

Estrogen Actions in the Brain: A Symposium  
to Honor the Contributions of Roger A. Gorski.  
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This meeting, a satellite symposium to the Society for Neuroscience meeting in Los Angeles, was opened by its organizer, Paul Micevych. He thanked the UCLA sponsors (the Brain Research Institute, the Laboratory of Neuroendocrinology, and the School of Medicine) and welcomed Roger's former students, postdoctoral fellows, and collaborators and friends. He highlighted several of Roger Gorski's most notable contributions to date. Among these were the conceptualization of the control of luteinizing hormone release, as tonic in males and cyclic in females, and the description of structural sexual dimorphisms in the brains of rodents and humans. Both of these contributions have profoundly impacted the thinking of several generations of neuroscientists.

**SESSION ONE:  
ESTROGENS AND HYPOTHALAMIC FUNCTION**

**Y. Arai** (Juntendo Univ., Tokyo, Japan) chaired the first session and opened with an overview of how Roger Gorski's research has influenced the field of neuroendocrinology. Although previous groups had identified ties between the hypothalamus and pituitary and gonadal synchronization, Gorski was among the first to suggest that ovulation was controlled by a pulse generator for luteinizing hormone that was located in the medial preoptic area (MPO) of the hypothalamus. This has become the basis of our current understanding of ovulatory regulation. His subsequent studies to understand the sexual differentiation of the mammalian brain not only delineated that formation of a male phenotype required the availability of testicular hormones during specific critical periods in prenatal and perinatal development, but also identified a number of structural anatomical sex differences, such as the sexually dimorphic nucleus of the preoptic area (SDN-POA), an area

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of discovery for which he is most widely known. An ongoing focus of the research in Gorski's laboratory has been determining whether sex-specific anatomical differences can be correlated with sex-specific behaviors in the rodent; more recent work has extended these animal studies to parallel work with human subjects which asks whether hormone-dependent sexual differentiation of the brain may play a role in the development of differences in cognitive performance, partner preference, or gender identity.

**R. Simerly** (Oregon Regional Primate Center, Portland, USA) presented work using anatomical techniques and organotypic explants to determine the cellular mechanisms that underlie the development of sexually dimorphic neural pathways and structures. The focus was on the formation and characterization of the anteroventral periventricular nucleus (AvPV) in the rostral hypothalamus, a sexually dimorphic area that has been identified as developmentally sensitive to levels of perinatal gonadal steroids. The AvPV has a larger nuclear volume in the female rat than in the male rat and AvPV neurons send projections to gonadotropin releasing hormone (GnRH) neurons involved in timing ovulation. Studies identified the period during which high levels of circulating gonadal steroids masculinize and defeminize the AvPV. He observed similar results using genetically altered (estrogen receptor alpha knockout mice) or spontaneously mutant animals (testicular feminized male, *Tfm*) that are not sensitive to the effects of gonadal steroid hormones. His group has also observed that undifferentiated perinatal explants of the AvPV responded to acutely administered gonadal steroid hormones. In addition, when AvPV explants were co-cultured with the principal part of the bed nucleus of the stria terminalis (BNSTp), a nuclear area that innervates the AvPV in the adult rat, estrogen induced BNSTp innervation of the AvPV. Continuing studies are aimed at using this approach to identify the specific events that are hormonally directed during development and that result in the sexual differentiation of structural and chemical phenotypes in the hypothalamus.

**D. Torran-Allerand** (Columbia Univ., New York, USA) reviewed some of her pioneering work identifying the inductive effects of estrogen on neurite outgrowth during development and described current efforts to determine whether these effects are due, at least in part, to interactions between estrogens and neurotrophins. Both estrogen receptors and neurotrophin receptors require phosphorylation for activation and a variety of second messengers may be involved in this regulation. Indeed, the rapid phosphorylation of receptors, transcription factors, or enzyme elements in different second messenger pathways may function as points of convergence or co-regulation of the mechanisms by which neurotrophins and estrogens mediate neuronal outgrowth, providing the basis from which cell- or tissue-specific effects may be produced. For example, in PC12 cells, estrogens and neurotrophins can reciprocally activate estrogen receptors and neurotrophin receptors. NGF phosphorylated the unliganded estrogen receptor within 10 min and estradiol increased the phosphorylation of intracellular proteins for up to 4 h. In cortical explants, estrogen induced the phosphorylation of ERK1 and ERK2 within 15 min, suggesting a nongenomic effect of the steroid, which appeared to mimic neurotrophin's effects on cellular differentiation. Her laboratory has identified a number of neuronal populations in which estrogen receptors and neurotrophin receptors are co-localized, share similar intracellular activation pathways, and may produce similar intercellular effects. Many of these neural populations are outside the hypothalamic and limbic areas traditionally thought to be involved in gonadal regulation. Thus, Torran-Allerand hypothesized that estrogen may have wide-reaching trophic effects in the developing brain, maintain functional plasticity and have a neuroprotective role in adulthood.

**R. Weiner** (University of California, San Francisco, USA) described the development of the GT1 (gonadotropin releasing hormone expressing) hypothalamic cell line, which has allowed *in vitro* studies of the mechanisms involved in pulsatile GnRH release. These cells exhibit a coordinate, spontaneous GnRH release every 20 to 30 min, which is similar in frequency to that which is measured *in vivo* in rats. These pulses are dependent on electrical activity with intercellular communication of the timing occurring through gap junctions between cells. They have also used GT1 cells to study the regulation of GnRH release resulting from stimulation of the dopamine D<sub>1</sub> receptor, which activates an intracellular cAMP-regulated second messenger pathway. Dopamine treatment causes a rapid release of cAMP into the culture medium, which precedes the release of GnRH by approximately 5 to 10 min; this has led to the hypothesis that a nucleotide-gated cation channel, analogous to that which has recently been identified in the olfactory system, may be involved in the pulsatile release of GnRH. The nucleotide-gated cation channel would depolarize the GT1 cells after dopamine stimulation, leading to an opening of voltage-gated calcium channels that would further depolarize the cells, resulting in GnRH release. Current studies in his laboratory focus on identifying the phosphoproteins that are activated by the increased intracellular cAMP/PKA or inhibited through negative feedback pathways.

## SESSION TWO: SEX DIFFERENCES IN HUMANS

**R. Green** (University College, London, UK) introduced the second session by discussing the importance of applying measures of gender identity and sexual orientation to research in the field of sex differences in humans. While behavioral correlates of sexual differentiation are frequently studied in other animals, social relationships, environmental influences, and cognitive development make similar inferences very difficult to make for humans. His own work examining correlations between right- and left-handedness or finger-print ridge asymmetry with gender identity in transsexual, heterosexual, and homosexual subjects has suggested that genetic sex may be further influenced in adulthood not only by cyclical endogenous or exogenous steroid hormones, but also by other psychological and physiological elements to which an individual is exposed.

**M. Hines** (City Univ., London, UK) then spoke about her research on the influences of gonadal steroid hormones on human behavior, gender identity, and sexual orientation. To facilitate this, she studies groups of human subjects who have genetic mutations or had prenatal exposure to exogenous steroids that may have affected their sexual differentiation. For example, genetic females born with congenital adrenal hyperplasia (CAH) have increased levels of circulating adrenal steroids during critical periods of sexual differentiation, which result in masculinized external genitalia. To determine whether they are also behaviorally affected, young girls with CAH were observed during play with “gender-stereotypic” or “gender-neutral” toys, and preferences for each category were noted. As predicted, CAH girls played more with “boy-typical” toys and less with “girl-typical” toys than did control girls, suggesting that high levels of prenatal adrenal androgens both masculinize and defeminize the behavior of affected girls. Similar effects were seen in studies of women exposed in utero to diethylstilbestrol (DES), an exogenous steroid com-

pound that has deleterious masculinizing effects during development. In the general population, the majority of left-handed people are male, and most women are right-handed; if women are exposed to DES before the ninth week of fetal development, they are more likely to be left-handed than right-handed. A third population of women studied have Turner's syndrome, a condition characterized by a prenatal regression of the ovaries with intact female external genitalia. When these women were examined using a defined battery of cognitive tests on which performance is sexually dimorphic, their performance tended to be both demasculinized and defeminized, suggesting that low levels of both estrogen and testosterone during development can result in "undifferentiated" abilities on certain types of tasks. However, while gonadal steroid exposure contributes to human behavior and cognitive development, it is usually insufficient to account for the wide degree of individual variability that is observed when studying humans.

**D. Kimura** (Simon Fraser Univ., Vancouver, Canada) presented work designed to observe how cognitive function and lateralization of the cerebral hemispheres are influenced by sex and gonadal steroids. Using a number of cognitive tests to examine the verbal fluency and spatial abilities of people with localized brain lesions, she has correlated loss of skills with both the sex of the patient and the lesioned hemisphere. For example, men with left hemisphere lesions had deficits in verbal fluency, compared with control men, although similar lesions did not affect the verbal fluency of women. Alternatively, some skill losses are not correlated with sex, i.e., right-hemisphere lesions decreased the spatial abilities of men and women to a similar degree. Thus, it does not appear that wide generalizations can be made on the basis of hemispheric lateralization to describe sources of cognitive differences between men and women. It was also reported that, in normal subjects, additional correlations between gonadal steroid levels and cognitive function can be made across a short, dynamic time course. Men generally have higher circulating testosterone levels in the autumn than in the spring, and they tend to perform better on verbal tasks in the spring, when testosterone levels are lower. Conversely, women's verbal skills are better during phases of the menstrual cycle when estrogen levels are elevated. Their spatial abilities decline during cyclical periods of low circulating estrogens. These data suggest that gonadal steroids can have both long-term effects on cognitive abilities during development and dynamic effects on performance during adulthood.

In the final presentation of this session, **W. Byne** (Mt. Sinai Medical School, New York, USA) reviewed a number of conflicting studies that have attempted to locate anatomically dimorphic hypothalamic areas in humans that may be analogous to the SDN-POA of rats. While the hypothalamus is involved with regulating sexual behavior in most mammals, many authors, including the Gorski laboratory, have predicted that it may also govern sexual behavior and sexual orientation in humans. Thus, the anterior hypothalamus is being examined for sexually dimorphic structures in humans that may correlate with sexual orientation, e.g., the hypothalamic anatomy of homosexuals may be more structurally similar to that of heterosexual individuals of the opposite sex. The results of these studies have produced widely varying results, which are dependent on the source, preservation, and histological processing of the tissue, and are influenced by the nomenclature used by the researchers. Additionally, interpretation of the data has been confounded by incomplete medical histories of the subjects, because disease states or drug treatment paradigms may alter gonadal steroid levels, neural structure, or cell density. When Byne examined the hypothalamic cytoarchitectonics of autopsy material from homosexual and heterosexual men and women in which many of these variables were controlled, he saw no differences in neuronal density that correlated with sexual orientation;

however, males had more neurons in the interstitial nucleus of the hypothalamus 3 (INAH3), confirming the results of Allen and Gorski. In addition, he is attempting to identify other neural markers, such as neuropeptide expression or innervation, that may more accurately identify specific neural populations in the anterior hypothalamus. Thus, while sexual dimorphisms may exist in the human brain, the existing data are not sufficient to suggest that a biological characteristic can predict sexual orientation.

### SESSION THREE: ESTROGEN, MEMORY AND THE HIPPOCAMPUS

This session was chaired by **B. McEwen** (Rockefeller Univ., New York, USA). He introduced the topic of alteration of synaptic structure by reviewing pioneering work in his laboratory that demonstrated a rapid, estrogen-induced modulation of dendritic spines in the hippocampus. These changes in spines reflect altered synaptic inputs to hippocampal neurons and may underlie the neuroprotective actions of estrogens. This work has been expanded by several laboratories, and prominent among these are the laboratories of the speakers in this session.

**C. Woolley** (Northwestern Univ., USA) presented her work looking at how gonadal steroids influence hippocampal synapses in the adult female rat. Using Golgi-impregnated tissues, she has observed that the density of dendritic spines on the apical dendrites of pyramidal neurons in the CA1 region of the hippocampus changes during the estrous cycle, with approximately 25% fewer spines per unit area on the day of estrus, compared with the day of proestrus. Similarly, when ovariectomized female rats were treated with estrogen followed by progesterone, estrogen caused a gradual increase in spine density with progesterone inducing an additional rapid increase. Subsequently, progesterone caused a decrease in spine density, which paralleled the hormonal effects measured during the estrous cycle. Estrogen is also proconvulsive, females are more susceptible to seizures than males are and there is a decreased seizure threshold during proestrus. To determine the mechanisms, the functional anatomy of the hippocampus was studied after estrogen treatment of ovariectomized females. A 35% increase in  $^3\text{H}$ -glutamate binding to the NMDA receptor was observed, without concurrent changes in binding to the AMPA receptors in the area, and NMDA NR1 subunit immunoreactivity increased in the stratum radiatum following estrogen treatment. Electrophysiological correlates were also studied in hippocampal slices obtained from estrogen-treated, ovariectomized females. Electrical stimulation was applied to the stratum radiatum and recordings were made at CA1 with biocytin-filled pipettes, to allow subsequent anatomical identification of the neurons from which recordings were made. While estrogen treatment did not affect AMPA-mediated neuronal activity in CA1, there was increased sensitivity to NMDA-mediated receptor activity in estrogen-treated animals, which was correlated with increased estrogen-dependent dendritic spine density on the neurons from which recordings were made. Thus, estrogen can induce both rapid structural and functional changes in the adult hippocampus, indicating a high degree of synaptic plasticity that may reflect mechanisms through which estrogen promotes cognition and memory formation.

**D. Murphy** (Univ. Pennsylvania, USA) also discussed the intracellular mechanisms through which estrogen can alter hippocampal synaptic structure. Perinatal explants of the hippocampus show similar steroid-induced changes in spine density, allowing *in vitro* ma-

nipulation of the hippocampus. Treatment with  $17\beta$ -estradiol for 24 to 48 h increased the density of spines in culture, an effect that was prevented by the coadministration of tamoxifen, a mixed estrogen antagonist. Estrogen treatment also increases levels of phosphorylated CREB protein. This phosphorylation is a component of the estrogen-dependent spine formation, i.e., application to the explants of antisense oligonucleotides to CREB, which prevent CREB's phosphorylation, also prevented increased spine formation. Additionally, estrogen decreased GAD-immunoreactivity at 12 and 24 h post-treatment, reduced the levels of brain derived neurotrophic factor (BDNF), and reduced the number of spontaneous inhibitory postsynaptic currents (IPSCs). These effects may be related, since application of BDNF to serum-deprived cultures stimulates growth of GAD-immunoreactive interneurons, although this does not occur in estrogen-treated cultures. Further evidence of a relationship can be seen after administration of BDNF-antisense oligonucleotides, which reduces IPSCs that can be recorded from interneurons and mimics the effects of coadministration of estrogen and BDNF, with a concomitant increase in neuronal spine density. Thus, estrogen treatment of these cultures decreases the levels of BDNF, glutamate decarboxylase (GAD), and GABAergic activity to increase levels of intracellular calcium which, in turn, indirectly stimulates the phosphorylation of CREB and may activate formation of spine-associated proteins.

**G. Einstein** (Duke Univ., USA) further examined the effects of estrogen on hippocampal spine density in the aged rat to determine whether females may be affected by estrogen differently than males. It is known that women are at greater risk of developing Alzheimer's disease with age than are men, and the disease is characterized by a dramatic pruning of the hippocampal dendritic arbor and a severe reduction in the number of dendritic spines that can be seen in the dentate gyrus. She reported a series of studies in which animals were gonadectomized at approximately 3 m of age and implanted with either a blank capsule or a capsule containing high or low levels of estradiol benzoate to produce constant release of estrogen for an additional year. At that time, hippocampal slices were prepared and granule cells in the dentate gyrus were filled to allow anatomical identification. In all female treatment groups, large decreases were observed in dendritic spine density with increased pruning of branch arbor; the decrease in branching was protected by high levels of estrogen replacement. In males with blank or estrogen-filled capsules, however, very little decrement was seen in either dendritic spine density or branching. To examine the effects of a more acute paradigm of estrogen administration to gonadectomized animals, animals were deprived of estrogen for 6 m and then received either a bolus injection or a capsule replacement of estradiol benzoate and were examined 48 h later. In both groups of ovariectomized females, acute estrogen treatment produced an increased dendritic spine density, although few effects were seen in estrogen-treated males. These results suggest that males may have less cognitive requirement for estrogen than do females and may help explain the greater susceptibility of women to Alzheimer's disease that develops with age.

The meeting concluded with some observations by **R. Gorski** who thanked the organizers and speakers for this satellite symposium. He also gratefully acknowledged the wonderful contributions made to his career by his graduate students and postdoctoral fellows. He pointed out that his doctoral studies under the mentorship of Charles A. Barracough were initially focused on the neural control of ovulation, albeit using the androgenized female rat. In a "Eureka" moment, he realized that he had actually uncovered just the tip of the iceberg we now call the fundamental phenomenon of the sexual differentiation of the brain.