Annu. Rev. Neurosci. 2002. 25:507–36
doi: 10.1146/annurev.neuro.25.112701.142745

WIRED FOR REPRODUCTION: Organization and Development of Sexually Dimorphic Circuits in the Mammalian Forebrain

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Key Words hypothalamus, limbic, sexual differentiation, amygdala, hippocampus

■ **Abstract** Mammalian reproduction depends on the coordinated expression of behavior with precisely timed physiological events that are fundamentally different in males and females. An improved understanding of the neuroanatomical relationships between sexually dimorphic parts of the forebrain has contributed to a significant paradigm shift in how functional neural systems are approached experimentally. This review focuses on the organization of interconnected limbic-hypothalamic pathways that participate in the neural control of reproduction and summarizes what is known about the developmental neurobiology of these pathways. Sex steroid hormones such as estrogen and testosterone have much in common with neurotrophins and regulate cell death, neuronal migration, neurogenesis, and neurotransmitter plasticity. In addition, these hormones direct formation of sexually dimorphic circuits by influencing axonal guidance and synaptogenesis. The signaling events underlying the developmental activities of sex steroids involve interactions between nuclear hormone receptors and other transcriptional regulators, as well as interactions at multiple levels with neurotrophin and neurotransmitter signal transduction pathways.

INTRODUCTION

A principal goal of brain development is to produce the necessary neural architecture for integration of information from the external environment with internal cues that reflect important aspects of an animal's physiological state. This integration allows the elaboration of adaptive behavioral and physiological responses that are essential for an individual's survival, as well as for propagation of the species. From an evolutionary perspective, the most adaptive physiological responses are those that ensure successful reproduction. The long-term consequences of adaptive behavioral profiles that enhance survival are of little significance if an animal lacks the reproductive fitness necessary to pass on its genome. Moreover, the coordination of physiological events with behavior is a prerequisite to successful reproduction. For example, it is of no benefit to a mammalian species if females

display appropriate solicitation behaviors and successfully copulate with conspecific males but have not ovulated. Males have similar requirements for physiological coordination; an individual that has mature sperm and is ready to impregnate a female will not get the chance if he displays agonistic behaviors. Thus, the future of a species often rests with the ability of its members to coordinate behavioral responses with physiological processes in response to sexually relevant cues. This coordination of behavior and physiology must also be reliable, which depends in part on how consistently the neural circuits underlying neuroendocrine integration are constructed and regulated.

Mammals reproduce sexually; males and females of a species display distinct patterns of copulatory behaviors and neuroendocrine physiology (Gerall & Givon 1992, Gorski & Jacobson 1981). This array of sex-specific behaviors and physiological responses is so vital to the success of mammalian species that robust developmental mechanisms have evolved to produce distinct yet complimentary neural systems that ensure the coordinated expression of reproductive function in male and female mammals. In this review key aspects of sexually dimorphic neural systems in the rodent forebrain are examined to consider developmental mechanisms that may be responsible for specifying sex-specific aspects of these neural pathways. Although the regions dealt with in detail play major roles in reproduction, it is important to note that significant sexual dimorphisms have been documented throughout the central nervous system, from the cerebral cortex to spinal motor neurons; therefore, the process of sexual differentiation of the brain should be viewed as a widespread series of developmental events with functional significance for diverse behaviors and physiological responses.

The central tenet of sexual differentiation is that the brain is bipotential but develops differently in males and females under the influence of sex steroid hormones during the perinatal period. In male rats, secretion of androgen from the differentiated testis produces two perinatal elevations in plasma testosterone, the first of which occurs on day 18 of gestation, and the second at approximately 2 h after birth (Corbier et al. 1992, Weisz & Ward 1980). The resulting difference in exposure of the brain to testosterone, or to its metabolites dihydrotestosterone and estradiol (Simpson et al. 1994), causes the brain to change its structure and function. Thus, the perinatal steroid environment determines whether male or female copulatory behavior is expressed, or whether the pituitary gland is able to mount a preovulatory surge of gonadotropin secretion. Before a significant effort was made to identify sex differences in brain architecture, it was suspected that the biological basis of these functional dimorphisms is hormonal modification of the brain, and work carried out during the past two decades has provided strong support for this notion. A large number of morphological and neurochemical sexual dimorphisms that are dependent on exposure to sex steroid hormones during the perinatal period have been documented in the mammalian brain (De Vries & Simerly 2002, Gorski 1996, Madeira & Lieberman 1995, McEwen 2001). Although it is likely that genetic background influences the degree to which various sexually dimorphic regions develop and may influence expression of sexually dimorphic traits

that are independent of hormone action (Arnold 1997), it is clear that many brain dimorphisms can be completely reversed by hormone treatment alone.

Sexually Dimorphic Forebrain Pathways

The hypothalamus plays a critical role in coordinating expression of reproductive behaviors and physiological responses with environmental cues. Its close anatomical and physiological relationship with the pituitary gland provides an effective means for coordinating diverse homeostatic processes through neuroendocrine regulation of hormone secretion. The hypothalamus also shares strong connections with the limbic region of the forebrain so it can effectively coordinate neuroendocrine responses with sensory cues that regulate motivated behavior. The preoptic region of the hypothalamus was the historical focus of early studies on morphological sex differences, owing in part to its dominant role in the regulation of copulatory behavior and gonadotropin secretion (Gerall & Givon 1992, Larsson 1979, Pfaff 1980), but also because it contained a high density of neurons that were known to be targets for steroid hormones (see Simerly 1993 for summary). The modern era of sexual differentiation research was ushered in when Raisman and Field used electron microscopy to identify the first clear sex difference in neuronal connectivity (Raisman & Field 1971). Because it was generally believed that stereotypic patterns of behavior were dependent on the organization of neural connections, their finding that sexually dimorphic patterns of synaptology in the dorsal part of the medial preoptic area could be reversed by treatment with testosterone provided the first clear evidence that the developmental role of sex steroid hormones on behavior may have a structural basis. However, subsequent efforts were not successful to demonstrate that the observed sex difference in synaptic relationships were responsible for specific dimorphisms in behavior and gonadotropin secretion. Nevertheless, this work brought together the experimental paradigms used to study sexual differentiation of neuroendocrine physiology with modern neuroanatomical approaches to developmental plasticity.

The developmental actions of sex steroid hormones are not limited to relatively subtle alterations in synaptic organization. Soon after the publication of the paper by Raisman & Field, Gorski and colleagues reported a dramatic sexual dimorphism in the size of a group of cells in the medial preoptic area of the rat, which they designated the sexually dimorphic nucleus of the preoptic area (Gorski et al. 1978). Furthermore, they showed that perinatal exposure to testosterone or estrogen could cause a nucleus to form in females equal in size to that of males. This sensitivity to hormones declines after the first week of life (Gorski 1985). These observations had an enormous impact on the field because they demonstrated that sex steroid hormones can cause profound changes in regional anatomy, and they implied that testosterone and/or estrogen can specify cell number in hypothalamic nuclei. Because the number, size, or density of sexually dimorphic morphological features tended to be greater in males, a general principle emerged that steroid exposure during the perinatal period promoted neuronal development. However,

the anteroventral periventricular nucleus (AVPV) of the preoptic region was found to be larger in female rodents, suggesting that sexual dimorphisms may also favor females (Bleier et al. 1982). The demonstration that the AVPV contained a greater number of dopaminergic neurons in females, which can be reduced to that of males by a single injection of testosterone, indicated that sex steroid hormones may actually facilitate loss of neurons in certain regions (Simerly et al. 1985).

THE MEDIAL PREOPTIC NUCLEUS The sexually dimorphic nucleus of the preoptic area comprises neurons that are part of the medial preoptic nucleus (MPN), a nucleus known for its dominant role in expression of male sexual behavior (Gorski 1985, Larsson 1979). The MPN is a sexually dimorphic complex made up of three distinct subdivisions that can be distinguished on the basis of neurochemistry and cytoarchitecture (Simerly 1995b). The cell-dense central part of the MPN (MPNc) is the most dimorphic part of the nucleus with substantially more neurons in male rats than are found in females (Madeira et al. 1999). The medial part of the MPN (MPNm) is also larger in males and, like the MPNc, contains a high density of neurons that express large numbers of receptors for estrogen and androgen (Simerly et al. 1990). The sexually dimorphic nucleus identified by Gorski and collegues was not originally defined in anatomical terms but was later shown to correspond to the MPNc and subpopulations of cells in the MPNm. The observation that in males MPNc neurons display an accelerated decline in neuron number postnatally provided support for the notion that exposure to sex steroids enhanced their survival, which led to more neurons in the male MPNc (Dodson & Gorski 1993).

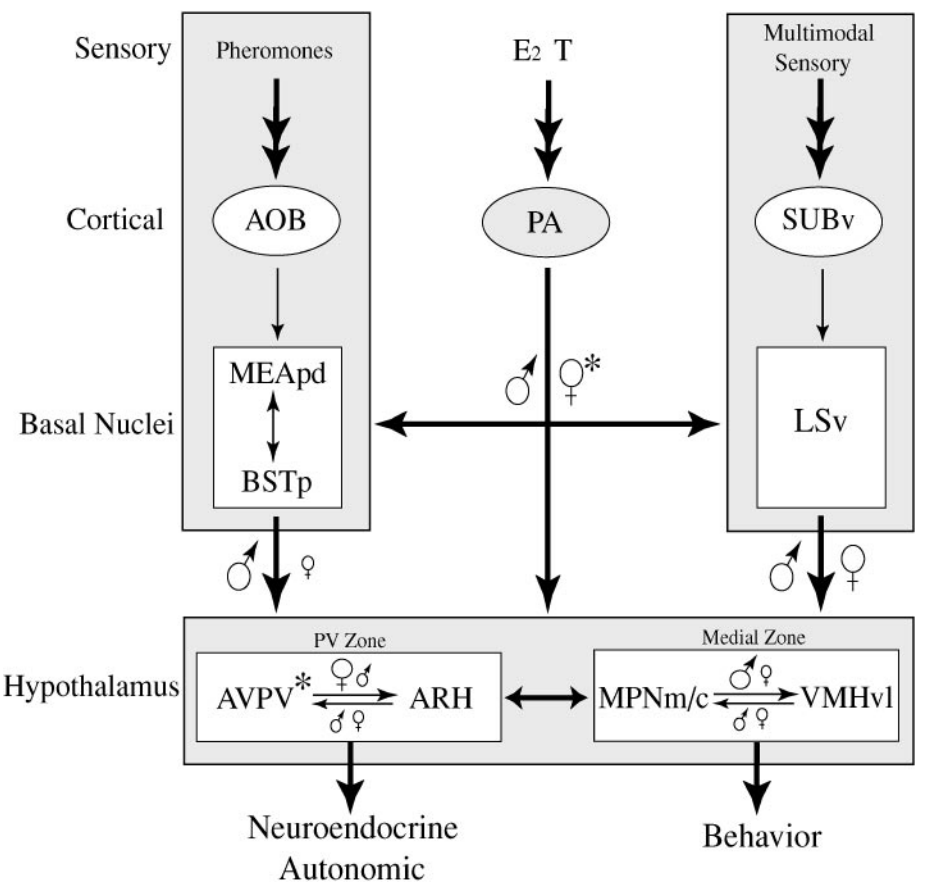
Each subdivision of the MPN shows a distinct pattern of connectivity: The MPNm sends its strongest projections to the periventricular zone of the hypothalamus, which is primarily involved in the control of hormone secretion from the anterior pituitary, while the MPNc sends its major projections to other sexually dimorphic forebrain nuclei (Simerly & Swanson 1988). Considering the widespread pattern of projections of the MPN, and the predominately bidirectional nature of these connections, it is clear why it proved so difficult to define discrete functional roles for the sexually dimorphic nucleus of the preoptic region or for the synaptic dimorphism studied by Raisman and Field. These regions do not function as centers but rather participate in interrelated functional neural systems that collectively integrate diverse aspects of reproductive function and contribute to multiple aspects of homeostasis. This problem is not unique to the study of neural mechanisms underlying reproduction but is being confronted by investigators studying how a variety of homeostatic functions are mediated by interrelated forebrain pathways (Kruk 1991, Sawchenko et al. 2000, Watts 2001), and by researchers interested in how distributed neural networks mediate cognition. Thus, the MPNc lies at the center of what can be viewed as a limbic-hypothalamic neural network of regions that develop under the influence of sex steroid hormones and collectively influence reproduction differently in males and females, as well as impacting other sexually dimorphic aspects of neuroendocrine function.

THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPV) Because gonadotropin secretion is perhaps the most significant sex difference in reproductive physiology, some of the earliest studies of sexual differentiation focused on the impact of sex steroid hormones on the phasic secretion of luteinizing hormone (LH), which initiates ovulation in female mammals (see Gerall & Givon 1992 for review). Treatment of ovariectomized adult female rats with estrogen causes a massive surge in LH secretion, yet similar treatments in males fail to induce a similar response. This sexually dimorphic response to hormone treatment can be reversed by castrating male rats at birth, and treatment of neonatal female rats with a single dose of testosterone results in permanent anovulatory sterility. Evidence from a variety of experimental approaches indicates that sex steroids act at the level of the preoptic region during postnatal life to organize the neural pathways controlling preovulatory gonadotropin secretion. The AVPV is a likely site of action because it plays a critical role in controlling the preovulatory LH surge and is sensitive to the developmental actions of sex steroid hormones (see Simerly 1998, Terasawa et al. 1980 for reviews). Consistent with its neuroendocrine role, neurons in the AVPV primarily innervate other parts of the periventricular zone and provide direct projections to gonadotropin releasing hormone (GnRH) neurons, as well as to tuberoinfundibular neurons (TIDA) in the arcuate nucleus that control secretion of prolactin (Gu & Simerly 1997). These projections appear to be more robust in females, which is not surprising given the larger volume of the AVPV in this sex, and is consistent with earlier ultrastructural studies that revealed a greater synaptic density in the arcuate nucleus of the hypothalamus in female rats (Matsumoto et al. 2000). Dendritic arborization of arcuate neurons also appears to be greater in female rats, although there are slightly more neurons in the nucleus in males (Leal et al. 1998).

The total number of neurons in the AVPV has not been determined in male and female rats, but cellular markers for dopaminergic neurons and peptidergic neurons (Simerly 1989, 1991) have identified subpopulations of AVPV neurons that are more numerous in females. It is surprising that neurons that contain the opioid peptide enkephalin are more abundant in male rats, which demonstrates that the developmental effects of sex steroids show considerable cell-type specificity, even within a single nucleus (Simerly 1991). Some of the strongest afferents to the AVPV are from other sexually dimorphic nuclei such as the MPNm/c. It is also heavily innervated by the accessory olfactory and septohippocampal pathways that innervate other sexually dimorphic parts of the hypothalamus (Simerly 1998). Thus, the AVPV is of interest not only because it illustrates the region and cell-type–specific activity of sex steroid hormones in regulating neuronal development, but also because it represents a site for sensory convergence at an interface between the limbic region of the telencephalon and neuroendocrine circuits.

PARALLEL LIMBIC-HYPOTHALAMIC SENSORY PATHWAYS In addition to the AVPV and MPN, several other forebrain regions are known to undergo sexual differentiation (Figure 1). Each of these regions contains high densities of neurons that express receptors for steroid hormones and are located in the hypothalamus or in

limbic nuclei that have strong connections with the sexually dimorphic hypothalamic nuclei. Sexually dimorphic nuclei of the hypothalamus are either in the periventricular zone of the hypothalamus, as are the AVPV and arcuate nucleus, or are in the medial zone. Medial zone nuclei such as the MPN and ventrolateral part of the ventromedial hypothalamic nucleus (VMHvl) play central roles in mediating reproductive behavior (Meisel & Sachs 1994, Pfaff et al. 1994). The medial and central parts of the MPN share bidirectional projections with the VMHvl, and anatomical links between the medial and periventricular zone sexually dimorphic nuclei provide a possible substrate for coordination of copulatory behavior with gonadotropin secretion and associated autonomic responses. The major routes for sensory activation of this core circuitry of reproduction are via projections from the ventral subiculum of the hippocampal formation to the ventral part of the lateral septal nucleus and via the accessory olfactory pathway. Of



particular importance to reproduction are pheromonal cues from the vomeronasal organ relayed centrally by the accessory olfactory bulb, which is itself sexually dimorphic with more projection neurons in males (Guillamon & Segovia 1996, Simerly 1990) and which functions as primary olfactory cortex for pheromonal information (see Swanson & Petrovich 1998 for references and a discussion of this issue). This olfactory information is transmitted to the hypothalamus primarily by two sexually dimorphic nuclei: the medial nucleus of the amygdala and principal nucleus of the bed nuclei of the stria terminalis (BSTp). The posterodorsal part of the medial amygdaloid nucleus (MEApd) and BSTp are both larger in males (Guillamon & Segovia 1996), contain high densities of hormone-sensitive neurons (Shughrue et al. 1997, Simerly et al. 1990), and provide strong projections to sexually dimorphic nuclei in the periventricular and medial zones of the hypothalamus (Canteras et al. 1995, Gu et al. 2001, Simerly 1990). The dense projections of the MEApd and BSTp to the AVPV and arcuate nucleus of the hypothalamus

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Figure 1 Overview of sexually dimorphic pathways in the forebrain of the rat. The cell groups are organized to emphasize the major routes for sensory information impacting sexually dimorphic nuclei in the hypothalamus and to illustrate the relative representations of major pathways between forebrain sexually dimorphic cell groups in male and female animals. Two interconnected functional sexually dimorphic subcircuits in the hypothalamus mediate behavioral aspects of reproduction (MPNm/c and VMHvl in the medial zone of the hypothalamus) and associated neuroendocrine/autonomic responses (AVPV and ARH in the periventricular zone of the hypothalamus). Descending projections from the AVPV to the ARH are more robust in females, and descending projections from the MPNm/c to the VMH predominate in males. A descending pathway that is more robust in males conveys pheromonal information from the vomeronasal organ (which is also larger in males) to the hypothalamus via the accessory olfactory bulb (AOB) and nuclei in the amygdala and bed nuclei of the stria terminalis. A parallel descending pathway conveying multimodal sensory information to the hypothalamus via the subiculum and lateral septum appears to be represented similarly in male and female rats. The PA responds to fluctuations in circulating levels of estradiol (E2) and testosterone (T) and provides what is presumably a stimulatory input to both descending sensory pathways as well as to the hypothalamic effector nuclei. This pathway has roughly equal representation in males and females, but the asterisks denote one exception: The AVPV receives only a minor input from the PA in female rats. Four levels in a hypothetical functional hierarchy are proposed that correspond to a model advanced by Swanson for comparing functional neural systems in the mammalian forebrain (Swanson 2000). According to this scheme pathways that impact effector regions of the hypothalamus consist of a triple descending pathway composed of a glutamatergic cortical stimulatory projection and overlapping GABAergic projections from basal nuclei in a pattern analogous to the classical isocortical-striatal-pallidal model. See text for details and abbreviations.

(ARH) in the periventricular zone, and to the MPNm/c and VMHvl in the medial zone, are among the strongest descending projections of these sexually dimorphic nuclei. Thus, these limbic-hypothalamic pathways provide a particularly robust route for carrying pheromonal information to sexually dimorphic hypothalamic nuclei mediating reproduction.

Other sensory information that may impact the activity of sexually dimorphic nuclei is derived from multimodal association cortex and passes to the hypothalamus via glutamatergic projections from the ventral part of the subiculum and region CA1 of the hippocampal formation to the ventral part of the lateral septal nucleus (LSv) (Canteras & Swanson 1992). The LSv is a distinct part of the septal complex that, like the MEApd and BSTp, contains high densities of neurons that express sex steroid receptors and can be viewed functionally as a basal nucleus for the ventral subiculum and CA1. Its projections appear to be largely GABAergic (Risold & Swanson 1997a) and overlap with those of the MEApd and BSTp, which provides strong inputs to the AVPV and ARH, as well as to the MPNm/c and VMHvl (Risold & Swanson 1997b). However, no part of this septohippocampal-hypothalamic sensory pathway appears to be sexually dimorphic (F. Varoqueaux & R. B. Simerly, unpublished observations), although rostral and caudal parts of the lateral septal nucleus do receive a sexually dimorphic vasopressinergic innervation (De Vries & Miller 1998). Thus, the sexually dimorphic nuclei in the periventricular and medial zones of the hypothalamus receive completely overlapping inputs from two separate sensory pathways: Pheromonal information is conveyed along a sexually dimorphic pathway from the accessory olfactory bulb, and multimodal sensory information from isocortex is transmitted along a ventral subiculoseptal pathway that appears to be similar in males and females (Figure 1).

According to a recent model for telencephalic projections onto hypothalamic motor regions proposed by Swanson (2000), components of the accessory olfactory pathway, or ventral subiculoseptal pathway, can be viewed as being analogous to the cerebral cortex and basal ganglia. According to this model virtually all parts of the cerebral cortex provide a triple descending projection to brainstem regions involved in behavioral control. The cortex sends excitatory inputs to the basal nuclei such as the striatum and pallidum, which have descending inhibitory and disinhibitory projections, respectively. Thus, the major glutamatergic projection from the AOB (olfactory cortex) to the MEApd (striatum), and the massive GABAergic projections from the MEApd to the BSTp (pallidum), can be viewed as analogous to key aspects of basal ganglia circuitry. The MEApd and BSTp, in turn, send overlapping GABAergic projections to sexually dimorphic nuclei in the hypothalamus in much the same way as projections of striatal and pallidal neurons converge onto the substantia nigra. The parallel ventral subiculoseptal pathway also has a hierarchical organization with the ventral subiculum providing excitatory cortical inputs to the ventral part of the lateral septal nucleus, which may function as a basal nucleus by sending GABAergic projections to sexually dimorphic parts of the hypothalamus. The utility of this model for generating new approaches to

understanding sensory integration and control of reproduction seems clear, but the accuracy of its predictions remains to be validated experimentally.

The accessory olfactory and ventral subiculoseptal pathways represent the major limbic-hypothalamic pathways impacting reproduction. The posterior nucleus of the amygdala (PA) (see Canteras et al. 1992a) is the only other part of the telencephalon that provides strong projections to sexually dimorphic hypothalamic nuclei. The PA is the caudal-most part of the amygdala and was previously considered to be a transition zone between the amygdala and hippocampal formation (see Swanson & Petrovich 1998 for review). An analysis of its projections, however, clearly indicates that this region should be included in the division of the amygdala that is primarily involved in relaying olfactory information to the hypothalamus because its only significant sensory afferents are indirect inputs from the accessory and main olfactory pathways. The PA appears to lack GABAergic neurons, which indicates that it provides primarily glutamatergic descending projections, and the pattern of its connections suggests that it occupies a cortical position in forebrain circuit hierarchies (Swanson 2000). Although virtually nothing is known with certainty about the functional role of the PA, its high density of neurons that express sex steroid receptors and massive projections to sexually dimorphic parts of the forebrain involved in reproduction suggest a key role in regulating sexually differentiated aspects of behavior and hormone secretion. Moreover, the specificity of its projections to sexually dimorphic forebrain regions might lead one to suspect that the PA itself is dimorphic and may provide differential projections to the hypothalamus in males and females, but this is not the case. A recent examination of PA morphology found that it is of approximately equal volume in male and female rats and lacks a clear sex difference in cell density (Gu et al. 2000). The descending projections of the PA are also remarkably similar in males and females. Thus, it provides massive inputs to the BSTp, MEApd, MPNm/c, and VMHvl that are similar in both male and female rats, but the projection to the AVPV is much more robust in males, similar to the inputs from the MEApd and BSTp (Gu et al. 2000, Hutton et al. 1998). Another unique aspect of the PA is the apparent lack of robust autoregulation of estrogen and androgen receptors by sex steroid hormones. In other parts of the forebrain, gonadectomy and steroid replacement have profound effects on cellular levels of sex steroid receptors, but in the PA expression of these receptors appears to be relatively stable, at least at the level of mRNA (Gu et al. 2000). One possible interpretation of this curious finding is that hormone-sensitive activity in the PA may accurately reflect dynamic changes in levels of circulating sex steroid hormones owing to this lack of autoregulation. In the future it will be important to establish whether neuronal activity in the PA changes as a function of sex steroid hormone levels in the blood, which would support a possible role for the PA as a stable central sensor of endocrine status.

Despite the robust innervation of sexually dimorphic nuclei in the hypothalamus by the BSTp and amgdala (MEApd and PA), neither the periventricular nor the medial zone dimorphic nuclei provide substantial return projections. Instead, feedback appears to be conveyed by the ventral premammillary nucleus (PMv), which

is itself sexually dimorphic, contains high densities of receptors for sex steroids, and receives a strong input from the MPNc (Akesson & Micevych 1995, Simerly et al. 1990, Simerly & Swanson 1988). The PMv receives massive inputs from the MEApd, PA, and BSTp and sends equally strong return projections, as well as providing dense inputs to the LSv (Canteras et al. 1992b). Although the neurotransmitter in these pathways is unknown, the projections of the PMv represent the strongest hypothalamic projection to telencephalic regions that innervate sexually dimorphic nuclei. The function of this robust hypothalamic projection remains a mystery, but the PMv has been implicated in the neural regulation of reproductive behavior and gonadotropin secretion from the anterior pituitary (Akesson & Micevych 1995, Beltramino & Taleisnik 1985).

The organization of the sexually dimorphic forebrain pathways discussed above suggests that they function as part of a broader forebrain circuitry that conveys sensory information to effector regions in the hypothalamus and brainstem. These effector regions then integrate this information with visceral and endocrine signals that ultimately impact neuroendocrine, visceromotor, and behavioral responses. Sensory cues, such as pheromones, are transmitted along sexually dimorphic pathways that presumably do not impact the hypothalamus in the same way in male and female animals. Other sensory information, such as the multiple modalities relayed from the ventral subiculum and CA1 via the LSv, reaches the hypothalamus along pathways that are similar in both sexes, which provides a way for information from the environment to impact hypothalamic circuits similarly in males and females. Visual or main olfactory cues related to foraging behavior may represent an example of sensory information that impacts both sexes equally.

However, even sensory influences transmitted to the hypothalamus along monomorphic pathways may contribute to sexually dimorphic responses because the hypothalamic regions innervated are sexually differentiated. For example, the LSv provides strong inputs to both the AVPV and MPNm/c, which may process the afferent multimodal information differently in each sex. Sexually dimorphic pathways such as the accessory olfactory pathway provide more robust sensory inputs to hypothalamic nuclei in males, which indicates that there is greater convergence of this information onto hypothalamic neurons in target nuclei. This convergence is even more profound in target regions with fewer neurons in males, as is the case with the AVPV. Alternatively, descending projections from the LSv appear to be more divergent in males since there are more neurons in target nuclei such as the MPNm/c in males relative to that of females.

Although at present it is difficult to confidently predict the functional impact of sexually dimorphic patterns of sensory convergence and divergence on specific reproductive functions, it appears likely that the sexually dimorphic representations of these sensory routes and hypothalamic targets impose a sex-specific bias on information processing at nodal points in these circuits. The emerging appreciation of the sexually dimorphic organization of sensory pathways, and a detailed understanding of the cellular relationships that define the signaling balance encoded in patterns of sensory convergence and divergence onto hypothalamic circuits, is a

prerequisite to an improved understanding of how these pathways function in the control of neuroendocrine physiology and behavior.

The recent clarification of anatomical relationships between sexually dimorphic parts of the forebrain and new theoretical proposals on information processing in cortico-hypothalamic pathways (Swanson 2000, Swanson & Petrovich 1998) has generated new insights into possible mechanisms underlying reproductive function. Investigators are currently applying a neural systems approach to this problem, and it will be useful to view these neural networks from the perspective of how descending forebrain pathways function generally. Thus, the hierarchical organization of other descending pathways, such as those from the cerebral cortex to the basal ganglia and brainstem motor systems, may have much in common with how descending sexually dimorphic pathways provide cortical input to neural systems mediating reproduction.

Development of Sexually Dimorphic Pathways

CELL NUMBER Numbers of cells in brain regions and the connections between them are major determinants of the functional properties of forebrain neural circuits. Although it is clear that sex steroid hormones play an important role in determining the size of sexually dimorphic nuclei and can alter patterns of connectivity, the cellular mechanisms underlying the developmental actions of these hormones are only now beginning to be elucidated. Nevertheless it is clear that exposure to high levels of testosterone during the first week of life can dramatically alter the numbers of neurons that occupy sexually dimorphic nuclei in the adult. Cellular mechanisms proposed to explain how sex steroids accomplish such dramatic changes in cell number fall into three main categories: neurogenesis, neuronal migration, and cell death.

Of the potential mechanisms underlying hormonal control of cell number during development, a significant alteration in neurogenesis has found the least experimental support. Indeed, neuronal birthdating studies using thymidine or 5-bromodeoxyuridine (BrdU) labeling have provided evidence to the contrary. In the MPN, this is surprising because many of the neurons in the MPNc are born during the prenatal surge in testosterone secretion. Dodson & Gorski addressed this question by labeling neurons in the MPNc with thymidine and found that treating females with testosterone before or after the testosterone surge had the same effect on the number of labeled neurons in the MPNc of 30-day-old animals (Dodson et al. 1988), which suggests that hormone treatment does not enhance or prolong neurogenesis. Using the same experimental approach they also demonstrated that perinatal exposure to testosterone prevented the loss of neurons that normally occurs in the MPNc of females during the first ten postnatal days (Dodson & Gorski 1993). Using similar methods Arai and colleagues arrived at the conclusion that testosterone does not alter neurogenesis in the AVPV (Arai & Murakami 1994). Neurons in the AVPV are born between embryonic day 13 (E13) and E17. Exposure of female rats to high levels of testosterone on E14–16 did not produce

a significant difference in the number of BrdU-labeled neurons in the AVPV of male, female, or androgen-treated female rats on E17 (Nishiuzuka et al. 1993). However, there was a significant reduction in the number of labeled neurons by E21 in males and in females exposed to T, indicating that the hormone exposure begins to cause a reduction in cell number after neurogenesis is complete. Although neurogenesis does not appear to be a major contributor to sexual differentiation of neuronal number in the MPN or AVPV, estrogen promotes numbers of newly generated neurons in the forebrain of avian species (Burek et al. 1995). Moreover, in mature rats estrogen appears to increase formation of new neurons in regions where neurogenesis continues well into adulthood, including the olfactory bulb (Kaba et al. 1988) and dentate gyrus of the hippocampal formation (Tanapat et al. 1999). There are more newborn neurons in the dentate gyrus of females relative to that of age-matched males, but this difference is transient and appears to be related to changes in circulating levels of estrogen that occur in the female during the estrous cycle.

Even less attention has been paid to whether sex differences in neuronal migration contribute to differences in neuronal number in sexually dimorphic nuclei in the mammalian forebrain. Thymidine labeling was used to examine the location of preoptic neurons that become postmitotic on E18 and are presumably destined to reside in the MPN (Jacobson et al. 1985). No significant differences were noted in the locations of labeled cells at various ages, so it was concluded that sexually dimorphic patterns of neuronal migration do not make a major contribution to the development of the sexually dimorphic nucleus of the preoptic area. Tobet and colleagues also addressed this question using BrdU labeling and subsequent evaluation of relative cell densities in ferrets killed at three different prenatal ages (Park et al. 1996). Consistent with the earlier findings in rats, they did not find evidence for sexually dimorphic patterns of migration in the preoptic region. Neither of these labeling methods is optimal for studying neuronal migration because it is difficult to determine when migrating neurons reach their targets and the course of individual cells cannot be traced. Optical recordings obtained by using time-lapse imaging of labeled cells provide a more direct appreciation for defining neuronal migratory routes, often with surprising results (O'Rourke et al. 1992). Tobet et al. (Henderson et al. 1999) used this approach on cultured brain slices to study migration in the preoptic region of the hypothalamus, and the results suggest that there is more medial to lateral migration in males than in females. The possibility that this observed difference in migratory orientation was related to differences in the orientation or density of radial glia was also examined, but again no significant sex differences were detected. The importance of glia in regulating patterns of neuronal migration has long been appreciated and steroid hormones clearly influence glial morphology in the hypothalamus (Garcia-Segura et al. 1996a). A sex difference in the morphology of astroglia was observed in the arcuate nucleus of neonatal rats, and this difference can be manipulated with testosterone treatment (Mong et al. 1999). However, such differences do not appear to be a general feature of sexually dimorphic regions because comparable differences were not found in the VMH.

Although the data to date do not support the notion that migration plays a major role in determining differences in neuronal number in sexually dimorphic nuclei, it remains possible that experimental limitations are largely responsible for this lack of information.

The major way that steroid hormones alter neuron number in sexually dimorphic regions is by influencing cell death. The earlier indirect assessments of hormone-induced changes in cell number have been confirmed by morphometric analyses utilizing a variety of differential cell-labeling procedures (Arai et al. 1996, Dodson & Gorski 1993, Forger & Breedlove 1987, Kay et al. 1999, Nunez et al. 2001, Yoshida et al. 2000). Thus, exposure to steroids during perinatal life enhances cell number in nuclei such as the MPN and BSTp, which are larger in males, and decreases the number of cells in the AVPV. It is less clear how steroids regulate cellular processes during development that lead to changes in cell number. Programmed cell death (PCD) occurs throughout the nervous system and is a major determinant of neuronal number (Burek & Oppenheim 1996, Vaux & Korsmeyer 1999). The notion is widely accepted that hormones influence the numbers of cells that reside in mature sexually dimorphic nuclei by altering the rate or duration of PCD, but this remains largely unproven. The problem of reliably determining that changes in cell number are due to PCD, as opposed to migration or necrotic mechanisms related to physiological insults, is difficult because cells that undergo PCD often are degraded quite rapidly, leading to underestimation of the amount of PCD that occurs in response to hormone exposure. An additional difficulty is that PCD is a dynamic process, and most experimental evaluations lack adequate temporal resolution to fully appreciate its impact on cell number; in addition, there are so many agents that are capable of initiating cell death independent of developmental programs that experimental results are often dependent on peculiar attributes of experimental model systems. The best genetic characterization of a cell-death process in the central nervous system is apoptosis (Yuan & Yankner 2000), which was first defined in morphological terms (Kerr et al. 1972) and was later linked to genetic pathways of PCD in the nematode *C. elegans* (Ellis & Horvitz 1986). Histochemical labeling of fragmented DNA with TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining suggests that at least some of the neurons in sexually dimorphic nuclei die by apoptosis (Arai & Murakami 1994). Although TUNEL labeling is consistent with an apoptotic mechanism of cell death and certainly detects DNA fragmentation (Kerr et al. 1972), morphological features of dying neurons together with the demonstrated involvement of caspases are generally thought to be more reliable indicators of apoptosis (Zhang et al. 1998).

Caspases are members of a family of cysteine proteases that exist in cells as inactive zymogens, which are activated by proteolytic cleavage. Once activated, caspases cleave a broad spectrum of proteins within cells that ultimately result in cell death (Thornberry & Lazebnik 1998). Mutation of Caspase 3 or 9 in mice are generally lethal before birth but do result in reduced apoptosis in the central nervous system (Ranger et al. 2001). However, in certain brain regions and cell types, PCD persists during prenatal development in caspase knockouts, which

suggests that the activity of caspases and the occurance of caspase-independent cell-death pathways may be a region-specific feature of early brain development (Oppenheim et al. 2001). Hormonal regulation of caspase expression during development of the mammalian forebrain has not been reported, nor has estrogen or testosterone been shown to alter caspase activation in sexually dimorphic nuclei, but recent preliminary findings suggest that estrogen may influence cell number in the AVPV through a caspase-dependent mechanism (Waters et al. 2000). Perhaps more problematic will be the definition of signaling pathways linking steroid hormones and apoptosis effector mechanisms that lead to alterations in apoptosis. Thus, although there is general agreement that sex steroid hormones regulate PCD, there is very little known about how these regulatory signals affect genetic pathways responsible for apoptosis. However, estrogen was recently reported to induce a neuroprotective activity in cultured fetal neurons that functions to inhibit caspase 6–mediated cell death through a receptor-dependent nongenomic pathway (Zhang et al. 2001). Given the variety of cellular signaling pathways that are apparently influenced by estrogen (see below), there may be multiple mechanisms for regulating the activity of effector caspases.

In addition to the caspases, several mammalian homologues of genes involved in PCD pathways have been identified (Nijhawan et al. 2000). Members of the antiapoptosis Bcl family such as Bcl2 and BclX interact with adapter proteins involved in activation of caspases to inhibit apoptosis and can themselves be regulated by proapoptotic family members like Bad and Bax that promote PCD (Conradt & Horvitz 1998). Thus, it appears that the ratio of antiapoptotic to proapoptotic proteins represents a critical molecular mechanism for determining whether a cell survives or undergoes apoptosis. Steroid hormones regulate cellular events most commonly by acting as ligand-activated transcription factors (Aranda & Pascual 2001, Zhang & Lazar 2000), so the possible hormonal regulation of antiapoptotic to proapoptotic proteins represents a likely mechanism for influencing cell death. Treatment of ovariectomized adult female rats with estrogen increased levels of bcl2 mRNA in the hypothalamus (Garcia-Segura et al. 2001), and estradiol also influenced bcl2 gene expression in the cerebral cortex of adult rats following experimentally induced ischemia (Wise et al. 2001). The rapidly expanding literature on the neuroprotective actions of estrogen in adult rats is complemented by numerous studies in vitro that more directly inform its possible roles during development (see Garcia-Segura et al. 2001, McEwen 2001, Wise et al. 2001 for reviews). For example, the ability of estrogen to enhance neuronal survival in primary cultures of hypothalamic and cortical neurons, or in cortical explants, can be blocked by estrogen receptor (ER) antagonists, which suggests a critical role for the ER in mediating the neurotrophic actions of estrogen (see Wise et al. 2001 for references). Stable transfection of PC12 cells with the alpha form of ER (ERα) increased the viability of estrogen-treated cultures following withdrawal of serum-containing medium (Gollapudi & Oblinger 1999a). ERα is presumably responsible for estrogen-induced increases in Bcl-xl mRNA and reduced expression of Bad mRNA in these cells (Gollapudi & Oblinger 1999b).

Although it remains to be shown definitively that the neuroprotective effects of estrogen in the brain are due to differential expression of apoptotic factors, deletion of Bcl2 or BclX leads to massive cell death in the CNS, whereas mice lacking the proapoptotic protein Bax have developmental defects due to failure of normal developmental cell death (Knudson & Korsmeyer 1997, White et al. 1998). Overexpression of Bcl2 has proven more informative and appears to function in a neuroprotective way following experimentally induced neuronal damage (Alkayed et al. 2001, Martinou et al. 1994, Parsadanian et al. 1998). The impact of these genetic manipulations on neuronal survival in sexually dimorphic nuclei has not been examined, but overexpression of Bcl2 appears to decrease programmed cell death in the spinal nucleus of the bulbocavernosus (SNB) in female mice. Nearly all of the motoneurons in the SNB of females normally die unless exposed to androgen during perinatal life, and this action of androgen appears to depend on expression of androgen receptors within the spinal motoneurons (Breedlove 1992, Forger et al. 1992). Overexpression of Bcl2 in neurons increased the number of motoneurons in the SNB by approximately 40% (Zup et al. 2001). The molecular mechanisms underlying the observed effects of Bcl2 on hormonally regulated neuroprotection are unknown, but the ability of estrogen to alter Bcl2 gene expression via protein-protein interactions with other transcription factors such as Sp-1 and Brn-3a (see Alkayed et al. 2001 for references), or the induction of Bcl2 expression by hormone-sensitive transcription factors such as the cAMP response element binding protein (CREB; see below), suggest possible regulatory mechanisms.

To date, analysis of PCD in the hawk moth represents the most completely characterized example of hormone-dependent neuronal survival and underscores some of the gaps in the mammalian literature (see Alkayed et al. 2001, Truman et al. 1992). During metamorphosis, motoneurons innervating specific proleg muscles in the hawk-moth larva die owing to a rapid fall in levels of the steroid hormone ecdysone, which signals the end of pupal life at adult emergence. These motoneurons express receptors for ecdysone and show segment-specific patterns of hormonally triggered PCD that are restricted to certain developmental stages. That this process occurs in isolated motoneuron cultures in vitro demonstrates cell autonomous regulation of neuronal survival (Zee & Weeks 2001). Given the conservation of PCD signaling mechanisms, it is likely that hormonal regulation of neuronal survival in mammalian systems is equally dependent on cell autonomous factors. Perinatal hormones appear to reduce cell death in some regions, such as the MPN and BSTp, while increasing the loss of cells in the AVPV. Moreover, these effects are cell-type–specific. For example, exposure to sex steroids perinatally decreased cell number and increased DNA fragmentation in the AVPV during the postnatal period but did not significantly alter the volume of the nucleus until much later (Davis et al. 1996). However, the same perinatal hormone treatment increases the number of enkephalinergic neurons that mature in the AVPV of rats, while decreasing numbers of dopaminergic or dynorphin-containing neurons (Simerly 1998). Thus, examinations of gross changes in the volume or neuron number of sexually dimorphic nuclei are likely to obscure cell-specific signaling events.

Sexual differentiation of distinct neuronal subpopulations in sexually dimorphic nuclei has been studied by using immunohistochemical markers (De Vries 1990), but visualization of neurotransmitter substances often requires severe colchicine pretreatment. Equally problematic is that neurochemical markers are often activationally regulated by sex steroids, which can confound interpretation of developmental events. Tyrosine hydroxylase (TH) has proven to be a reliable marker for dopaminergic neurons, is abundantly expressed in hypothalamic neurons, and is relatively resistant to acute regulation by sex steroid hormones. TH immunostaining was used to reveal that the AVPV contains a sexually dimorphic population of dopaminergic neurons that are more abundant in female rats and that sexually differentiate in response to perinatal sex steroids (see Simerly 1999 for review). This sex difference develops postnatally and can be completely reversed by exposing newborn animals to either testosterone or estrogen. Estrogen is as effective as testosterone in defeminizing the pattern of gonadotropin secretion, and the development of the sexually dimorphic dopaminergic neurons in the AVPV is dependent on the alpha form of the ER. The sex difference in dopamine neurons in the AVPV is conserved in C57Bl/6 mice, and most of the TH neurons transiently express the ERα during the first week of life (E.M. Waters & R.B. Simerly, unpublished observations). Mutation of the ERα prevents the loss of TH immunoreactive neurons normally seen in males, but TH neurons in the AVPV of Tfm mice, which have a naturally occurring mutation in the androgen receptor, develop normally, which indicates that sexual differentiation of these cells is independent of the androgen receptor (Simerly et al. 1997). Moreover, estrogen appears to act directly on the AVPV to specify the number of TH immunoreactive neurons that remain in adult animals because exposure of organotypic explants of the AVPV to either testosterone or estradiol for 24 hours under defined conditions results in a persistent loss of these cells (Ibanez et al. 1998). The effects of steroid exposure on TH immunoreactive neurons in the AVPV appear to be permanent, but hormone exposure is less effective if it is delayed until after 6 days in vitro, which suggests that estrogen exerts a cell-type– and receptor-specific action on the sexual differentiation of dopamine neurons in the AVPV during a restricted postnatal period. Although it is likely that estrogen can increase PCD in the AVPV (Arai et al. 1996), it is unclear whether it enhances death of dopaminergic neurons or simply effects a lasting change in neurotransmitter phenotype. An example of the latter pattern of development is found in the BST, where perinatal steroids appear to specify a subset of galanin-containing neurons to coexpress vasopressin (De Vries & Simerly 2002). Thus, in addition to morphological changes, the number of neurons in brain regions that control reproduction may undergo dramatic changes in gene expression during perinatal development and display sex-specific patterns of differentiation into mature phenotypes.

CONNECTIVITY The organization of neural connections between sexually dimorphic nuclei determines how information is transmitted through these pathways and therefore has a profound influence on their functional output. In the developing

brain, axons must navigate through complex environments under the influence of local cues to reach their appropriate targets to accurately establish patterns of connectivity that determine the display of adaptive behavioral and physiological responses. Over 25 years ago Toran-Allerand and colleagues (Toran-Allerand 1976) demonstrated the neurotrophic effects of estrogen on neurite outgrowth from hypothalamic explants that were remarkably similar to the trophic action of nerve growth factor on neurite outgrowth from dorsal root ganglion neurons (Levi-Montalcini 1987). This similarity did not go unappreciated by Toran-Allerand who went on to show that estrogen synergized with insulin to promote neurite extension of both hypothalamic and cortical explants, and she correctly predicted a role for the insulin-like growth factor I (IGF-I) (Toran-Allerand et al. 1988). Although its role in sexual differentiation remains to be defined, IGF-I has a clear neuroprotective role that is dependent on estrogen (Garcia-Segura et al. 1996b). In addition, the IGF-I receptor is necessary for hormonal regulation of synaptic plasticity in the arcuate nucleus of the hypothalamus by estrogen (Cardona-Gomez et al. 2000). Common elements in the IGF-I signal transduction pathway and nongenomic mechanisms for estrogen signaling such as those mediated by the mitogen-activated protein kinase (MAPK) pathway (see below) may underlie many of the interactions between estrogen and IGF-I on neural development (Garcia-Segura et al. 2001). In addition to neurotrophic factors, other proteins involved in axonal growth and synaptogenesis, such as the growth-associated protein-43 (GAP-43) (Skene 1989), may participate in hormone-induced neurite outgrowth. Expression of GAP-43 is regulated by estrogen in the hypothalamus of postnatal and adult rats, and males have significantly higher levels of GAP-43 in the preoptic region during the postnatal period (Shughrue & Dorsa 1992, 1994).

SYNAPTOGENESIS There is now compelling evidence that estrogen represents a powerful neurotrophic agent that promotes synaptogenesis in a variety of functional neural systems during development and in adulthood. Estrogen treatment increases the density of axodendritic contacts in the hypothalamus and hippocampus (Matsumoto et al. 2000, Woolley & McEwen 1992), and some of these effects are accompanied by changes in neuronal signaling (Woolley 1999, Woolley et al. 1997). In the arcuate nucleus of the hypothalamus, the number of both spine and somatic synapses in female rats is approximately twice that of males, but there do not appear to be differences in the incidence of synapses on dendritic shafts (Matsumoto et al. 2000). Whether the greater innervation of the arcuate nucleus by the AVPV contributes to this sex difference is unknown, but exposure to testosterone perinatally caused a significant reduction in the density of axospinous synapses by the second postnatal day (Mong et al. 2001). In contrast, the ventrolateral part of the VMH has more synapses in male rats, relative to that of females (Matsumoto et al. 2000), which may reflect its innervation by more neurons in the MPN and other sexually dimorphic cell groups. The sexually dimorphic synaptic pattern in the VMH is localized to its ventrolateral part, which contains most of the neurons that express estrogen receptors.

Estrogen receptors are coexpressed with neurotrophin receptors, such as the trkA and trkB receptors in forebrain neurons, and estrogen regulates expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the cortex, hippocampus, and basal forebrain. Estrogens and neurotrophins tend to exert reciprocal regulation of their receptors, and both the trkA and p75NTR genes contain estrogen response elements, which suggests that at least some of this regulation may be mediated directly by estrogen receptors at the level of gene transcription (see Toran-Allerand 1995 for review). Neurotrophins also appear to participate in the hormonally induced changes in dendritic spine density that occur in the hypothalamus and hippocampus. In primary cultures of embryonic hippocampal neurons, estrogen causes a twofold increase in spine density. BDNF and GABA appear to be involved in this induction of dendritic spines because suppression of BDNF expression by estrogen appears to reduce γ-aminobutyric acid (GABA) activity in inhibitory interneurons (Segal & Murphy 2001). The resulting increase in the activity of pyramidal neurons is thought to lead to formation of new dendritic spines, a process that appears to involve phosphorylation of transcription factor CREB (Murphy et al. 1998). CREB may also participate in the regulation of sexual differentiation by GABA. Treatment of newborn males with the GABA agonist muscimol increased CREB phosphorylation in sexually dimorphic nuclei, whereas only decreases in phospho-CREB were seen in females (Auger et al. 2001b). Thus, neuronal signaling initiated by exposure to excitatory GABA during postnatal development may exert a sex-specific activation of CREB with enduring consequences on neuronal survival and differentiation (McCarthy et al. 1997). That CREB phosphorylation is increased by estrogen in sexually dimorphic nuclei such as the AVPV, VMH, and BSTp (Auger et al. 2001a, Gu et al. 1996, Zhou et al. 1996), in vivo as it is in the hippocampal cultures, supports the notion that increased activation of CREB by estrogen promotes synaptogenesis in sexually dimorphic forebrain circuits. Estrogen may also signal through the estrogen receptor to influence spine formation locally through receptors expressed in or near the spine (Blaustein et al. 1992, McEwen et al. 2001, Milner et al. 2001). Taken together with direct imaging experiments that show dynamic interactions between dendritic spines and afferent axons during synaptogenesis (Dailey et al. 1994, Zhai et al. 2001, Ziv & Smith 1996), these observations suggest the possibility that steroid hormones influence synapse formation in sexually dimorphic nuclei by regulating the formation of dendritic spines, which then promote synapse formation with afferent axons.

AXONAL GUIDANCE Little is known about whether sex steroids direct axons to their targets or simply determine the number of neurons in sexually dimorphic nuclei that provide inputs to other parts of the forebrain. In general, regions that contain more neurons in one sex have more neurons that provide inputs to other parts of the forebrain. Thus, there are more neurons in the MPN in males that project to the periaqueductal gray (Lisciotto & Morrell 1994) and more neurons in the AVPV of females that project to the arcuate nucleus of the hypothalamus (Gu &

Simerly 1997). On a similar note, the projection from the BSTp to the AVPV is approximately an order of magnitude more robust in male rats compared with the homologous pathway in females, a sex difference determined by perinatal exposure to testosterone (Gu et al. 2001, Hutton et al. 1998). However, the magnitude of this sex difference is surprising given that the AVPV is smaller and has fewer neurons in males. In most neural systems the number of projection neurons tends to be developmentally regulated so as to promote registration with target fields. Therefore, one might expect that, although there are approximately twice as many neurons in the BSTp of males, the reduction in the number of target neurons in the AVPV would produce a compensatory reduction in the density of inputs to the AVPV from the BSTp. Indeed the opposite is true: The magnitude of the sex difference in the projection from the BSTp to the AVPV is much greater than that of other BSTp terminal fields, which suggests that there is a considerable degree of site-specific regulation in the development of BSTp projections (Gu et al. 2001). Thus, the projection from the BSTp to the AVPV represents a direct neural pathway between two regions with divergent developmental histories: Exposure to sex steroids increases the number of cells in the BSTp, with robust projections to the AVPV, yet has the opposite effect on AVPV neurons, resulting in a massive convergence of information relayed by the BSTp onto the remaining AVPV neurons in males. It is intriguing to speculate that such target-dependent control of the development of connections between sexually dimorphic nuclei provides great flexibility in determining circuit architecture and that the functional outcome may be that the same sensory cue can have profoundly different effects on reproductive function in males and females.

Evidence from a variety of model systems indicates that axons respond to regionally specific contact-mediated guidance cues and are either attracted or repelled by diffusible factors that influence the behavior of individual growth cones as they seek their targets (see Goodman 1996, Goodman & Shatz 1993, Song & Poo 1999, Tessier-Lavigne & Goodman 1996 for reviews). The response of a particular axon depends on its complement of receptors for chemotropic molecules and on its sensitivity to activity-dependent developmental activities. Although it is common for axons to form exuberant terminal fields that are then restricted through regressive events such as those that are responsible for localizing retinal projections to specific parts of the tectum (Cowan et al. 1984, O'Leary 1992), the BSTp to AVPV pathway develops in a sexually dimorphic pattern that suggests a directed mechanism of axonal guidance (Hutton et al. 1998). Moreover, sex steroids act on the AVPV in a target-dependent way to direct development of BSTp inputs despite the fact that both the AVPV and BSTp express high levels of receptors for sex steroids both in vivo and in vitro (Hutton & Simerly 1997). Reconstitution of the BSTp to AVPV pathway in vitro by preparing heterochronic cocultures revealed that a high density of neurites extend from the BSTp to the AVPV explant only when the AVPV explant is derived from a male animal or from a female that was treated with testosterone perinatally (Ibanez et al. 2001). Only a few neurites extend from the BSTp explant toward the AVPV explant when the

cocultures are prepared with an AVPV explant derived from a female rat, regardless of whether the BSTp explant is taken from a male or female rat. Therefore, even though many sexually dimorphic nuclei express steroid hormone receptors during development, the development of connections between them may be directed in a site-specific way, rather than by a concomitant action on several interconnected sexually dimorphic nuclei.

Because the cocultures are suspended in a collagen matrix, the target-dependent developmental activity specifying formation of BSTp to AVPV connections is likely to be a diffusible factor that acts on BSTp neurons. Moreover, when cocultures are prepared with AVPV explants derived from rats that are significantly younger or older than P10–P12, the time when BSTp to AVPV projections normally form in vivo, there is a marked reduction in the density of neurites that grow between the explants. This observation suggests that the diffusible factor is expressed in a temporally specific pattern. Thus, the sexual differentiation of the BSTp to AVPV pathway is likely a target-dependent developmental event mediated by the hormonal induction of chemotropic factors that act specifically on BSTp neurons during a defined postnatal critical period (Ibanez et al. 2001). Proof of this hypothesis awaits isolation of differentially expressed factors that display the appropriate developmental activity. Netrins, semaphorins, and slit proteins are certainly candidates (Brose & Tessier-Lavigne 2000, Raper 2000, Tessier-Lavigne & Goodman 1996), and members of the ephrin family of factors (O'Leary & Wilkinson 1999) may influence migration of BSTp axons through the preoptic region prior to reaching the AVPV. Although there is rapid progress in identifying molecular cues that direct formation of neural connections, none have been shown to be developmentally regulated by sex steroids. As outlined above, the projections of the AVPV are also sexually dimorphic, and it is unknown if the development of sexually dimorphic projections to GnRH or TIDA neurons are the result of a direct action of sex steroids on AVPV neurons or are induced by target-derived factors. GnRH neurons contain ERβ but do not express the ERα (Herbison & Pape 2001, Hrabovszky et al. 2001, Shivers et al. 1983), and a substantial number of TIDA neurons express ERα (Sar 1984). Therefore, estrogen may direct development of projections from the AVPV to these neuroendocrine neurons through either a neurotrophic action on AVPV neurons, a target-dependent mechanism, or a combination of the two. Alternatively, estrogen may act on GnRH neurons through an ER-independent mechanism or through transynaptic regulation of factors that specify synapse formation.

Sex Steroid Signaling During Development

One of the major advantages of studying developmental events regulated by sex steroid hormones is that much is known about the detailed signaling pathways utilized by these hormones to alter cellular processes. Thus, a key developmental factor in controlling sexual differentiation of forebrain pathways is the expression of receptors for estrogen and androgen by neurons in sexually dimorphic nuclei. Indeed all of the major sexually dimorphic nuclei in the mammalian forebrain contain high densities of neurons that express ERα, AR, and, possibly, the ERβ

(Shughrue et al. 1997, Simerly 1995b). The demonstration by Toran-Allerand and colleagues that the ability of estrogen to induce neurite outgrowth from brain explants appeared to be limited to tissue that contained high densities of estrogen receptors (Toran-Allerand et al. 1980) supports this concept. On a similar note, the failure of dopamine neurons in the AVPV to sexually differentiate in ERα knockout mice (Simerly et al. 1997) is a good example of how important these nuclear proteins can be for neuronal sexual differentiation. The major role of steroid receptors is to function as ligand-activated transcription factors that regulate diverse patterns of gene expression (see Aranda & Pascual 2001, Zhang & Lazar 2000 for reviews). These receptors bind specific sequences that function as *cis*-acting hormone response elements located within or near hormone responsive genes to influence promoter activity. The promoter regions of several genes known to effect neural development contain putative estrogen response elements, including brain-derived neurotrophic factor (Sohrabji et al. 1994). Coexpression of the ERα and ERβ in sexually dimorphic nuclei (Greco et al. 2001) adds considerable range to potential regulatory actions of estrogen because the two receptors appear to play complementary but not redundant roles in modulating gene expression, show distinct patterns of autoregulation, and have unique pharmacological properties (see Woolley 1999 for references).

Similarities between hormone response elements for different hormone receptors can lead to functional interactions. For example, thyroid hormone receptors can inhibit estrogen induction of transcription (Glass et al. 1988), which may account for similar interactions between estrogen and thyroid hormone in the modulation of behavior (see Pfaff et al. 2000 for review). Because thyroid hormone receptors are expressed abundantly in sexually dimorphic nuclei, their coexpression with ERs within individual neurons (Kia et al. 2001) may allow estrogen to directly suppress or enhance the profound developmental activities of thyroid hormone on neuronal development. Neonatal treatment with thyroid hormone results in elevated levels of choline acetyltransferase in male, but not female, rats (Westlind-Danielsson et al. 1991). The choline acetyltransferase gene contains a putative ERE (Miller et al. 1999), and the sexually dimorphic response to thyroid hormone supports the idea that sex steroids can alter brain development through interactions with thyroid hormone receptors. However, the same hormonal treatments did not alter the morphology of hippocampal neurons (Gould et al. 1991). More central to the action of sex steroid hormone receptors are coregulatory proteins that function as coactivators to increase transactivation by steroid hormone receptors, or they act as corepressors to lower transcriptional activity of target genes (McKenna et al. 1999, Shibata et al. 1997). Expression of these coregulator proteins in cell-type–specific patterns has a major impact on how cells respond to sex steroids and may allow different populations of neurons to display divergent responses to perinatal hormone exposure. Differential expression of nuclear receptor coregulators may also be responsible for specifying critical periods for the developmental activities of sex steroid hormones, such as the temporally specific loss of dopamine neurons in the AVPV or the target-dependent induction of afferents from the BSTp.

In addition to the direct genomic actions of nuclear hormone receptors, steroid hormones can regulate transcriptional events by altering expression of other transcription factors such as the protooncogene Fos (Insel 1990) or by coupling to second-messenger pathways (Kelly & Levin 2001). Thus, hormonal regulation of transcription factors or second-messenger signaling to the nucleus may mediate the induction of hormone-sensitive genes that lack conventional hormone response elements. Estrogen elicits a rapid and sustained phosphorylation of mitogen-activated protein kinase (MAPK) (Singer et al. 1999, Singh et al. 1999, Watters et al. 1997) as well as activates extracellular signal–regulated kinases (see Toran-Allerand et al. 1999 for review). In addition, rapid phosphorylation of Akt following exposure to estrogen may impact neuronal survival (Datta et al. 1999, Singh 2001) as well as contribute to the stimulation of CREB phosphorylation that promotes synaptogenesis in the hypothalamus and hippocampus. Thus, hormonal regulation of multiple signaling cascades with both rapid and sustained consequences provides a means of integrating and coordinating the action of sex steroids on diverse developmental events. A clear role for this type of developmental regulation in sexual differentiation is lacking, but estrogen induces a receptor-dependent increase in phosphorylation of CREB in the AVPV of adult female, but not male, rats (Gu et al. 1996; G. Gu & R.B. Simerly, unpublished data). One possible interpretation of this sex difference is that the cells that undergo CREB activation have been lost in males. However, neurons in the ventromedial nucleus of the hypothalamus show sexually dimorphic patterns of CREB phosphorylation (Auger et al. 2001a) despite similar numbers of cells in this nucleus (Madeira et al. 2001), which suggests that sexual differentiation may extend to differences in signal transduction pathways. An alternative interpretation is that the organization of local circuits in the VMH (Flanagan-Cato 2000), or sexually dimorphic afferents from other parts of the forebrain, are responsible for sex differences in transynaptic activation of CREB. CREB regulates gene transcription by acting directly on calcium/cAMP response elements but also through interactions with CREB binding protein (CBP) (Shaywitz & Greenberg 1999). In addition to CREB, CBP has binding sites for a wide variety of transcription factors including Fos and Jun (Vo & Goodman 2001). CBP can also bind estrogen and thyroid hormone receptors, which indicates that these protein-protein interactions represent a powerful means of integrating diverse molecular signals at the transcriptional level.

CONCLUSION

Despite many unresolved issues, it is now clear that steroid hormones effect permanent changes in the development of multiple interconnected regions of the mammalian forebrain that participate in the neural control of reproduction and influence other homeostatic functions as well. Estrogen and testosterone regulate most major developmental events including neurogenesis, neuronal migration, cell death, and neurotransmitter plasticity. In addition, sex steroid hormones specify sex-specific patterns of neuronal connectivity by affecting axonal guidance and

synaptogenesis. The signaling events mediating these developmental activities interact at multiple levels with neurotrophin and neurotransmitter signal transduction pathways. In addition, sex steroid hormones signal to the nucleus through their ligand-activated receptors to influence a broad array of gene-expression events that contribute to the important developmental role of these hormones in specifying the architecture of forebrain pathways that are fundamental to propagation of mammalian species.

ACKNOWLEDGMENTS

I would like to acknowlege my many collegues for discussions that aided in the development of the ideas presented here and members of my laboratory for contributing their data and insight. I also thank Drs. Eva Polston and Bradley Cooke for helpful comments on the manuscript. Work in the author's laboratory is supported by grants from the NIH (NS37952, DK55819, and RR00163).

The *Annual Review of Neuroscience* is online at http://neuro.annualreviews.org

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