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An Oxytocinergic Projection of the Paraventricular Nucleus of the Hypothalamus to the Sexually Dimorphic, Lumbosacral Spinal Cord of the Rat

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AN OXYTOCINERGIC PROJECTION OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO THE SEXUALLY DIMORPHIC, LUMBOSACRAL SPINAL CORD OF THE RAT

By

Anthony Edwin Ackerman

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ABSTRACT

AN OXYTOCINERGIC PROJECTION OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO THE SEXUALLY DIMORPHIC, LUMBOSACRAL SPINAL CORD OF THE RAT

By

Anthony Edwin Ackerman

Oxytocin (OT) has been implicated in many psychophysiological activities: contractile effects on smooth muscle, sexual satiety, alterations in autonomic functions associated with sexual behavior, and the possible formation of social bonding. Previous studies have demonstrated that OT-containing neurons, found within the paraventricular nucleus (PVN) of the hypothalamus, project throughout the CNS, including the spinal cord. In both males and females, small dosages of centrally administered OT, or somewhat larger peripheral amounts, have facilitatory effects on sexual behavior, whereas relatively large dosages, administered centrally, have inhibitory effects.

In the present study, we extended the analysis of PVN projections to sexually dimorphic regions of lower lumbar spinal cord (L_5-L_6), a region known to contain motoneurons of the spinal nucleus of the bulbocavernosus (SNB) and their dendritic arborization as well as autonomic cell bodies. The distribution of OT-like-IR, PVN neurons and density of OT-like-IR fibers within L_5-L_6 were compared in male and female rats. No sex differences were found in the distribution of OT-like-IR, PVN neurons that project to L_5-L_6.

The majority of these spinal-projecting cells were located in the lateral parvocellular subnucleus. It was also demonstrated that OT-like-IR fibers, presumably originating in the PVN, project to L_5-L_6 in both male and female rats. Subtle sex differences in the density of OT-like-IR fibers were found in L_5. However, this dimorphism was more pronounced in L_6. OT-like-IR fibers and putative terminals were found in the region of SNB.

Finally, n-methyl-d-aspartic acid (NMDA) lesions, which have been shown to destroy parvocellular PVN neurons while leaving magnocellular neurons intact, were used to evaluate the role of parvocellular neurons in controlling male copulatory behavior and seminal emissions. NMDA lesions of the PVN reduced OT-like-IR fibers in lower lumbar spinal cord, whereas mount, intromission, and ejaculatory latencies were unaffected by these chemical lesions. However, significant decreases were found in seminal emission, as measured by seminal plug weights.

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Bruce and Lu Ackerman

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LIST OF ABBREVIATIONS

3V, third ventricle
V-X, laminae of spinal cord
AHA, anterior hypothalamic area
AP, anterior parvocellular subnucleus of the PVN
BC, bulbocavernosus muscle
CC, central canal
cp, cerebral peduncle
DAB, diaminobenzidine
DHT, dihydrotestosterone
DLN, dorsal lateral nucleus
DP, dorsal parvocellular subnucleus of the PVN
E, estrogen
EL, ejaculatory latency
FG, Fluorogold
fx, fornix
ic, internal capsule
IL, intromission latency
iml, intermediolateral column
-IR, immunoreactive
IC, ischiocavernosus muscle
ICV, intracerebroventricular
L_{1-6}, levels of lumbar spinal cord
LA, levator ani muscle
LHA, lateral hypothalamic area
LP (PVPO), lateral parvocellular subnucleus of the PVN
ML, mount latency
MP, medial parvocellular subnucleus of the PVN
MPOA (MPO), medial preoptic area of the hypothalamus
NMDA, n-methyl-d-aspartic acid
NP, neurophysin
ot, optic tract
OT, oxytocin
PEI, postejaculatory interval
PM, posterior magnocellular subnucleus of the PVN
PRV, pseudorabies virus

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SNB
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T, tes
VMH
VP, v
ZI, zo

PVN, paraventricular nucleus of the hypothalamus
PVPO (LP), posterior subnucleus of the PVN
Re, nucleus reuniens
S_1, first level of sacral spinal cord
SNB, spinal nucleus of the bulbocavernosus
SON, supraoptic nucleus of the hypothalamus
T, testosterone
VMH, ventromedial nucleus of the hypothalamus
VP, vasopressin
ZI, zona incerta

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INTRODUCTION

The sexually dimorphic motor nuclei of the lower lumbosacral cord have been a model for the study of sexual differentiation within the CNS due to their perinatal responsiveness to gonadal steroids. During the past decade, much attention has been given to supraspinal afferents to this region of spinal cord. The characterization of these projections has been important in the understanding of neural circuits involving steroid-sensitive neurons and their role in regulating sexually dimorphic behaviors. Wagner and Clemens (1993) recently described a projection from the paraventricular nucleus of the hypothalamus (PVN) to lumbosacral levels of spinal cord (L_5-S_1). This projection may be part of a circuit responsible for the modulation of male sexual behaviors, including the regulation of penile reflexes and autonomic control of seminal emission. Anatomical and pharmacological studies support the idea of an oxytocin (OT)-containing, hypothalamic projection to the L_5-S_1 region of spinal cord. The purpose of the present study is to characterize the PVN projection to lower spinal cord and to identify any functional relation of this circuit to male sexual behavior.

Neuromuscular System of the SNB

Anatomy of the SNB and Its Target Musculature

The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic population of motoneurons located in the dorsal medial region of the ventral horn in the fifth and sixth lumbar levels of spinal cord in the rat (Breedlove and Arnold, 1980; Schroder, 1980) and the

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mouse (Wagner and Clemens, 1989a) (Figure 1). Because of its location, the SNB is often referred to as the dorsal medial nucleus. These neurons, along with a subpopulation of neurons in the dorsal lateral nucleus (DLN) of the ventral horn at the same level of spinal cord, innervate striatal perineal muscles in both sexes and are sexually dimorphic (Breedlove and Arnold, 1981; Breedlove, 1984; Jordan *et al.*, 1982; Tobin and Payne, 1990). The retrodorsal lateral nucleus and the ventral medial nucleus are two other neuronal populations which are also located in lower lumbar cord. Unlike the previous two populations, the retrodorsal lateral nucleus and the ventral medial nucleus are not sexually dimorphic.

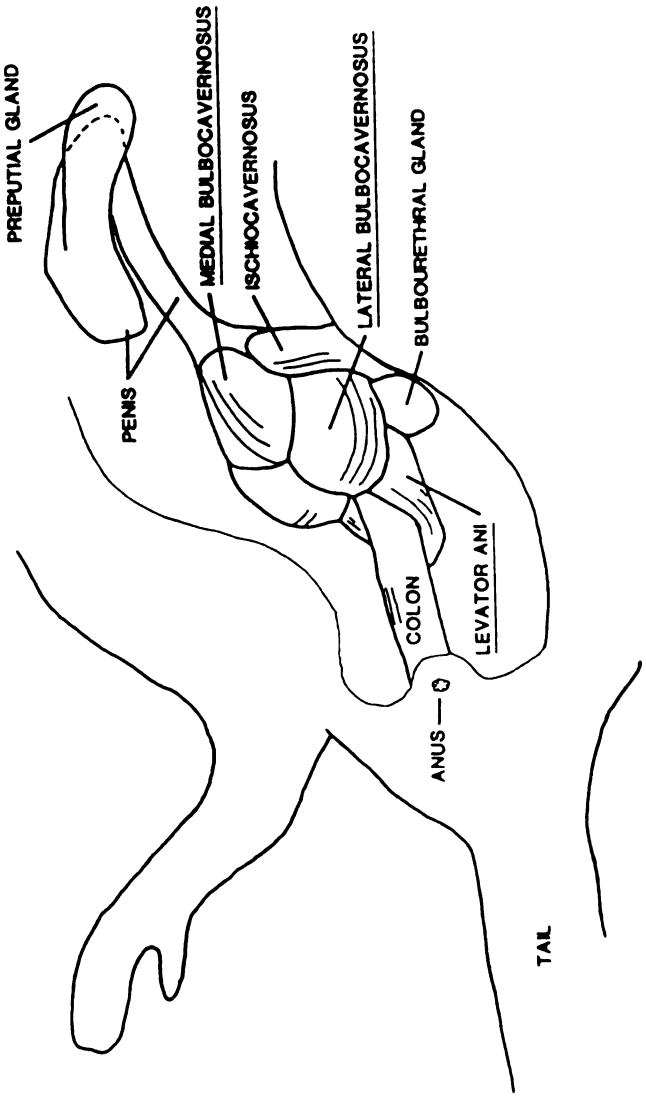
The four striatal perineal muscles in the male rat are the lateral and medial bulbocavernosus (BC), the levator ani (LA), and the ischiocavernosus (IC) (Figure 2). The IC is attached to the ischium and the base of the penis, while the remaining muscles, the BC and LA, are attached exclusively to the penis. The same muscles are found in the female. However, they are atrophic in comparison. This neuromusculature system, the neurons and their targets, is steroid-sensitive during development, as well as adulthood, and serves as a well-studied model for sexual dimorphism in the nervous system.

Functional Aspects

SNB motoneurons innervate the BC muscles which mediate penile reflexes during male copulatory behavior and are necessary for successful reproduction. These reflexes have been characterized in the dog (Hart and Kitchell, 1966; Hart, 1967a; Hart, 1968) and in the rat by Hart (1967b), Breedlove and Arnold (1980) and others (Sachs, 1982; Hart and Melese-d'Hospital, 1983). Penile flips are the result of the contraction of IC muscles innervated by the DLN, while the flared, cup-like erection of the glans penis is produced by the contraction of the BC muscles innervated by the SNB. Dendrites from the SNB and DLN form elaborate

Figure 1 Schematic of lower lumbar segments of spinal cord, L_5 **(A)** and L_6 **(B)**, in the rat. Based on Molander, *et al.* (1984).

Figure 2 Schematic drawing of the perineal region of a male rat. (From Breedlove, 1985)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and large bundles that run between these nuclei (Schroder, 1980; McKenna and Nadelhaft, 1986). Ultrastructural evidence has demonstrated that terminals are found within these bundles, each terminal contacting several dendrites (Anderson et al, 1976). It is thought that these dendritic bundles have a role in the integration of function between cells of these nuclei (Rooney et al, 1979; Rose and Collins, 1985). In a review, Breedlove (1984) describes three tasks accomplished by these penile reflexes in rats. First, they facilitate transport of sperm through the uterus by means of helping to form a proper copulatory plug against the female's cervix. Second, the cup removes from the cervix any copulatory plugs left by previous males. And third, these reflexes provide vagino-cervical stimulation which is essential for signalling the release of prolactin from the pituitary, which potentiates the release of progesterone secretion from the corpus luteum, thereby facilitating pregnancy.

Development of the SNB

During perinatal development, the motoneurons of the SNB are located in the dorsal lateral region of the ventral horn and share a common population of cells with the DLN (Sengelaub and Arnold, 1986). Between embryonic day 22 and postnatal day 10, the cells of the SNB migrate medially toward the dorsomedial region of the ventral horn (Sengelaub and Arnold, 1986). The sex difference in SNB motoneuron numbers is not present during this cell migration. In both males and females, the number of motoneurons in the SNB increases until the day before birth, at which time there are more cells than there are in adult rats (Nordeen, *et al.*, 1984). A critical period of differential cell death occurs, in both sexes, from the day before birth (prenatal day 22) to postnatal day 10. This critical period in the morphology of the SNB corresponds well with the endogenous peak in testostereon (T) as measured perinatally on days 18 and 19 in male and female rats. These T levels are

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significantly higher in males (Weisz and Ward, 1980). It is during this time that sexual differentiation occurs in motoneuron number and size and in the masculinization of perineal musculature (Breedlove and Arnold, 1983b). Females lose a greater number of motoneurons and do not exhibit the permanent enlarging effects of testosterone on SNB motoneurons. In addition, their BC, IC, and LA begin to atrophy as compared to males. The anatomical specificity of the SNB and DLN neuromuscular system is affected by the presence of steroids during development (Breedlove, 1985a and 1985b). During prenatal life, the SNB of the female contains the same number of motoneurons as the SNB of the male (Nordeen, *et al.*, 1985). And, the female's SNB motoneurons make functional synapses with the target musculature (Rand and Breedlove, 1987). However, in the female, these motoneurons undergo naturally occurring cell death in the absence of endogenous T (Nordeen, *et al.*, 1985); whereas in the male, SNB neurons remain, resulting in a sex difference in the number of cells in this nucleus in adulthood. Cell loss is reduced in females treated with androgens and induced in males treated with the anti-androgen, flutamide (Sengelaub, *et al.*, 1989; Nordeen, *et al.*, 1984 and 1985; Breedlove and Arnold, 1983a, 1983b, 1983c). At maturation, the male SNB contains five times more motoneurons than the same region in the female (Breedlove and Arnold, 1980; Breedlove, 1984; Marson and McKenna, 1990).

Hormonal Control in Adulthood

Gonadal hormones have been shown to influence somal size, in both rats (Breedlove and Arnold, 1981) and mice (Wee and Clemens, 1987). However, SNB cell number, in adult rats, is believed to be unaffected by castration (Breedlove and Arnold, 1981). Wagner and Clemens (1989a) demonstrated a decrease in number of thionin-stained neurons in adult mice following castration. But because thionin, a Nissl stain, reacts with nuclear DNA and

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cytoplasmic RNA, they suggest that this decrease in thionin-stained neurons may reflect the decrease in protein synthetic activity of some SNB motoneurons following castration. In the adult rat, castration has been shown to reduce gap junctions and synaptic coverage of SNB motoneurons. This effect was reversed by T replacement (Leedy, *et al.*, 1987; Matsumoto, *et al.*, 1988a, 1988b). Dendritic morphology of SNB motoneurons may also be regulated by androgens, in that castration reduced dendritic length (Kurz, *et al.*, 1986) and arborization (Goldstein *et al.*, 1990).

Penile reflexes, which are controlled by the neuromusculature mentioned previously, are also androgen dependent. This androgen dependency may be observed in two test situations: *ex copula*, in which the penile reflexes are observed while the male is restrained outside a copulatory context; and *in copula*, in which the reflexes are viewed ventrally during copulation. Castration has been shown to reduce the incidence of penile reflexes, while T replacement restored this function to intact levels. (Bradshaw, *et al.*, 1981; Davidson, *et al.*, 1978; Hart, 1967, 1973; Rodgers and Alheid, 1972). It has been shown that the administration of estrogen (E) failed to facilitate penile responses in the castrate during an *ex copula* situation (Hart, 1979) but did restore them during an *in copula* situation (Meisel, *et al.*, 1984; O'Hanlon, *et al.*, 1981). These tests suggest that E may be modulating male sexual behavior by acting at a supraspinal level. It has been documented that, in the adult, SNB motoneurons contain T and dihydrotestosterone (DHT) receptors (Krieg, *et al.*, 1974), but not E receptors (Breedlove and Arnold, 1980), and that motoneuronal size is increased in the presence of both T and DHT (Hall, *et al.*, 1984). These androgen-concentrating SNB motoneurons innervate the BC muscle, which contains androgen and estrogen receptors (Krieg, *et al.*, 1974; Dube, *et al.*, 1976; Dionne, *et al.*, 1979). Interestingly, SNB

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motoneurons do not accumulate either T, DHT or E during pre- or early postnatal life (Fishman, *et al.*, 1990). These authors have suggested that steroidal effects during this time take place via steroid action on the target musculature.

Some PVN neurons which project to the region of the SNB contain E receptors (Sar and Stumpf, 1980; Wagner, *et al.*, 1993) but relatively few androgen receptors (Sar and Stumpf, 1975). In addition, T can be metabolized to E by the P450 cytochrome enzyme. Hagihara and colleagues (1990) have identified sex-specific cytochrome P450 on many oxytocin-like-immunoreactive (OT-like-IR) PVN neurons. They conclude that these sex-specific hydroxylase systems could be involved in the metabolism of steroids.

Because T can restore penile reflexes within a relatively short period of time (6-12 hours), T may be acting on the CNS, rather than peripheral tissue (Gray, *et al.*, 1980; Hart, *et al.*, 1983). It is possible that the elicitation of penile reflexes *ex copula* may involve only a local, androgen-dependent, lumbosacral spinal circuit; whereas, these reflexes *in copula* may involve hypothalamic circuits as well (Clemens, *et al.*, 1993). And because these circuits are the target of steroid hormone action, the activity of these pathways may play a key role in the display of sexually dimorphic behavior.

Afferents to the SNB

Supraspinal sites that control sexual reflexes in male rats have been recently identified. Retrograde tracing, as well as neurotoxic and electrolytic lesions, have served to identify the paragigantocellular reticular nucleus in the ventral medulla as one source of tonic inhibition of spinal sexual reflexes (Marson and McKenna, 1990). This same study employed anterograde tracers to locate descending fibers from this region. Fibers and presumptive terminals were found in the region of the DLN and the SNB, areas containing pudendal and

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pelvic nerve afferents from pelvic organs, and in the region of lamina X surrounding the central canal, an area important for the integration of visceral and somatic information (Honda, 1985). Another study used the retrograde tracer, Fluorogold (FG), to identify other afferents of the SNB (Shen, *et al.*, 1990). The greatest number of cells was present in the medulla oblongata, the lateral vestibular nucleus, and the gigantocellular reticular nucleus (including the ventral and alpha divisions). Cells were also found in the medullary raphe nuclei, the ventral medullary nucleus, and the spinal vestibular nucleus. In the pons, cells were found in the nucleus locus coeruleus, nucleus subcoeruleus, and the caudal pontine reticular nucleus. These results, along with immunohistochemical analysis of descending projection to the region of the SNB, have implicated three, monosynaptic, bulbospinal sources: catecholaminergic input from the nuclei locus coeruleus and/or subcoeruleus (Kojima, *et al.*, 1985), substance P input from the raphe nuclei (Bowker, *et al.*, 1982a; Micevych, *et al.*, 1986), and serotonergic input from the paragigantocellular nucleus (nPGi) (Marson and McKenna, 1990) and other supraspinal sources (Bowker, *et al.*, 1982b, 1982b). Shen and colleagues (1990) found no labelled afferents of the SNB present in the cerebellum, rostral pons, mesencephalon, and cerebral cortex. However, SNB afferents were found in the medial parvicellular division of the hypothalamic paraventricular nucleus.

Oxytocin in the PVN/Lower Lumbar Spinal Cord Pathway

PVN and Oxytocin

The chemical nature of the hormones of the hypothalamo-neurohypophysial system was investigated and identified by Du Vigneaud and colleagues in the early 1950s. These two hormones, OT and vasopressin (VP), are closely related nonapeptides, characterized by a six amino acid ring structure with a three amino acid tail and are associated closely with other,

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supposedly inactive compounds: the neurophysins (Gainer, *et al.*, 1988). By the early 1960s, it was shown that neurohypophysial hormones and their associated neurophysins, neurophysin I (OT) and neurophysin II (VP), were produced as parts of a common precursor. The genes encoding VP and OT are structurally similar. Both have three exons, containing the amino acids for the precursors, and are separated by two introns. The first exon contains the nucleotide bases encoding the signal peptide, followed by the nonapeptide, then a three amino acid spacer which contains the signal for the endoprotease cleavage of the precursor, and then the first nine amino acids of the N-terminal of the neurophysin (NP). The second exon contains the first 66 amino acids of the NP. The third exon contains the C-terminal of this protein. This C-terminal is followed by a single arginine that separates the NP from a C-terminal 39 amino acid glycopeptide in the VP precursor. The OT precursor contains only the single arginine and is similar in internal sequence to provasopressin, except that it lacks the C-terminal glycopeptide (Brownstein, 1983).

Biosynthesis takes place in the hypothalamic perikarya of separate OT- and VP-containing neurons. From the endoplasmic reticulum, the prohormones make their way to the Golgi apparatus and are then packaged into secretory granules. During axonal transport of the granules, the prohormones are cleaved enzymatically to yield VP and neurophysin II, OT and neurophysin I, and a glycopeptide. The compounds are then released within the CNS as neuropeptides or into the periphery as neurohormones, via the posterior pituitary. Currently, the neurophysins and glycopeptide have no known endocrine function.

The half-life of peripheral or centrally injected OT is species specific (Pfaff and Schwartz-Giblin, 1988). The half-time for clearance in CSF is approximately 19 minutes in rats (Mens, *et al.*, 1983). OT's long half-life is beneficial to its capacity to act as both a

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neurohormone as well as a neuropeptide in the periphery and within the CNS, respectively. It appears that OT does not pass easily through the blood-brain barrier (Mens, *et al.*, 1983). However, Mens and colleagues (1983) did observe small amounts being transported bidirectionally between the general circulation and the CSF. The biological significance of this transport is not well understood (Ermisch, *et al.*, 1985; Landgraf, *et al.*, 1979). The occurrence of numerous, conflicting behavioral effects of OT, when delivered peripherally versus centrally, may be explained partially by OT's inability to cross the blood-brain barrier.

Within the hypothalamus, PVN and supraoptic (SON) cells possess gap junctions and chemical synapses (Hatton, 1988; Hatton and Tweedle, 1982). Astroglial processes isolate OT cells, while retraction of these glial cells increase cell-cell contact, forming multiple synapses and dendritic bundles in a steroid dependent manner (Cobbett, *et al.*, 1987; Montagnese, *et al.*, 1987; Montagnese, *et al.*, 1990). These electrical couplings between PVN neurons may synchronize pulsatile release of OT and facilitate the OT surge at the time of lactation and ejaculation. It has been also shown that OT can stimulate its own release (Falke, *et al.*, 1989; Freund-Mercier, and Richard, 1981; Freund-Mercier, *et al.*, 1984; Moos, *et al.*, 1984; Theodosis, *et al.*, 1985). This observed positive feedback may underlie the pulsatile release that is characteristic of this neuropeptide.

Release of OT is dependent on the interaction of steroidal hormones. The PVN contains cells that concentrate radioactively labelled T and its metabolites (Sar and Stumpf, 1975; Stumpf, *et al.*, 1975), as well as cells that produce mRNA for androgen and estrogen receptor proteins (Simerly, *et al.*, 1990). Recently, Wagner, *et al.* (1993) demonstrated efferent projections from E-sensitive PVN neurons to the SNB. Gonadal steroids have been shown to influence the number of electrical couplings (gap junctions) between neurons.

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Castration reduced incidence of dye coupling among magnocellular PVN neurons (Cobbett, *et al.*, 1987), while androgens increased the incidence of gap junctions in the SNB of castrated males (Matsumoto, *et al.*, 1988a). Furthermore, gonadal steroids affected the general morphology of PVN neurons in the adult mouse through organizational effects that occur in the neonate (Perez-Delgado, *et al.*, 1987). Electrophysiological studies have demonstrated that the firing rate of tonically firing, oxytocinergic neurons of the PVN in anesthetized male rats is increased two days after systemic injections of T, but not E (Akaishi and Sakuma, 1985b). But in females, they observed that estrogen selectively and directly excited the tonically firing, presumably oxytocinergic cells (Akaishi and Sakuma, 1985a). Both E and T can increase OT receptor number in some receptor sites (e.g., medial preoptic area and ventral medial nucleus) (see review, Carter, 1992; De Kloet, *et al.*, 1986) and can increase OT mRNA levels (Caldwell, *et al.*, 1989; Miller, *et al.*, 1989). However, these effects may differ across species. For example, Witt, D.M., *et al.* (1991) found that within the VMN of prairie voles, OT receptors may not be E-dependent.

Anatomy of the PVN

The heterogeneous PVN consists of subdivisions that are distinct cytoarchitectonically and can be characterized by their projections (Armstrong, *et al.*, 1980; Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983). The three magnocellular subnuclei of the PVN, the anterior, medial, and posterior magnocellular subnuclei, are known to project to the neurohypophysis (Bargmann and Scharrer, 1951). The PVN also projects to other areas of the CNS (Silverman, *et al.*, 1981) including the brainstem and spinal cord (Kuypers and Maiskay, 1975; Conrad and Pfaff, 1976; Hancock, 1976; Saper, *et al.*, 1976; Swanson, 1977; Ono, *et al.*, 1978; Hosoya, 1980; Schwanzel-Fukuda, *et al.*, 1984). The PVN cells that

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project to cervical, thoracic, and lumbar regions of the spinal cord originate in the four parvicellular subnuclei, the anterior, medial, dorsal, and lateral (also referred to as the posterior) parvicellular subnuclei (Swanson and Kuypers, 1980; Armstrong, *et al.*, 1980). The majority of the neurons that project to the lower regions of lumbar spinal cord, which contain the SNB, arise from the dorsal and lateral parvicellular subnuclei (Monaghan and Breedlove, 1991). These afferents to lower lumbar spinal cord are also sexually dimorphic and androgen-dependent (Wagner and Clemens, 1991).

Oxytocin in Lumbosacral Spinal Cord

The PVN is the major source of OT in the brain and spinal cord. Retrograde tract tracing studies of projections from brain to spinal cord demonstrated that the PVN was the only site, containing neurophysin (NP), found to project to spinal cord (Cechetto and Saper, 1988; Shen *et al.*, 1990; Wagner and Clemens, 1993). Lesions of the PVN resulted in decreases in OT-like-IR at all levels of spinal cord (Hawthorne, *et al.*, 1985; Lang, *et al.*, 1983). The NP present in the sexually dimorphic regions of lower lumbosacral cord probably reflects the presence of OT. Immunohistochemical studies have shown that there are fewer VP-like-IR fibers than OT-like-IR fibers in spinal cord (Buijs, 1978; Sofroniew, 1983). Radioimmunoassay detection has demonstrated that VP levels are lower than OT levels in spinal cord (Hawthorne, *et al.*, 1985; Lang, *et al.*, 1983; Valiquette, *et al.*, 1985). And, there are very few VP-like-IR fibers in the region of the sexually dimorphic regions of the lumbar cord (Newton, *personal correspondence*). Since OT has been shown to regulate male sexual behavior (Argiolas, *et al.*, 1986; 1987a, 1987b; Melis, *et al.*, 1986, 1987, 1989; and see review, Carter, 1992), it is possible that this OT-containing projection from the PVN to lumbosacral spinal cord is involved with some aspects of male sexual responses.

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Role of Oxytocin in Penile Reflexes and Autonomic Functions

The peripheral and central effects of OT on male sexual behavior have been well-summarized in two recent articles (Argiolas and Gessa, 1991; Carter, 1992). Peripherally, plasma OT levels have been shown to increase significantly after ejaculation, as compared to pre-ejaculatory levels (Hughes, *et al.*, 1987). OT released from the neurohypophysis facilitates smooth muscle contractions in pelvic organs (Niemi and Kormano, 1965). OT receptors have been localized in the tunica albuginea, epididymis, and vas deferens (Maggi, *et al.*, 1987). Further, systemic delivery of OT shortened the postejaculatory interval (PEI) and ejaculatory latency (EL) (Arletti, *et al.*, 1985) and decreased the number of intromissions preceding ejaculation (Stoneham, *et al.*, 1985). Centrally, increased CSF levels of OT were measured after ejaculation (Hughes, *et al.*, 1987). Intracerebroventricular (ICV) administration of OT shortened PEI and EL (Arletti, *et al.*, 1985) and increased penile erection frequency (Argiolas, *et al.*, 1987a), as did injections into the PVN (Melis, *et al.*, 1986). In addition, Stoneham and colleagues (1985) found that OT infused ICV increased ML, intromission latency (IL), and PEI. When the potent OT antagonist, $d(CH_2)_5$Try(Me)-Orn^8-vasotocin, was administered ICV, a general decrease in male sexual behavior was observed: 1) mounts decreased and most ejaculations were eliminated (Argiolas, *et al.*, 1989); 2) a dose-dependent decrease in OT- or apomorphine-increased penile erections was seen (Argiolas, *et al.*, 1987b); and 3) increased ML, IL, and PEI were reported (Stoneham, *et al.*, 1985). Electrolytic lesions of the PVN by Hughes and colleagues (1987) eliminated the CSF increases in OT that typically followed ejaculation; these lesions also decreased PEI. However, electrolytic lesions by Monaghan and colleagues (1993) destroyed virtually all OT-like-IR in lumbar spinal cord, but showed little effect on penile reflexes, except for an increase

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latency to the first erection. The latter study seems to suggest that OT may play only a minor role in the modulation of penile reflexes.

It appears that two systems may be involved in male sexual behavior: 1) the peripheral, hypothalamo-neurohypophyseal system, where peripheral OT may exert its effects on the peripheral genitalia and seminal emission (as suggested by Hughes, *et al.*, 1987), and 2) the central system, where OT-containing PVN efferents may exert their effects on PEI and modulate sympathetic and parasympathetic outflow to internal and external genitalia (as also suggested by Hughes, *et al.*, 1987). In her review, Carter (1992) observed that low levels of OT appear to facilitate or accelerate the onset of ejaculatory behavior while high levels of OT inhibit sexual behavior. The segregation of these two oxytocinergic systems would be difficult, at this time, due to technical complexity of employing a peripheral anti-OT, the partial permeability of OT across the blood-brain barrier, and "leaking" OT resulting from a neurohypophysectomy, just to name a few of the difficulties. OT's effects on autonomically mediated, male sexual behavior include erection and seminal emission (parasympathetic) and ejaculation followed by penile detumescence (sympathetic). Central OT, reaching the level of the spinal cord, may participate in tachycardia that accompanies sexual arousal, while systemic release of OT (present at orgasm) may play a role in postcoital bradycardia. The majority of recent investigations of OT's role in seminal emission and prostatic modulation focus on the peripheral effect of OT (Bodanszky, *et al.*, 1992; Sharaf, *et al.*, 1992). However, little attention has been given to OT's role in the central control of seminal emission, especially in lumbar and sacral cord. PVN efferents are known to project to autonomic structures in spinal cord (Luiten, *et al.*, 1985). Swanson (1977) demonstrated a NP-containing autonomic pathway originating in the PVN. While Rhodes and colleagues

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(1981) found that the distribution of E-concentrating oxytocinergic cells in the PVN suggests that they project mostly to autonomic centers rather than to the pituitary.

Summary

The effects of OT on male sexual behavior have been well documented. These effects may be summarized by the following general characteristics: 1) OT is released during pre- and post-ejaculatory behaviors; 2) behavioral actions of OT may be dose- and/or time-dependent; and 3) endogenously released OT may function first to enhance pre-ejaculatory components of sexual behavior, then later inhibit it.

Both anatomical and pharmacological studies suggest that OT from the PVN may regulate penile reflexes and autonomic control of seminal emission. The following experiments describe two OT-like-IR projections from the PVN to lower lumbosacral cord. The first may be monosynaptic, terminating directly on SNB motoneurons. The second may be less direct, forming synapses onto preganglionic parasympathetic cells and their collaterals and/or interneurons involved in modulating parasympathetic control of seminal emission within the vas deferens, coagulating gland, and/or the prostate.

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EXPERIMENT 1: THE DISTRIBUTION OF OT-LIKE-IR NEURONS OF THE PVN THAT PROJECT TO LOWER LUMBAR SPINAL CORD

OT-like-IR neurons have been found within the PVN of the hypothalamus and project throughout the CNS, including the spinal cord (Cechetto and Saper, 1988). Recently, a loss of neurophysin-immunoreactive fibers has been demonstrated in the sexually dimorphic lower lumbar cord (L_5-L_6) following PVN lesions in male rats (Wagner and Clemens, *unpublished observations*).

In this experiment, the analysis of PVN projections to L_5-L_6 was extended by comparing the distribution of PVN, OT-like-IR neurons in male and female rats. This comparison was made using PVN tissue from rats injected with Flurogold into segments L_5-L_6. The objective of this experiment was to determine whether OT-like-IR neurons in the PVN of male and female rats project to sexually dimorphic levels of lower lumbar spinal cord (L_5-L_6), which are known to contain SNB motoneurons.

METHODS

General Methods

Animals used in Experiments I and II were 60-70 day old, Sprague-Dawley, albino male and female rats (Sasco Laboratories, Omaha, Nebraska). They were housed in wire mesh cages in a 14:10 light:dark cycle with food (Wayne rodent blox) and were given tap water *ad lib.* Animals were anesthetized with 60 mg/kg sodium pentobarbital, delivered intraperitoneally, for all surgical procedures.

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Animal Preparation

A laminectomy was performed at the level of the lumbosacral enlargement. A 2 mm incision was made into the meninges with a #11 scalpel blade, and a glass micropipette with an inner diameter of 30-40 μm was stereotaxically-held and visually guided into the cord directly adjacent to the dorsal blood vessel, and then lowered 1.5 mm. During a period of two minutes, 0.8 μl of 4% FG were unilaterally injected into the region of the spinal nucleus of the bulbocavernosus, in 6 males and 6 females.

Tissue Preparation and Histology

Following a survival time of 2-3 weeks, animals were perfused with physiological saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains and spinal cords were then cryoprotected with 20% sucrose in 0.1 M phosphate buffer. Spinal cords were sectioned at 50 μm in the horizontal plane. Alternate sections were mounted on gelatin coated slides and were either counterstained with thionin or left unstained. Sections through the PVN of the hypothalamus were taken at 30 μm in the coronal plane. Alternate sections were either mounted on gelatin coated slides and counterstained with thionin, or left unmounted and processed for immunohistochemistry.

Immunocytochemistry

Immunohistochemistry was performed using antisera against OT (Chemicon International, Inc.). For detection using fluorescent marker, the immunohistochemistry was performed using a biotinylated secondary antibody followed by incubation with rhodamine-tagged avidin (Vector Labs, Inc.). Following immunohistochemistry, sections were mounted onto gelatin coated slides. Unstained sections were dehydrated in 100% ethanol for one

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minute and cleared in xylene for two minutes. Slides were coverslipped with a DPX (Fluka Chemika), a non-autofluorescent mountant.

Photomicroscopy

Two black and white photographs (TMax, Eastman Kodak, Inc.) were taken of each section through the PVN, for each animal, using epifluorescence (FG: 480nm; rhodamine: 570nm). The distribution of FG-labelled cells was drawn on acetate sheets overlaying the photographs. The acetate sheets were then overlaid on the photographs of the oxytocin-containing cells and the distribution was drawn. Those cells appearing to contain FG and rhodamine were confirmed as double-labelled cells on the microscope using epifluorescence. Using adjacent thionin stained sections as reference sections, the distribution and number of FG-labelled, oxytocin-containing, and double-labelled cells within the PVN subnuclei were determined.

RESULTS

Three males and three females were successfully injected with FG into the lumbosacral spinal cord and within the rostro-caudal extent of the SNB (Figure 3). OT-like-IR neurons in the PVN of both male and female rats were shown to project to the sexually dimorphic levels of lower lumbar spinal cord (L_5-L_6) (Figure 4).

The quantification of double labelled cells within the PVN in male and females is summarized in Table 1. Approximately 15% of all FG-labelled cells in the PVN also contained OT. And, the majority of these double labelled cells were located in the lateral parvocellular (lp) subnucleus. The distribution of OT-like-IR parvocellular

Figure 3 Schematic of FG injection placement **(SHADED)** in a horizontal section through lumbosacral spinal cord of the rat. The central canal (cc) is drawn for general orientation; however, the horizontal section is taken ventral to the cc.

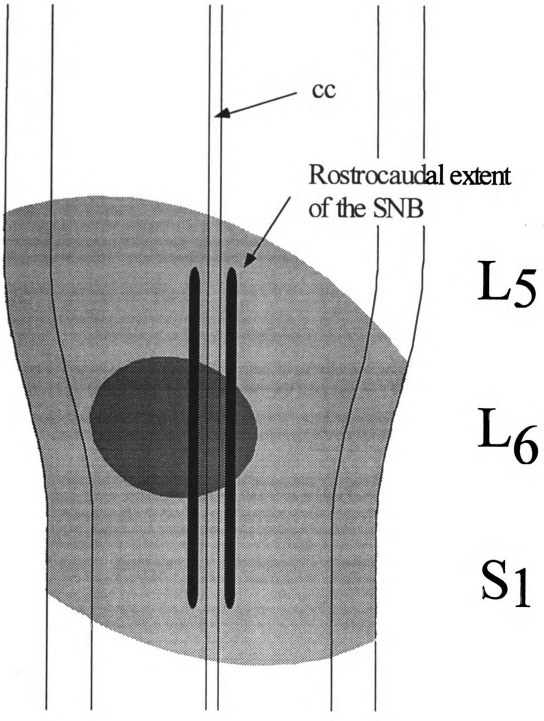
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Figure 4 Epifluorescent photomicrographs showing FG-labelled neurons **(A)** and OT-like-IR neurons **(B)** indicated by the presence of rhodamine in the lp subnucleus of the PVN in a female rat. **Arrows** indicate neurons that contain both FG and OT-like-IR, indicating that some neurons in the PVN that project to L_5-L_6 contain OT. Bar = 100 μm.

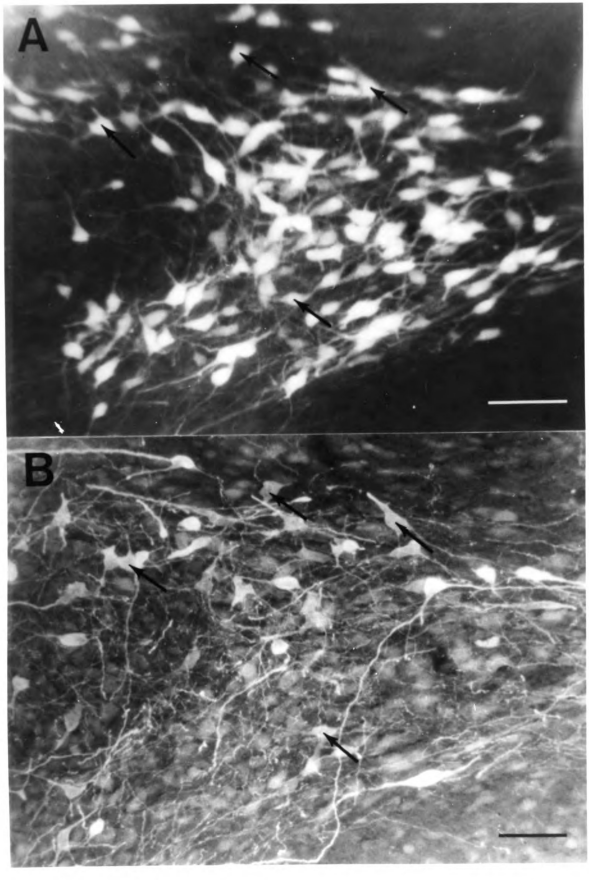
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Table 1 Mean percent (s.e.m.) of OT-like-IR PVN neurons projecting to lower lumbar spinal cord, of double labelled neurons in each subnucleus, and of lower lumbar projecting PVN neurons that contain OT.

CHARACTERIZATION OF OT-LIKE-IR, PVN NEURONS WHICH PROJECT TO LOWER LUMBAR SPINAL CORD

SEX	% OF ALL FG CELLS CONTAINING OXYTOCIN	% DOUBLE LABELLED THAT ARE IN EACH SUBNUCLEUS					% OF FG CELLS IN EACH SUBNCLEUS THAT CONTAIN OXYTOCIN				
		AP	DP	MP	PM	LP	AP	DP	MP	PM	LP
MALE	14.0	2.2	27.4	9.4	21.3	39.7	13.5	13.1	14.6	16.8	15.9
(n = 3)	(3.0)	(1.1)	(4.3)	(2.2)	(8.1)	(12.7)	(6.8)	(2.4)	(3.7)	(3.3)	(5.6)
FEMALE	15.0	3.1	16.8	7.8	21.2	51.0	10.0	11.1	13.9	17.3	17.1
(n = 3)	(2.2)	(0.5)	(4.9)	(2.5)	(4.1)	(5.1)	(3.2)	(0.8)	(5.2)	(2.6)	(2.8)

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neurons that project to lower lumbar cord, was similar to other reports of lower lumbar projecting, NP-like-IR neurons in males (Armstrong, *et al.*, 1980; Wagner and Clemens, *unpublished observations*). Interestingly, there were no sex differences in the number of retrogradely labelled, OT-like-IR parvocellular neurons within the entire PVN or within individual PVN subnuclei.

DISCUSSION

The results demonstrate that approximately 15% of the neurons in PVN that project to lower lumbar spinal cord contain OT. And, the majority of these OT-like-IR neurons were found in the lp subnucleus. These results are similar to previous findings which have characterized NP and OT projections from PVN to cervical (Hoyosa, 1980), thoracic (Armstrong, *et al.*, 1980; Sawchenko and Swanson, 1982; Cechetto and Saper, 1988), and lumbar levels (Wagner and Clemens, 1991) of spinal cord. Sawchenko and Swanson (1982) found that 11-16% of PVN neurons that project to thoracic levels of spinal cord also contain OT. Cechetto and Saper (1988) found a more substantial representation, approximately 20-25%. However, the discrepancy between these two experiments may be due to the use of colchicine in the latter study. This toxin disrupts the axonal transport system and causes the accumulation of peptides in the perikarya. The results of Experiment 1 are also consistent with those of Wagner and Clemens (1991), who showed that approximately 17% of the neurons in the PVN that project to lower lumbar levels of spinal cord contain NP. And, the distribution of lumbar-projecting, OT-like-IR neurons in each the PVN subnucleus was similar to that of the NP-like-IR neurons, which project to the same region of spinal cord (Wagner and Clemens, 1991).

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It is interesting to note the lack of sexual dimorphism in any of the parameters quantified in Experiment 1, especially in a brain region which may be involved in modulating a sexual dimorphic behavior such as penile reflexes. Because both E and T have been shown to alter firing rate of tonically firing, oxytocinergic neurons of the PVN (Akaishi and Sakuma, 1985a and 1985b), increase OT receptor number in MPOA and VMN receptor sites (see review, Carter, 1992; De Kloet, *et al.*, 1986), and increase OT mRNA levels (Caldwell, *et al.*, 1989; Miller, *et al.*, 1989), the dimorphism may lie in differential, hormonal action on OT-containing PVN neurons, which project to sexually dimorphic levels of spinal cord.

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EXPERIMENT 2: CHARACTERIZATION OF OT-LIKE-IR FIBERS AND PUTATIVE TERMINALS IN THE LOWER LUMBER SPINAL CORD

This experiment extended the analysis of the OT-containing projection identified in the previous experiment. The objectives of Experiment 2 were: to identify OT-like-IR fibers in lower lumbar spinal cord of males and females; to compare the distribution and density of OT-like-IR fibers in lower lumbar spinal cord in males and females; and to determine whether OT-like-IR fiber are found in the region of SNB motoneurons and their dendritic fields.

METHODS

Part 1: Distribution and Sexual Dimorphism of OT-like-IR in the Lower Lumbar Spinal Cord

Tissue Preparation, Histology, and Immunocytochemistry

Seven males and seven females were perfused, as described previously. Their spinal cords were sectioned at 30 μm in the coronal plane and then processed for immunohistochemistry, using a DAB chromogen for detection of OT-like-IR. Sections were mounted onto gelatin coated slides and counterstained with thionin. Camera lucida drawings or photographic prints were made of every fourth section through L_5 (camera lucida) and L_6 (photographic prints). The distribution, length, and number of OT-like-IR fibers, exhibiting putative terminals, were quantified using a bioquant system (MegM, R&M Biometrics, Inc., Nashville, TN), and were compared between the sexes.

Part 2: Relationship of OT-like-IR Fibers and Putative Terminals to Bulbocavernosus Motoneurons in Males

Animal Preparation

Two males received 4 µl of 4% FG injected bilateral into the bulbocavernosus using a 10 µl Hamilton syringe. Each injection was made in several smaller injections in various parts of the BC to maximize the number of terminals exposed to the FG.

Tissue Preparation, Histology, and Immunocytochemistry

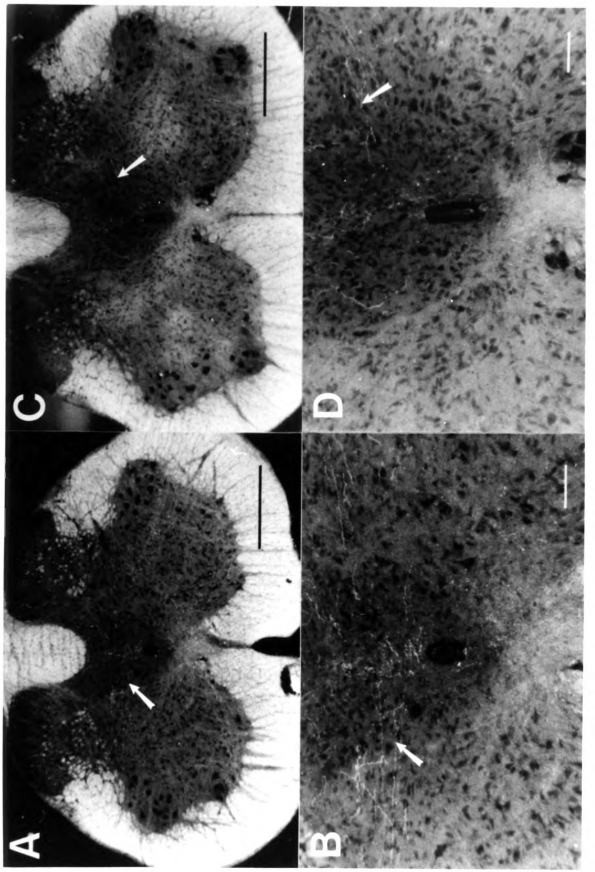
Following a survival time of 48 hours, the animals were perfused, as described previously. Spinal cords were sectioned at 30 µm in the coronal plane and processed for immunohistochemistry for oxytocin using the rhodamine detection (see Appendix). Sections were mounted onto gelatin coated slides and left unstained. They were then viewed using epifluorescence microscopy.

RESULTS

Part 1: Distribution and Sexual Dimorphism of OT-like-IR in the Lower Lumbar Spinal Cord

OT-like-IR fibers and putative terminals were identified throughout lower lumbar spinal cord of both sexes (Figures 1 and 5). These regions included the apex of the dorsal horn (marginal zone and substantia gelatinosa), the intermediate zone [the dorsal gray commissure (laminae V and VI) and the region of the intermediolateral cell column], and lamina X. Very few fibers were seen in the ventral horn, with the exception of the region of the SNB. Fibers were rarely found in the region of the DLN. However, OT-like-IR fibers and putative terminals were found in the region of the SNB. The *en passant* terminals on these fibers appeared to contact cell bodies of SNB motoneurons and their

Figure 5 Darkfield photomicrographs showing the distribution of OT-like-IR fibers **(ARROWS)** within laminae V & VI in coronal sections of lower lumbar (L_6) spinal cord in female **(A and B)** and male **(C and D).** The region above the central canal, in the upper two photomicrographs (Bar = 500 µm), has been enlarged in the lower two photomicrographs (Bar = 100 µm).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proximal dendrites (Figure 6). And although many SNB motoneurons were surrounded by OT-like-IR fiber, others received no contact.

As summarized in Table 2, females were generally found to have a larger number of OT-like-IR fibers and greater length in OT-like-IR fibers than males throughout lumbosacral cord. This dimorphism was more apparent between L_6 and S_1. However, the analysis was only limited to lower lumbar cord, because the SNB of females does not extend into sacral levels of cord. Sex differences, within L_5, were observed in the number of OT-like-IR fibers in laminae V and VI ($t = -2.972$, $p < 0.01$) and in the length of OT-like-IR fibers in laminae VII and VIII ($t = -2.257$, $p < 0.05$). Sex differences were also observed in both the number ($t = -2.729$, $p < 0.05$) and the length ($t = -2.735$, $p < 0.05$) of OT-like-IR fibers in laminae V and VI within L_6.

Part 2: Relationship of OT-like-IR Fibers and Putative Terminals to Bulbocavernosus Motoneurons in Males

Because the SNB innervates both the BC and the anal sphincter, it could not be determined in Experiment 2, Part 1 whether OT-like-IR fibers contacted specifically motoneurons which control penile reflexes. However, following the injection of FG in the BC, it was found that OT-like-IR fibers and putative terminals did approach and appeared to contact SNB motoneurons. And like the thionin-stained tissue, terminals on these fibers appeared to contact cell bodies and their proximal dendrites (Figure 7).

DISCUSSION

The results of Experiment 2 demonstrate that OT-like-IR fibers and putative terminals are distributed throughout lower lumbar spinal cord. Their distribution is consistent with other reports describing OT and NP (OT-specific) in both cervical, thoracic, and lumbar cord

Figure 6 Photomicrograph showing OT-like-IR fibers and putative terminals **(ARROWS)** that appear to contact the thionin stained soma of a male SNB motoneuron. Bar = 50 μm.

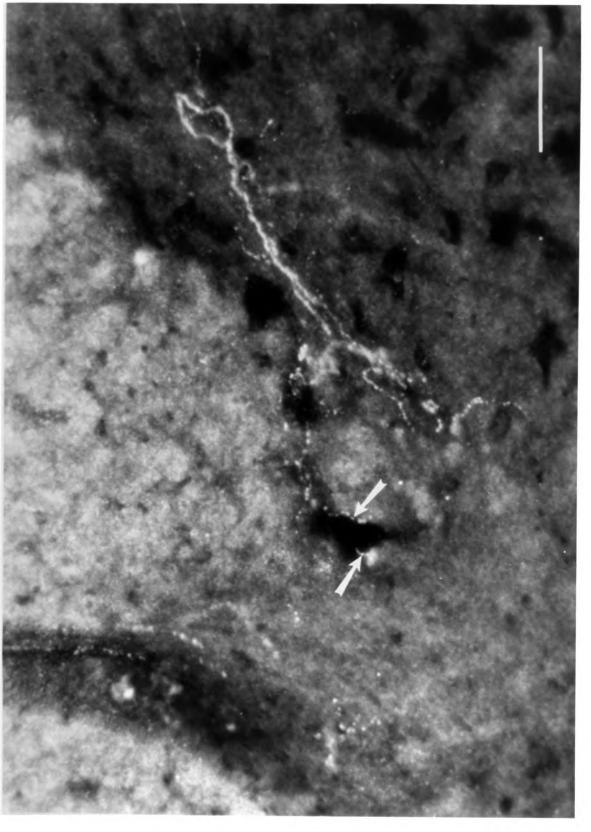

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Table 2 Mean number (s.e.m.) and length in microns (s.e.m.) of OT-like-IR fibers with putative terminals in laminae V & VI, VII & VIII, and X in L_5 and in L_6 of male (n = 7) and female rats (n = 7). Student *t*-test; asterisks indicate significant difference, **(*)** = $p < 0.05$, **(**)** = $p < 0.01$.

MEAN NUMBER AND LENGTH OF OT-LIKE-IR FIBERS WITH PUTATIVE TERMINALS IN L_5 AND L_6

	SEX	MEAN NUMBER (S.E.M.)			MEAN LENGTH IN MICRONS (S.E.M.)		
		V & VI	VII & VIII	X	V & VI	VII & VIII	X
L_5	MALE	53.71	21.95	22.14	0.34	0.06	0.16
	(n=7)	(3.84)	(1.77)	(3.70)	(0.03)	(0.02)	(0.02)
	FEMALE	75.86	17.29	28.71	0.61**	0.15*	0.19
	(n=7)	(11.33)	(3.82)	(2.73)	(0.09)	(0.03)	(0.02)
L_6	MALE	89.14	68.71	65.14	0.61	0.60	0.37
	(n=7)	(12.52)	(10.80)	(7.04)	(0.06)	(0.09)	(0.03)
	FEMALE	180.14*	78.43	105.14	0.88*	0.60	0.32
	(n=7)	(30.91)	(11.84)	(18.63)	(0.08)	(0.08)	(0.03)

* $p < 0.05$

** $p < 0.01$

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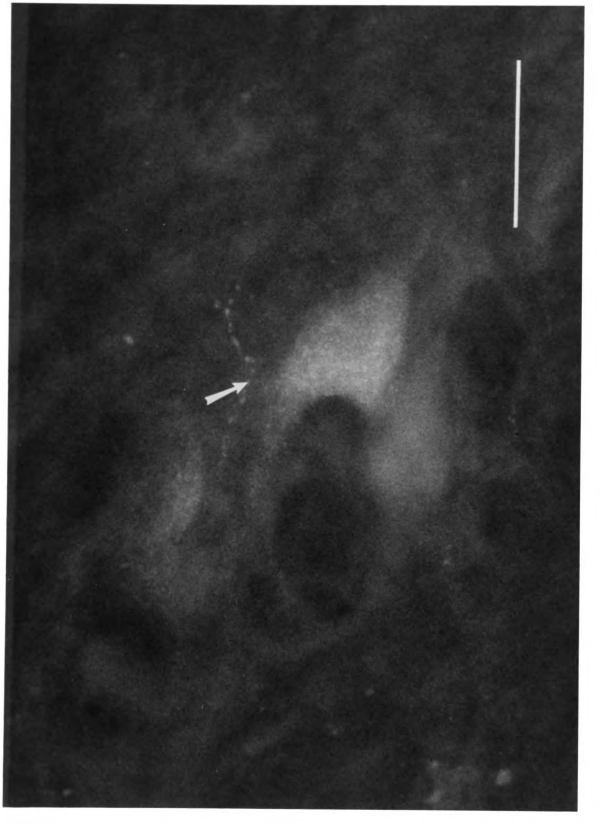
Figure 7 Double exposure photomicrograph using epifluoresence showing FG labelled motoneurons **(LARGE ARROWS)** in the SNB following an injection of FG into the BC muscle and OT-like-IR fibers **(SMALL ARROWS)** indicated by the presence of rhodamine. OT-like-IR fibers approach and appear to contact these motoneurons. Bar = 50 μm.



(Swanson and McKellar, 1979; Wagner and Clemens, 1991). In addition, the location of fibers is consistent with the distribution of anterogradely labelled fibers following *Phaseolus vulgaris* leuco-agglutinin (PHA-L) injections into the PVN (Luiten, *et al.*, 1985). Virtually all of these OT-like-IR fibers within the gray matter, and including the few located within the ventral funiculus and the ependymal cells around the central canal, include putative terminals, approximately 1-2 μm in diameter. The majority of these fibers and putative terminals are generally restricted to the apex of the dorsal horn, the intermediate zone (including the region of the iml and the dorsal gray commissure), and the dorsomedial region of the ventral horn. As mentioned previously, the latter two regions contain the sexually dimorphic SNB and its extensive dendritic field. In addition, OT-like-IR fibers and their putative terminals appear to contact SNB cell bodies and proximal dendrites. However, it must be stressed that, although terminal boutons and *en passant* terminal swellings are thought to be presynaptic structures, ultrastructural verification would be required for determining whether or not these OT-like-IR terminals are actually making chemical synapses onto SNB motoneurons.

The present experiment demonstrated a direct OT input, presumably from the PVN, to lower lumbar spinal cord. OT fibers, as well as being found in the region of the SNB, were also located in other sexually dimorphic areas, such as terminal areas for the sensory branch of the pudendal nerve (McKenna and Nadelhaft, 1986). There was only a subtle sexual dimorphism in the distribution of OT-like-IR fibers in L_5. However, this dimorphism became much more pronounced in the intermediate zone of L_6 and continuing into S_1, inclusive of the region containing preganglionic parasympathetic neurons and their collaterals (Morgan, *et al.*, 1991). It may be important to note the observation by Wagner and Clemens (1991) that castrated males had more NP-like-IR fibers in the region of the SNB as compared to intact

males. The means of these two groups were quite distinct, however, the variability between animals within a group was quite high, especially in the castrate group. Albeit the lack of statistical significance, this general trend appears to be consistent with the sexual dimorphism found in the present experiment.

If this sex difference in OT-like-IR is functionally relevant, it could indicate several possible effects by hormones on the differential regulation of this central, OT-containing pathway. An increase in OT-like-IR fibers could result from a lack of release by the terminals of these fibers. If cell activity is low, release may decrease, but axonal transport may continue at the same rate. This may result in a "back-up" of OT within the axon. If release occurred at the same rate as axonal transport, OT-containing fibers would empty and therefore become undetectable. A second possibility is that the rate of synthesis may be altered. Steroids are known to alter the levels of OT mRNA in the hypothalamus (Caldwell, *et al.*, 1989). The third possibility is that increased OT-like-IR may reflect a hyperinnervation of the SNB in the absence of T. Synaptic input onto SNB motoneurons decreases with castration (Matsumoto, *et al.*, 1988b; Leedy, *et al.*, 1987). In addition, *in vitro* studies show that androgens act additively with nerve growth factor to increase neurite outgrowth, branching, and arborization, thereby increasing the likelihood for interneural contact (Lustig, *et al.*, 1994).

EXPERIMENT 3: EFFECT OF NMDA LESIONS OF THE PVN ON PENILE REFLEXES AND SEMINAL EMISSION

Oxytocinergic neurons of the PVN have been implicated in the modulation of male sexual responses in rats. Cerebrospinal fluid (CSF) levels of OT increased following ejaculation (Hughes, *et al.*, 1985); ICV administration of OT increased ML, IL, and the PEI (Stoneham, *et al.*, 1985); electrolytic lesions of the PVN increased ML and IL and increased PEI (Hughes, *et al.*, 1985). We have shown that OT-like-IR neurons in the parvocellular submuclei of the PVN project to lower levels of spinal cord (Ackerman and Clemens, 1992). In this experiment, n-methyl-d-aspartic acid (NMDA) lesions, which have been shown to destroy parvocellular PVN neurons while leaving magnocellular neurons intact (Tang and Sisk, 1992), were used to evaluate the role of parvocellular neurons on male copulatory behavior and seminal emissions. The objectives of this experiment were:

1. to determine whether NMDA lesions of the PVN reduce OT-like-IR fibers in lower lumbar spinal cord (L_5-L_6), which are known to contain SNB motoneurons and their dendritic arborizations as well as autonomic cell bodies;
2. to evaluate the effect of NMDA lesions of parvocellular PVN neurons on male copulatory behavior; and
3. to evaluate the effect of NMDA lesions of parvocellular PVN neurons on seminal emission, as measured by dried copulatory plug weights.

METHODS

Animal Preparation and Copulatory Testing

Animals used in Experiment 3 were 60-70 day old, Long Evans male and female rats (Charles River, Wilmington, Massachusetts). Both sexes were separately housed in the same room. The ovariectomized females were brought into behavioral estrus by sequential, intramuscular injections of estradiol benzoate (50 μg once a day, 3 days prior to testing) and PG (0.5 mg, 4-5 hours prior to testing), both administered in a sesame oil vehicle. Males were placed individually into 10 gallon aquaria, which served as testing arenas, for 10 minutes prior to the onset of testing. Testing began by placing a hormone-treated, stimulus female into the male's testing arena. Males were allowed to copulate to exhaustion with his paired female; after which, the animals were returned to their home cages. After two separate copulatory exposures to a receptive female, a pre-lesion test of each male's sexual behavior was recorded. The following behavioral parameters were recorded: ML, time from introduction of the stimulus female into the test arena to first mount; IL, time from introduction of the stimulus female into test arena to first intromission; and EL, time from first intromission to ejaculation. A detailed description of these copulatory behaviors may be found in a review by Sach and Meisel (1988).

Thirty males were anesthetized and placed in a stereotaxic apparatus. An incision was made through the skin and muscle of the head to expose the skull. Coordinates from bregma for PVN were as follows: A-P: -2.1, M-L: ±0.6, D-V: -7.7. Holes were drilled through the skull, and a pair of 24g stainless steel cannulae were lowered into the brain until the tip of the cannulae were 0.1 mm dorsal to the PVN. Bilateral lesions of parvocellular PVN neurons were made by injecting 0.6 μl of either 0.3M NMDA in 0.1 M phosphate buffer (pH 7.4) (n

= 12) or of phosphate buffer alone (n = 18) for 30 minutes. Holes in the skull were filled with bone wax, the incision was closed with wound clips, and the animals were allowed to recover. Males were again tested for copulatory behaviors on day 15 post-surgery. After each pre- and post-surgery copulatory test, the male's paired female was anesthetized with sodium pentabarbital (50 mg/kg). The seminal plug was removed, allowed to air-dry for 14 days, and then weighed (Lange, *et al.*, 1993).

Tissue Preparation, Histology, and Immunocytochemistry

Animals were perfused with physiological saline, 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and cryoprotected with 20% sucrose in 0.1 M phosphate buffer (pH 7.4). Brains and spinal cords were sectioned on a freezing microtome, mounted onto gelatin coated slides, and were counterstained with thionin or processed for immunohistochemistry. Lesion placement was evaluated using 50 µm sections through the PVN. Immunohistochemistry was performed on alternate 30 µm sections through L_5-L_6, using antisera against OT (Chemicon International, Inc.). For detection using a chromogen, the avidin-biotin method (Vector Labs, Inc.) was performed using the diaminobenzidine/H_2O_2 (DAB) reaction. Following immunohistochemistry, chromogen treated sections were counterstained with thionin.

RESULTS

The NMDA bilateral lesions of the PVN were relatively large. A typical lesion from one animal may be seen in Figure 8. Only data from those animals in which the entire PVN was significantly damaged were used in the experiment's analyses. OT-like-IR fibers were virtually eliminated in lower lumbar spinal cord following NMDA lesions of parvocellular PVN neurons (Figure 9). Interestingly, these chemical lesions of the PVN did not

TH

Figure 8 Schematic of lesion placement **(SHADED)** in successive coronal sections **(A-F)** through the PVN in the rat. Based on Swanson and Kuypers (1980).

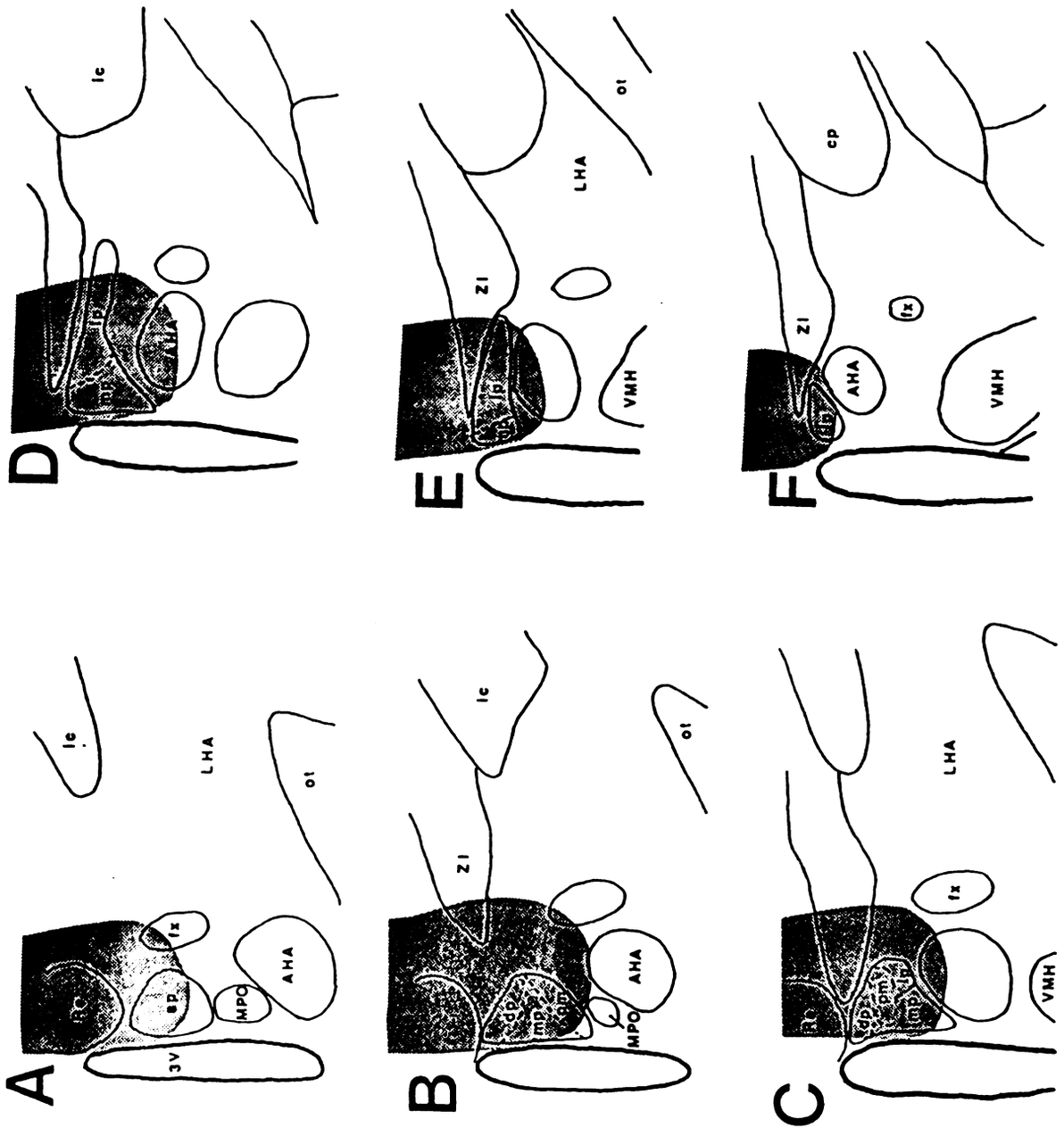
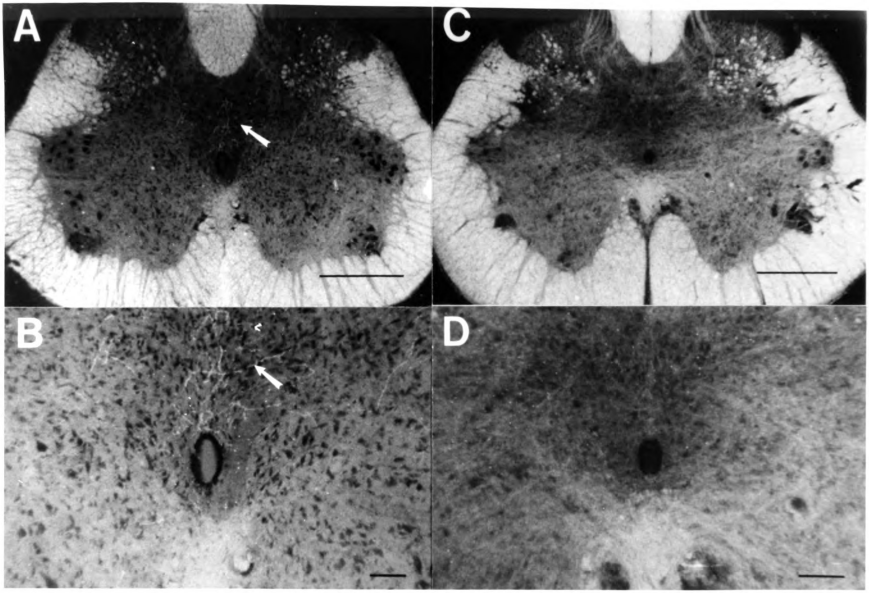

A
B
C
D
E
F
ic
LHA
ot
fx
3V
MPO
AHA
ZI
cp
VMH

Figure 9 Darkfield photomicrographs showing the distribution of OT-like-IR fibers **(ARROWS)** within coronal sections of lower lumbar (L_6) spinal cord in unlesioned **(A and B)** and lesioned **(C and D)** males. The region above the central canal, in the upper two photomicrographs (Bar = 500 μm), has been enlarged in the lower two photomicrographs (Bar = 100 μm).

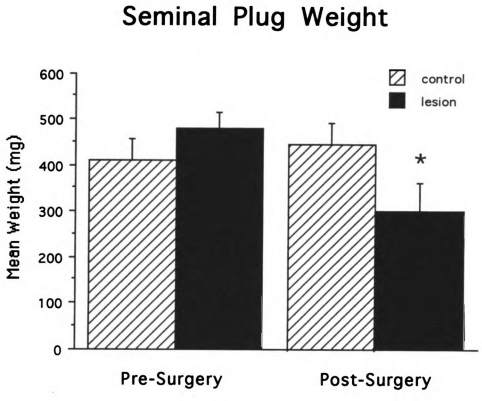

A
C
B
D

significantly affect ML, IL, or EL. However, seminal plug weight was significantly reduced following the NMDA lesions (Repeated Measures ANOVA, $F[1,11] = 11.404$, $p < 0.01$; Tukey's Test, $p < 0.01$; Figure 10).

DISCUSSION

The results of Experiment 3 demonstrate that bilateral destruction of the PVN destroys virtually all OT-like-IR in lower lumbar spinal cord. This finding is consistent with the electrolytic lesion studies using radioimmunoassay (Hawthorn, *et al.*, 1985) and immunocytochemistry (Monaghan, *et al.*, 1993) to measure decreases in OT-like-IR in spinal cord. In Hawthorn and colleagues'study, PVN lesions resulted in a dramatic decrease in the levels of OT and VP at all levels of spinal cord. In Monaghan and colleagues' study, lesions destroyed virtually all OT-like-IR in lumbar spinal cord, but showed little effect on penile reflexes, except for an increase latency to the first erection. Unlike electrolytic lesions, NMDA lesions selectively destroy parvocellular PVN neurons while, presumably, leaving fibers of passage and magnocellular PVN neurons intact. In addition, the PVN is the only OT-containing efferent in the hypothalamus that projects to lower lumbar cord. None of the other areas known to contain OT, such as the SON, suprachiasmatic nucleus, MPOA, bed nucleus of the stria terminalis, and the lateral septum, do not project to L_5-L_6. Together, these results, as well as those from Experiment 1, suggest that the parvocellular subnuclei of the PVN are the sources of OT in lower lumbar cord and that OT plays only a minor role in the modulation of penile reflexes and may not be necessary for normal copulation. This OT projection to the iml of the spinal cord is thought to modulate sympathetic and parasympathetic outflow to the internal and external genitalia (Gilby, *et al.*, 1982). Decreases in lumbar OT concentrations and in seminal plug weights, following parvocellular PVN

Figure 10 Mean weight of seminal plugs from pre- and post-surgery control (n = 7) and lesioned (n = 6) males. Asterisk (*) indicates a significant difference (Repeated Measures ANOVA, $F[1,11] = 11.404$, $p < 0.01$; Tukey's Test, $p < 0.01$).


Seminal Plug Weight
control
lesion
Mean Weight (mg)
600
500
400
300
200
100
0
*
Pre-Surgery
Post-Surgery

lesions, seem to support the idea that these OT efferents to lower lumbar cord may modulate autonomic regulation of emission-related structures. OT's effect on these structures may be central (i.e., OT may modulate preganglionic neurons in lower lumbar cord) and/or peripheral (i.e., plasma OT released from the neurohypophysial may facilitate smooth muscle contractions in these pelvic organs by acting directly on the tissue). Hughes and colleagues (1982) have proposed a possible coordination of co-released OT centrally and peripherally which has been suggested in the context of parturition and induction of maternal responsiveness in rats (Pederson, *et al.*, 1982).

GENERAL DISCUSSION

The present study demonstrates, in both male and female rats, an OT-like-IR projection from parvocellular neurons of the PVN to sexually dimorphic regions of lower lumbar spinal cord (L_5-L_6), a region known to contain motoneurons of the spinal nucleus of the bulbocavernosus (SNB) and their dendritic arborization as well as autonomic cell bodies. The majority of these spinal-projecting cells are located in the lateral parvocellular subnucleus. The distribution of OT-like-IR, PVN neurons that project to L_5-L_6 is identical in males and females. However, the density (e.g., number and length) of OT-like-IR fibers with putative terminals appears to be higher in the lower lumbar spinal cord of females. This sex difference appears subtle in L_5 but becomes more pronounced in L_6. In both sexes, OT-like-IR fibers and putative terminals are found in the region of SNB. Axon-sparing, NMDA lesions, which have been shown to destroy parvocellular PVN neurons while leaving magnocellular neurons intact, virtually abolishes all OT-like-IR fibers and their putative terminals in lower lumbar spinal cord, thereby implicating the PVN as the source of lumbosacral OT. These selective lesions significantly reduced seminal emission, however, measures of male copulatory behaviors (i.e., ML, IL, and EL) were unaffected.

Neural Control of Male Sexual Behavior

The neural circuitry of male sexual behavior is not well understood. However, before the role of the PVN and OT play in penile reflexes can be elucidated, it may be instructive to

consider how this system, together with other neural circuits, might coordinate the display of male copulatory behavior, from the initiation and maintenance of sexual behavior to the successful impregnation of the female.

Integrative Role of the MPOA and Other Supraspinal Circuits

For the initiation of male copulatory behavior, the male must integrate auditory, olfactory, gustatory, and tactile sensory cues. The MPOA appears to be critical for processing this sensory information. Olfaction is the sensory modality that most directly influences the MPOA. Afferents from the accessory olfactory bulb to the posterior cortical and medial nuclei of the amygdala (Scalia and Winans, 1975) may be relayed to the central and medial part of the MPOA via the BNST (Krettek and Price, 1978a and 1978b) or may be relayed directly from the medial amygdala to the MPOA through the stria terminalis. Lesions of these areas and the A14 dopaminergic region, disrupt specific aspects of male sexual behavior but do not abolish it completely (Chiba and Murata, 1985). Auditory, visual, and tactile sensory information are also integrated into the MPOA, however their influence is not as direct as that from olfaction. Sensory information is known to reach the neocortex and may influence MPOA function through intracortical projections from the appropriate cortical regions to the perirhinal area (Deacon, *et al.*, 1983), which in turn projects to the ventral subiculum (Kosel, *et al.*, 1983). Thus, the ventral subiculum may relay sensory information from the neocortex to the MPOA through its projection to the ventrolateral septal nucleus (Swanson and Cowan, 1977), or directly through the corticohypothalamic tract (Krettek and Price, 1978a and 1978b).

Generally speaking, the lateral part of the medial preoptic nucleus (MPN) within the MPOA receives ascending projections from the brainstem, including serotonergic inputs,

which may exert significant effects on sexual behavior (Crowley and Zemlan, 1981) and gonadotropin release (Kalra and Kalra, 1983) at the level of the MPOA. Similarly, ascending gustatory and visceral inputs to the medial part of the MPN from the nucleus of the solitary tract and A1 region appear to relay vagal and glossopharyngeal sensory information (Sawchenko and Swanson, 1982b) and may contain norepinephrine, which is also thought to modulate reproductive physiology and behavior. Other brainstem regions that appear to provide modest somatomotor inputs include the ventral tegmental area, lateral (central) tegmental field, periaqueductal gray, pedunculopontine nucleus, and the peripeduncular nucleus. Sparse innervation is received from infralimbic and insular cortical areas, the nucleus accumbens, and the substantia innominata. Intrahypothalamic projections to and from the MPOA are widespread. These included most of the major hypothalamic regions: AHA, VMH, DMH, PVN, lateral POA, LHA, tuberomamillary and supramammillary nuclei (see review, Swanson, 1987). It is through these projections that the MPOA can directly influence neuroendocrine and autonomic responses.

The primary efferents of the MPOA project to the midbrain via the medial forebrain bundle (Swanson, 1976). Elimination of copulation by lesions of the MPOA is generally considered to result from the interruption of efferents to the midbrain. Lesions of the medial forebrain bundle rostral to the MPOA have no effect on copulation, whereas lesions caudally along the extent of this fiber pathway eliminate copulation (Hendricks and Scheetz, 1973). Tegmental regions, receiving MPOA input, have been implicated in male sexual behavior (Hansen and Gummesson, 1982; Brackett and Edward, 1984). Lesions of the lateral (central) tegmental field, dorsal and medial to the substantia nigra, eliminated copulation in male rats (Hansen and Gummesson, 1982; Brackett and Edwards, 1984). Similar results were found

when lesions were placed more medially, sparing the peripeduncular nucleus (Brackett and Edwards, 1984). It appears that the peripeduncular nucleus may not play a critical role in the execution of copulation but may serve as a sensory relay from the pudendal nerve (Carrer, 1978) to the MPOA, via the amygdala (Mascó and Carrer, 1980). Lesions of the dorsal tegmental area (Clark, *et al.*, 1975; Walker, *et al.*, 1981), ventrolateral to the midbrain central gray, or of the ventral tegmental area (VTA) (Barfield, *et al.*, 1975) substantially accelerate copulation by reducing PEI. These VTA lesions also damage fibers of the dorsal noradrenergic bundle, ascending from the locus coeruleus, thus reducing a source of noradrenergic inhibition to the forebrain. Chemical lesions of the dorsal tegmental area, which leave the noradrenergic bundle intact, also speed up pacing of copulation (Hansen, *et al.*, 1982). The coordination of sympathetic, vascular, sensory, and reflexive functions by the MPOA is largely unknown. Projections important to these functions are undoubtedly relayed between MPOA and brainstem (Chiba and Murata, 1985). This is illustrated by the MPOA efferent to the paragigantocellularis nucleus of the reticular formation, a region known to send inhibitory efferents to SNB motoneurons (Marson and McKenna, 1990).

Reciprocal projections between the MPOA, the ventral striatum, and the basal ganglia may relay information concerning the motivational state of the animal. In the model proposed by Mogenson and colleagues (1980), the forebrain transmits the motivational state of the animal to the ventral tegmental area, were it is relayed to the ventral striatum and nucleus accumbens. The integration of this information forms the basis for the motivational state of the animal. The accumbens, in turn, projects to the basal ganglia, forming an neural interface between the regions assessing the animal's motivational state and motor systems capable of organizing movements that permit the animal to execute behaviors.

To summarize, the MPOA is influenced by sensory information. Although the olfactory and visceral modalities appear to reach it relatively directly, other modalities must first pass through multimodal association areas of the cerebral cortex, including limbic regions. Projections from the MPOA also directly influence neuroendocrine, autonomic, and somatomotor responses. Because these pathways are bidirectional, the MPOA may influence both motor responses, as well as the processing of sensory information and the accompanying cognitive activities.

Large lesions of the preoptic area (including the anterior AHA) eliminated copulation in sexually experienced male rats, even with chronic T administration (Heimer and Larsson, 1964), and therefore, deficits were not the indirect result of diminished gonadal output. However, behavior in most POA lesioned animals was restored following the administration of the non-specific monoamine receptor agonist, lisuride (Hansen, *et al.*, 1982). Smaller lesions have produced less severe deficits (Heimer and Larsson, 1964; Ginton and Merari, 1977; Arendash and Gorski, 1983). This has been of interest because the MPOA can be partitioned into several subnuclei, which show different connectivity and cytoarchectiture. Restricted lesions, in some regions, have been shown to increase IL and EL, whereas others have spared copulation (Arendash and Gorski, 1983). Interestingly, animals receiving these restricted lesions showed behavioral inconsistencies from test to test (Heimer and Larsson, 1964), and in some cases, showed recovery of normal copulatory ability over time (Ginton and Merari, 1977).

Spinal Regulation of Penile Reflexes

Penile erection is regulated by three major pathways: 1) the lumbosacral system, which travels via the pelvic nerve to the penile corpora and vasculature; 2) the pudendal

nerve, which innervates the striated penile muscles, is the second pathway; and 3) the thoracolumbar system, which is the primary route by which sexual stimuli, received by suprasegmental receptors, cause erection. The dorsal penile nerve, a sensory branch of the pudendal nerve, is the route of tactile sensory information from the penis, although other genital afferent fibers are carried to the spinal cord by the pelvic nerve (Purinton, *et al.*, 1973 and 1981). Both the dorsal penile nerve and the pudendal nerve originate in the same spinal segments, which contain SNB and DLN motoneurons, and project to the same spinal laminae (Núñez, *et al.*, 1986). These anatomical features, as well as the overlapping pudendal and pelvic afferent and efferent spinal projections, may represent a circuit for rapid sensorimotor coordination, characteristic of the intromissive pattern observed in rats (Roppolo, *et al.*, 1985; Rose and Collins, 1985; Núñez, *et al.*, 1986). Hypogastric efferent activity has been shown to be highly synchronized in sexual reflexes with pudendal and pelvic nerve activity (McKenna, et al., 1991). Nadelhaft and McKenna (1987) demonstrated that the hypogastric nerve has relatively few afferent fibers. Therefore, this synchronization must be mediated by intraspinal pathways. The band of medially located, pseudorabies (PRV)-labelled neurons which extends from lower thoracic to upper sacral segments in Marson and colleagues' (1993) study would be appropriate for such a role.

Overall influence of the brain upon penile reflexes is inhibitory, as demonstrated in spinal transection (Sachs and Garinello, 1980) and pharmacological blockade studies (Sachs and Bitran, 1990). Reflexes are more reliably elicited and more frequent after mid-thoracic transection of spinal cord (Hart, 1968; Sachs and Garinello, 1980). Also, enhanced electromyographic activity in perineal muscles is observed following spinal transection (Marson and McKenna, 1990). Although lesions of the MPOA drastically reduce male

copulatory behavior (Heimer and Larsson, 1966), removal of the MPOA's indirect input to spinal cord, by means of a knife cut through the medial forebrain bundle, do not affect penile reflexes (Szechtman, et al, 1978). However, investigations involving supraspinal transections and electrolytic and kainic acid lesions in specific areas of the ventral medulla indicate that the paragigantocellular reticular nucleus and the raphe nuclei mediate this supraspinal inhibition (Marson and McKenna, 1990). It should also be noted that some regions (e.g., lateral vestibular nucleus) may facilitate expression of penile reflexes, suggesting that multiple supraspinal efferents may be involved in the production of reflexes (Monaghan, *et al.*, 1993). Therefore, situational variables may influence the activity and hence the strength of each projection. For example, if a male rat is exposed to an estrous female, activity of inhibitory projections to spinal regions involved in penile activity might decrease, whereas activity in facilitatory regions might increase. The brain determines when a given spinal reflex is appropriate and transmits this information to the spinal cord.

It has been assumed that the coordination underlying penile functions occurs and is mediated by interneurons in the lumbosacral spinal cord (Hart, 1968; Sachs and Garinello, 1980). Sachs and Garinello (1980) suggest that a spinal pacemaker regulates the rate at which penile reflexes occur and may also contribute to the pacing of the male's attempts to copulate. Details of this circuitry remain unknown. However, it could be inferred from several lines of evidence that penile interneurons, located in the dorsal gray commissure around the central canal in L5-S1, may be involved in this circuitry. Electrophysiological studies and immunohistochemical staining have identified neurons around the central canal in the sacral spinal cord of cats which were responsive to pelvic visceral and/or somatic sensory stimulation, including genital stimuli (Honda, 1985). As previously mentioned, the

dorsal gray commissure of L5-S1 is a site of extensive terminal fields for both pelvic and pudendal nerve afferents. The dorsal gray commissure is therefore likely to contain interneurons coordinating pelvic function. This region also contains neurons which project supraspinally (Burstein, *et al.*, 1990) and has reciprocal connections with medullary regions shown to modulate sexual reflexes (Marson and McKenna, 1990 and 1992). Some neurons in this region project to the parasympathetic preganglionic neurons (Sasek and Elde, 1985). Sacral preganglionic neurons have extensive axon collaterals, with an especially heavy innervation in lamina X, surrounding the central canal (Morgan, *et al.*, 1991). The wide range of axon collaterals of preganglionic neurons may explain the large numbers of putative interneurons identified in the present study. A transneuronal study using injections of wheatgerm agglutinin conjugated horseradish peroxidase (WGA-HRP) into the bulbospongiosus muscle, also resulted in labelled presumptive interneurons in the central gray of the lumbosacral cord (Collins, *et al.*, 1991). There is much less evidence regarding the location of pelvic interneurons outside the L5-S1 segments.

Role of Central OT from the PVN in Male Copulatory Behavior

As previously mentioned, the MPOA is innervated by dopaminergic axons of the A14 area. In addition, it sends efferents to the PVN (Silverman, *et al.*, 1981; Chiba and Murata, 1985) and receives reciprocal afferents from the PVN (Simerly and Swanson, 1986). This relationship of the MPOA to the PVN may help explain why the infusion of apomorphine into the MPOA facilitates penile reflexes (Pehek, *et al.*, 1989). These effects of apomorphine on penile responses, following central administration, are prevented by electrolytic lesion of the PVN (Argiolas, *et al.*, 1987a). And since the effects of systemically administered apomorphine are blocked by central administration of an OT antagonist (Argiolas, *et al.*,

1987b), these lesions appear to disrupted OT processes. The concomitant administration of apomorphine and OT, both of which induce penile responses when given alone, did not produce an additive effect on the incidence of the responses (Melis, *et al.*, 1989) and led to the conclusion that apomorphine induces penile responses by releasing OT within the CNS. These studies suggest that the PVN may be an additional site by which the MPOA modulates SNB motoneurons activity. However, the role of OT in regulating penile reflexes has been called into question, by recent lesion studies. Although electrolytic PVN lesions reduce dramatically OT-like-IR in lower lumbar cord, they had little effect on *ex copula* penile reflexes, except for latency to first erection (Monaghan, *et al.*, 1993).

As noted earlier, OT may affect temporal measures of male copulatory behavior. Discrete electrolytic lesions to the lp subnucleus of the PVN abolish ejaculation-associated increases in CSF OT, prolong ML and IL, and reduce the absolute PEI (Hughes, *et al.*, 1987). However, the effects of chemical lesions on measures of male copulatory behavior are inconsistent with those of electrolytic PVN lesions (Hughes, *et al.*, 1987). NMDA lesions of the PVN do not affect *in copula* displays of penile reflexes or most temporal measures of male copulatory behavior, however, the effect of OT on PEI could not be determined. Whereas chemical lesions selectively destroy parvocellular PVN neurons, electrolytic lesions destroy both magnocellular and parvocellular PVN neurons, as well as axons *en passant*. Differences in sample size may have also contributed to the inconsistency between the the electrolytic lesions of Hughes and colleagues (1987) and our chemical lesions. The effects of chemical PVN lesions could not be determined in the present experiment's behavioral tests because of the removal of the seminal plug immediately following ejaculation.

The PVN's central OT-containing neural circuitry may also serve as a sensory and motor relay to the MPOA, coordinating temporal aspects of motor patterns of male sexual behavior with penile reflexes. In both sexes, the PVN receives inputs from neurons within the dorsal gray commissure and surrounding the central canal at lumbosacral levels of spinal cord (Akaishi, *et al.*, 1988; Burstein, *et al.*, 1990; Yang and Clemens, *unpublished observations*).. The same region receives both pelvic and pudendal sensory afferents (Honda, 1985; Burstein, *et al.*, 1990), as well as interneurons, whose circuitry may constitute part of Sachs and Garinello's (1980) hypothetical, spinal pacemaker.

Swanson (1977) has identified "autonomic cells" in the PVN which project to brain stem and spinal cord. Descending fibers from these neurons travel to the dorsal motor nucleus of the vagal nerve and the iml column of the spinal cord where they innervate parasympathetic and sympathetic preganglionic cells respectively (Sawchenko and Swanson, 1982a). The hypogastric and pelvic nerves are major sources autonomic influence over bladder activity, erection, ejaculation, and penile detumescence (De Groat and Booth, 1980; Kihara, *et al.*, 1991; Kihara, *et al.*, 1992; Sato, *et al.*, 1991). Sympathetic preganglionic neurons have been localized in T_{13}-L_3 (Nadelhaft and McKenna, 1987), and sacral parasympathetic preganglionic neurons have been localized in L_6-S_1 (Nadelhaft and Booth, 1984; Morgan, *et al.*, 1991). In both these cell groups, preganglionic neurons and their axon collaterals have been located in regions of spinal cord (iml, dorsal commissure, and lamina X) where OT-like-IR fibers have been identified. By injecting one of two retrograde tracers, FG or cholera toxin ß, into the hypothalamus and injecting the other tracer into the pelvic ganglion, Burstein and colleagues (1990) have found two populations of parasympathetic preganglionic neurons in the sacral spinal cord of rats: one that projects through the pelvic

nerve and another that projects to the hypothalamus. This latter projection may be a reciprocal connection for a descending paraventriculo-preganglionic neuron projection and may modulate parasympathetic control of seminal emission.

The projections from PVN and other hypothalamic nuclei (lateral hypothalamus and dorsal hypothalamic area) to the lumbosacral region of spinal cord in females (Wagner and Clemens, 1991), which do not possess the BC and IC neuromuscular system, may involve reproductive functions specific to females. The pudendal nerve, which originates in L_5-S_1 of the spinal cord, innervates structures important in both female copulatory behavior and parturition (Pacheco, *et al.*, 1989). OT has been shown to have central effects on sexual receptivity in female rats (Brinton, *et al.*, 1984). Electrophysiological evidence demonstrates that tonically firing OT neurons in PVN receive input from the uterus (Akaishi, *et al.*, 1988).

Future Directions

Because the present findings show that approximately one-sixth of neurons in the PVN that project to lumbosacral spinal cord contain OT-like-IR, further identification of other neuroactive substances would be important in the neurochemical characterization of this paraventriculo-spinal projection. Met-enkephalin has been found in approximately 10% of the spinal-projecting PVN neurons (Cechetto and Saper, 1988) and has been demonstrated to coexist with OT and VP in some parvocellular PVN neurons (Martin and Voight, 1981). Small populations of spinal-projecting cells of the PVN have been shown to contain tyrosine hydroxylase (Swanson, *et al.*, 1981), angiotensin II (Lind, *et al.*, 1985), corticotropin releasing hormone (Sawchenko and Swanson, 1985), thyrotropin releasing hormone, and neurotensin (Swanson, *et al.*, 1986). Only a few galanin-IR, cholecystokinin-IR, and substance P-IR neurons have been identified in the paraventriculo-spinal projection (Cechetto

and Saper, 1988). Met-enkephalin, galanin, and substance-P are found in all parvocellular neurons of the PVN (Cechetto and Saper, 1988; Ljungdahl, *et al.*, 1978) and in fibers in the region of the SNB (Micevych, *et al.*, 1986; Coquelin, *et al.*, 1991; Newton, 1992). High densities of substance P-IR fibers have been also described in the region of autonomic preganglionic neurons (Ljungdahl, *et al.*, 1978). In addition to these paraventriculo-spinal projections, other hypothalamic nuclei (lateral hypothalamus and dorsal area of the hypothalamus) have been shown to project to lower lumbosacral spinal cord (Wagner and Clemens, 1991). The possible function of these projections is unclear. However, 90-100% of neurons in the lateral hypothalamus that project to thoracic spinal cord contain þ-melanocyte stimulating hormone and 50-90% of neurons in the dorsal area of the hypothalamus contain tyrosine hydroxylase (Cechetto and Saper, 1988). Both of these neuroactive substances have been shown to affect penile reflexes (Argiolas, *et al.*, 1987a, 1987b; Bitran, *et al.*, 1988; Ferrari, *et al.*, 1963; Pehek, *et al.*, 1988).

As discussed in Experiments 1 and 2, the lack of sexual dimorphism in OT-like-IR PVN neurons that project to lower lumbar spinal cord, together with the sexual dimorphism seen in lumbosacral cord, poses an interesting question: How are gonadal hormones involved in the differential regulation of this pathway? Considering that E and T alter firing rates of OT neurons in the PVN (Akaishi and Sakum, 1985a and 1985b), increases OT mRNA levels (Caldwell, *et al.*, 1989; Miller, *et al.*, 1989), decreases synaptic density and number on SNB motoneurons altered by castration (Leedy, *et al.*, 1987), and increases in OT-like-IR fibers, as seen in female lumbosacral spinal cord, could result from several factors: 1) decreased OT release from terminals; 2) alteration of protein synthesis rate; 3) hyperinnervation of the SNB

in the absence of T; 4) regulation of the synaptic coverage of SNB motoneurons from afferents originating in the PVN.

Although terminal boutons and *en passant* terminal swellings appear to contact SNB cell bodies and their proximal dendrites, ultrastructural verification and the identification of OT-receptor sites on SNB motoneurons would be necessary for determining whether oxytocinergic terminals are, indeed, making synapses onto SNB motoneurons. Likewise, the identification of oxytocinergic terminals, forming chemical synapses onto preganglionic neurons and/or their axon collaterals and onto interneurons connected with this neuronal circuit, will strengthen the notion that OT is involved in the regulation of seminal emission, via contraction of the vas deferens and/or prostate. The location of these synapses will be of great importance in understanding the role of the PVN on both penile reflexes and seminal emission. Terminals located directly on the soma would imply that OT is having a more direct, controlling effect on the firing of these cells due to basic cable (passive) properties exhibited by neurons (i.e., current will decrease as the distance from the point of stimulation increases). This effect may be less if terminals are located on dendrites, or in the case of preganglionic neuronal circuit, on interneurons.

As previously mentioned, OT appears to affect the PEI in male rats. Both systemic and ICV administration of OT have been shown to shorten PEI. Conversely, ICV infusion of the potent OT antagonist, $d(CH_2)_5Try(Me)\text{-}Orn^8\text{-}$vasotocin, increased PEI. Unfortunately, removal of the seminal plug immediately following ejaculation did not permit the measurement of PEI in the present experiment's behavioral tests. If high post-ejaculatory OT levels induce sexual satiety, one would suspect that NMDA lesions of the PVN would also shorten PEI.

Another, perhaps less direct, method for investigating OT's role in the modulation of SNB motoneurons and seminal emission, would be the use of PRV injected into the BC muscle or into emission-related structures, such as the seminal vesicles, coagulating gland, vas deferens, and prostate. OT-containing terminals co-localizing PRV would suggest a modulatory role of OT in these circuits. The major advantage of using PRV over other transneuronal markers, such as WGA-HRP, is its ability to replicate within the neuron and thus function as a self-amplifying cell marker.

The antibody used in the present experiments was neat, polyclonal antisera to OT. Because the antibody was not monoclonal or affinity purified, cross-reactivity with VP could have been possible. However, the probability of cross-reactivity is considered to be minimal, less than 2% according to the antibody's manufacturer, Chemicon International, Inc. Further support for this idea is provided by the following data: there are fewer VP fibers than OT fibers in spinal cord as detected by immunohistochemistry (Buijs, 1978; Sofroniew, 1983); VP levels are lower than OT levels in the spinal cord, as detected by radioimmunoassay (Lang, *et al.*, 1983; Hawthorn, *et al.*, 1985; Valiquette, *et al.*, 1985); and there are very few VP-like-IR fibers in sexually dimorphic regions of the lumbar cord (Bruce Newton, *personal communication*). According to Bruce Newton, VP-containing fibers are found in laminae VII and X and seem to be associated with preganglionic autonomic neurons. There is also a small fiber tract that lies at the dorsal aspect of the ventral fissure. These fibers wrap around blood vessels which arise from the anterior spinal artery and are most prominent in the lumbosacral regions of the spinal cord. Although there is not much VP-IR in the SNB or DLN, nevertheless, an occasional fiber is seen.

Recently, Thomas Insel (*personal communication*) and Tribollet and colleagues (1995) have localized VP receptors on SNB motoneurons but were unable to identify OT receptors. These preliminary data may suggest a greater role of VP than previously thought. However, centrally administrated VP shows little effect on male sex behavior in the rat (Sodersten, *et al.*, 1983). In addition, it has been demonstrated that NP-II (VP-associated) can act independently to alter prolactin release in estrogen-primed male rats (Shin, *et al.*, 1989). The possibility that NP may act as a neurotransmitter, as Christine Wagner (*personal communication*) has suggested, cannot be completely dismissed. Autoradiographic binding studies using radiolabelled NP would be necessary to clarify this highly controversial idea.

Summary and Conclusions

The findings of the present experiments suggest that OT, from the PVN, projects directly to steroid-sensitive levels of lumbosacral spinal cord including the region of the SNB. At supraspinal levels, this OT-like-IR projection to lower lumbar cord is not different between sexes. Males and females also show a similar distribution of OT-like IR fibers with putative terminals in lower lumbar spinal cord. These fibers are seen primarily in the apex of the dorsal horn, lamina X, and the dorsal commissure. However, the density of OT-like-IR in lower lumbar spinal cord appears to be higher in females. In addition, OT-like-IR fibers and their putative terminals are also seen adjacent to retrogradely traced, SNB motoneurons, suggesting possible innervation by parvocellular, OT-containing PVN neurons. Selective lesions of parvocellular PVN neurons virtually eliminate OT-like-IR from lower lumbar spinal cord but do not effect *in copula* penile reflexes. However, seminal emission is significantly reduced following these chemical lesions.

The PVN appears to serve as a relay center within the hypothalamus, integrating intrahypothalamic, brainstem, and spinal information associated with male copulatory behaviors. Based on these present experiments, as well as previous studies, central OT appears to autonomically mediate male sexual behavior. Its associated effects include the parasympathetic control of pre-ejaculatory behaviors, such as erection and seminal emission, and the sympathetic control of ejaculation and post-ejaculatory behaviors, such as penile detumescence and sexual satiety. Generally, low levels of endogenously released OT enhance pre-ejaculatory components of sexual behavior. Later, at the time of ejaculation and for several minutes afterwards when OT levels are at their highest, OT assumes an inhibitory role.

Unfortunately, the majority of paraventriculo-spinal projecting neurons are still unidentified in terms of their neurochemistry. In addition, there is still relatively little known about the organization and neurochemical specificity of intrahypothalamic integration. But with continued refinement of neuroanatomical techniques, future investigations of the PVN will continue to dissect and elucidate the connectivity of its highly differentiated circuitry and its functional roles in the mediation of autonomic and endocrine factors that regulate sexual behavior.

APPENDIX

APPENDIX

Protocol for Oxytocin Immunohistochemistry using the Avidin-Biotinylated HRP Complex (ABC)/3,3'-Diaminobenzidine (DAB) Detection Method

1. Rinse with 0.87% saline in 0.05M tris buffer (pH 7.4) with 0.2% Triton X100 (TBS/TX), 3 X 5 min. at R.T.

2. Incubate in 3% Normal goat serum in TBS/TX for 30 min. at R.T.

3. Rinse 3 X 5 min. with TBS/TX at R.T.

4. Incubate in 1:200 rabbit anti-oxytocin serum in TBS/TX for 48 hrs. at 4°C.

5. Rinse 3 X 5 min. with TBS/TX at R.T.

6. Incubate in 300 µl goat anti-rabbit serum in 10 ml TBS/TX for 60 min. at R.T.

7. Rinse 3 X 5 min. with TBS/TX at R.T.

8. Incubate in a solution of the ABC (Vector Laboratories) (2 drops A: 2 drops B in 10 ml TBS/TX) for 90 min. at R.T.

9. Rinse 4 X 5 min. with TBS/TX at R.T.

10. React for approximately 10 min. with 10 ml DAB solution containing 300 µl GOD solution.

DAB Solution:
7.32 g Trizma HCl
3.45 g Trizma Base
500 mg 3,3'-diaminobenzidine
1000 mg B-D(+)Glucose
200 mg NH_4Cl
190 mg imidazole

Q.S. to 500 ml dH_2O; pH 7.4; Store at -20_oC until use.

LIST OF REFERENCES

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Ackerman, A.E. and Clemens, L.G. (1992) Oxytocin projections from the paraventricular nucleus of the hypothalamus to lower lumbar spinal cord and the distribution of oxytocin fibers in the region of the spinal nucleus of the bulbocavernosus in male and female rats. *Soc. Neuroscience Abstr.* **18**: 358.

Ackerman, A.E., Lang, G.M. and Clemens, L.G. (1993) Effects of n-methyl-d-aspartic acid lesions in the paraventricular nucleus of the hypothalamus on oxytocin projections to lower lumbar spinal cord, on male sex behavior, and on seminal emission in the rat. *Soc. Neurosci. Abstr.* **19**: 174.

Akaishi, T. and Sakuma, Y. (1985a) Estrogen excites oxytocinergic, but not vasopressinergic cells in the paraventricular nucleus of the female rat hypothalamus. *Brain Res.* **335**: 302-305.

Akaishi, T. and Sakuma, Y. (1985b) Gonadal steroid actions on the paraventricular magnocellular neurosecretory cells of the male rat. *Neurosci. Lett.* **54**: 91-96.

Akaishi, T., Robbins, A., Sakuma, Y. and Sato, Y. (1988) Neural inputs from the uterus to the paraventricular magnocellular neurons in the rat. *Neurosci. Lett.* **84**: 57-62.

Anderson, W.J., Stromberg, M.W. and Hinsman, E.J. (1976) Morphological characteristics of dendrite bundles in the lumbar spinal cord of the rat. *Brain Res.* **110**: 215-227.

Arendash, G.W. and Gorski, R.A. (1983) Effects of discrete lesions of the sexually dimorphic nucleus of the preoptic area or other medial preoptic regions on the sexual behavior of male rats. *Brain Res.* **10**: 147-154.

Argiolas, A. and Gessa, G.L. (1991) Central Functions of oxytocin. *Neurosci. Biobehav. Rev.* **15**: 217-231.

Argiolas, A., Melis, M.R. and Gessa, G.L. (1986) Oxytocin: an extremely potent inducer of penile erection and yawning in male rats. *Eur. J. Pharmacol.* **130**: 265-272.

Argiolas, S., Melis, M.R. and Gessa, G.L. (1987a) Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. *Brain Res.* **421**: 349-352.

Argiolas, A., Melis, M.R., Vargiu, L. and Gessa, G.L. (1987b) d(CH_2)$_5$Tyr(Me)-[Orn^8]vasotocin, a potent oxytocin antagonist, antagonizes penile erection and yawning induced by oxytocin and apomorphine, but not by ACTH-(1-24). *Europ. J. Pharmacol.* **134**: 221-224.

Argiolas, A., Collu, M., Gessa, G.L., Melis, M.R. and Serra G. (1988) The oxytocin antagonist d(CH2)5Tyr(Me)-Orn8-vasotocin inhibits male copulatory behavior in rats. *Eur. J. Pharmacol.* **149**: 389-392.

Argiolas, A., Collu, M., D'Aquila, P., Gessa, G.L., Melis, M.R. and Serra, G. (1989) Apomorphine stimulation of male copulatory behavior is prevented by the oxytocin antagonist d(CH_2)$_5$Tyr(Me)-Orn^8-vasotocin in rats. *Pharmacol. Biochem. Behav.* **33**: 81-83.

Arletti, R., Bazzani, C., Castelli, M. and Bertolini, A. (1985) Oxytocin improves male copulatory performance in rats. *Horm. Behav.* **19**: 14-20.

Armstrong, W.E., Warach, S., Hatton, G.I. and McNeill, T.H. (1980) Subnuclei in the rat hypothalamic paraventricular nucleus: A cytoarchitectural, horseradish peroxidase and immunocytochemical analysis. *Neuroscience* **5**: 1931-1958.

Barfield, R.J., Wilson, C., and McDonald, P.G. (1975) Sexual behavior: Extreme reduction of postejaculatory refractory period by midbrain lesions in male rats. *Science* **189**: 147-149.

Bargmann, W. and Scharrer, E. (1951) The site of origin of the hormones of the posterior pituitary. *Am. Scient.* **39**: 255-259.

Bitran, D., Hull, E.M., Holmes, G.M., and Lookingland, K.J. (1988) Regulation of male rat copulatory behavior by preoptic incertohypothalamic dopamine neurons. *Brain Res. Bull.* **20**: 323-331.

Bodanszky, M., Sharaf, H., Roy, J.B., and Said, S.I. (1992) Contractile activity of vasotocin, oxytocin, and vasopressin on mammalian prostate. *Eur. J. Pharmacol.* **216**: 311-313.

Bowker, R.M., Westlund, K.N., and Coulter, J.D. (1982a) Origins of serotonergic projections to the lumbar spinal cord in the monkey using a combined retrograde transport and immunocytochemical technique. *Brain Res. Bull.* **9**: 271-278.

Bowker, R.M., Westlund, K.N., Sullivan, M.C., and Coulter, J.D. (1982b) Transmitters of the raphe-spinal complex: immunocytochemical studies. *Peptides* **3**: 291-298.

Brackett, N.L. and Edwards, D.A. (1984) Medial preoptic connections with the midbrain tegmentum are essential for male sexual behavior. *Physiol. Behav.* **32**: 79-84.

Bradshaw, W.G., Baum, M.J. and Awh, C.C. (1981) Attenuation by a 5alpha-reductase inhibitor of the activational effect of testosterone propionate on penile erections in castrated male rats. *Endocrinology* **109**: 1047-1051.

Breedlove, S.M. (1984) Steroid influences on the development and function of a neuromuscular system. *Prog. Brain Res.* **61**: 147-170.

Breedlove, S.M. (1985a) Cellular analyses of hormone influence on motoneuronal development and function. *J. Neurobio.* **17**: 157-176.

Breedlove, S.M. (1985b) Hormonal control of the anatomical specificity of motoneuron-to-muscle innervation in rats. *Science* **227**: 1357-1359.

Breedlove, S.M. and Arnold, A.P. (1980) Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. *Science* **210**: 564-566.

Breedlove, S.M. and Arnold, A.P. (1981) Sexually dimorphic motor nucleus in the rat lumbar spinal cord: Response to adult hormone manipulation, absence in androgen-insensitive rats. *Brain Res.* **225**: 297-307.

Breedlove, S.M. and Arnold, A.P. (1983a) Hormonal control of a developing neuromuscular system I. Complete demasculinization of the male rat spinal nucleus of the bulbocavernosus using the anti-androgen flutamide. *J. Neurosci.* **3**: 417-423.

Breedlove, S.M. and Arnold, A.P. (1983b) Hormonal control of a developing neuromuscular system II. Sensitive periods for the androgen-induced masculinization of the rat spinal nucleus of the bulbocavernosus. *J. Neurosci.* **3**: 424-432.

Breedlove, S.M. and Arnold, A.P. (1983c) Sex differences in the pattern of steroid accumulation by motoneurons of the rat lumbar spinal cord. *J. Comp. Neurol.* **215**: 211-216.

Brinton, R.E., Wamsley, J.K., Gee, K.W., Wan, Y.-P. and Yamamura, H.I. (1984) [^{3}H]Oxytocin binding sites in the rat brain demonstrated by quantitative light microscope autoradiography. *Europ. J. Pharmacol.* **102**: 365-367.

Brownstein, M.J. (1983) Biosynthesis of vasopressin and oxytocin. *Ann. Rev. Physiol.* **45**: 129-135.

Buijs, R.M. (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell. Tiss. Res.* **192**: 423-435.

Burstein, R., Wang, J., Elde, R.P., and Giesler, G.J. Jr., (1990) neurons in the sacral parasympathetic nucleus that project to the hypothalamus do not also project through the pelvic nerve--a double labelling study combining Fluoro-gold and cholera toxin B in the rat. *Brain Res.* **506**: 159-165.

Carrer, H.F. (1978) Mesencephalic participation in the control of sexual behavior in the female rat. *J. Comp. Physiol. Psychol.* **92**: 877-887.

Carter, C.S. (1992) Oxytocin and Sexual Behavior. *Neurosci. Biobehav. Rev.* **15**: 131-144.

Caldwell, J.D., Brooks, P.J., Jirikowski, G.F., Barakat, A.S., Lund, P.K. and Pedersen, C.A. (1989) Estrogen alters oxytocin mRNA levels in the preoptic area. *J. Neuroendocrinol.* **1**: 273-278.

Cechetto, D.F. and Saper, C.B. (1988) Neurochemical organization of the hypothalamic projection to the spinal cord in the rat. *J. Comp. Neurol.* **272**: 579-604.

Chiba, T. and Murata, Y. (1985) Afferent and efferent connections of the medial preoptic area in the rat: A WGA-HRP study. *Brain Res. Bull.* 14: 261-272.

Clark, T.K., Caggiula, A.R., McConnell, R.A., and Antelman, S.M. (1975) Sexual inhibition is reduced by rostral midbrain lesions in the male rat. *Science* **190**: 169-171.

Clemens, L.G., Wagner, C.K., and Ackerman, A.E. (1993) A sexually dimorphic motor nucleus: Steroid sensitive afferents, sex differences and hormonal regulation. *The Development of Sex Differences and Similarities in Behavior*, M. Haug, R.E. Whalen, C. Aron, and K.L. Olsen, eds., Kluwer Academic Publishers, Dordrecht/Boston/London, pp. 19-31.

Cobbett, P., Yang, Q.Z. and Hatton, G.I. (1987) Incidence of dye coupling among magnocellular paraventricular nucleus neurons in male rats is testosterone dependent. *Brain Res. Bull.* **18**: 365-370.

Collins, W.F. III, Erichsen, J.T., and Rose, R.D. (1991) Pudendal motor and premotor neurons in the male rat: a WGA transneuronal study. *J. Comp. Neurol.* **308**: 28-41.

Conrad, L.C.A. and Pfaff, D.W. (1976) Efferents from medial basal forebrain and hypothalamus in the rat - II. An autoradiographic study of the anterior hypothalamus. *J. Comp. Neurol.* **169**: 221-262.

Coquelin, A., Micevych, P.E., and Arnold, A.P. (1991) Sexually dimorphic, androgen sensitive, enkephalinergic afferents to a lumbar motor nucleus of rats. *J. Neurobiol.* **22**: 873-881.

Crowley, W.R. , Popolow, H.B., and Ward, O.B. Jr. (1973) From dud to stud: Copulatory behavior elicited through conditioned arousal in sexually inactive male rats. *Physiol. Behav.* **10**: 391-394.

Davidson, J.M., Stefanick, M.L., Sachs, B.D. and Smith, E.R. (1978) Role of androgen in sexual reflexes of the male rat. *Physiol. Behav.* **21**: 141-146.

Deacon, T.W., Eichenbaum, H., Rosenberg, P., Eckmann, K.W. (1983) Afferent connections of the perirhinal cortex in the rat. *J. Comp. Neurol.* **220**: 168-190.

De Groat, W.C. and Booth, A.M. (1980) Physiology of male sexual function. *Ann. Intern. Med.* **92**: 329-331.

De Kloet, E.R., Voorhuis, Th.A.M., Boschma, Y., and Elands, J. (1986) Estradiol modulates density of putative oxytocin receptors in discrete brain regions. *Neuroendocrinol.* **44**: 415-421.

Dionne, F.T., Dube, J.Y. Lesage, R.L. and Tremblay, R.R. (1979) In vivo androgen binding in rat skeletal and perineal muscles. *Acta Endocrinol.* **91**: 362-372.

Dube, J.Y., Lesage, R. and Tremblay, R.R. (1976) Androgen and estrogen binding in rat skeletal and perineal muscle. *Can. J. Biochem.* **54**: 50-55.

Ermisch, A., Barth, T., Ruhle, H.J., Okopkova, J., Hrbas, P. and Landgraf, H. (1985) On the blood-brain barrier to peptides: accumulation of labelled vasopressin, desglyNH_2-vasopressin and oxytocin by brain regions. *Endocrinol. Exp.* **19**: 29-37.

Falke, N. (1989) Oxytocin stimulates oxytocin release from isolated nerve terminals of rat neural lobes. *Neuropeptides* **14**: 269-274.

Ferrari, W., Gessa, G.L., and Vargiu, L. (1963) Behavioural effects induced by intracisternally injected ACTH and MSH. *Ann. N.Y. Acad. Sci.* **104**: 330-334.

Fishman, R.B., Chism, L., Firestone, G.L. and Breedlove, S.M. (1990) Evidence for androgen receptors in sexually dimorphic perineal muscles of neonatal male rats: Absence of androgen accumulation by the perineal motoneurons. *J. Neurobiol.* **21**: 694-704.

Freund-Mercier, M.J. and Richard, P. (1981) Excitatory effects of intraventricular injections of oxytocin on the milk ejection reflex in the rat. *Neurosci. Lett.* **23**: 193.

Freund-Mercier, M.-J. and Richard, P. (1984) Electrophysiological evidence for facilitatory control of oxytocin neurones by oxytocin during suckling in the rat. *J. Physiol.* **352**: 447-466.

Gainer, H., Altstein, M. and Whitnall, W.S. (1988) The biosynthesis and secretion of oxytocin and vasopressin. *The physiology of reproduction*. Knobil, E., Neill, J., *et al.* eds., Raven Press, New York, 2265-2281.

Gilbey, M.P., Coote, J.H., Fleetwood-Walker, S., and Peterson, D.F. (1982) The influence of the paraventriculo-spinal pathway, and oxytocin and vasopressin on sympathetic preganglionic neurones. *Brain Res.* **251**: 283-290.

Ginton, A. and Merari, A. (1977) Long range effects of MPOA lesion on mating behavior in the male rat. *Brain Res.* **120**: 158-163.

Goldstein, L.A., Kurz, E.M. and Sengelaub, D.R. (1990) Androgen regulation of dendritic growth and retraction in the development of a sexually dimorphic spinal nucleus. *J. Neurosci.* **10**: 935-946.

Gray, G.D., Smith, E.R. and Davidson, J.M. (1980) Hormonal regulation of penile erection in castrate male rats. *Physiol. Behav.* **24**: 463-468.

Hagihara, K., Shiosaka, S., Lee, Y., Kato, J., Hatano, O., Takakusu, A., Emi, Y., Omura, T., and Tohyama, M. (1990) Presence of sex difference of cytochrome P-450 in the rat preoptic area and hypothalamus with reference to coexistence with oxytocin. *Brain Res.* **515**: 69-78.

Hall, D.S., Fishman, R.B., and Breedlove, S.M. (1984) Non-aromatizable androgen alters SNB motoneuronal morphology in adult rats. *Soc. Neurosci. Abstr.* **11**: 531.

Hancock, M.B. (1976) Cells of origin of hypothalamo-spinal projections in the rat. *Neurosci. Lett.* **3**: 179-184.

Hansen, S. and Gummesson, B.M. (1982) Participation of the lateral midbrain tegmentum in the neuroendocrine control of sexual behavior and lactation in the rat. *Brain Res.* **251**: 319-325.

Hansen, S., Kohler, C. and Ross, S.B. (1982) On the role of the dorsa mesencephalic tegmentum in the control of masculine sexual behavior in the rat: Effects of electrolytic lesions, ibotenic acid and DSP4. *Brain Res.* **240**: 311-320.

Hart, B.L. (1967a) Sexual reflexes and mating behavior in the male dog. *J. Comp. Physiol. Psych.* **64**: 388-399.

Hart, B.L. (1967b) Testosterone regulation of sexual reflexes in spinal male rats. *Science* **155**: 1283-1284.

Hart, B.L. (1968) Sexual reflexes and mating behavior in the male rat. *J. Comp. Physiol. Psych.* **65**: 453-460.

Hart, B.L. (1973) Effects of testosterone propionate and dihydrotestosterone on penile morphology and sexual reflexes of spinal male rats. *Horm. Behav.* **4**: 239-246.

Hart, B.L. (1979) Activation of sexual reflexes of male rats by dihydrotestosterone but not estrogen. *Physiol. Behav.* **23**: 107-109.

Hart, B.L. and Kitchell, R.L. (1966) Penile erection and contraction of penile muscles in the spinal and intact dog. *Am. J. Physiol.* **210**: 257-262.

Hart, B.L. and Melese-d'Hospital, P.Y. (1983) Penile mechanisms and the role of the striated penile muscles in penile reflexes. *Physiol. Behav.* **31**: 807-813.

Hart, B.L., Wallach, S.J.R. and Melese-d'Hospital, P.Y. (1983) Differences in responsiveness to testosterone of penile reflexes and copulatory behavior of male rats. *Horm. Behav.* **17**: 274-283.

Hatton, G.I. (1988) Cellular reorganization in neuroendocrine secretion. *Current Topics in Neuroendocrinology, Vol. 9*, Ganten, D. and Pfaff, D., eds., Springer-Verlag, Berlin, 1-27.

Hatton, G.I. and Tweedle, C.D. (1982) Magnocellular neuropeptidergic neurons in hypothalamus: Increases in membrane apposition and number of specialized synapses from pregnancy to lactation. *Brain Res. Bull.* **8**: 197-204.

Hawthorn, J., Ang, V.T.Y. and Jenkins, J.S. (1985) Effects of lesions in the hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei on vasopressin and oxytocin in rat brain and spinal cord. *Brain Res.* **346**: 51-57.

Heimer, L. and Larsson, K. (1964) Drastic changes in the mating behavior of male rats following lesions in the junction of the diencephalon and mesencephalon. *Experientia* **20**: 460-461.

Hendricks, S.E. and Scheetz, H.A. (1973) Interaction of hypothalamic structures in the mediation of male sexual behavior. *Physiol. Behav.* **10**: 711-716.

Honda, C.N. (1985) Visceral and somatic afferent convergence onto neurons near the centra canal in the sacral spinal cord of the cat. *J. Neurophysiol.* **54**: 1059-1078.

Hosoya, Y. (1980) The distribution of spinal projection neurons in the hypothalamus of the rat, studied with the HRP method. *Exp. Brain Res.* **40**: 79-87.

Hughes, A.M., Everitt, B.J., Lightman, S.L. and Todd, K. (1987) Oxytocin in the central nervous system and sexual behavior in male rats. *Brain Res.* **414**: 133-137.

Jordan, C.L., Breedlove, S.M., and Arnold, A.P. (1982) Sexual dimorphism and the influence of neonatal androgen in the dorsolateral motor nucleus of the rat lumbar spinal cord. *Brain Res.* **249**: 309-314.

Kalra, S.P. and Kalra, P.S. (1983) Neural regulation of luteinizing hormone secretion in the rat. *Endocrine Rev.* **4**: 311-351.

Kihara, K., Sato, K., Ando, M., Sato, T., and Oshima, H. (1991) Lumbosacral sympathetic trunk as a compensatory pathway for seminal emission after bilateral hypogastric nerve transections in the dog. *J. Urol.* **145**: 640-643.

Kihara, K., Sato, K., Ando, M., Sato, T., and Oshima, H. (1992) Ability of each lumbar splanchnic nerve and disability of thoracic ones to generate seminal emission in the dog. *J. Urol.* **147**: 260-263.

Kojima, M., Matsuura, T., Tanaka, A., Amagai, T., Imanishi, J., and Sano, T. (1985) Characteristic distribution of noradrenergic terminals on the anterior horn motoneurons innervating the perineal striated muscles in the rat. *Anat. Embryol. Berl.* **171**: 267-273.

Kosel, K.C., Van Hoesen, G.W., and Rosene, D.L. (1983) A direct projection from the perirhinal cortex (area 35) to the subiculum in the rat. *Brain Res.* **269**: 347-351.

Krettek, J.E. and Price, J.L. (1978a) Amygdaloid projections to subcortical structures within the basalforebrain and brainstem in rat and cat. *J. Comp. Neurol.* **178**: 225-254.

Krettek, J.E. and Price, J.L. (1978b) A description of the amygdaloid complex in the rat and cat with obserations on intra-amydaloid axonal connections. *J. Comp. Neurol.* **178**: 255-280.

Krieg, M., Szalay, R. and Voigt, K.D. (1974) Binding and metabolism of testosterone and 5þ-dihydrotestosterone in bulbocavernosus/levator ani (BCLA) of male rats: *in vivo* and *in vitro* studies. *J. Steroid Biochem.* **5**: 453-459.

Kurz, E.M., Sengelaub, D.R. and Arnold, A.P. (1986) Androgens regulate the dendritic length of mammalian motoneurons in adulthood. *Science* **232**: 395-398.

Kuypers, H.G.J.M. and Maisky, V.A. (1975) Retrograde axonal transport of horseradish peroxidase from spinal cord to brainstem cell groups in the cat. *Neurosci. Lett.* **1**: 9-14.

Landgraf, R., Ermisch, A. and Heb, J. (1979) Indications for brain uptake of labelled vasopressin and oxytocin and the problem of the blood-brain barrier. *Endokrinologie* **73**: 77-81.

Lang, R.E., Heil, J., Ganten, D., Hermann, K., Rascher, W. and Unger, Th. (1983) Effects of lesions in the paraventricular nucleus of the hypothalamus on vasopressin and oxytocin contents in brainstem and spinal cord of rat. *Brain Res.* **260**: 326-329.

Lange, G.M., Ackerman, A.E., and Clemens, L.G. (1993) Effects of n-methyl-d-aspartic acid lesions in the paraventricular nucleus of the hypothalamus in the rat (*Rattus norvegicus*): A comparison of male copulatory behaviors and seminal emissions. Presented at the Conference on Reproductive Behavior, Michigan State University, East Lansing, MI.

Leedy, M.G., Beattie, T.S. and Bresnahan, J.S. (1987) Testosterone-induced plasticity of synaptic inputs to adult mammalian motoneurons. *Brain Res.* **424**: 386-390.

Lind, R.W., Swanson, L.W. and Sawchenko, P.E. (1985) Anatomical evidence that neural circuits related to the subfornical organ of the rat. *Brain Res. Bull.*, **15:** 79-82.

Ljungdahl, A., Hökfelt, T. and Nilsson, G. (1978) Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. *Neuroscience*, **3:** 861-943.

Luiten, P.G.M., ter Horst, G.J., Karst, H. and Steffens, A.B. (1985) The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. *Brain Res.* **329**: 374-378.

Lustig, R.H., Hua, P., Smith, L.S., Wang, C., and Chang, C. (1994) Androgen induction of neurite outgrowth and arborization in androgen receptor (AR)-transfected PC12 cells *in vitro*. *Soc. Neurosci. Abstr.* **20**: 871.

Maggi, M., Makozowski, S., Kassis, S., Guardabassao, V., and Rodbard, D. (1987) Identification and characterization of two classes of receptors for oxytocin and vasopressin in porcine tunica albuginea, epididymis, and vas deferens. *Endocrinology* **120**: 986-994.

Marson, L. and McKenna K.E. (1990) The identification of a brainstem site controlling spinal sexual reflexes in male rats. *Brain Res.* **515**: 303-308.

Marson, L. and McKenna K.E. (1992) A role for 5-hydroxytryptamine in descending inhibition of spinal sexual reflexes. *Expl. Brain Res.* **88**: 313-320.

Marson,L., Platt, K.B., and McKenna, K.E. (1993) Central Nervous system innervation of the penis as revealed by the transneuronal transport of pseudorabies virus. *J. Neurosci.* **55**: 263-280.

Martin, R. and Voigt, G.J. (1981) Enkephalins co-exist with oxytocin and vasopressin in nerve of rat neurohypophysis. *Nature,* **289**: 502-504.

Mascó, D.H. and Carrer, H.F. (1980) Sexual receptivity in female rats after lesion or stimulation in different amygdaloid nuclei. *Physiol Behav.* **24**: 1073-1080.

Matsumoto, A., Arnold, A.P., Zampighi, G.A. and Micevych, P.E. (1988a) Androgenic regulation of gap junctions between motoneurons in the rat spinal cord. *J. Neurosci.* **8**: 4177-4183.

Matsumoto, A., Micevych, P.E. and Arnold, A.P. (1988b) Androgen regulates synaptic input to motoneurons of the adult rat spinal cord. *J. Neurosci.* **8**: 4168-4176.

McKenna, K.E. and Nadelhaft, I. (1986) The organization of the pudendal nerve in the male and female rat. *J. Comp. Neurol.* **248**: 532-549.

McKenna, K.E., Chung, S.K., and McVary, K.T. (1991) A model for the study of sexual function in anesthetized male and female rats. *Am. J. Physiol.* **261**: R1276-1285.

Meisel, R.L., O'Hanlon, J.K. and Sachs, B.D. (1984) Differential maintenance of penile responses and copulatory behavior by gonadal hormones in castrated male rats. *Horm. Behav.* **18**: 56-64.

Melis, M.R., Argiolas, A. and Gessa, G.L. (1986) Oxytocin-induced penile erection and yawning: Site of action in the brain. *Brain Res.* **398**: 259-265.

Melis, M.R., Argiolas, A., and Gessa, G.L. (1987) Apomorphine increase plasma oxytocin concentration in male rats. *Brain Res.* **415**: 98-102.

Melis, M.R., Argiolas, A. and Gessa, G.L. (1989) Evidence that apomorphine induces penile erection and yawning by releasing oxytocin in the central nervous system. *Europ. J. Pharmacol.* **164**: 565-570.

Mens, W.B.J., Witter, A. and Van Wimersam Greidanus, T.B. (1983) Penetration of neurohypophyseal hormones from plasma into cerebrospinal fluid (CSF): half-times of disappearance of these neuropeptides from CSF. *Brain Res.* **262**: 143-149.

Micevych, P.E., Coquelin, A. and Arnold, A.P. (1986) Immunohistochemical distribution of substance P, serotonin, and methionine enkephalin in sexually dimorphic nuclei of the rat lumbar spinal cord. *J. Comp. Neurol.* **248**: 235-244.

Miller, F.D., Ozimek, G., Milner, R.J. and Bloom, F.E. (1989) Regulation of neuronal oxytocin mRNA by ovarian steroids in the mature and developing hypothalamus. *Proc. Natl. Acad. Sci.* **86**: 2468-2472.

Mogenson, G.J., Jones, D.L., and Yim., C.Y. (1980) From motivation to action: Functional interface between the limbic system and the motor system. *Prog. Neurobiol.* **14**: 69-97.

Molander, C., Xu, Q. and Grant, G. (1984) The cytoarchitectonic organization of the spinal cord of the rat. I. The lower thoracic and lumbosacral cord. *J. Comp. Neurol.* **230**: 133-141.

Monaghan, E.P. and Breedlove, S.M. (1991) Brain sites projecting to the spinal nucleus of the bulbocavernosus. *J. Comp. Neurol.* **307**: 370-374.

Monaghan, E.P., Arjomand, J., and Breedlove, S.M. (1993) Brain lesions affect penile reflexes. *Horm. Behav.* **27**: 122-131.

Montagnese, C.M., Poulain, D.A., Vincent, J.-D., and Theodosis, D.T. (1987) Structural plasticity in the rat supraoptic nucleus during gestation, post-partum lactation and suckling-induced psudogestation and lactation. *J. Endocrinol.* **115**: 97-105.

Montagnese, C.M., Poulain, D.A., and Theodosis, D.T. (1990) Influence of ovarian steroids on the ultrastuctural plasticity of adult rat supraoptic nucleus induced by central administration of oxytocin. *J. Neuroendocrinol.* **2**: 225-230.

Moos, F., Freund-Mercier, M.J., Guerne, Y., Guerne, J.M., Stoekel, M.E. and Richard, P. (1984) Release of oxytocin and vasopressin by magnocellular nuclei in vitro: specific facilitatory effect of oxytocin on its own release. *J. Endocrinol.* **102**: 63.

Morgan, C.W., de Groat, W.C., Felkins, L.A., and Zhang, S.J. (1991) Axon collateral indicate broad intraspinal role for sacral preganglionic neurons. *Proc. Natl. Acad. Sci.* **88**: 6888-6892.

Nadelhaft, I. and Booth, A.M. (1984) The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: A horseradish peroxidase study. *J. Comp. Neurol.* **226**: 238-245.

Nadelhaft, I. and McKenna, K.E. (1987) Sexual dimorphism in sympathetic preganglionic neurons of the rat hypgastric nerve. *J. Comp. Neurol.* **256**: 308-315.

Newton, B.W. (1992) A sexually dimorphic population of galanin-like neurons in the rat lumbar spinal cord: functional implications. *Neurosci. Lett.* **137**: 119-122.

Niemi, M. and Kormano, B. (1965) Contractility of the seminiferous tubules of the rat testis and its response to oxytocin. *Ann. Med. Exp. Biol. Fenn.* **43**: 40-42.

Nordeen, E.J., Nordeen, K.W., Sengelaub, D.R. and Arnold, A.P. (1984) Ontogeny of sexual dimorphism in a rat spinal nucleus. I. Hormonal control of neuron number. *Soc. Neurosci. Abstr.* **10**: 453.

Nordeen, E.J., Nordeen, K.W., Sengelaub, D.R. and Arnold, A.P. (1985) Androgens prevent normally occurring cell death in a sexually dimorphic spinal nucleus. *Science* **229**: 671-673.

Núñez, R. , Gross, G.H., and Sachs, B.D. (1986) Origin and central projections of rat dorsal penile nerve: Possible direct projection to autonomic and somatic neurons by primary afferents of nonmuscle origin. *J. Comp. Neurol.* **247**: 417-429.

O'Hanlon, J.K., Meisel, R.L. and Sachs, B.D. (1981) Estradiol maintains castrated male rats' sexual reflexes in copula, but not ex copula. *Behav. Neural Biol.* **32**: 269-273.

Ono, T., Nishino, H., Sasaka, K., Muramoto, K., Yano, I. and Simpson, A. (1978) Paraventricular nucleus connections to spinal cord and pituitary. *Neurosci. Lett.* **10**: 141-146.

Pacheco, P.M., Martinez-Gomez, M., Whipple, B., Beyer, C. and Komisurak, B.R. (1989) Somato-motor components of the pelvic and pudendal nerves of the female rat. *Brain Res.* **490**: 85-94.

Pederson, C.A., Ascher, J.A., Monroe, Y.L, and Prange, A.J. (1982) Oxytocin induces maternal behaviour in virgin female rats. *Science*, **216**: 648-649.

Pehek, E.A., Thompson, J.T., Eaton, R.C., Bazzett, T.J., and Hull, E.M. (1988) Apomorphine and haloperidol, but not domperidone, affect penile reflexes in rats. *Pharmacol. Biochem. Behav.* **31**: 201-208.

Pehek, E.A., Thompson, J.T. and Hull, E.M. (1989) The effects of intracranial administration of the dopamine agonist apomorphine on penile reflexes and seminal emission in the rat. *Brain Res.* **500**: 325-332.

Perez-Delgado, M.M., Serrano-Aguilar, P.G., A. Castaneyra-Perdomo, R. Ferres-Torres, J. and Gonzales-Hernandez, T. (1987) Topographic organization of the karyometric response to neonatal castration of the male mouse in the paraventricular and ventromedial hypothalamic nuclei. *Acta Anat.* **129**: 67-73.

Pfaff, D.W. (1988) Multiplicative responses to hormones by hypotalamic neurons. *Recent progress in posterior pituitary hormones*, Yoshida, S. and Share, L., eds., Excerpta Medica, Amsterdam, 257-267.

Pfaff, D.W. and Schwartz-Giblin, S. (1988) Cellular mechanisms of female reproductive behaviors. *The physiology of reproduction*, Knobil, E., Neill, J., *et al.*, eds., Raven Press, New York, 1487-1568.

Purinton, P.T., Fletcher, T.F., and Bradley, W.E. (1973) Gross and light microscopic features of the pelvic plexus in the rat. *Anat. Rec.* **175**: 697-706.

Purinton, P.T., Oliver, J.E. Jr., and Bradley, W.E. (1981) Differences in routing of pelvic visceral afferent fibers in the dog and cat. *Exp. Neurol.* **73**: 725-731.

Rand, M.N. and Breedlove S.M. (1987) Ontogeny of functional innervation of bulbocavernosus muscles in male and female rats. *Dev. Brain Res.* **33**: 150-152.

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

Rodgers, C.H. and Alheid. G. (1972) Relationship of sexual behavior and castration to tumescence in the male rat. *Physiol. Behav.* **9**: 581-584.

Rooney, K.J., Scheibel, A.B. and Shaw, G.L. (1979) Dendritic bundles: survey of anatomical experiments and physiological theories. *Brain Res. Rev.* **1**: 225-271.

Roppolo, J.R., Nadelhaft, I., and de Groat, W.C. (1985) The organization of pudendal motoneruaons and primary afferent projections in the spinal cord of the rhesus monkey revealed by horseradish peroxidase. *J. Comp. Neurol.* **234**: 475-488.

Rose, R.D. and Collins III, W.F. (1985) Crossing dendrites may be a substrate for synchronized activation of penile motoneurons. *Brain Res.* **337**: 373-377.

Sachs, B.D. (1982) Role of striated penile muscles in penile reflexes, copulation and induction of pregnancy in the rat. *J. Reprod. Fert.* **66**: 433-443.

Sachs, B.D. and Britran, D. (1990) Spinal block reveals roles for brain and spinal cord in the mediation of reflexive penile erections in rats. *Brain Res.* **528**: 99-108.

Sachs, B.D. and Garinello, L.D. (1980) Hypothetical spinal pacemaker regulating penile reflexes in rats: Evidence from transection of spinal cord and dorsal penile nerves. *J. Comp. Physiol. Psychol.* **94**: 530-535.

Saper, C.B., Loewy, A.D., Swanson, L.W. and Cowan, W.M. (1976) Direct hypothalamo-autonomic connections. *Brain Res.* **117**: 305-312.

Sar, M. and Stumpf, W.E. (1975) Distribution of androgen-concentrating neurons in rat brain. In *Anatomical Neuroendocrinology*, W.E. Stumpf and L.D. Grant, eds. pp. 120-133, S. Karger-Basal, Munchen.

Sar, M. and Stumpf, W.E. (1980) Simultaneous localization of [^{3}H]estradiol and neurophysin I or arginine vasopressin in hypothalamic neurons demonstrated by a combined technique of dry-mount autoradiography and immunohistochemistry. *Neurosci. Lett.* **17**: 179-184.

Sasek, C.A. and Elde, R.P. (1985) Distribution of neuropeptide Y-like immunoreactivity and its relationship to FMRF-amide-like immunoreactivity in the sixth lumbar and first sacral spinal cord segments fo the rat. *J. Neurosci.* **5**: 1729-1739.

Sato, K., Kihara, K., Ando, M., Sato, T., and Oshima, H. (1991) Seminal emission by electrical stimulation of the spermatic nerve and epididymis. *Int. J. Androl.* **14**: 461-467.

Sawchenko, P.E. and Swanson, L.W. (1982a) Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J. Comp. Neurol.* **205**: 260-272.

Sawchenko, P.E. and Swanson, L.W. (1982b) The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Res. Rev.* **4**: 275-325.

Sawchenko, P.E. and Swanson, L.W. (1985) Localization, colocalization, and plasticity of corticotropin-releasing factor immunoreactivity in rat brain. *Fed. Proc.*, **44:** 221-227.

Scalia.F. and Winaus, S.W. (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *J. Comp. Neurol.* **161**: 31-56.

Schroder, H.D. (1980) Organization of the motoneurons innervating the pelvic muscles of the male rat. *J. Comp. Neurol.* **192**: 567-587.

Schwanzel-Fukuda, M., Morrell, J.I. and Pfaff, D.W. (1984) Localization of forebrain neurons which project directly to the medulla and spinal cord of the rat by retrograde tracing with wheat germ agglutinin. *J. Comp. Neurol.* **226**: 1-20.

Sengelaub, D.R. and Arnold, A.P. (1986) Development and loss of early projections in a sexually dimorphic rat spinal nucleus. *J. Neurosci.* **6**: 1613-1620.

Sengelaub, D.R., Nordeen, E.J., Nordeen, K.W. and Arnold, A.P. (1989) Hormonal control of neuron number in sexually dimorphic spinal nuclei of the rat: III. Differential Effects of the androgen dihydrotestosterone. *J. Comp. Neurol.* **280**: 637-644.

Sharaf, H., Foda, H.D., and Said, S.I. (1992) Oxytocin and related peptides elicit contractions of prostate and seminal vesicle. *Oxytocin in Maternal, Sexual, and Social Behaviors, Annals of the New York Academy of Sciences*, Vol. **652**, Pedersen, C.A., *et al.*, eds., New York Academy of Sciences, New York, 474-477.

Shen, P., Arnold, A.P. and Micevych, P.E. (1990) Supraspinal projections to the ventromedial lumbar spinal cord in adult male rats. *J. Comp. Neurol.* **300**: 263-272.

Shin, S.H., Obonsawin, M.C., and Stirling, R. (1989) Bovine neurophysin-II stimulates prolactin release without involvement of dopaminergic prolactin-release inhibiting factor receptor in the estradiol-primed male rat. *Acta Endocrinol.* **121**: 411-416.

Silverman, A.J., Hoffman, D.L. and Zimmerman, E.A. (1981) The descending afferent connections of the paraventricular nucleus of the hypothalamus (PVN). *Brain Res. Bull.* **6**: 47-61.

Simerly, R.B. and Swanson, L.W. (1986) The organization of neural inputs to the medial preoptic nucleus of the rat. *J. Comp. Neurol.*, **246**: 312-342.

Simerly, R.B., Chang, C., Muramatsu, M. and Swanson, L.W. (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat Brain: An in situ hybridization study. *J. Comp. Neurol.* **294**: 76-95.

Sodersten, P., Henning, M., Melin, P. and Ludin, S. (1983) Vasopressin alters female sexual behavior by acting on the brain independently of alterations in blood pressure. *Nature* **301**: 608-610.

Sofroniew, M.V. (1883) Vasopressin and oxytocin in the mammalian brain and spinal cord. *Trends Neurosci.* **378**: 467-472.

Stoneham, M.D., Everitt, B.J., Hansen, S., Lightman, S.L. and Todd, K. (1985) Oxytocin and sexual behavior in the male rat and rabbit. *J. Endocrinol.* **107**: 97-106.

Stumpf, W.E., Sar, M. and Keefer, D.A. (1975) Atlas of estrogen target cells in rat brain. In *Anatomical Neuroendocrinology*, W.E. Stumpf and L.D. Grant, eds. pp. 104-119, S. Karger-Basal, Munchen.

Swanson, L.W. (1976) An autoradiographic study of the efferent connections of the preoptic region of the rat. *J. Comp. Neurol.* **167**: 227-256.

Swanson, L.W. (1977) Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus. *Brain Res.* **128**: 346-353.

Swanson, L.W. (1987) The hypothalamus. *Handbook of Chemical Neuroanatomy. Vol. 5: Integrated Systems of the CNS, Part I*, Björklund, A., Hökfelt, T. and Swanson, L.W., eds., Elsevier Science Publishers B.V., 1-124.

Swanson, L.W. and Cowen, W.M. (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J. Comp. Neurol.* **172**: 49-84.

Swanson, L.W. and Kuypers, H.G.J.M. (1980) The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J. Comp. Neurol.* **194**: 555-570.

Swanson, L.W. and McKellar, S. (1979) The distribution of oxytocin-and neurophysin-stained fibers in the spinal cord of the rat and monkey. *J. Comp. Neurol.* **196**: 271-285.

Swanson, L.W. and Sawchenko, P.E. (1983) Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Ann. Rev. Neurosci.* **6**: 269-324.

Swanson, L.W., Sawchenko, P.E., Berod, A., Hartman, B.K., Helle, K.B. and VanOrden, D.E. (1981) An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. *J. Comp. Neurol.*, **196:** 271-285.

Swanson, L.W., Mogenson, G.J., Simerly, R.B. and Wu, M. (1986) Anatomical and electrophysiological evidence for a projection from the medial preoptic area to the 'mesencephalic and subthalamic locomotor regions' in the rat. *Brain Res.*, **405:** 108-122.

Swenson, K.L. and Sladek, C.D. (1995) Effects of castration and testosterone on vasopressin release. *Soc. Neurosci. Abstr.* **21**: 875.

Szechtman, H., Caggiula, A.R., and Wulkan, D. (1978) Precoptic knife cuts and sexual behavior in male rats. *Brain Res.* **150**: 569-591.

Tang, Y.P. and Sisk, C.L. (1992) Neurochemical lesions of the hypothalamic parventricular nucleus accelerate pubertal gonadal growth in male ferrets. *Soc. Neurosci. Abs.*, **18**:1222.

Theodosis, D.T. (1985) Oxytocin-immunoreactive terminals synapse on oxytocinergic neurones in the supraoptic nuclei. *Nature* **313**:682.

Tobin, A.M. and Payne A.P. (1990) Perinatal androgen administration and the maintenance of sexually dimorphic and nondimorphic lumbosacral motor neuron groups in female Albino Swiss rats. *J. Anat.* **177**: 47-53.

Tribollet, E., Kato, G., and Arsenijevic, Y. (1995) Localization of vasopressin receptors, but not of oxytocin receptors, in putendal motor nuclei in both male and female rats. *Soc. Neurosci. Abstr.* **21**: 1623.

Valiquette, G., Haldar, J., Abrams, G.M., Nilaver, G. and Zimmerman, E.A. (1985) Extrahypothalamic neurohypophysial peptides in the rat central nervous system. *Brain Res.* **331**: 176-179.

Wagner, C.K. and Clemens, L.G. (1989a) Anatomical organization of the sexually dimorphic perineal neuromuscular system in the house mouse. *Brain Res.* **499**: 93-100.

Wagner, C.K. and Clemens, L.G. (1989b) Perinatal modification of a sexually dimorphic motor nucleus in the spinal cord of the B6D2F1 house mouse. *Physiol. Behav.* **45**: 831-835.

Wagner, C.K. and Clemens, L.G. (1991) Projections of the paraventricular nucleus of the hypothalamus to the sexually dimorphic lumbosacral region of the spinal cord. *Brain Res.* **539**: 254-262.

Wagner, C.K. and Clemens, L.G. (1993) A neurophysin-containing pathway from the paraventricular nucleus of the hypothalamus to a sexually dimorphic motor nucleus in lumbar spinal cord. *J. Comp. Neurol.* **336**: 106-116.

Wagner, C.K., Sisk, C.L., and Clemens, L.G. (1993) The spinal nucleus of the bulbocavernosus receives estrogen-sensitive afferents from the paraventricular nucleus of the hypothalamus in the male rat. *J. Neuroendocrinol.* **5**: 545-551.

Walker, L.C., Gerall, A.A., and Kostrzewa, R.M. (1981) Rostral midbrain lesions and copulatory behavior in male rats. *Physiol. Behav.* **26**: 349-353.

Wallach, S.J. and Hart, B.L. (1983) The role of the striated penile muscles of the male rat in seminal plug dislodgement and deposition. *Physiol. Behav.* **31**: 815-821.

Wee, B.E.F. and Clemens, L.G. (1987) Characteristics of the spinal nucleus of the bulbocavernosus are influenced by genotype in the house mouse. *Brain Res.* **424**: 305-310.

Weisz, J. and Ward, I.L. (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinol.* **106**: 306-316.

Witt, D.M., Carter, C.S., and Insel, T.R. (1991) Oxytocin receptor binding in female prairie voles: effects of endogenous and exogenous estradiol stimulation. *J. Neuroendocrinol.* **3**: 155-161.