hormones.

References

Brink, E. E., Morrell, J. I., and Pfaff, Donald W. (1979) Localization of lumbar epaxial motoneurons in the rat. *Brain Research*, 170: 23–41.

Brink, E. E., and Pfaff, D. W. (1980) Vertebral muscles of the back and tail of the albino rat (*Rattus norvegicus albinus*). *Brain Behav. Evol.*, 17: 1–47.

Brink, E. E., and Pfaff, D. W. (1982) Supraspinal and segmental input to lumbar epaxial motoneurons in the rat. *Brain Research*, 226: 43–60.

Ceccatelli, S., Grandison, L., Scott, R. E. M, Pfaff, D. W., and Kow, L.-K. (1996) Estradiol regulation of nitric oxide synthase mRNAs in rat hypothalamus. *Neuroendocrinology*, 64: 357–363S.

Choleris, E., Gustafsson, J.-A., Korach, K. S., Muglia, L. J., Pfaff, D. W., Ogawa, S. O. (2003) An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-alpha and -beta knockout mice. *Proc Natl Acad Sci USA* 213;100(10): 6192–197.

Chung, S. R., and Pfaff, D. W., and Cohen, R. S. (1988) Estrogen-inducted alterations in synaptic morphology in the midbrain central gray. *Experimental Brain Research*, 69: 522–530.

Chung, S. R., Pfaff, D. W., and Cohen, R. S. (1990a) Projections of ventromedial hypothalamic neurons to the midbrain central gray: An ultrastructural study. *Neuroscience*, 38: 395–407.

Chung, S. R., Pfaff, D. W., and Cohen, R. S. (1990b) Transneuronal degeneration in the midbrain central gray following chemical lesions in the ventromedial nucleus: A qualitative and quantitative analysis. *Neuroscience*, 38(2): 409–426.

Chung, S. R., McCabe, J. T., and Pfaff, D. W. (1991) Estrogen influences on oxytocin mRNA expression in preoptic and anterior hypothalamic regions studied by in situ hybridization. *J. Comp. Neurol.*, 307: 281–295.

Cohen, M. S., Schwartz-Giblin, S., and Pfaff, D. W. (1987) Brainstem reticular stimulation facilitates back muscle motoneuronal responses to pudendal nerve input. *Brain Research*, 405: 155–158.

Cohen, M. S., Schwartz-Giblin, S., and Pfaff, D. W. (1987) Effects of total and partial spinal transections on the pudendal nerve-evoked response in rat lumbar axial muscle. *Brain Research*, 401: 103–112.

Cohen, R., and Pfaff, D. W. (1981) Ultrastructure of neurons in the ventromedial nucleus of the hypothalamus in ovariectomized rats with or without estrogen treatment. *Cell and Tissue Research*, 217: 451–470.

Cottingham, S. L., and Pfaff, D. W. (1986) Interconnectedness of steroid hormone-binding neurons: Existence and implications. In *Current Topics of Neuroendocrinology*, eds. D. Ganten and D. W. Pfaff, Vol. 7, 223–250. Springer-Verlag.

Cottingham, S. L., Femano, P. A., and Pfaff, D. W. (1987a) Electrical stimulation of the midbrain central gray facilitates reticulospinal activation of axial muscle EMG. *Experimental Neurology*, 97: 704–724.

Cottingham, S. L., and Pfaff, D. W. (1987b) Electrical stimulation of the midbrain central gray facilitates lateral vestibulospinal activation of back muscle EMG in the rat. *Brain Research*, 421: 397–400.

Davis, P. G., Krieger, M. S., Barfield, R. J., McEwen, B. S., and Pfaff, D. W. (1982) The site of action of intrahypothalamic estrogen implants in feminine sexual behavior: An autoradiography analysis. *Endocrinology*, 111: 1581–1586.

Davis, P. G., McEwen, B., and Pfaff, D. W. (1979) Localized behavioral effects of tritiated estradiol implants in the ventromedial hypothalamus of female rats. *Endocrinology*, 104: 898–903.

Davis, R. E., Morrell, J. I., and Pfaff, D. W. (1977) Autoradiography localization of sex steroid-concentrating cells in the brain of the teleost Macropodus opercularis (Osteichthyes: Belonttidae). *General and Comparative Endocrinology*, 33: 496–505.

Dupré, C., Lovett-Barron, M., Pfaff, D. W., and Kow L. M. (2010) Histaminergic responses by hypothalamic neurons that regulate lordosis and their modulation by estradiol. *Proc Natl Acad Sci USA*, 107(27): 12311–12316.

Easton, A., Jasnow, A., Norton, J., Goodwillie, A., and Paff, D. W. (2004) Sex differences in mouse behavior following pyrilamine treatment: Role of histamine 1 receptors in arousal. *Pharmacology, Biochemistry, and Behavior*, 79: 563–572.

Faustino, L. C., Gagnidze, K., Ortiga-Carvalho, T. M. and Pfaff, D. W. (2015) Impact of thyroid hormones on estrogen receptor α-dependent transcriptional mechanisms in ventromedial hypothalamus and preoptic area. *Neuroendocrinology*, 101(4): 331–346.

Gagnidze, K., Weil, Z. M., Faustino, L. C., Schaafsma, S. M., and Pfaff, D. W. (2013) Early histone modifications in the ventromedial hypothalamus and preoptic area following oestradiol administration. *J. Neuroendocrinol.*, 25(10): 939–955.

Gagnidze, K., Hunter, R., and Pfaff, D. W. (2018) Molecular endocrinology of female reproductive behavior. *Molecular and Cellular Endocrinology*, 467: 14–20.

Imwalle, D. B., Gustafsson, J.-A., and Rissman, E. F. (2005) Lack of functional estrogen receptor beta influences anxiety behavior and serotonin content in female mice. *Physiol Behavior*, 84(1): 157–163.

Jones, K. J., Pfaff, D. W., and McEwen, B. S. (1985) Early estrogen-induced nuclear changes in rat hypothalamic ventromedial neurons: An ultrastructural and morphometric analysis. *J. Comparative Neurology*, 239: 255–266.

Jones, K. J., Chikaraishi, D. M., Harrington, C. A., McEwen, B. S., and Pfaff, D. W. (1986) In situ hybridization detection of estradiol-induced changes in ribosomal RNA levels in rat brain. *Molecular Brain Research*, 1: 145–152.

Jones, K. J., Harrington, C. A., Chikaraishi, D. M., and Pfaff, D. W. (1990) Steroid hormone regulation of ribosomal RNA in rat hypothalamus: Early detection using *in situ* hybridization and precursor-product ribosomal DNA probes. *J. Neuroscience*, 10(5): 1513–1521.

Koehler, K. F., Helguero, L. A., Haldosén, L.-A., Warner, M., and Gustafsson, J.-A. (2005) Reflections on the discovery and significance of estrogen receptor beta. *Endocrine Reviews*, 26(3): 465–478.

Kow, L.-M., and Pfaff, D. W. (1973) Effects of estrogen treatment on the size of receptive field and response threshold of pudendal nerve in the female rat. *Neuroendocrinology*, 13: 299–313.

Kow, L.-M., Montgomery, M., and Pfaff, D. W. (1977) Effects of spinal cord transections on lordosis in female rats. *Brain Research*, 123: 75–88.

Kow, L.-M., and Pfaff, D. W. (1976) Sensory requirements for lordosis in female rats. *Brain Research*, 101: 47–66.

Kow, L.-M., and Pfaff, D. W. (1979) Responses of single units in sixth lumbar dorsal root ganglion of female rats to mechanostimulation relevant for lordosis reflex. *J. Neurophysiol.*, 42: 203–213.

Kow, L.-M., Zemlan, F. P., and Pfaff, D. W. (1980) Responses of lumbosacral spinal s to mechanical stimuli related to analysis of lordosis reflex in female rats. *J. Neurophysiol.*, 43: 27–45.

Kow, L.-M.,and Pfaff, D. W. (1982) Responses of medullary reticulospinal and other reticular neurons to somatosensory and brainstem stimulation in anesthetized or freely-moving ovariectomized rats with or without estrogen treatment. *Experimental Brain Research*, 47: 191–202.

Kow, L.-M., and Pfaff, D. W. (1985) Estrogen effects on neuronal responsiveness to electrical and neurotransmitter stimulation: an in vitro study on the ventromedial nucleus of the hypothalamus. *Brain Research*, 347: 1–10.

Kow, L., Devidze, N., Pataky, S., Shibuya, I., and Pfaff, D. W. (2006) Acute estradiol application increases inward and decreases outward whole-cell currents of neurons in rat hypothalamic ventromedial nucleus. *Brain Research*, 1116(1): 1–11.

Krieger, M. S., Conrad, L. C. A., and Pfaff, D. W. (1979) An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. *J. Comp. Neurol.*, 183: 785–816.

Kudwa, A. E., Bodo, C., Gustafsson, J.-A., and Rissman, E. F. (2005) A previously uncharacterized role for estrogen receptor beta: defeminization of male brain and behavior. *Proc Natl Acad Sci USA*, 22;102(12): 4608–4612.

Lauber, A. H., Romano, G. J., Mobbs, C. V., Howells, R. D., and Pfaff, D. W. (1990) Estradiol induction of proenkephalin messenger RNA in hypothalamus: dose-response and relation to reproductive behavior in the female rat. *Molecular Brain Research*, 8: 47–54.

Lauber, A. H., Mobbs, C. V., Muramatsu, M., and Pfaff, D. W. (1991) Estrogen receptor mRNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. *Endocrinology*, 129(6): 3180–3186.

Lee, A. W., Kyrozis, A., Chevaleyre, V., Kow, L. M., Zhou, J., Devidze, N., Etgen, A. M., and Pfaff, D. W. (2008a) Voltage-dependent calcium channels in ventromedial hypothalamic neurones of postnatal rats: Modulation by oestradiol and phenylephrine. *J. Neuroendocrinol*. 20(2): 188–198.

Lee, A. W., Kyrozis, A., Chevaleyre, V., Kow, L. M., Devidze, N., Zhang, Q., Etgen, A. M., and Pfaff, D. W. (2008b) Estradiol modulation of phenylephrine-induced excitatory responses in ventromedial hypothalamic neurons of female rats. *Proc Natl Acad Sci USA*, 105(20): 7333–7338.

Martin, E. M., Devidze, N., Shelley, D. N., Westberg, L., Fontaine, C., and Pfaff, D. W. (2011) "Molecular and neuroanatomical characterization of single neurons in the mouse medullary gigantocellular reticular neurons. *J. Comparative Neurology*, 519(13): 2574–2593.

Meisel, R., and Pfaff, D. W. (1985) Brain region specificity in estradiol effects on neuronal ultrastructure in rats. *Molecular and Cellular Endocrin.*, 40: 159–166.

Mobbs, C. V., Harlan, R. E., Burrous, M. R., and Pfaff, D. W. (1988) An estradiol-induced protein synthesized in the ventral medial hypothalamus and transported to the midbrain central gray. *J. Neuroscience*, 8: 113–118.

Mobbs, C., Fink, G., Johnson, M., Welch, W., and Pfaff, D. W. (1989) Similarity of an estrogen-induced protein and a luteinizing hormone releasing hormone induced protein. *Molecular and Cellular Endocrinology*, 62: 297–306.

Mobbs, C. V., Fink, G., and Pfaff, D. W. (1990) HIP-70: A protein induced by estrogen in the brain and LH-RH in the pituitary. *Science*, 247: 1477–1479.

Morrell, J. I., and Pfaff, D. W. (1982) Characterization of estrogen-concentrating hypothalamic neurons by their axonal projections. *Science*, 217: 1273–1276.

Morrell, J. I., Wolinsky, T. D., Krieger, M. S., and Pfaff, D. W. (1982) Autoradiographic identification of estradiol-concentrating cells in the spinal cord of the female rat. *Experimental Brain Research*, 45: 144–150.

Musatov, S., Chen, W., Pfaff, D. W., Kaplitt, M. G., and Ogawa, S. (2006) RNAi-mediated silencing of estrogen receptor (alpha) in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. *Proc Natl Acad Sci USA*, 103(27): 10456–10460.

Nomura, M., Durbak, L., Chan, J., Smithies, O., Gustafsson, J.-A., Korach, K. S., Pfaff, D. W., and Ogawa, S. (2002) Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor beta knockout (betaERKO) male mice. *Horm Behavior*, 41(3): 288–296.

Nomura, M., Andersson, S., Korach, K. S., Gustafsson, J.-A., Pfaff, D. W., and Ogawa, S. (2006) Estrogen receptor-beta gene disruption potentiates estrogen-inducible aggression but not sexual behaviour in male mice. *Eur. J. Neuroscience*, 23(7): 1860–1868.

Ogawa, S., Olazabal, U. E., Parhar, I. S., and Pfaff, D.W. (1994) Effects of intrahypothalamic administration of antisense DNA for progesterone receptor mRNA on reproductive behavior and progesterone receptor immunoreactivity in female rat. *J. Neuroscience*, 14: 1766–1774.

Ogawa, S., Taylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1996) Reversal of sex roles in genetic female mice by disruption of estrogen receptor gene. *Neuroendocrinology*, 64: 467–470.

Ogawa, S., Eng, V., Taylor, J., Lubahn, D., Korach, K., and Pfaff, D. W. (1998) Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology*, 139: 5070–5081.

Ogawa, S., Chan, J., Chester, A.E., Gustafsson, J., Korach, K., and Pfaff, D. W. (1999) Survival of reproduction-related behaviors in male and female estrogen receptor b deficient (bERKO) male and female mice. *Proc Natl Acad Sci USA*, 96: 12887–12892.

Ogawa, S., Chester, A. E., Hewitt, S. C., Walker, V. R., Gustafsson, J.-A., Smithies, O., Korach, K. S., Pfaff, D. W. (2000) Abolition of male sexual behaviors in mice lacking estrogen receptors α and ß (αßERKO). *Proc Natl Acad Sci USA*, 97(26): 14737–14741.

Pan, J.-T., Kow, L.-M., Kendall, D. A., Kaiser, E. T., and Pfaff, D. W. (1986) Electrophysiological test of an amphiphilic B-structure in LHRH action. *Molecular and Cellular Endocrinology*, 48: 161–166.

Parhar, I. S., Iwata, M., Pfaff, D. W., and Schwanzel-Fukuda, M. (1995) Embryonic development of gonadotropin-releasing hormone neurons in the sockeye salmon. *J. Comparative Neurology*, 362: 256–270.

Parhar, I. S., Iwata, M., Pfaff, D. W., and Schwanzel-Fukuda, M. (1996) Gonadotropin-releasing hormone gene expression in teleosts. *Molecular Brain Research*, 41: 216–227.

Parsons, B., MacLusky, N. J., Krieger, M. S., McEwen, B. S., and Pfaff, D. W. (1979) The effects of long-term estrogen exposure on the induction of sexual behavior and measurements of brain estrogen and progestin receptors in the female rat. *Hormones and Behavior*, 13: 301–313.

Parsons, B., McEwen, B. S., and Pfaff, D. W. (1982) A discontinuous schedule of estradiol treatment is sufficient to activate progesterone-facilitated feminine sexual behavior and to increase cytosol receptors for progestins in the hypothalamus of the rat. *Endocrinology*, 110: 613–619.

Parsons, B., Rainbow, T., Pfaff, D. W., and McEwen, B. S. (1982) Hypothalamic protein synthesis essential for the activation of the lordosis reflex in the female rat. *Endocrinol*, 110: 620–624.

Pettersson, K., and Gustafsson, J.-A. (2001) Role of estrogen receptor beta in estrogen action. *Annual Review of Physiology*, 63: 165–192.

Pfaff, D. W. (1965) Cerebral implantation and autoradiography studies of sex hormones. In *Sex Research: New Developments*, ed. J. Money, 219–234. Holt, Rinehart & Winston.

Pfaff, D. W. (1968) Autoradiographic localization of radioactivity in rat brain after injection of tritiated sex hormones. *Science*, 161: 1355–1356.

Pfaff, D. W. (1969) Olfactory and hormonal influences on the basal forebrain of the male rat. *Brain Research*, 15: 137–156.

Pfaff, D. W. (1973) Luteinizing hormone releasing factor (LRF) potentiates lordosis behavior in hypophysectomized ovariectomized female rats. *Science*, 182: 1148–1149.

Pfaff, D. W. (1980) *Estrogens and Brain Function: Neural Analysis of a Hormone-Controlled Mammalian Reproductive Behavior*. Springer-Verlag.

Pfaff, D. W. (1999) *Drive: Neurobiological and Molecular Mechanisms of Sexual Motivation*. MIT Press.

Pfaff, D. W. (2006) *Brain Arousal and Information Theory: Neural and Genetic Mechanisms*. Harvard University Press.

Pfaff, D. W. (2017) *How the Vertebrate Brain Regulates Behavior: Direct from the Lab*. Harvard University Press.

Pfaff, D. W. (2019) *How Brain Arousal Mechanisms Work: Paths Toward Consciousness*. Cambridge University Press.

Pfaff, D. W. (2020) *Origins of Human Socialization*. Academic Press/Elsevier.

Pfaff, D. W., and Banavar, J. R. (2007) Hypotheses: A theoretical framework for CNS arousal. *BioEssays*, 29(8): 803–810.

Pfaff, D. W., Gagnidze, N., and Hunter, R. (2017) Estrogen-induced histone modifications. *Molecular and Cellular Endocrinology*, 467: 14–20.

Pfaff, D. W., and Keiner, M. (1973) Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J. Comp. Neurol.*, 151: 121–158.

Pfaff, D. W., and Lewis, C. (1974) Film analyses of lordosis in female rats. *Hormones and Behavior*, 5: 317–335.

Pfaff, D. W., Montgomery, M., and Lewis, C. (1977) Somatosensory determinants of lordosis in female rats: Behavioral definition of the estrogen effect. *J. Comp. Physiol. Psychol.*, 91: 134–145.

Pfaff, D. W., Phillips, M. I., and Rubin, R. T. (2004) *Principles of Hormone/Behavior Relations*. Elsevier/Academic Press.

Pfaff, D. W., Rosello, L., and Blackburn, P. (1984) Proteins synthesized in medial hypothalamus and transported to midbrain in estrogen-treated female rats. *Experimental Brain Research*, 57: 204–208.

Pfaff, D. W., with Sherman, S. (2014) *The Altruistic Brain: How We Are Naturally Good*. Oxford University Press.

Pfaff, D. W., and Sherman, S. (2024) *Laws of Human Behavior: Steps Toward Hard Science*. MIT Press.

Pfaff, D. W., Volkow, N. D., and Rubenstein, J. L., eds. (2022) *Neuroscience in the 21st Century: From Basic to Clinical*. 3rd ed. Springer Verlag. (This five-volume text is free electronically in developing countries.)

Pfaff, D. W, Volkow, N. D., and Rubenstein, J. L., eds. (2023) *Neuroscience in the 21st Century*. 3rd ed. Springer.

Quiñones-Jenab, V., Jenab, S., Ogawa, S., Funabashi, T., Weesner, G. D., and Pfaff, D. W. (1996) Estrogen regulation of gonadotropin-releasing hormone receptor messenger RNA in female rat pituitary tissue. *Molecular Brain Research*, 38: 243–250.

Quiñones-Jenab, V., Jenab, S., Ogawa, S., Adan, R. A. M., Burbach, P. H., and Pfaff, D. W. (1997) Effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the uterus, pituitary and forebrain of the female rat. *Neuroendocrinology*, 65: 9–17.

Quiñones-Jenab, V., Ogawa, S., Jenab, S., and Pfaff, D. W. (1996) Estrogen regulation of preproenkephalin messenger RNA in the forebrain of female mice. *J. Chem. Neuroanatomy*, 12: 29–36.

Rachman, I., Unnerstall, J., Pfaff, D. W., and Cohen, R. (1998) Regulation of neuronal nitric oxide synthase mRNA in lordosis-relevant neurons of the ventromedial hypothalamus following short-term estrogen treatment. *Molecular Brain Research*, 59: 105–108.

Rhodes, C. H., Morrell, J. I., and Pfaff, D. W. (1981) Distribution of estrogen-concentrating, neurophysin-containing magnocellular neurons in the rat hypothalamus as demonstrated by a technique combining steroid autoradiography and immunohistology in the same tissue. *Neuroendocrinology*, 33: 18–23.

Ribeiro, A. C., Musatov, S., Shteyler, A., Simanduyev, S., Arrieta-Cruz, I., Ogawa, S., Pfaff, D. W. (2012) siRNA silencing of estrogen receptor-α expression specifically in medial preoptic area neurons abolishes maternal care in female mice. *Proc Natl Acad Sci USA*, 109(40): 16324–16329.

Rissman, E. F., Heck, A. L., Leonard, J. E., Shupnik, M. A., and Gustafsson, J.-A. (2002) Disruption of estrogen receptor beta gene impairs spatial learning in female mice. *Proc Natl Acad Sci USA*, 99(6): 3996–4001.

Robbins, A., Schwartz-Giblin, S., and Pfaff, D. W. (1990) Ascending and descending projections to medullary reticular formation sites which activate deep lumber back muscles in the rat. *Experimental Brain Res.*, 80: 463–474.

Romano, G. J., Mobbs, C. V., Lauber, A., Howells, R. D., and Pfaff, D. W. (1990) Differential regulation of proenkephalin gene expression by estrogen in the ventromedial hypothalamus of male and female rats: implications for the molecular basis of a sexually differentiated behavior. *Brain Research*, 536: 63–68.

Rothfeld, J. M., Hejtmancik, J. F., Conn, P. M., and Pfaff, D. W. (1987) LHRH messenger RNA in neurons in the intact and castrate male rat forebrain studied by in situ hybridization. *Experimental Brain Research*, 67: 113–118.

Rothfeld, J., Hejtmancik, J. F., Conn, P. M., and Pfaff, D. W. (1989) In situ hybridization for LHRH mRNA following estrogen treatment. *Molecular Brain Research*, 6: 121–125.

Sakuma, Y., and Pfaff, D. W. (1979) Facilitation of female reproductive behavior from mesencephalic central gray in the rat. *American Journal of Physiology*, 237: R278–R284.

Sakuma, Y., and Pfaff, D. W. (1979) Mesencephalic mechanisms for integration of female reproductive behavior in the rat. *American Journal of Physiology*, 237: R285–R290.

Sakuma, Y., and Pfaff, D. W. (1981) Electrophysiologic determination of projections from ventromedial hypothalamus to midbrain central gray: Differences between female and male rats. *Brain Research*, 225: 184–188.

Sakuma, Y., and Pfaff, D. W. (1982) Properties of ventromedial hypothalamic neurons with axons to midbrain central gray. *Experimental Brain Research*, 46: 292–300.

Schwanzel-Fukuda, M., and Pfaff, D. W. (1989a) Origin of luteinizing hormone-releasing hormone neurons. *Nature*, 338: 161–164.

Schwanzel-Fukuda, M., Bick, D., and Pfaff, D. W. (1989b) Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. *Molecular Brain Research*, 6: 311–326.

Schwanzel-Fukuda, M., Abraham, S., Crossing, K. L., Edelman, G. M., and Pfaff, D. W. (1992) Immunocytochemical demonstration of neural cell adhesion molecule (NCAM) along the migration route of luteinizing hormone-releasing hormone (LHRH) neurons in mice. *J. Comp. Neurology*, 321: 1–18.

Schwanzel-Fukuda, M., Abraham, S., Reinhard, G. R., Crossin, K. L., Edelman, G. M., and Pfaff, D. W. (1994) Antibody to neural cell adhesion molecule (NCAM) can disrupt the migration of luteinizing hormone-releasing hormone (LHRH) nurons into the mouse brain. *J. Comparative Neurology*, 342: 174–185.

Schwanzel-Fukuda, M., Crossin, K. L., Pfaff, D. W., Bouloux, P. M. G., Hardelin, J.-P., and Petit, C. (1996) Migration of LHRH neurons in early human embryos: Association with neural cell adhesion molecules. *J. Comp. Neurol.*, 366: 547–557.

Scott, R. E. M., Wu-Peng, S., and Pfaff, D. W. (2002) Regulation and expression of progesterone receptor mRNA isoforms A and B in the male and female rat hypothalamus and pituitary following estrogen treatment: a comparison. *J of Neuroendocrinology*, 14(3): 175–83.

Tabansky, I., Liang, Y., Frankfurt, M., … Friedman, J., Stern, J. N. H., and Pfaff, D. W. (2018) Molecular profiling of reticular gigantocellularis neurons indicates that eNOS modulates environmentally dependent levels of arousal. *Proc Natl Acad Sci USA*, 115: E6900–6909.

Tomihara, K., Soga, T., Nomura, M., Korach, K. S., Gustafsson, J.-A., Pfaff, D. W., and Ogawa, S. (2009) Effect of ER-beta gene disruption on estrogenic regulation of anxiety in female mice. *Physiol Behavior*, 96(2): 300–306.

Wu, H., Stavarache, M., Pfaff, D. W., and Kow, L. (2007) Arousal of cerebral cortex electroencephalogram consequent to high-frequency stimulation of ventral medullary reticular formation. *Proc Natl Acad Sci USA*, 104(46): 18292–18296.