Night Session Sexual Differentiation

Estrogen Receptor and Brain Sex Differentiation

Yasuo Sakuma

Nippon Medical School, Japan

Sexual behavior in many mammals is characterized by its dependence on circulating titer of sex hormones and sexual dimorphism. Sexual dimorphism in female and male behavior depends, not on genetic sex but on early actions of sex hormone at a specific period during ontogenesis in each species. The period, termed the critical period for the brain sex differentiation, spans over gestational day 18 to neonatal day 5 in the rat. Testosterone or its aromatization product estradiol organizes or androgenizes the rat brain toward male phenotype. Thus, according to the aromatization hypothesis, binding of circulating estrogen by alpha-fetoprotein prevent this hormone to cross over the blood-brain barrier and female type of rat brain develops in the absence of estrogen. In the males, testosterone enters into the brain and converted to estrogen by an enzyme aromatase. Colocalization of aromatase and estrogen receptor (ER) has been demonstrated in neurons in the preoptic area (POA) and bed nucleus of the stria terminals regions. Ability to exhibit male-like sexual behavior of the testicular feminized rat, that develops as an anatomic female despite its male karyotype due to an androgen receptor deficiency, support that male development of the brain is mediate by ER. Conversely, the male mice genetically altered so that they lack ER alpha generally took the female attitude, showing a high open-field activity and the lack of aggression to intruders in their territory. Endocrine disruptors with estrogenic activity would thus interfere with the process of brain sex differentiation.

Brain sex differences can be detected by several different measures. Morphometry revealed sexual dimorphism in the rat POA in its synaptic organization, dendritic branching patterns and the size of particular neuronal groups or the number of neurons it contains. In females more than in males, a significantly larger number of ER beta positive cells has been visualized in the anteroventral periventricular nucleus (AVPV) of the rat POA. Orchidectomy of male neonates or estrogen treatment of female pups reversed the brain sex. Simultaneous visualization of ER beta and ER alpha revealed that 83% of ER beta positive cells in the female AVPV co-express ER alpha, suggesting that the developmental effect of estrogen depends on ER alpha. Aside from brain morphology, sexual difference in the properties of individual neurons effectively dictates sex-specific functions. Neuronal physiology provides clues to subtle effects of organizational effects of sex hormones that defy morphological identification. Estrogen changes thresholds and refractory periods for antidromic activation of certain efferents of forebrain, hypothalamus and limbic structures in site- and sex-specific manner. These parameters reflect changes in neuronal excitability. In general, estrogen effectively alters neuronal excitability in females or neonatally castrated males, but is ineffective in males or androgenized. Thus, estrogen would determine neural impulses to flow through particular neuronal circuitry in the females or neonatally castrated males but such selection would not occur in the males or androgenized females.

Sex-specific behavior patterns, most of which are associated more or less with reproductive functions, manifest brain sex difference. Among female rat sexual behavior, proceptive and receptive components can be distinguished. The lordosis reflex, an estrogen-sensitive postural reflex with a dorsiflexion of the vertebral column, is by far the most closely scrutinized in the female rat receptive behavior. This reflex let the female allow penile insertion and insemination to occur. Somatosensory pressure stimulation of the skin of the flank perineum region elicits this reflex. In the laboratory, similar cutaneous stimuli given manually by the experimenter elicits the reflex. No other sensory inputs, such as visual, olfactory or acoustic inputs, are needed for the induction of the reflex. On the other hand, the initiation of the proceptive components of the female rat sexual behavior depends on visual and olfactory cues. Several discrete brain regions, most of which are associated with main or accessory olfactory system, have been implicated in the regulation of the male rat sexual behavior. However, neural substrates for components of masculine sexual behavior that include territorial marking, cognition of estrous females, mounting, to name a few, remain largely unknown.

Sexual Differentiation of Sexual Behavior

James G. Pfaus
Concordia University, Canada

The presence or absence of steroid hormones during perinatal development is believed to differentiate the central nervous system into distinct "male" and "female" brains, respectively. This organization or "sculpting" of the brain leads to the differentiation of behavior following the subsequent hormonal activation of differentiated brain regions during puberty and in adulthood. Sexual differentiation of the brain is therefore believed to underlie differences in behavior observed between genders. This is most readily observed in the gender-specific sexual behaviors. For example, in mammals, females control the timing of sexual contact by a complex interplay of sexually proceptive behaviors, such as solicitation and pacing, and sexually receptive behaviors, such as lordosis. Males respond to these behaviors by courting and chasing the females, and mounting with intromission when the female is stationary until ejaculation is achieved. Previous research has shown that the presence of androgens or estrogens in the female during critical perinatal periods can both defeminize (i.e., decrease the probability of displaying female-typical behavior), and masculinize (i.e., increase the probability of displaying male-typical behavior) their behavior. Conversely, removal of these hormones from the male during the same critical period can demasculinize (i.e., decrease the probability of displaying male-typical behavior) and in some notable cases feminize (i.e., increase the probability of displaying female-typical behavior) their behavior. The neural and neurochemical processes through which androgens exert their masculinizing actions on behavior have been studied in detail, and involve a differentiation of hypothalamic and limbic structures that control sexual behavior. Thus, the differentiation of these brain regions is thought to control different behavioral outputs that generate female-typical and male-typical sexual behaviors. Environmental endocrine disruptors can therefore alter sexual behavior as well as reproductive functions by altering the functional activity of these differentiated brain regions.

Is the relationship between brain differentiation and behavioral differentiation one of motor output for behavior, or might this relationship lie in the differentiation of sensory inputs that generate behavior? Recent work from our laboratory has shown that females of two distinct species, rats and Japanese macaques, mount other females and males of their species. This mounting behavior occurs *without* any perinatal androgenization, does not influence reproductive success, appears to be driven by estrogen, certain sensory cues, and is motivated by sexual reward. The fact that these females display a male-typical pattern of sexual behavior despite having fully differentiated brains calls into question the traditional role believed for androgenic differentiation of brain and behavior, and suggests that sensory inputs rather than motor outputs are the targets of differentiation. Accordingly, disruption of such sensory pathways by environmental toxins may play a critical role in the disruption of sexual behavior and reproductive function.

Hypothalamic Development and Sexual Differntiation

Stuart A. Tobet, A. M. Davis, H. J. Walker, and M. L. Seney,
University of Massachusetts Medical School, USA

Steroid hormones dramatically influence the development of numerous sites in the nervous system. Basic mechanisms in neural development provide foci for understanding how factors related to sex can alter the ontogeny of these regions. Sex differences in neurogenesis, cell migration, differentiation, and death, as well as synaptogenesis are being addressed by laboratories around the world. All of these mechanisms serve as likely targets for genetic or gonadal steroid-dependent targets of sexual differentiation throughout development. Although the majority of sexually dimorphic characteristics in brain have been described in older animals, many hormonal mechanisms that determine sexually differentiated brain characteristics occur during critical perinatal periods. Genes suggested to contribute to the development of specific hypothalamic nuclear groups have rarely been examined in the context of sex. The identification of sex differences in the expression of some of these genes may suggest early and likely transient molecular events that set the stage for later amplification by hormone actions. Sex differences in the positioning of cells in the developing hypothalamus further suggest that cell migration may be one key target for early gene actions that impact long-term susceptibility to brain sexual differentiation.

Sex Determination and Gonadal Sex Differentiation in Fish

Yoshitaka Nagahama

Okazaki National Research Institutes, Japan

Recent evidence from studies on fish suggests that the periods of sex determination and gonadal sex differentiation are potential targets for endocrine disrupting chemicals (EDCs) present in the environment. To determine causation and the mechanisms of disruption, it is critical to have a working knowledge of normal gonadal sex differentiation and its regulation. Here I will review some of our recent work on the regulation of gonadal sex differentiation in tilapia and the identification of the sex-determining gene of medaka.

Using all-female and all-male tilapia, we have shown that in this species gonadal sex becomes distinct at 20-25 days post-hatching. Steroid-producing cells in gonads of all-female prior to sex differentiation express all of the steroidogenic enzymes required for estradiol-17 β production. Transcripts of estrogen receptors (ER) α and β first appear in both female and male gonads of fry prior to sex differentiation (5-10 days post-hatching). These results, together with evidence of masculinization of genetic females by an aromatase inhibitor (fadrozole) or an estrogen receptor antagonist (tamoxifen) suggest that endogenous estrogens act as the natural inducers of ovarian differentiation in tilapia. Our results also suggest a potential role for Ad4BP/SF-1 in the transcriptional regulation of aromatase gene. In contrast, the ability of steroid-producing cells to synthesize steroid hormones in all-male fry appears after testicular differentiation. *DMRT1* is expressed male-specifically in the gonads (Sertoli cells) during sex differentiation, suggesting an important role of *DMRT1* in testicular differentiation in tilapia.

The medaka has two major advantages for genetic research: a large genetic diversity within the species and the existence of several inbred strains. We used positional cloning to identify the sex-determining gene of medaka. Chromosome walking mapped the sex-determining region to a 530 kb strech of the Y chromosome. We found a congenic XY female medaka lacking 250 kb of this region, further shortening the probable sex-determining region. Shotgun-sequencing of this region predicted 27 genes. Three of these genes were expressed in embryos during sex differentiation; however, only one gene was Y-chromosome specific. The protein encoded by this gene, named *DMY* (DM-related gene on the Y-chromosome), contains a DNA-binding motif, the DM-domain, found in other genes involved in sexual development in both vertebrates and invertebrates. Expression analysis shows that *DMY* is found in the somatic cells (Sertoli cells) of the XY gonads at the time when sex determination occurs. We then screened wild medaka populations for naturally-occurring mutants and found two XY females with distinct mutations in *DMY*. One of these mutants contained an insertion that causes premature termination in the *DMY* protein. When mated, all XY offspring with the mutant Y were female. The other mutant had a severe depression in *DMY* expression in the embryo and 60% of its XY offspring with the mutant Y developed as females. These results demonstrate that the *DMY* gene is required for male development and likely represents the sex-determining gene of medaka. *DMY* can be used as an excellent genetic marker to detect the effects of EDCs on the genetic sex in medaka.