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Studying Neurotransmitter Systems to Understand the Development and Function of Sex Differences in the Brain: The Case of Vasopressin

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GEERT J. DE VRIES

Introduction

For neuroscientists, the study of sex differences in the brain promises at least two benefits. Investigations of their development can elucidate the processes that form brain structure during ontogeny that generates specific functions and behaviors, while investigations of the functional significance of these sex differences can reveal how brain morphology and function are related. Except for the fact that sex-related differences in the number of spinal motoneurons have been linked to sex-related differences in the number of specific muscle cells (Kelley 1988; Breedlove 1992), these benefits have been difficult to achieve, however. The complexity of the neuroanatomical connections to and from the brain regions where these differences are found and technical difficulties in manipulating specific sexually dimorphic elements in these areas have delayed the desired result.

This complexity, however, can be exploited. Given that all brain areas contain heterogeneous populations of cells and inputs, focusing on the neurotransmitter content of cells and inputs could reveal whether sexual differentiation selectively affects particular cell populations. This, in turn, could facilitate our understanding of the cellular processes underlying differentiation. Focusing on the neurotransmitter content may also help to reveal the anatomical connections of sexually dimorphic areas, and therefore to assess the impact of a particular dimorphism on other brain areas. Finally, knowing the neurotransmitter systems involved would allow specific manipulation of sexually dimorphic elements by applying specific agonists and antagonists (De Vries 1990). To illustrate some of these advantages, this chapter will discuss a particularly well characterized, sexually dimorphic system, the vasopressin projections of the bed nucleus of the stria terminalis (BNST) and the medial amygdaloid nucleus (MeA).

Sexually dimorphic vasopressin projections in the brain

Discovery of the sexually dimorphic vasopressin innervation of the brain

Both the BNST and MeA show some marked morphological sex differences. In rats, subregions of the BNST are bigger in males than in females (Del Abril et al. 1987; Hines et al. 1992) and contain more neurons in males than in females (Guillamon et al. 1988). Similar differences are found in the MeA (Mizukami et al. 1983; Hines et al. 1992). There are also differences in the number of synapses in the MeA (Nishizuka and Arai 1983) and in specific neurotransmitters other than vasopressin. For example, the posterodorsal area of the MeA and the encapsulated part of the BNST contain more cholecystokinin-immunoreactive cells and receive a denser substance P–immunoreactive innervation in males than in females (Malsbury and McKay 1987, 1989; Micevych et al. 1988).

Although vasopressin-immunoreactive (vasopressin-ir) cells in the BNST and MeA are present in the posterodorsal area of the MeA and the encapsulated area of the BNST, which are the subregions that show the most extreme sex-related differences in size (Hines et al. 1992), these cells are mostly found lateral and ventral to these areas (Caffé and Van Leeuwen 1983; Van Leeuwen and Caffé 1983). Therefore, they appear to contribute only partly to the sex differences in these areas. The sex differences in the projections of these cells were discovered serendipitously, before any other sex difference in a specific neurotransmitter system had been detected anatomically and, in fact, before it was known that these projections came from the BNST and MeA. While studying the development of what was then seen as the projections of the suprachiasmatic nucleus in rats, we stumbled on a large variation in the density of the vasopressin innervation of the lateral septum and lateral habenular nucleus in rats 12 days of age and older. A repeat experiment – with subjects separated according to sex – revealed that males have a vasopressin-ir fiber plexus from the twelfth postnatal day onward, while females show such a plexus only after the twenty-first postnatal day. In adulthood, males still have a denser vasopressin-ir fiber network than females (Fig.11.1; De Vries et al. 1981).

Vasopressin-ir projections in the brain have now been extensively traced. Vasopressin is synthesized by several cell groups, each projecting to distinct areas in the brain (Fig. 11.2). In addition to hypothalamic vasopressin-ir cells, there are several cell groups that are not readily stained for vasopressin unless animals are pretreated with the axonal transport blocker colchicine, most notably in the BNST and the MeA (Caffé and Van Leeuwen 1983; Van Leeuwen and Caffé 1983). The BNST and MeA cells project to several limbic structures, such as the lateral septum and lateral habenular nucleus, and to several midbrain structures, such as
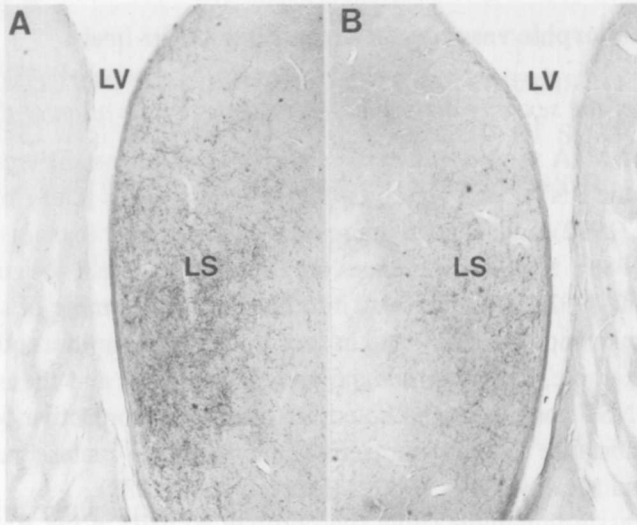
Figure 11.1. Vasopressin-ir fibers in the lateral septum (LS) of a male (A) and female.(B) rat; LV, lateral ventricle.
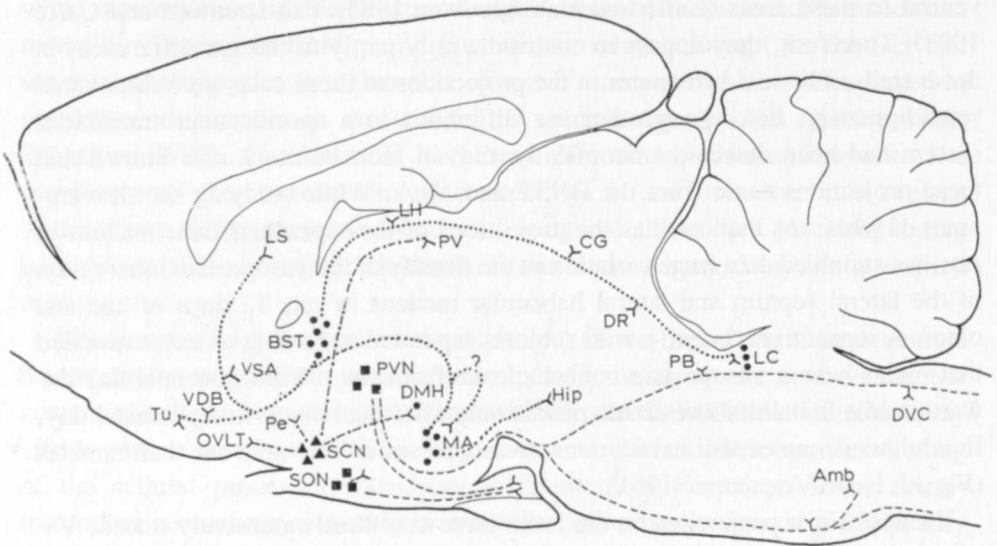
Figure 11.2. Scheme of the most prominent vasopressin-ir pathways. (······) BNST and MeA projections to the lateral septum (LS), ventral septal area (VSA), perimeter of the diagonal band of Broca (VDB), olfactory tubercle (Tu), lateral habenular nucleus (LH), midbrain central gray (CG), dorsal raphe nucleus (DR), locus coeruleus (CR), and ventral hippocampus (Hip). (•—•) suprachiasmatic nucleus (SCN) projections to the perimeter of the organum vasculosum laminae terminalis (OVLT), the periventricular (Pe) and dorsomedial nucleus of the hypothalamus (DMH). (- - -) paraventricular nucleus (PVN) projections to the parabrachial nucleus (PB), dorsal vagal complex (DVC), and ambiguus nucleus (Amb); (- - -) and (- - -) PVN and supraoptic nucleus (SON) projections to the neurohypophysis. (Adapted from De Vries et al. 1985.)
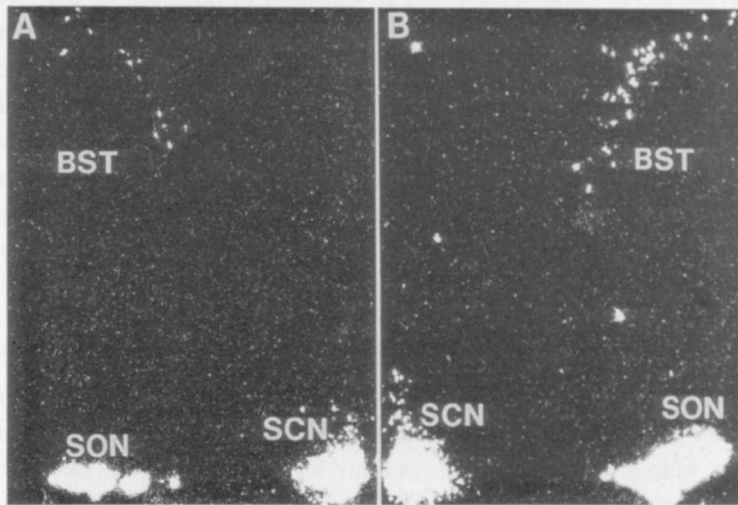
Figure 11.3. Dark-field-illuminated sections displaying cells labeled for vasopressin mRNA in the BNST of a female (A) and a male (B) rat treated with similar levels of testosterone. Labeled cells also show up in the supraoptic nucleus (SON) and the supra-chiasmatic nucleus (SCN).

midbrain central gray, dorsal raphe nucleus, pontine peripeduncular nucleus, and the locus coeruleus (De Vries and Buijs 1983; Caffé et al. 1987, 1989).

In rats, all vasopressin-ir projections of the BNST and MeA appear to be denser in males than in females (De Vries and Al-Shamma 1990). Consistent with these differences, males have about two or three times more vasopressin-ir cells in the BNST than females (Van Leeuwen et al. 1985; De Vries and Al-Shamma 1990; Wang et al. 1993) and a similar difference in the number of cells that can be labeled for vasopressin mRNA (Fig. 11.3; Miller et al. 1989b; De Vries et al. 1994). Although a similar trend was found in the number of vasopressin-ir cells in the MeA in two studies (De Vries and Al-Shamma 1990; Wang et al. 1993), these differences were not significant, possibly because of the larger variation in the staining of MeA cells than in BNST cells. With *in situ* hybridization, nearly twice as many MeA cells labeled for vasopressin mRNA are present in males than in females (Szot and Dorsa 1993).

Such widespread sex-related differences in cell number and density of the projections have not been found in other vasopressin-ir projections, although some of these have sexually dimorphic portions. For example, in gerbils, the vasopressin-ir projections to the sexually dimorphic area of the preoptic/anterior hypothalamic area (SDA) are much denser in males than in females, but the projections to the periventricular nucleus of the hypothalamus do not differ (Crenshaw et al. 1992), even though both appear to come from the suprachiasmatic nucleus (Crenshaw

and De Vries 1991). The widespread sex-related differences in the BNST and MeA projections might be related to the difference in the number of vasopressin-ir cells in the male and female BNST and MeA, whereas the sex differences in the presumed projections of the suprachiasmatic nucleus may be related to the presence or absence of sex differences in the areas they innervate; in this case, they may be related to the presence of a difference between the male and female SDA and the absence of such an obvious difference in the periventricular nucleus of the hypothalamus (Commins and Yahr 1984).

Gonadal hormone effects on vasopressin cells in the BNST and MeA

The vasopressin-ir projections of the BNST and MeA are extremely sensitive to steroid. After gonadectomy, BNST and MeA cells and their projections lose their vasopressin immunoreactivity and can no longer be labeled for vasopressin mRNA; treatment with gonadal steroids prevents these changes from occurring (Fig. 11.4; De Vries et al. 1984, 1985; Van Leeuwen et al. 1985; Miller et al. 1989a). In males, vasopressin immunoreactivity disappears gradually from BNST and MeA projections in about 2–3 months (De Vries et al. 1984); in mice, a similarly gradual decline was observed in females as well as males (Mayes et al. 1988). Biosynthesis of vasopressin declines much faster than vasopressin immunoreactivity. Only 1 day after castration, vasopressin mRNA levels in individual cells were significantly decreased, while the number of cells that could be labeled for vasopressin mRNA were reduced by 90% after 1 week (Miller et al. 1992). These findings suggest that although vasopressin biosynthesis is almost instantly reduced by gonadectomy, vasopressin remains present in projections for several weeks, possibly still capable of influencing brain function. The dramatic reduction of vasopressin biosynthesis, however, suggests that castration also dramatically reduces vasopressin release.

In this regard, there is indirect evidence that vasopressin release is reduced after castration. Following intracerebroventricular (icv) injection of vasopressin, rats showed motor disturbances following a second vasopressin injection given 2 days later (Poulin and Pittman 1991). Injections of hypertonic saline, which stimulates septal vasopressin release (Demotes-Mainard et al. 1986; Landgraf et al. 1988), sensitize rats just as well to the motor effects of vasopressin as does an icv vasopressin injection. In castrated rats, however, vasopressin injections can still sensitize rats to the motor effects of subsequent injections, though intraperitoneal injections of hypertonic saline cannot do so, suggesting that castration eliminates endogenous vasopressin release in the septal area (Poulin and Pittman 1991).

Although the sex-related differences in, and hormonal effects on, the vasopressin-ir projections of the BNST and MeA most likely influence vasopressin release, they do not seem to influence receptor density or sensitivity as do changes
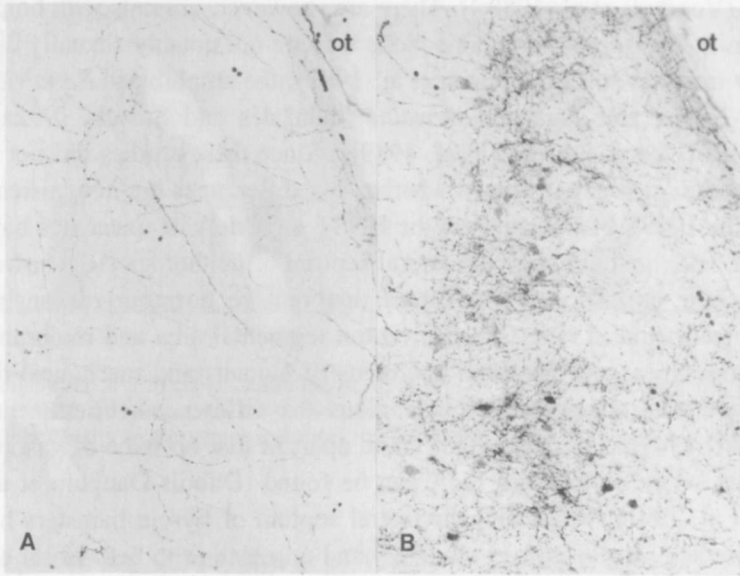
Figure 11.4. Vasopressin-immunoreactive cells and fibers in the medial amygdaloid nucleus of a castrated (A) and an intact male (B) rat. Note that after castration only a few nonbranching, thick-caliber fibers remain (ot, optic tract).

in other peptide systems (Catt et al. 1979). Long-term castration does not affect the distribution of vasopressin binding sites (Tribollet et al. 1988), nor does it affect the number and affinity of vasopressin receptors, vasopressin-stimulated phosphoinositol hydrolysis in septal tissue, or the ability of vasopressin to sensitize septal tissue to the motor effects of a subsequent vasopressin injection (Poulin and Pittman 1991).

Homologous sex differences in other vertebrates

Sexual dimorphism and hormone responsiveness appear to be conserved features of the vasopressin-ir projections of the BNST and MeA. They have been found not only in various rodent species [European (Buijs et al. 1986) and Djungarian hamsters (Bittman et al. 1991), Mongolian gerbils (Crenshaw et al. 1992), prairie and meadow voles (Bamshad et al. 1993), and mice (Mayes et al. 1988)] but also in other mammalian species [ferrets (G. De Vries and M. Baum, unpublished results)]. Similar dimorphism and hormonal responsiveness have been found in homologous, vasotocin-ir projections in nonmammalian vertebrates [amphibians: rough-skinned newts (Moore 1992) and bullfrogs (Boyd et al. 1992); reptiles: the lizards *Gekko gecko* (Stoll and Voorn 1985) and *Anolis carolinensis* (Propper et al. 1992), the turtle *Pseudemys scripta elegans*, and the snake *Python regius* (Smeets et al. 1990); birds: Japanese quails (Viglietti-Panzica et al. 1992) and

canaries (Voorhuis et al. 1988)]. There are, however, animals with homologous vasotocin-ir or vasopressin-ir projections that are not notably sexually dimorphic [rainbow trouts (Van Den Dungen et al. 1982), the amphibians *Rana ridibunda, Xenopus-laevis*, and *Pleurodeles waltii* (Gonzales and Smeets 1992a,b), and guinea pigs (Dubois-Dauphin et al. 1989)]. Since these studies did not quantify immunostaining, it is not known whether sex differences are nonexistent or just subtle. The BNST of humans and the BNST and MeA of macaques have vaso-pressin-ir cells, and although the lateral septum of neither species showed dense vasopressin-ir projections, other areas that receive hormone-responsive vaso-pressin innervation in rats (i.e., the ventral tegmental area and midbrain central gray of macaques, and the locus coeruleus of humans and macaques) did show such projections, although without obvious sex differences (Fliers et al. 1986; Caffé et al. 1989). Syrian hamsters stand apart in that no trace of vasopressin-ir projections of the BNST and MeA can be found (Dubois-Dauphin et al. 1989; Albers et al. 1991). Ironically, the lateral septum of Syrian hamsters has vaso-pressin binding sites (Ferris et al. 1993) and is sensitive to behavioral effects of vasopressin (Irvin et al. 1990). These variations in vasopressin/vasotocin pro-jections may be exploited to reveal the processes underlying their sexual differen-tiation and their functional significance.

Sexual differentiation of the vasopressin projections of the BNST and MeA

Most research on the sexual differentiation of these projections has utilized rats. In these animals, gonadal hormones determine sexual differentiation of centrally regulated functions and behaviors presumably by influencing the development of specific neuronal systems during a restricted – often referred to as critical – pe-riod around birth (Gorski 1984; Yahr 1988). A first attempt to test whether go-nadal hormones also influence the sexual differentiation of the vasopressin-ir pro-jections indicated that gonadal hormones influence these projections not only in the first but also in the third postnatal week (De Vries et al. 1983), which is later than predicted from the critical periods of other sexually dimorphic neural sys-tems (Gorski 1984; Yahr 1988). In retrospect, this first study did not examine whether perinatal levels of gonadal hormones permanently influence the sexual differentiation of vasopressin-ir projections, since the subjects were killed within 4 weeks after birth. This is considerably less than the time required for vaso-pressin immunoreactivity to disappear completely from these fibers after gonadec-tomy in adulthood, which takes more than 8 weeks (De Vries et al. 1984). Conse-quently, it was impossible from these studies to distinguish between permanent and temporary effects of gonadal steroids.

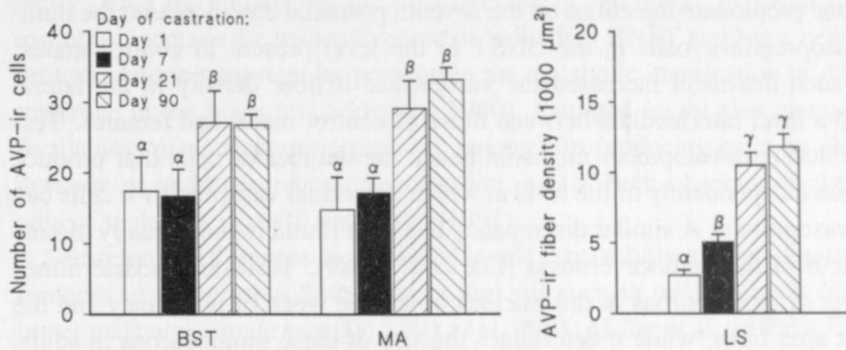We reconsidered whether perinatal levels of gonadal hormones permanently

Figure 11.5. Effects of castration at 1, 7, 21, or 90 days after birth on the number (±SEM) of vasopressin-ir (AVP-ir) cells in the BNST and MeA and the vasopressin-ir fiber density in the lateral septum (LS). Greek letters indicate significant differences by ANOVA.

influence the sexual differentiation of vasopressin-ir projections, by comparing the effects of neonatal manipulations on the appearance of these projections in 3-month-old rats that had been treated with similar testosterone levels for 4 weeks before death. Male rats that were castrated at 3 months of age (control males) showed more vasopressin-ir cells in the BNST and a higher density of vaso-pressin-ir fibers in the lateral septum than neonatally castrated male rats, whose cell numbers and fiber density did not differ from those of female rats that were ovariectomized neonatally or at 3 months of age (control females). This suggested that testicular secretions after birth permanently influence the development of the vasopressin-ir projections of the BNST. A second experiment showed that male rats castrated on the day of birth or at postnatal day 7 had fewer vasopressin-ir cells in the BNST and MeA and a lower vasopressin-ir fiber density in the lateral septum than did either male rats castrated at postnatal day 21 or control males (Fig. 11.5), suggesting that testicular secretions influenced the differentiation of vasopressin-ir projections around day 7. A third experiment confirmed that tes-tosterone propionate treatment on the seventh postnatal day significantly in-creased vasopressin-ir fiber density in the lateral septum of neonatally gonadec-tomized male and female rats and fully restored the number of vasopressin-ir cells in the BNST of neonatally castrated males, but not of females (Wang et al. 1993).

Some discrepancies in the effects of hormonal manipulation on the vasopressin-ir cell number and fiber density may provide clues to the mechanism by which differentiation of this system occurs. There were, for example, no differences of vasopressin-ir cell number in the BNST and MeA of males castrated on postnatal day 1 or 7, or of control females. However, differences were found in the vaso-pressin-ir fiber density in the lateral septum, which was lower in neonatally cas-trated males than in males castrated on postnatal day 7 (Fig. 11.5). In addition,

testosterone propionate injections on the seventh postnatal day increased the number of vasopressin-ir cells in the BNST to the level present in control males, whereas such treatment increased the vasopressin-ir fiber density in the lateral septum to a level intermediate between those of control males and females. Testosterone during development might influence the number of cells that produce vasopressin independently of the level at which individual vasopressin-ir cells can produce vasopressin. A similar discrepancy has been found in the sexually dimorphic nucleus of the bulbocavernosus (Lee et al. 1989). Testosterone determines the number of motoneurons in this nucleus in the last week of pregnancy and the first week after birth, while it determines the size of these motoneurons in adulthood during the first two weeks after birth. Recent studies of the effects of testosterone metabolites on vasopressin mRNA expression suggest that BNST and MeA cells display different sexually dimorphic features, which are determined by separate critical periods.

Cellular basis of sex-related differences in vasopressin expression

Testosterone influences vasopressin production by androgen as well as by estrogen receptor–mediated mechanisms. In castrated male rats, estradiol, which is metabolite of testosterone generated by aromatization (Naftolin et al. 1975), partially restores vasopressin immunostaining in castrated male rats, while dihydrotestosterone, which is a nonaromatizable, androgenic metabolite of testosterone generated by reduction (Lieberburg and McEwen 1975), does not by itself restore vasopressin immunostaining. However, if dihydrotestosterone is given in combination with estradiol, it enhances vasopressin immunostaining (De Vries et al. 1986). Since virtually all vasopressin-ir cells in the BNST and MeA in males are immunoreactive for estrogen as well as androgen receptors (Axelson and Van Leeuwen 1990; Zhou et al. 1994), androgens and estrogens may influence vasopressin production by directly acting on vasopressin ir cells.

Because there are no clearly recognizable androgen- and estrogen-responsive elements in the promoter region of the vasopressin gene (Young 1992; Adan and Burbach 1992), it is not clear whether androgens and estrogens can directly influence the transcription of vasopressin messenger RNA. It is even questionable whether they influence arginine vasopressin (AVP) mRNA transcription at all, since a nuclear run-on assay of BNST tissue did not show any effect of castration on AVP gene transcription. Since the same study showed that castration decreased, while testosterone increased, the length of the polyadenylate tail of AVP mRNA, which may enhance its stability, gonadal hormones might influence AVP mRNA levels at a post-transcriptional level (Carter and Murphy 1993).

Androgens and estrogens might also influence vasopressin production indi-

rectly, each by influencing the effectiveness of the other. Estrogen might, for example, increase the responsiveness of individual BNST and MeA cells to dihydrotestosterone treatment by preventing the metabolic inactivation of dihydrotestosterone in the brain (cf. Södersten 1980). Estrogen might also increase the effectiveness of androgen receptors of vasopressin-producing cells by altering the duration of androgen receptor occupation, as has been observed in the preoptic area of male rats (Roselli and Fasasi 1992).

Sex-related differences in androgen levels cannot fully explain sex-related differences in vasopressin fiber staining and vasopressin mRNA levels observed in intact male and female rats (De Vries et al. 1981; Miller et al. 1989b). If male and female rats are treated with similar levels of testosterone, males show more vasopressin-ir and vasopressin mRNA–labeled cells in the BNST and MeA and denser vasopressin-ir fiber projections from the BNST and MeA than do females (De Vries and Al-Shamma 1990; Wang et al. 1993; De Vries et al. 1994). This sex difference, however, can be caused by differences in the metabolism of testosterone, since the BNST of male rats has a higher level of aromatase, the enzyme that catalyzes the aromatization of testosterone into estradiol, than the BNST of female rats (Roselli 1991). Testosterone could, therefore, stimulate BNST cells more effectively in male than in female rats.

Whether or not a sex-related difference in testosterone metabolism contributes to the sex-related differences in vasopressin cells, it cannot be the only factor, since there are still differences in the effects of estradiol and dihydrotestosterone on vasopressin mRNA expression in male and female gonadectomized rats. More BNST cells respond to estrogen stimulation in males than in females (Fig. 11.6). In addition, these BNST cells show more labeling per cell in males than females, suggesting that in addition to a difference in the number of cells that express vasopressin, there are also sex-related differences in estrogen responsiveness of individual vasopressin-producing cells. Sex-related differences in androgen responsiveness might contribute even more to the sex differences of vasopressin-producing cells in the BNST. Dihydrotestosterone, when given by itself, did not raise vasopressin mRNA levels over those of gonadectomized animals, but when given together with estradiol, it significantly increased the number of vasopressin mRNA labeled cells in males, but not in females (Fig. 11.6).

Since it is not known at which level androgens and estrogens act on vasopressin synthesis, it is not yet clear which factor contributes to the sex differences in androgen and estrogen responsiveness of vasopressin cells. One factor may be steroid receptor levels of individual cells. Although no sex-related differences of estrogen receptors in the BNST have been reported (Brown et al. 1992), the number of androgen receptors associated with the cell nuclear fraction in the BNST is higher in males than in females (Roselli 1991). Such a difference might
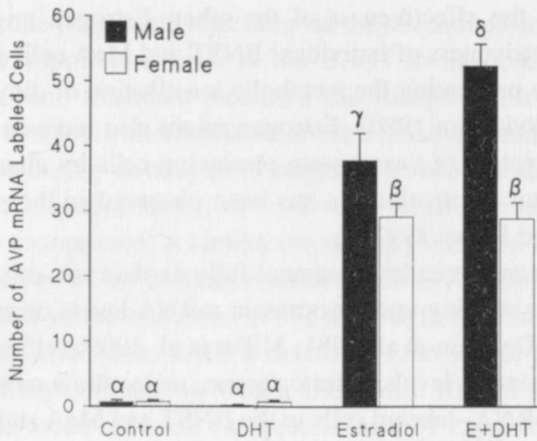
Figure 11.6. Differences in the number ($\pm$ SEM) of BNST cells labeled for vasopressin (AVP) mRNA in male and female rats that were gonadectomized (control) or gonadectomized and treated with dihydrotestosterone (DHT), estradiol, or a combination of estradiol and DHT (E + DHT). Greek letters indicate significant differences by ANOVA.

explain why dihydrotestosterone increases the number of vasopressin mRNA–labeled cells in males but not in females.

Ontogeny of the vasopressin-ir cells of the BNST and MeA

To understand which cellular features make the developing vasopressin-ir cells sensitive to the differentiating influences of gonadal hormones, it would be desirable to study the development of these cells. This is hampered, however, by the late onset of vasopressin mRNA expression in the BNST and MeA, which is detected at postnatal days 3 and 5 in males and at postnatal days 21 and 35 in females, respectively (Szot and Dorsa 1993). This makes it impossible to recognize vasopressin-ir cells during the period when gonadal steroids influence their sexual differentiation. However, these vasopressin cells can be distinguished from surrounding cells in the adult BNST using the thymidine analog bromo-2-deoxy-5-uridine (BrdU) as a "birth marker." Most vasopressin-ir cells appear to be born on embryonic days 12 and 13 (counting the day that sperm plugs were found as embryonic day 1), which places them in the earliest cohort of cells that will survive to form the adult forebrain (H. Al Shamma and G. De Vries, unpublished results). This was surprising, since the majority of the surrounding cells in the BNST and MeA are born on embryonic days 14–16 (Bayer 1980, 1987). This difference in cell birth may be exploited to study the sexual differentiation of the vasopressin-ir cells by following the development of cells labeled by BrdU injections on embryonic days 12 and 13.

Functional significance of sex differences in vasopressin systems

Sexual behavior

One can safely assume that the vasopressin-ir projections are involved in sexually dimorphic functions or at least in functions that are influenced by gonadal steroids, such as sexual behavior. Lesion studies have indeed implicated the BNST and MeA in male sexual behavior (Harris and Sachs 1975; Valcourt and Sachs 1979). Furthermore, the involvement of these vasopressin-ir projections in male sexual behavior fits with the gradual effects of castration on male sexual behavior (Davidson 1966) and with the similarity in the effects of testosterone metabolites on male sexual behavior and on the density of vasopressinergic innervation of the lateral septum (Baum and Vreeburg 1973; Larsson et al. 1973). Treating rats with a centrally acting vasopressin analog, in fact, reversed the decline of copulatory behavior following castration (Bohus 1977). However, injecting vasopressin directly into the lateral septum did not affect male sexual behavior (Koolhaas et al. 1991).

Lesion and stimulation studies have implicated areas innervated by the sexually dimorphic vasopressin fibers in female sexual behavior as well (Nance et al. 1974; Zasorin et al. 1975). In fact, intraventricular injections of vasopressin stimulate female sexual behavior, and similar injections of vasopressin antagonist inhibit it (Södersten et al. 1985). However, a direct link between the vasopressin innervation of the lateral septum and female sexual behavior has never been tested. See De Vries (1990) and De Vries et al. (1992) for more extensive reviews of a possible involvement of these fibers in sexual behavior. This review will concentrate on functions in which septal vasopressin has been directly implicated.

Nonreproductive functions

Vasopressin was one of the first peptides found to influence behavior and was, in fact, among the first to be called a neuropeptide (De Wied 1969). A great many papers have addressed the effects of vasopressin on centrally regulated functions such as learning and memory (De Wied 1969), cardiovascular regulation (Versteeg et al. 1983), thermoregulation (Kasting 1989), and motor behaviors (Kasting et al. 1980). Many of these studies involved injecting vasopressin or its analogs into the ventricles or into areas that do not necessarily receive vasopressin-ir fibers. Since there are several different vasopressin systems (Fig. 11.2), the results of such studies cannot always be related to any of these systems in particular. More recent studies, undertaken with the anatomy of the central vasopressin pathways in mind, have indicated a number of functions in which BNST and MeA

projections could be involved. For example, the vasopressin innervation of the septal area has been implicated in thermoregulation, osmoregulation, social memory, motor disturbances, and aggressive behavior (see later). Competition studies using nonradioactive vasopressin analogs (Dorsa et al. 1988), electrophysiological studies (Raggenbass et al. 1988), *in situ* hybridization studies (Ostrowski et al. 1992), and functional studies suggest that vasopressin receptors in this area resemble vasopressor (V1) receptors and not the antidiuretic receptor or the vasopressin–oxytocin receptor that is found in the hippocampus (Audigier and Barberis 1985).

One of the first functions attributed to septal vasopressin was reduction of fever (Cooper et al. 1979). Since then, fever-reducing effects of vasopressin and fever-enhancing effects of $V1_a$ receptor antagonists injected into the ventral septal area have been demonstrated repeatedly for a variety of mammals including rats (Kasting 1989). The ventral septal area probably receives vasopressin-ir innervation from the BNST. This innervation is denser in males than in females (De Vries and Al-Shamma 1990) and disappears after castration (De Vries et al. 1985) as well as after lesioning of the BNST (De Vries and Buijs 1983). Similarly, electrical stimulation of the BNST alters the electrical activity of ventral septal neurons, as does exogenous application of vasopressin (Disturnal et al. 1985a,b). In addition, stimulation of the BNST attenuates pyrogen-induced fever, presumably by enhancing vasopressin release, since this effect can be prevented by administering a $V1_a$ receptor antagonist into the ventral septal area (Naylor et al. 1988). Castration, which apparently reduces vasopressin release in the lateral septum, lengthens pyrogen-induced fever (Pittman et al. 1988). Vasopressin treatment also reversed the effects of castration on the capacity of the pyrogen interleukin-1 to induce "sickness behavior" (increased sleepiness, lethargy, reduced social activities, reduced food intake). Using social investigation of juvenile conspecifics as an index of sickness behavior, castrated rats were observed to be more sensitive to the fever-inducing and behavioral effects of interleukin-1. Furthermore, vasopressin more effectively attenuated the effects of interleukin-1 in castrated than in intact rats, and, conversely, administration of a $V1_a$ receptor antagonist potentiated the effects of interleukin-1 in intact but not in castrated rats (Dantzer et al. 1991; Bluthe and Dantzer 1992a).

Although vasopressin release in the ventral septal area apparently reduces fever but does not affect body temperature in other ways (Kasting 1989), vasopressin release in the dorsal lateral septum may prevent hypothermia in European hamsters. In this species, the density of the vasopressin-ir fiber plexus in the lateral septum is high during the summer, when testosterone levels are elevated, and low during fall and winter, when testosterone levels are reduced and the animals hibernate (Buijs et al. 1986). Chronic infusions of vasopressin into the lateral septum prevented the bouts of hypothermia that are associated with hibernation

(Hermes et al. 1989). In addition, Silastic implants containing testosterone given at the beginning of the hibernation season kept the density of vasopressin-ir fibers in the lateral septum high and prevented hibernation, possibly by sustaining the release of vasopressin in the lateral septum.

Septal vasopressin could be involved in other homeostatic functions as well. Injections with hypertonic saline or hypovolemia in rats increased the release of vasopressin from the lateral septum (Demotes-Mainard et al. 1986; Landgraf et al. 1988), and dehydration reduced vasopressin immunoreactivity in the lateral septum (Epstein et al. 1983). Although the functional significance of these effects is unclear, they suggest that septal vasopressin may be involved in the regulation of body fluids, which is sexually dimorphic and influenced by gonadal steroids as well (Chow et al. 1992).

Septal vasopressin has been implicated in social behaviors that are influenced by gonadal steroids. Intermale aggression, for example, declines gradually after castration, as does male sexual behavior (DeBold and Miczek 1984). Injections of vasopressin into the lateral septum (or the MeA) reversed this decline (Koolhaas et al. 1990, 1991). Such studies also suggest that septal vasopressin enhances social recognition, defined as the ability of rats to recognize conspecifics they had previously investigated (Dantzer et al. 1988). However, social recognition is not impaired in long-term castrated rats and is even increased in female versus male rats. Social recognition of long-term castrated males and intact females appears to depend less, or not at all, on vasopressin, since it was not affected by $V1_a$ antagonists (Bluthe et al. 1990; Bluthe and Dantzer 1990).

Orchestration of steroid-responsive functions

Given the many actions of the sexually dimorphic vasopressin projections of the BNST and MeA [a list that is likely to grow, since regions innervated by these projections, such as the lateral septum, have multimodal inputs and equally diverse outputs (Jakab and Leranth 1991)], perhaps the most significant action of these projections is to change a set of functions in a coordinated fashion depending on the physiological condition. A good example of such coordinated changes are the physiological and behavioral transitions displayed by seasonal breeders, which also show dramatic changes in the density of the vasopressin-ir projections of the BNST and MeA (Buijs et al. 1986; Bittman et al. 1991).

Reproduction comprises another set of functions that in female rodents demand dramatic changes both in gonadal hormone secretions and in physiological processes such as the regulation of water and energy balance, and body temperature (Numan 1988). Vasopressin-ir projections of the BNST and MeA also exhibit reproduction-related changes in females. For example, vasopressin-ir fibers in the lateral septum are more intensely stained in pregnant than in nonpregnant guinea

pigs (Merker et al. 1980). In addition, the lateral habenular nucleus contains higher levels of assayable vasopressin in lactating than in pregnant rats (Caldwell et al. 1987), and the release of vasopressin from the septum is markedly higher in pregnant and parturient, than in sexually naive, female rats (Landgraf et al. 1991). These results suggest that vasopressin-ir projections of the BNST and MeA are involved in behaviors or functions that change in pregnant and parturient rats, such as maternal behavior, which is altered by icv injections of vasopressin (Pedersen et al. 1982).

Parental behavior

To help differentiate between physiological and behavioral changes in which vasopressin-ir projections may be involved, we compared males and females that become parents. Specifically, we compared the vasopressin innervation of sexually naive with parental animals of two closely related species of voles that differ dramatically in their reproductive strategies: prairie voles (*Microtus ochrogaster*), a monogamous species in which fathers as well as mothers provide parental care, and meadow voles (*Microtus pennsylvanicus*), a promiscuous species in which only mothers provide parental care (Wilson 1982). In prairie vole males, the density of the vasopressin-ir innervation of the lateral septum and lateral habenular nucleus was lower in parental males than in sexually naive males. Since similar differences were not observed in meadow vole males, they might be related to the regulation of paternal behavior. Parental behavior did not affect the density of vasopressin-ir fibers in prairie vole females, which suggests that the involvement of vasopressin may be sexually dimorphic as well (Fig. 11.7, Bamshad et al. 1993). In a follow-up experiment designed to examine when the vasopressin-ir projections change, we showed that pairing male and female prairie voles dramatically influences the density of the vasopressin-ir projections in males but not in females. The density of these projections decreased after mating in males, then increased gradually during the gestation period only to decrease again after the birth of pups (Fig. 11.8; Bamshad et al. 1994). The initial reduction of vasopressin-ir fiber density in the lateral septum and lateral habenular nucleus could reflect an increase in vasopressin release, since it coincides with higher levels of vasopressin messenger RNA in the BNST and with higher levels of testosterone (Wang et al. 1994).

The changes in the pattern of vasopressin innervation of male prairie voles during the reproductive period might be related to changes in social behaviors as well. After mating, prairie voles form stable pair bonds and display increased aggression toward unfamiliar conspecifics and increased paternal responsiveness (Getz et al. 1981; Bamshad et al. 1994). Winslow et al. (1993) showed that icv injections of a $V1_a$ antagonist inhibit the mating-induced increase in aggression
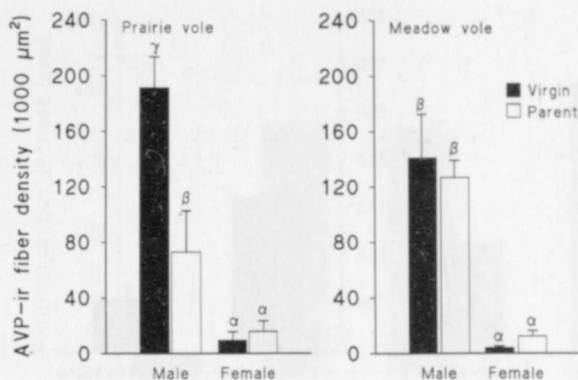
Figure 11.7. Vasopressin-ir fiber (AVP-ir) density ($\pm$ SEM) in the lateral septum of prairie and meadow voles that were either sexually naive or parental. Greek letters indicate significant differences by ANOVA.
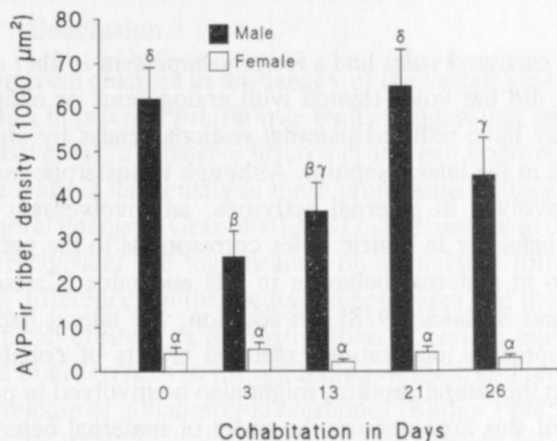
Figure 11.8. Vasopressin-ir (AVP-ir) fiber density ($\pm$ SEM) in the lateral septum before (0) and after 3, 13, 21, and 26 days of cohabitation in males and females. Greek letters indicate significant differences by ANOVA.

and pair bonding in males, suggesting that endogenous release of vasopressin stimulates the increase of aggression and pair bonding.

To test whether septal release of vasopressin also affects parental responsiveness, we injected saline, vasopressin, or the $V1_a$ receptor antagonist d(CH$_2$)$_5$-Tyr(Me) AVP into the lateral septum of sexually naive male prairie voles and recorded the four most prominent paternal activities displayed. Vasopressin stimulated grooming, crouching over, and contacting pups, while the $V1_a$ receptor antagonist blocked these behaviors (Fig. 11.9; Wang et al. 1994). We also observed that castrated voles spent less time grooming, contacting, and crouching over pups, and testosterone treatment reversed these changes (Fig. 11.10; Wang

Figure 11.9. Time spent on paternal behavior during a 10-minute testing period by prairie vole males in which saline was injected into the septum followed by vasopressin (AVP) or in which a $V1_a$ antagonist was injected 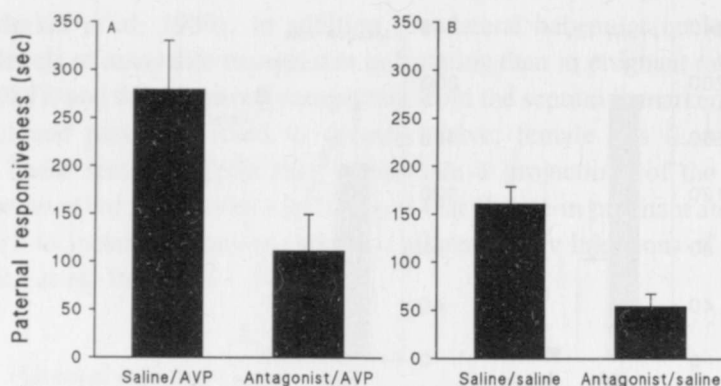followed by vasopressin (t-test, $p \leq .05$), and by prairie voles injected twice with saline or with a $V1_a$ antagonist followed by saline (t-test, $p \leq .001$).

and De Vries 1993). Since castrated voles had a lower vasopressin-ir fiber density in the lateral septum than did the voles treated with endogenous or exogenous testosterone, castration may have reduced paternal responsiveness by changing the vasopressin innervation in the lateral septum. Although testosterone may also influence other systems involved in paternal activities, an involvement of the lateral septum in paternal behavior in prairie voles corresponds to the pattern of involvement of the septum in maternal behavior in rats and mice (Carlson and Thomas 1968; Fleischer and Slotnick 1978). In addition, the lateral habenular nucleus, wherein the vasopressin innervation exhibited effects of cohabitation similar to those observed in the lateral septum, might also be involved in paternal behavior, since lesioning of this area impairs the onset of maternal behavior in rats (Corodimas et al. 1983).

The role of the vasopressin-ir projections of the BNST and MeA in social behaviors could be related to vomeronasal influences on the brain. Pheromones play an important role in the changes of reproductive physiology induced by pairing male and female prairie voles (Dluzen et al. 1981; Lepri and Wysocki 1987). Although it is unknown whether pheromones play a similar role in male reproductive physiology, the aforementioned effects of septal vasopressin on social recognition in rats depend on an intact vomeronasal system. Lesioning of the vomeronasal system temporarily impaired social recognition, which was no longer impaired by injections of $V1_a$ antagonist after recovery, suggesting that the vasopressin-ir projections of the BNST and MeA relay information from the vomeronasal organ to systems involved in social behaviors in intact animals (Bluthe and Dantzer 1992b). This could also occur in voles.
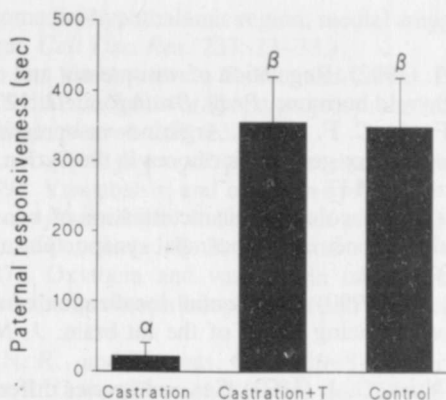
Figure 11.10. Time (± SEM) spent on paternal behavior during a 10-minute testing period by castrated voles, castrated voles treated with testosterone (T), and intact voles. Greek letters indicate significant differences by ANOVA.

Conclusion

Although the changes in the density of the vasopressin-ir projections of the BNST and MeA in paternal prairie vole males suggest that these fibers influence parental responsiveness, aggressive behavior, and pair bonding, it is puzzling why mating does not affect the density of these projections in females, which exhibit the same behavioral changes (Getz et al. 1981). The answer to this question may encourage us to reconsider the significance of sex-related differences in the brain. Sex-related differences in the medial preoptic area and the hypothalamus (e.g., in the size of cell clusters or the distribution of certain transmitters) are thought to be related to differences in the regulation of male and female sexual behavior and/or to the release of gonadotropic hormones (Kelley 1988; Yahr 1988; De Vries 1990; Breedlove 1992). Given the functions identified with the areas where such differences are found, these speculations make sense. They tend, however, to strengthen the notion that sex-related differences in the brain serve mainly to generate sex differences in physiology and behavior. Functional studies of the sexually dimorphic vasopressin-ir projections of the BNST and MeA suggest that the opposite may be equally true – that is, that differences in the brain may enable males and females to display certain hormone-sensitive behaviors in remarkably similar ways even though their hormonal and other physiological conditions differ dramatically.

Acknowledgment

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