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## Identification of neural circuits involved in female genital responses in the rat: A dual virus and anterograde tracing study

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### Abstract

The spinal and peripheral innervation of the clitoris and vagina are fairly well understood. However, little is known regarding supraspinal control of these pelvic structures. The multisynaptic tracer pseudorabies virus (PRV) was used to map the brain neurons that innervate the clitoris and vagina. In order to delineate forebrain input onto PRV labeled cells, the anterograde tracer biotinylated dextran amine (BDA) was injected into the medial preoptic nucleus (MPO), ventromedial nucleus of the hypothalamus (VMN) or the midbrain periaqueductal gray (PAG) 10 days prior to viral injections. These brain regions have been intimately linked to various aspects of female reproductive behavior. Four days after viral injections, into the vagina and clitoris PRV labeled cells were observed in the paraventricular nucleus, Barrington's nucleus, the A5 region, and the nucleus paragigantocellularis. At 5 days post-viral administration, additional PRV labeled cells were observed within the preoptic region, VMN, PAG and lateral hypothalamus. Anterograde labeling from the MPO terminated among PRV positive cells primarily within the dorsal paraventricular nucleus of the hypothalamus (PVN), ventrolateral VMN (VMNvl), caudal PAG and nucleus paragigantocellularis (nPGi). Anterograde labeling from the VMN terminated among PRV positive cells in the MPO and lateral/ventrolateral PAG. Anterograde labeling from the PAG terminated among PRV positive cells in the PVN, ventral hypothalamus and nPGi. Transynaptically labeled cells in the lateral hypothalamus, Barrington's nucleus and ventromedial medulla received innervation from all three sources. These studies, together, identify several CNS sites participating in the neural control of female sexual responses. They also provide the first data demonstrating a link between the MPO, VMNvl and PAG and CNS regions innervating the clitoris and vagina, providing support that these areas play a major role in female genital responses.

### Indexing Terms

medial preoptic area; ventromedial hypothalamus; PAG; clitoris vagina; PRV

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Numerous anatomical, physiological and behavioral studies have been conducted to delineate the essential neural substrates that mediate female reproductive behavior [13,15,56,58,<sup>60,70</sup>, 71,72,83,84]. These studies, conducted primarily in rodents, have identified several CNS regions involved in the sensory, autonomic and/or motor aspects of the lordosis reflex, a receptive behavior essential for vaginal penetration and pregnancy (e.g. 70). More recent studies examining proceptive or solicitation behavior are being utilized to identify neural

circuits involved in pacing behavior [21,25]. Together, these studies have identified at least three supraspinal regions essential to various aspects of female reproductive behavior, including the ventromedial nucleus of the hypothalamus (VMN), the medial preoptic area (MPO), and the midbrain periaqueductal gray (PAG). The VMN is considered an integral component of the lordosis reflex; stimulation of the VMN in estrogen-primed animals facilitates the display of the lordosis reflex and lesions of this region significantly disrupt it [56,68,69,70]. In contrast to the VMN, bilateral lesions of MPO increase the occurrence of lordosis [e.g. 31,64,79] while stimulation of the MPO attenuates the display of the posture [57,70]. The MPO is also identified as a crucial area for the mediation of female pacing behavior [25,102], and activation of the MPO results in increased blood flow to the vagina and increases vaginal wall tension [24]. Both the VMN and MPO send descending projections to the PAG [26,41,52,63,69], and the PAG itself has been shown to play a facilitatory role in female reproductive behavior [13,19,46,58,84,85,89]. The PAG sends dense projections to the nucleus paragigantocellularis (nPGi), which subsequently projects to motoneurons in the thoracolumbar spinal region, that innervate the axial musculature as well as to lumbosacral neurons that innervate the pelvic organs [17,18,27,29,51,61]. The PAG also receives direct inputs from the lumbosacral spinal cord [34,37,38,62,95], and may function to integrate ascending and descending information from multiple brain regions related to sexual behavior.

While the MPO, VMN and PAG have been implicated in some aspects of female sexual behavior, the role of these regions in regulating genital arousal and sexual climactic-like responses are unknown. Vaginal vasocongestion, muscle contractions and clitoral engorgement occur during genital arousal and sexual climax [5,24,35,43]. However, the CNS pathways that mediate these responses remain unknown. The MPO, VMN and PAG do not project directly to spinal regions that innervate spinal motor and preganglionic neurons involved in sexual responses. Therefore, modulation of genital responses by these regions must involve a multisynaptic pathway that relays in the brain. To delineate the anatomical pathways linking regions implicated in female sexual function (MPO, VMN and PAG) with the descending circuits that innervate the vagina and clitoris, the transneuronal tracer pseudorabies virus (PRV) was injected into the vagina and clitoris in combination with injections of the anterograde tracer biotin dextran amine into the MPO, VMN or PAG. The results of these studies identified several supraspinal-spinal circuits that may provide coordinated sensory, autonomic and hormonal modulation over female sexual responses.

## METHODS AND MATERIALS

### Viral and Anterograde Tracer Injections

All methods were performed in strict compliance with the Institutional Animal Care and Use Committee at University of Maryland, Baltimore. Female Sprague Dawley rats (Zivic Miller, 250–350 g) were deeply anesthetized with chloral hydrate (4% w/v; i.p.) and placed in a stereotaxic apparatus. The skull was adjusted so that bregma and lambda were on a horizontal plane. A small craniotomy was made and a glass micropipette (25–50  $\mu$ m) filled with biotinylated dextran amine (BDA; 10% solution, 10,000 molecular weight, Sigma Chemicals) was lowered into one of the following regions: MPO (Bregma  $-0.15$  mm,  $0.1$  mm lateral and  $7.1$  mm ventral;  $n = 8$ ), VMN (Bregma  $3.5$  mm,  $0.75$  mm lateral and  $9$  mm ventral;  $n=5$ ) or PAG (Lambda  $+1.20$ ,  $1.20$  mm lateral,  $-3.5$  mm ventral;  $n=8$ ). For the PAG injections, the manipulator was placed at a  $7.5^\circ$  angle to avoid the sagittal sinus. Following BDA injection (100 nl), the glass pipette remained in place for 10 min prior to removal to prevent backflow up the injection tract.

Ten days later, animals were reanesthetized with chloral hydrate and pseudorabies virus (PRV,  $2-3 \times 10^7$  plaque forming units/ml [74], a gift from Dr. L. Enquist) was injected into the clitoris and vagina using a 25 gauge Hamilton syringe. Each female received a single injection into

the clitoris (0.5–1  $\mu$ l) and two injections into the ventral region of the vagina (~1cm from the vaginal orifice, 1  $\mu$ l each). The needle remained in place for 1 min post-injection. After withdrawal of the needle, pressure was applied to the injection site using a cotton tip applicator to prevent leakage of the virus to the surrounding muscle. Both organs were injected in order to label the majority of CNS neurons that are engaged during genital arousal and climactic-like responses [5,26,37,45].

### Immunocytochemistry

At the end of the survival period, animals were given an overdose of sodium pentobarbital and perfused transcardially (descending aorta clamped) with 250 ml of 0.9% sodium chloride containing 2% sodium nitrite solution followed by 300 ml of 4% paraformaldehyde in 0.1M phosphate buffer containing 2% acrolein (Polyscience). A final rinse with the sodium chloride/sodium nitrite solution was used to remove any residual acrolein from the animal. Brains were removed and placed in 30% sucrose solution until sectioned. Sections were cut using a freezing microtome at 25  $\mu$ m, collected in cryoprotectant-antifreeze solution and stored at  $-20^{\circ}\text{C}$  until immunocytochemical processing.

A 1:6 series through the rostrocaudal axis of the brain was processed for BDA and PRV immunoreactivity. BDA was always visualized first as a black reaction product using nickel-enhanced diaminobenzadine (Ni-DAB); PRV was always visualized second as a brown reaction product using non-enhanced DAB (see below). Spinal cord sections (1:4 series) through the L6/S1 level were processed for PRV immunoreactivity.

Sections were removed from the cryoprotectant-antifreeze solution, rinsed extensively in potassium phosphate-buffered saline (0.1M KPBS, pH 7.4) and then reacted for 15 min in 1% sodium borohydride to remove excess aldehydes. Sections were incubated in goat anti-biotin antibody (1:60,000 dilution, Vector Labs, Burlington, CA) in KPBS containing 0.4% triton  $\times$  for 1 hr at room temperature followed by 48 hr at  $4^{\circ}\text{C}$ . After rinsing, the tissue was incubated for 1 hr in rabbit anti-goat (1:600 dilution, Vector Labs), followed by a 1 hr incubation in avidin-biotin peroxidase complex (1:10; ABC Elite Kit, Vector Labs). After rinsing, BDA was visualized as a blue-black reaction product using a nickel-sulfate intensified 3,3'-diaminobenzadine solution containing 0.08% hydrogen peroxide in 0.175M sodium acetate buffer. The reaction product was terminated after 7–25 min by rinsing in sodium acetate buffer.

Cells containing the pseudorabies virus were identified using a rabbit anti-PRV antibody (1:200,000 dilution, kindly donated by Dr. Lynn Enquist). Sections were rinsed several times in KPBS and then incubated for 1 hr in primary antibody directed against PRV at room temperature followed by 48 hrs at  $4^{\circ}\text{C}$ . Secondary and ABC reactions were as stated above. PRV was visualized as a brown reaction product using a 3,3'-diaminobenzadine solution containing 0.08% hydrogen peroxide in tris buffer. Sections were mounted onto gelatin-subbed slides and coverslipped using DPX.

### Data Presentation

The distribution of PRV neurons were plotted using a Nikon Drawing Tube attached to a Nikon Optiphot microscope. Plots were imported into the computer using the Wacom Drawing Tablet and Adobe Illustrator 10.0 software. Color photomicrographs were generated using a Synsys camera attached to a Nikon Eclipse E800 microscope. Images were captured using Adobe Photoshop 7.0. Alterations to images were strictly limited to enhancement of brightness/contrast.

## RESULTS

### Distribution of PRV labeled neurons in the spinal cord

Following a 4 day survival period spinal PRV labeling was confined to the region containing the sacral parasympathetic nucleus and dorsal gray commissure with an occasional PRV positive cell in the dorsal horn and intermediate gray (Figure 1, spinal cord, green dots). After 5 days survival PRV positive neurons were widely distributed in the spinal cord (Figure 1, spinal cord black dots). Regions containing PRV labeling included the superficial (laminae I and II), medial and lateral regions of the dorsal horn, lateral gray including the sacral parasympathetic nucleus, the dorsal gray commissure and intermediomedial nucleus, the intermediate gray (laminae V, VI and VII) and ventral horn.

### Distribution of PRV labeled neurons in the brain

Injections of PRV into the clitoris and vagina resulted in viral positive cells in selective regions of the brain. Following a 4 day survival period, PRV containing cells (Figure 1, green dots) were primarily observed in the dorsolateral region of the paraventricular nucleus (PVN) (Figure 2A), Barrington's nucleus, A5 (Figure 2B), rostral ventromedial medulla (RVM) including the raphe magnus and the ventral gigantocellular reticular formation, and the ventral lateral medulla primarily in the nucleus paragigantocellularis (nPGi) (Figure 2C). In addition, PRV positive cells were occasionally localized in the lateral hypothalamus (dorsal and lateral to the fornix), caudal ventrolateral PAG, raphe pallidus and raphe obscurus (Figure 1, green dots).

After 5 day survival, more PRV containing cells were observed in the areas described above as well as additional brain regions (Figure 1, black dots). In the forebrain numerous PRV positive cells were noted throughout the lateral hypothalamus including PRV cells that were clustered around the fornix and in the parvocellular PVN. In addition, PRV positive cells were present in the MPO including the medial preoptic nucleus (MPN), retrochiasmatic area and ventrolateral VMN (VMNvl). Fewer PRV cells were observed scattered in the ventral portion of the bed nucleus of the stria terminalis, zona inerceta, caudal medial amygdala and dorsal and ventral hypothalamic area. In the midbrain and pons, numerous PRV positive cells were found in the dorsal, lateral and ventrolateral PAG, Barrington's nucleus, A7 / Kolliker- Fuse region, lateral lemniscus and the pontine reticular formation including the area containing the subcoeruleus. In the medulla, PRV containing neurons were located in A5, RVM, nPGi and nucleus tractus solitarii (NTS). PRV labeling in the locus coeruleus (LC) was always inconsistent.

### MPO output onto PRV labeled cells

**MPO projections**—MPO injection sites spread from 0.3 mm to 1.4 mm caudal to bregma and did not spread into the lateral preoptic area. The data are described from analysis of 3 cases that had overlapping injection sites that were centered 0.8 mm caudal to bregma (Figure 4A). BDA injections resulted in dense anterograde fiber labeling similar to that previously described [52, 63, 77, 78, 90, 91]. Briefly, ascending BDA fibers ran dorsal to the MPO and innervated the bed nucleus of the stria terminalis. BDA fibers in the ventral hypothalamus coursed dorsal to the optic tract and innervated the caudal medial amygdala. A few BDA fibers and varicosities were found in the dorsal and medial PVN. A dense projection of BDA fibers was observed in the VMNvl. Descending fibers coursed through the posterior hypothalamus and periventricular fiber system. In the brainstem, BDA fibers were localized in the ventral tegmental region and dorsal to the substantia nigra. Numerous fibers were found running vertically through the medial and central PAG, and BDA fibers and terminals innervated the caudal dorsal, lateral and ventrolateral PAG. A dense projection from the MPO was observed in Barrington's nucleus. A moderate projection from the MPO was noted in the dorsal raphe and RVM. A few

scattered fibers were always present in the ventral part of the LC, subcoeruleus, A5, nPGi and NTS.

**MPO output onto PRV labeled cells**—In the hypothalamus numerous BDA fibers were intermingled with PRV positive cells in the dorsal, lateral and posterior hypothalamic areas. A close association between BDA fibers and varicosities and PRV labeled neurons was noted in the perifornical region of the lateral hypothalamus, dorsal PVN and VMNvl (Figure 4B). In the rostral PAG, BDA fibers were located primarily medial to the PRV labeled cells. More caudally in the PAG, MPO projections preferentially terminated among PRV labeled cells in the lateral and ventrolateral PAG, in Barrington's nucleus and the subcoeruleus region. While fewer BDA fibers projected to the A5, nPGi (Figure 4C) and rostral ventromedial medulla (Figure 4D), BDA fibers were consistently found in close apposition to PRV cells in these regions. No association of BDA fibers and PRV labeled cells was noted in the locus coeruleus, raphe obscurus or raphe pallidus.

#### **VMNvl output onto PRV labeled cells**

**VMNvl output**—Projections from the VMN were mapped in two animals that had similar injection sites in the ventral part of the VMN (~2.4–3.8 mm caudal to bregma) that did not diffuse into the more dorsal VMN (Figure 5A). BDA labeled ascending fibers terminated primarily in the BNST, MPO, including the MPN and the ventral preoptic area. BDA fibers were also found in the medial amygdala, lateral hypothalamus, retrochiasmatic area and paraventricular thalamic nuclei. Only a few BDA fibers were found in the ventral PVN near the 3<sup>rd</sup> ventricle. These studies confirm previous mapping of VMN outputs [45, 87]. The descending fiber system was not as pronounced as the ascending fiber system. Fibers traveled close to the 3<sup>rd</sup> ventricle in the periventricular fiber system. A few fibers were also noted in the ventral/posterior hypothalamus. In the midbrain, BDA labeled fibers terminated densely in the dorsal, lateral and ventrolateral PAG. More caudally, BDA labeling was found in the lateral dorsal tegmental region and as well as the ventral tegmental area. Occasional BDA fibers were found in Barrington's nucleus, RVM and nPGi.

**VMNvl output onto PRV labeled cells**—BDA fibers and PRV positive cells consistently overlapped in the MPO (Figure 5C), including the MPN, and the lateral hypothalamus. A moderate overlap was observed in the ventral preoptic area. In the midbrain, VMNvl fibers terminated heavily among PRV positive cells in the lateral and ventrolateral PAG and Barrington's nucleus (Figure 5B). A minor overlap was also present in the retrochiasmatic area and RVM including the raphe magnus and parapyramidal region of the medulla. No overlap of BDA fibers and PRV labeled cells was found in other brain regions including the PVN, arcuate nucleus, amygdala, A5 and nPGi.

#### **PAG output onto PRV cells**

**PAG output**—In 3 animals the BDA spread through the dorsal, lateral and ventral PAG but was confined to one side; in 2 animals the BDA injection site was more localized to the dorsal and dorso-lateral PAG and did not spread to the ventrolateral and lateral portions of the PAG. All injection sites mapped were centered ~6.0–7.6 mm caudal to bregma (Figure 6A) and resulted in anterograde labeling within the lateral preoptic area, ventral hypothalamic area, lateral hypothalamus and zona incerta. Fibers were also noted in the anterior and dorsal hypothalamus and many BDA fibers ran dorsal to the optic tract coursing towards the amygdala. A small innervation of BDA fibers was present in the MPO including the MPN and the dorsal PVN. In the brainstem, heavy anterograde labeling was observed in Barrington's nucleus, subcoeruleus, A5, ventrolateral medulla including the nPGi and RVM. A small projection to locus coeruleus and NTS was also observed.

Injections that were confined to the caudal dorsal/dorsolateral PAG (Figure 6A) produced a similar labeling pattern to that described above, however there were some differences. BDA fibers were primarily localized in the lateral preoptic area, lateral hypothalamus, dorsal hypothalamus, zona incerta, dorsal to the optic tract, and anterior hypothalamic area. Very few fibers were present in the MPO and PVN. BDA fibers were also observed in the ventral tegmental area and dorsal to the substantia nigra and geniculate nucleus. Similar to that noted above, descending BDA fibers were present primarily in the A5, RVM and nPGi. Projections from the dorsal/dorsolateral PAG did not result in a large innervation of the sexual dimorphic MPO, subcoeruleus region or Barrington's nucleus suggesting that these areas receive input primarily from the ventrolateral PAG. These studies confirm previous neuroanatomical studies reporting specific topographically defined PAG inputs and outputs [1,2,3,6].

**PAG output onto PRV cells**—Anterograde labeling from the PAG terminated among PRV labeled cells in the lateral and ventral hypothalamic area. While few PRV cells were found in the lateral preoptic area, they were always closely apposed to BDA fibers (Figure 6 B and C). A minor overlap of BDA fibers and PRV positive cells was observed in the caudal PVN. The major overlap in the brainstem was found in Barrington's nucleus, subcoeruleus, A5, RVM (Figure 6D) and nPGi (Figure 6D). In some animals a few PRV labeled cells and BDA fibers were observed in the locus coeruleus.

Injections into the caudal dorsal/dorsolateral PAG resulted in a large overlap of BDA fibers and PRV positive cells in the lateral hypothalamus and the dorsal and posterior hypothalamus. A consistent overlap was also found within the lateral preoptic area with a smaller overlap in the caudal PVN. In the brainstem, a large population of PAG output fibers terminated heavily among PRV neurons in the RVM, nPGi, and A5. However, anterogradely labeled PAG fibers primarily ran medial to PRV positive cells in Barrington's nucleus.

## DISCUSSION

Transneuronal tracing with PRV was used to identify supraspinal regions involved in the central regulation of the clitoris and vagina that may be associated with sexual responses. Anterograde tracing from the MPO, VMNvl and PAG, in combination with PRV, was used to determine if neurons transynaptically labeled from the sexual organs receive preferential inputs from brain regions previously shown to mediate various aspects of female reproductive behavior. The present studies provide the first data demonstrating a direct link between the MPO, VMNvl and PAG with CNS regions innervating the clitoris and/or vagina, providing support that these areas play a major role in female genital responses. The present results also demonstrate that several brain sites, including the lateral hypothalamus, PVN, Barrington's nucleus, A5, RVM and nPGi, provide input to the clitoris and/or vagina, and receive input from more than one brain site involved in sexual behavior. These brain regions may participate in the integration of hormonal and sensory signals related to female arousal and muscle responses that accompany sexual climactic-like reflexes.

### PRV labeling in the brain

The retrograde transynaptic tracer (PRV) was used to identify CNS regions that innervate the clitoris and/or vagina [7,8,9,74,79]. These data confirm and extend previous studies describing CNS pathways that innervate female pelvic organs. Brain areas labeled with PRV following a 4 day survival time included the dorsolateral PVN, Barrington's nucleus, A5, raphe magnus and ventrolateral medulla suggesting that these regions make direct contacts with spinal efferent pathways mediating genital organ function. These results confirm anatomical tracing studies using conventional tracers showing that these regions provide direct input to parasympathetic and sympathetic preganglionic regions, motoneurons and/or interneurons in

the lumbosacral spinal cord that are part of the circuit regulating sexual reflexes [12,18,27, 28,<sup>44,45</sup>,51,52,59,100]. Brain regions which were only labeled following a 5 day survival included the bed nucleus of the stria terminalis, MPO, medial amygdala, retrochiasmatic area, VMNvl, dorsal and lateral PAG, and A7, and most likely represent sites projecting to the brain regions that innervate spinal circuits that regulate the clitoris and/or vagina.

The majority of transynaptically labeled neurons were found in brain regions previously shown to be involved in the descending innervation of the back muscles, uterus, clitoris and cervix [17,42,51,67]. However, some differences were found in the present study compared to previous viral tracing studies of the reproductive organs. Transynaptically labeled neurons were not observed in the VMNvl after PRV injections into the clitoris [50], suggesting that the PRV positive neurons in the VMNvl in the present study may have been labeled from the vagina, although further studies examining vaginal projections are required to confirm this hypothesis. Injections of PRV into the corpus cavernosum, prostate or perineal muscles in the male rat did not result in PRV labeled neurons in the VMNvl even when many forebrain structures were labeled [54,55,65]. Therefore, PRV labeling of the VMNvl appears to be specific to females. The dorsal motor nucleus of the vagus was labeled after injections of PRV into uterine cervix but not labeled in the present study [42,66,67], labeling of this structure following uterine, but not clitoral and/or vaginal injections, supports the anatomical and physiological studies demonstrating vagal input to the uterus [32,66].

Several brain regions labeled with PRV in the present study have been shown previously to be activated during female sexual behavior. Vaginal stimulation, induced by artificial mechanical stimuli or during mating, resulted in c-fos expression in the bed nucleus of the stria terminalis, medial amygdala, MPO, PVN, lateral hypothalamus, PAG, and nPGi [21,72,73, 75,83,94,97]. Further studies examining the specific role of each of the brain sites are required to understand their contribution to specific aspects of sexual function.

**Brain areas containing PRV neurons that receive multiple inputs—**In the forebrain, PRV neurons were consistently found in the lateral hypothalamus in close association with outputs from the MPO, VMNvl and the PAG. The densest overlap between anterograde labeling and PRV+ cells was within the caudal lateral hypothalamus, surrounding the fornix. The lateral hypothalamus is involved in visceral sensory information, somatomotor control, feeding behavior and wakefulness [11,22,36,76]. The LH may also be important for some aspects of male sexual behavior. For example, serotonin released into the LH during ejaculation appears to regulate the post ejaculatory refractory period and injection of selective serotonin reuptake inhibitors into the LH delays copulation and ejaculation [33,47,48]. In both males and females, neurons in the LH are activated with sexual behavior [21,72,73,75,83,94]. No direct evidence for a role of the lateral hypothalamus in female sexual behavior has been documented to date. However, we propose that the LH may integrate hormonal, sensory and autonomic inputs in order to modulate the arousal level of the animal during reproductive behavior. This hypothesis is based on several factors (1) the dense PRV labeling in the LH from the clitoris and/or vagina, (2) inputs from the MPO, VMNvl and PAG that terminated in close apposition to the PRV labeled cells in the LH and (3) the role of the LH in sleep/wakefulness.

Transynaptically labeled PRV neurons that received inputs from both the MPO and the VMNvl were primarily localized to the lateral and ventrolateral PAG, confirming previous reports that the MPO and the VMNvl project to specific regions of the PAG [41,53,63,77,87]. The lateral and ventrolateral PAG have long been implicated in lordosis [14,59,84,85], and previous studies using PRV injected into the clitoris, uterus or back muscles reported similar labeling of ventrolateral PAG neurons at early survival times [17,51,67]. Very few neurons in the lateral PAG project directly to the spinal cord [52,61], therefore the majority of PRV labeled neurons in the PAG were most likely transynaptically labeled from the ventral medulla, including the

nPGi and raphe magnus, regions that show a dense direct spinal projection using conventional anatomical tracing techniques [27,28,44,45,49,52,63,96]. Interestingly, anterograde labeling from the MPO and VMNvl was localized predominantly in PAG regions containing PRV positive cells, suggesting a direct MPO/VMN – PAG – nPGi- spinal cord circuit that modulates female sexual responses. Some PAG areas containing PRV positive cells also receive direct inputs from the lumbosacral spinal cord [34,62,95], suggesting a direct feedback circuit for the coordination of somatosensory input with descending modulatory input from the forebrain.

Pseudorabies virus positive cells in the PVN, particularly the parvocellular region, received moderate inputs from the MPO and the PAG. PVN neurons are consistently labeled after injections of PRV into the reproductive organs [42,50,55,67] and are also labeled after injection of PRV into the heart and kidney etc. [e.g. 88,92]. The parvocellular neurons have long been linked to cardiovascular regulation, and these neurons have also been shown to regulate other autonomic functions. PVN neurons are activated during sex [e.g. 21,72,73,75] and may be involved in the increased circulating levels of oxytocin reported during sexual behavior [10, 22]. The PVN is also reportedly activated with orgasm in women with spinal cord injury [38]. The firing rate of PVN neurons increases with uterine distension, which may account for the release of circulating hormones associated with pregnancy and parturition. Oxytocin PVN neurons project directly to the lumbosacral spinal cord [86,98,99,100], and are transneuronally labeled after injections of PRV into the vagina, further suggesting the PVN may be directly involved in the regulation of genital organ function.

Barrington's nucleus also received innervation from all three sources. The primary role of Barrington's nucleus is to regulate continence and voiding [4,93]. Since this basic function would interfere with the process of sexual behavior, it is likely that overlapping pathways function to coordinate micturition and sexual behavior. Further studies are required to confirm this observation and to address the role of Barrington's nucleus in female sexual responses. Other areas that project to the clitoris/vagina and receive inputs from the MPO, VMNvl and PAG include the ventromedial and ventrolateral medulla. Neurons in the ventrolateral medulla, including the nPGi, mediate contractions of the skeletal muscles of the back, respond to genital stimulation and regulate the descending inhibition of the urethro-genital reflex [15,30,59,70, 81,82,103]. The raphe magnus, and its descending projections to the dorsal horn of the spinal cord, constitute the endogenous descending analgesia circuit; therefore, forebrain and PAG input to the raphe magnus may serve to increase somatosensory thresholds during reproductive behavior [16,39,41,101].

## Summary

Transneuronal tracing with PRV was used to identify brain regions involved in the regulation of clitoral and vaginal responses during sexual arousal and climactic-like responses. The results of these studies are the first to link input from the MPO, VMNvl and PAG with neurons transynaptically labeled from the clitoris and vaginal, and establish a potential circuit for the elaboration of female sexual responses. Input from the MPO, VMN and PAG may also allow for the coordination of hormonal input with sensory, autonomic and motor output. Further studies examining the specific contribution of these brain circuits and their neurotransmitters in regulating female sexual responses is required.

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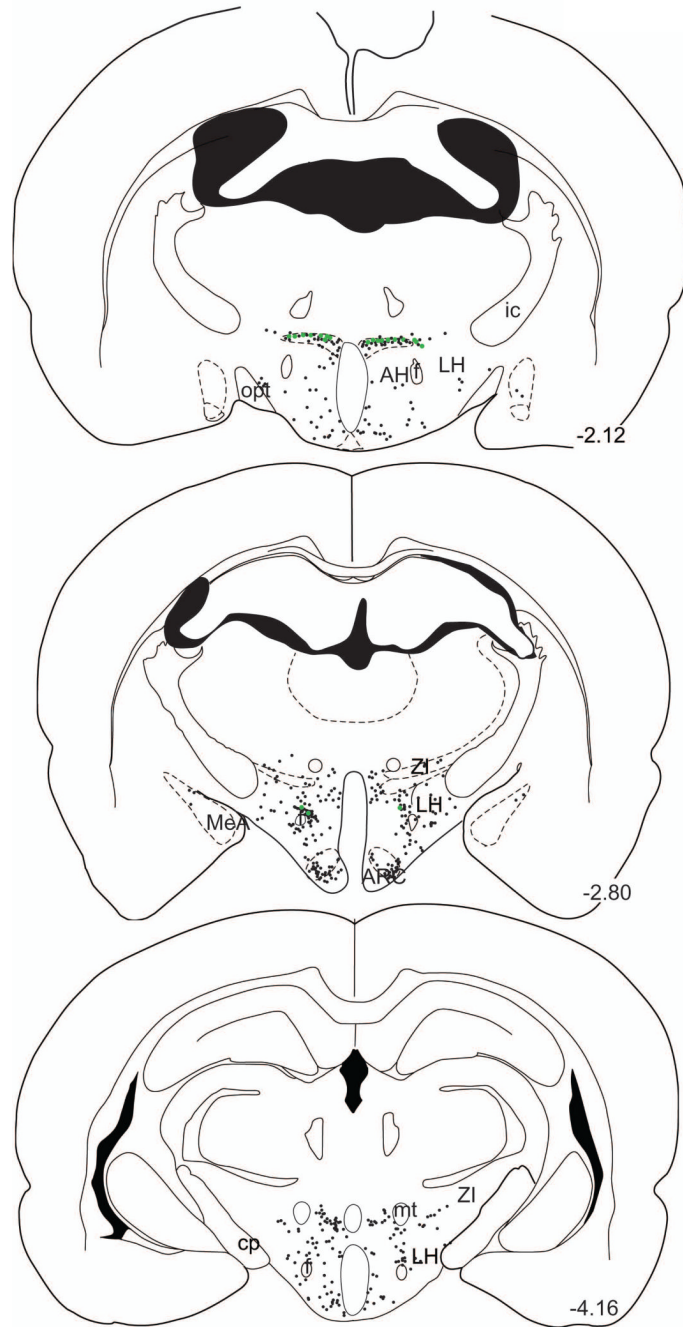
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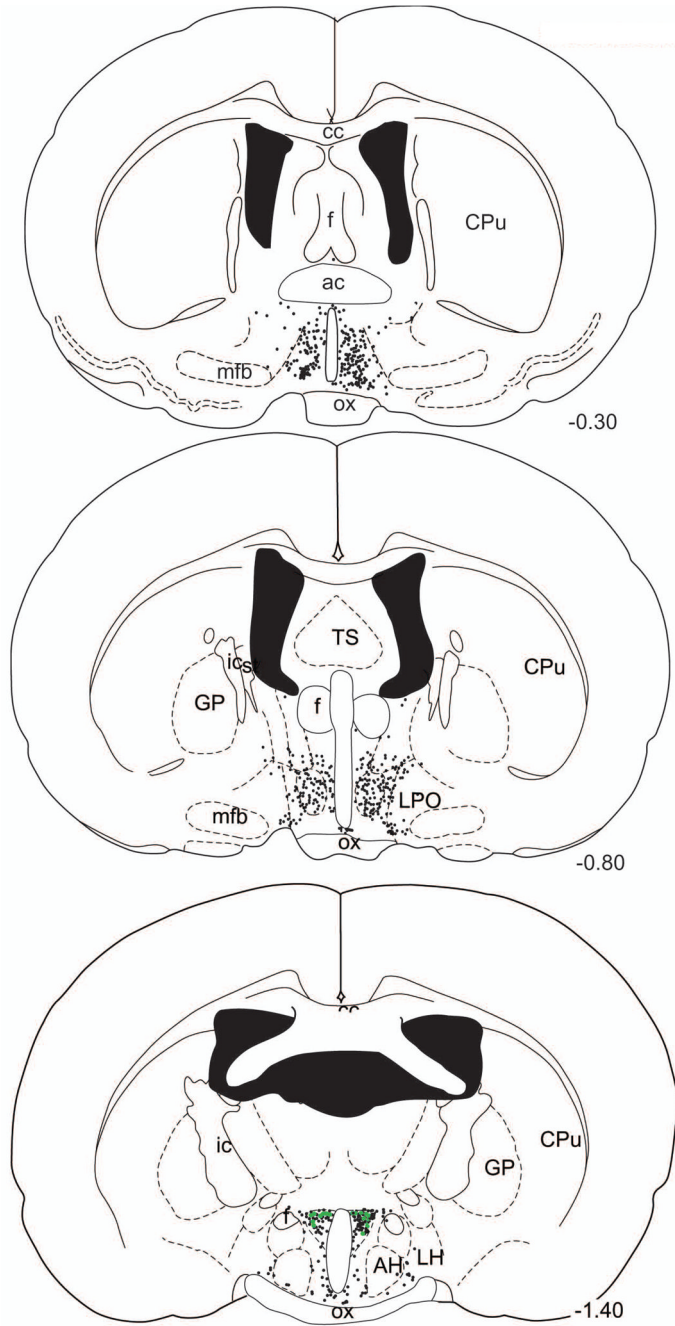
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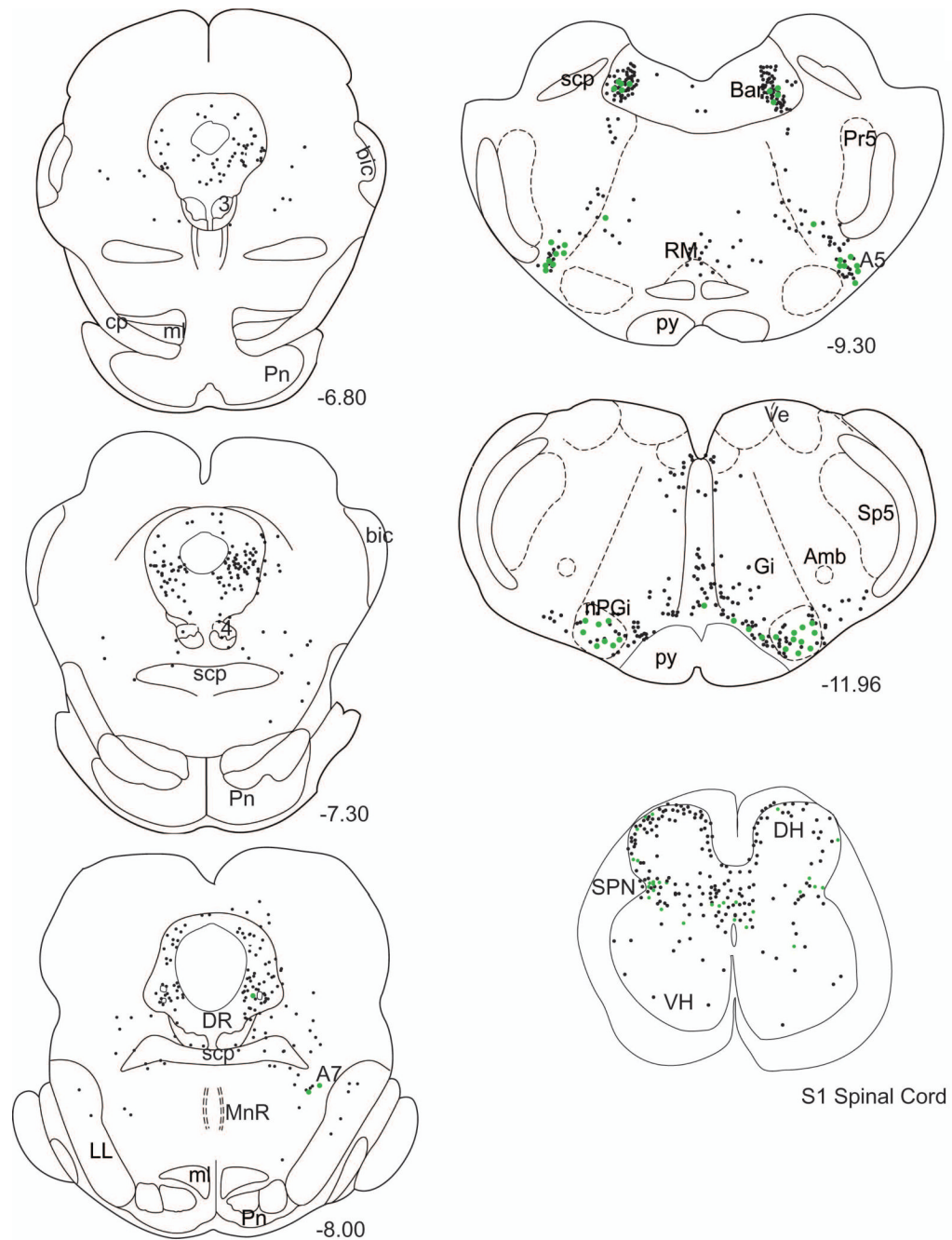
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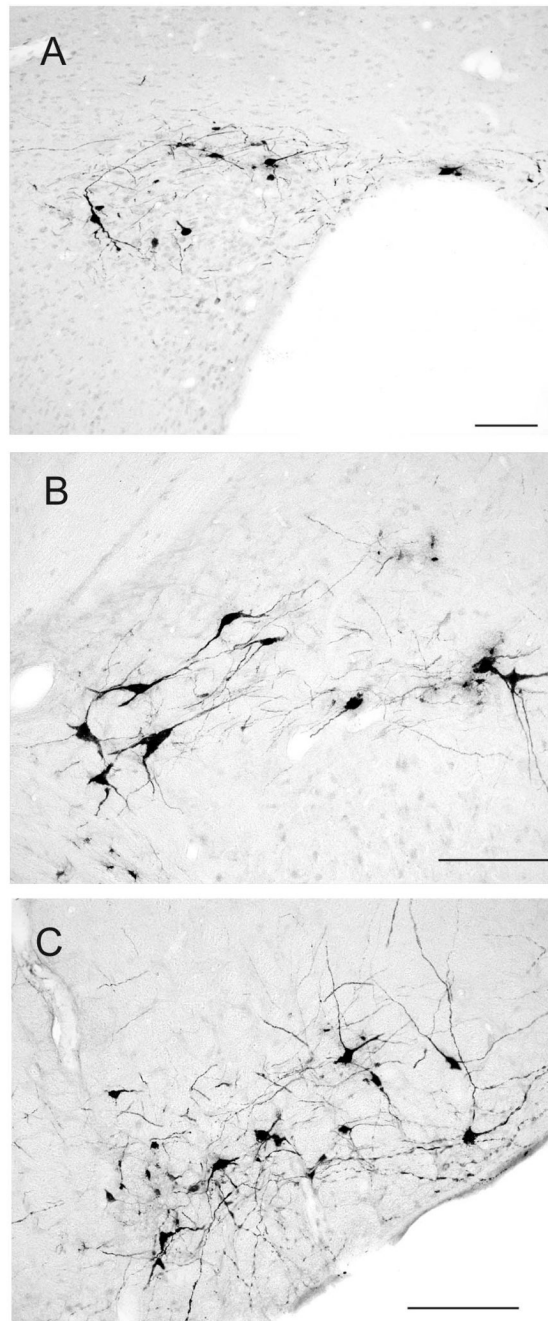


**Figure 1.**

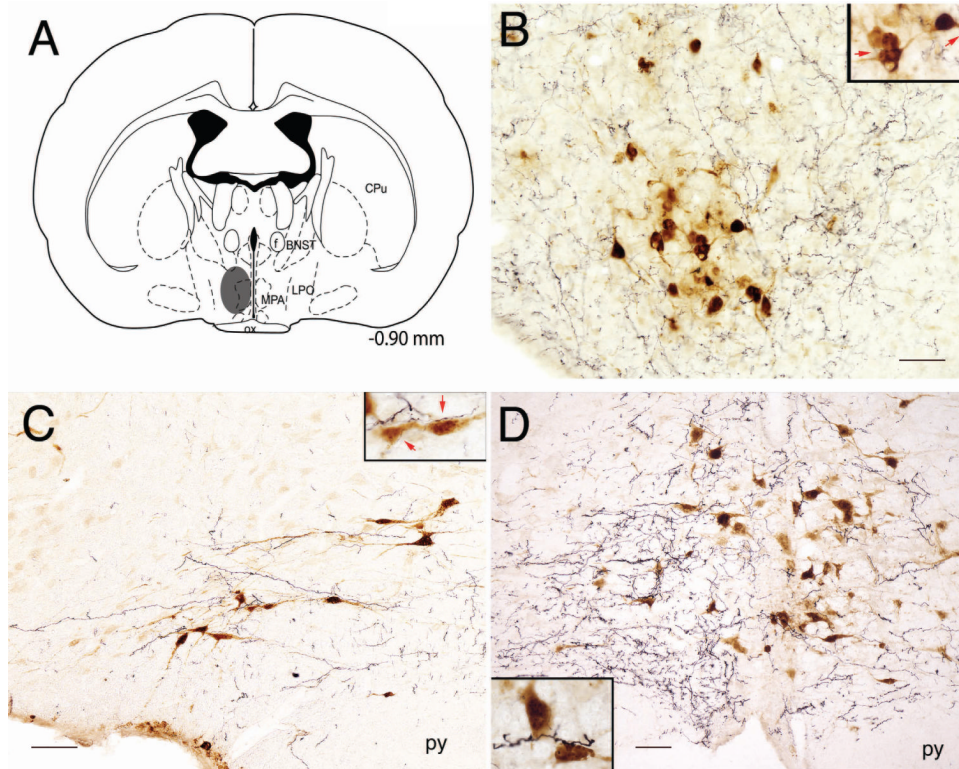
Distribution of PRV transynaptically labeled cells through the brain and spinal cord 4 days [green dots] and 5 days [black dots] after injection into the clitoris and vagina. These drawings were made from an animal which had a moderate amount of labeling. The number at the bottom of each drawing indicates the distance (mm) from Bregma. Abbreviations:- 3 - oculomotor nucleus, 4 - trochlear nucleus, A5 and A7 – noradrenergic cell groups, AH – anterior hypothalamic area, Ac - anterior commissure, Amb - ambiguus nucleus, ARC – arcuate hypothalamic nucleus, Bar - Barrington’s nucleus, bic - brachium of the inferior colliculus, cc – corpus callosum, cp - cerebral peduncle, Cpu caudate putamen, DH - dorsal horn, DR – dorsal raphe nucleus, Ic - internal capsule, f – fornix, Gi - gigantocellular reticular nucleus, GP- globus



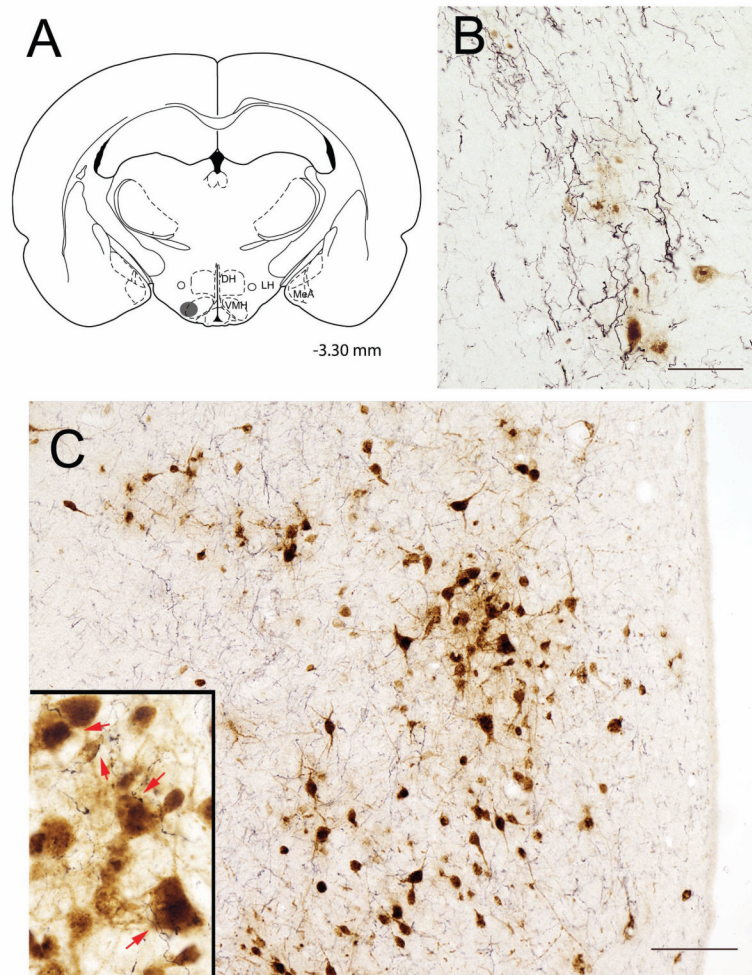
pallidus, LH - lateral hypothalamic area, LL - lateral lemniscus, LPO - lateral preoptic area, MeA - medial amygdaloid nucleus, mfb - medial forebrain bundle, ml - medial lemniscus, MnR - median raphe nucleus, mt - mammillothalamic tract, nPGi - nucleus paraventricularis, opt - optic tract, ox - optic chiasm, Pr5 - principal sensory trigeminal nucleus, Pn - pontine nucleus, py - pyramids, RM - raphe magnus, scp - superior cerebellar peduncle, Sp5 - spinal trigeminal tract, SPN - sacral parasympathetic nucleus, st - stria terminalis, TS - triangular septal nucleus, Ve - vestibular nuclei, VH - ventral horn, ZI - zona incerta.



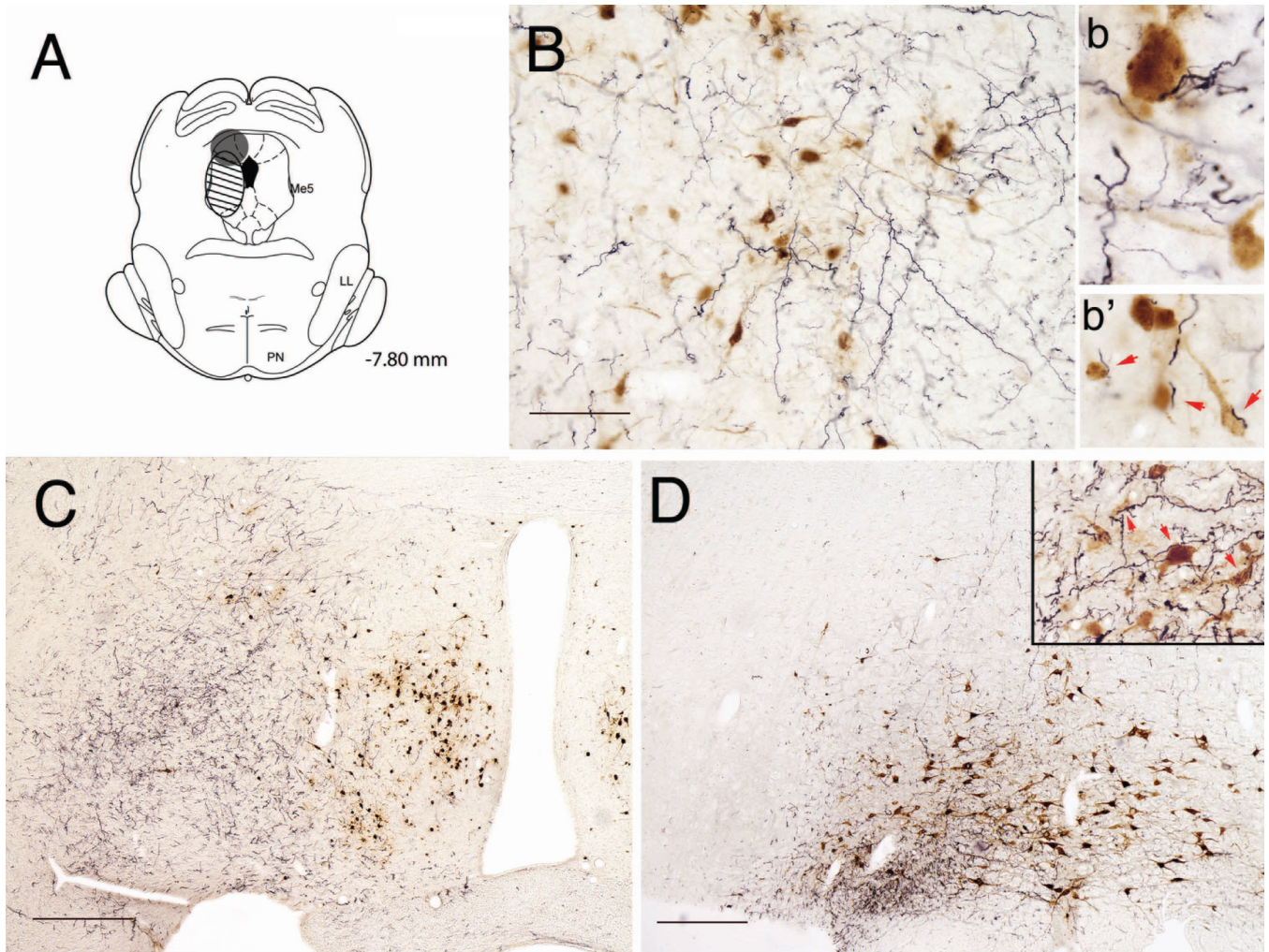
**Figure 2.** Photomicrographs showing PRV labeled cells in the brain 4 days after injection into the clitoris and vagina. [A] PRV labeled cells in the paraventricular nucleus of the hypothalamus. [B] PRV labeled cells in the A5 region. [C] PRV labeled cells in the nucleus paragigantocellularis. Scale bar =150  $\mu$ m



**Figure 3.** Photomicrographs showing medial preoptic area (MPO, [A]) output to the ventromedial nucleus [B], nucleus paragigantocellularis [C] and raphe magnus [D] and PRV labeled cells in the brain 5 days after injection into the clitoris and vagina. Figure A shows the location of the center of the BDA injection site in the MPO at  $-0.9$  mm caudal to bregma. Inserts in each photomicrograph show high power examples of the close association of the PRV neurons with BDA labeled fibers and varicosities (arrows). Abbreviations:- BNST – bed nucleus of stria terminalis, Cpu – caudate putamen, f- fornix, LPO – lateral preoptic area, MPA – medial preoptic nucleus, ox – optic chiasm. Scale bar =  $100\ \mu\text{m}$



**Figure 4.** Photomicrographs showing ventromedial nucleus (VMN, [A]) output to Barrington's nucleus [B] and the preoptic area [C] and PRV labeled cells in the brain 5 days after injection into the clitoris and vagina. Figure A shows the location of the center of the BDA injection site in the ventromedial nucleus of the hypothalamus at  $-3.30$  caudal to bregma. Insert [C] shows an example of the close association of the PRV neurons with BDA labeled fibers and varicosities (arrows). Abbreviations:- DH – dorsal hypothalamus, LH – lateral hypothalamus, MeA – medial amygdala, VMH – ventromedial hypothalamus. Scale bar =  $150\ \mu\text{m}$



**Figure 5.**

Photomicrographs showing periaqueductal gray (PAG, [A]) output to the lateral preoptic area [B, high magnification; and C low magnification] and to the ventral medulla [D] and PRV labeled cells in the brain 5 days after injection into the clitoris and vagina. Figure A shows the location of the BDA injection sites which included either the dorsal (solid circle) or lateral/ventrolateral (hatched oval) PAG. Inserts [b and b' and insert in figure D] show examples of the close association of PRV neurons with BDA labeled fibers and varicosities (arrows). Abbreviations:- LL – lateral lemniscus, Me5 - mesencephalic trigeminal nucleus, PN – pontine nucleus. Scale bar = 150  $\mu\text{m}$  [C and D]; Scale bar = 500  $\mu\text{m}$  [B].