

Georgia State University

ScholarWorks @ Georgia State University

---

Biology Dissertations

Department of Biology

---

4-26-2010

## Anatomy and Physiology of the Nucleus Paragigantocellularis: Neural Regulation of Genital Reflexes in Male and Female Rats

Joseph Jeremy Normandin  
*Georgia State University*

Follow this and additional works at: [https://scholarworks.gsu.edu/biology\\_diss](https://scholarworks.gsu.edu/biology_diss)



Part of the [Biology Commons](#)

---

### Recommended Citation

Normandin, Joseph Jeremy, "Anatomy and Physiology of the Nucleus Paragigantocellularis: Neural Regulation of Genital Reflexes in Male and Female Rats." Dissertation, Georgia State University, 2010.  
doi: <https://doi.org/10.57709/1332149>

This Dissertation is brought to you for free and open access by the Department of Biology at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Biology Dissertations by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact [scholarworks@gsu.edu](mailto:scholarworks@gsu.edu).

ANATOMY AND PHYSIOLOGY OF THE NUCLEUS PARAGIGANTOCELLULARIS:  
NEURAL REGULATION OF GENITAL REFLEXES IN MALE AND FEMALE RATS

By

JOSEPH J. NORMANDIN

Under the Direction of Dr. Anne Z. Murphy

ABSTRACT

The supraspinal control of descending inhibition of genital reflexes (such as ejaculation) is poorly understood but is important in our global comprehension of how neural signals are integrated to produce sexual behavior, and in our understanding of sexual dysfunction. Sexual dysfunctions, such as premature ejaculation/delayed ejaculation in men, and involuntary vaginal spasms, dyspareunia, and anorgasmia in women, are common. An underlying dysregulation of genital reflexes may produce these dysfunctions, especially in those individuals being treated for depression and anxiety with serotonergic drugs. The nucleus paragigantocellularis (nPGi) of the rat medulla has been described as a descending inhibitory system for genital reflexes in rats, and a homologue is known in humans. Through retrograde tracing of nPGi afferents with the tracer Fluorogold in rats, we found that a number of brain regions implicated in sexual behavior, such as the medial preoptic area, paraventricular nucleus of the hypothalamus, and periaqueductal gray (PAG) provide sexually dimorphic projections to the nPGi, and that many of these regions contain receptors for gonadal steroids and are active during sexual behavior. We also

found that excitotoxic lesions of the nPGi with N-methyl-D-aspartate facilitate male sexual behavior by reducing the number of intromissions required for ejaculation, and decreasing ejaculation latency. In females, such lesions attenuated sexual behavior by reducing the amount of time the female spent mating and reducing the reinforcement value of vaginocervical stimulation. Lastly, we found that by removing the source of serotonin to the nPGi (from the ventrolateral PAG) with the serotonergic neurotoxin 5,7-DHT in male rats, we were able to mimic the effects of nPGi lesions and facilitated male sexual behavior indicating that serotonin neurotransmission at the level of the nPGi is critical for genital reflex control. Taken together our results indicate that the nPGi is an important site of integration of internal signals for the regulation of sexual behavior that is sexually dimorphic and under serotonergic control. Our understanding of normal and dysfunction genital reflex control, and possible treatment options in people, is complemented by these results.

INDEX WORDS: Sex differences, Fos, Estrogen, Androgen, Brainstem, Penis, Vagina, Orgasm

ANATOMY AND PHYSIOLOGY OF THE NUCLEUS PARAGIGANTOCELLULARIS:  
NEURAL REGULATION OF GENITAL REFLEXES IN MALE AND FEMALE RATS

by

JOSEPH J. NORMANDIN

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2010



ANATOMY AND PHYSIOLOGY OF THE NUCLEUS PARAGIGANTOCELLULARIS:  
NEURAL REGULATION OF GENITAL REFLEXES IN MALE AND FEMALE RATS

by

JOSEPH J. NORMANDIN

Committee Chair: Anne Murphy

Committee: Andrew Clancy

Charles Derby

Aras Petrulis

James Pfaus

Electronic Version Approved:

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

May 2010

## DEDICATION

*"Ocean in view O! The Joy!"*

-Lewis and Clark Expedition, November, 1805

An academic life is an endless journey. Approaching the end of this phase of my career, I was struck by this quote from the Lewis & Clark expedition and how it spoke to me of finding the end of one's journey (in their case, the Pacific Ocean), only to be confronted with a great expanse of possibility. This work is dedicated to the mentors that have inspired and supported me (and in some cases, taken a chance on me) throughout my academic life, and who have given me the opportunity to forever be looking over the horizon.

*Dr. Richard C. Pillard*

*Dr. Mary S. Erskine*

*Dr. Anne Z. Murphy*

## ACKNOWLEDGEMENTS

Science is not a one-man job, and there have been many who have helped with this work both directly and indirectly. Thank you to Dr. Anne Murphy for her love, guidance, and fervent support. Thank you to my dissertation committee for all your help and encouragement: Dr. Andrew Clancy, Dr. Charles Derby, Dr. Aras Petrulis, and Dr James Pfaus. I am indebted to Dr. Laura Carruth, Dr. Matthew Grober, Dr. Tim Bartness, and Dr. Elliott Albers for their advice, assistance, teachings, and support. I also wish to thank my army of minions (Murphy lab undergraduates) for their technical contributions to my work: Tayisha Vilceus, Hau P. Bui, Alex Iskhakov, Erica Famojore, Brittany Grandy, Amal Abdulahi, Hila Eichenbaum, and Vincent Laufer.

I enjoyed the excellent administrative support of Latesha Morrison-Warren of the Department of Biology, and Dixie Elmore and Fatima Adams of the Neuroscience Institute. Thank you to all of the members of the Animal Resource Program especially Dr. Michael Hart, Dean Blake, and Matthew Davis.

Moral support provided by Lori Eidson, Nicole Victoria, Elizabeth Jeffress, Rick Hanberry, Pam Maras, Laura Been, Marc Badura, Amanda Arnold, Luis Martinez, Kyle Gobrogge, Dr. Kelli Duncan, Dr. Dayna Loyd, and Dr. Jamie LaPrairie. Unconditional love provided by my mother Patricia Normandin, my brother and brother-in-law Michael Normandin and Rob Villegas, and mon coeur Patrick Colot.

This work would not have been possible without the financial support of the National Institutes of Health (Grant DA16272 to Anne Z. Murphy) the National Science Foundation (Grant IBN9876754 to the Center for Behavioral Neuroscience) and the Georgia State University William M. Suttles Fellowship to Joseph J. Normandin.

Thank you



## TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS .....</b>	<b>v</b>
<b>LIST OF TABLES .....</b>	<b>ix</b>
<b>LIST OF FIGURES .....</b>	<b>x</b>
<b>1 GENERAL INTRODUCTION .....</b>	<b>1</b>
<b>1.1 The Study of Genital Reflexes.....</b>	<b>1</b>
<b>1.2 Neural control of Genital Reflexes .....</b>	<b>2</b>
<b>1.2.1 Anatomy &amp; Physiology.....</b>	<b>2</b>
<b>1.2.2 The Nucleus Paragigantocellularis .....</b>	<b>3</b>
<b>1.2.3 The Influence of Serotonin on Sexual Behavior.....</b>	<b>4</b>
<b>1.2.4 Serotonin and the Nucleus Paragigantocellularis.....</b>	<b>4</b>
<b>1.3 Implications for Human Health .....</b>	<b>5</b>
<b>1.4 Experimental Rationale .....</b>	<b>6</b>
<b>1.4.1 Experiment 1: Nucleus paragigantocellularis afferents in male and female rats: organization, gonadal steroid receptor expression, and activation during sexual behavior .....</b>	<b>6</b>
<b>1.4.2 Experiment 2: Excitotoxic lesions of the nucleus paragigantocellularis facilitate male sexual behavior but attenuate female sexual behavior in rats.....</b>	<b>6</b>
<b>1.4.3 Experiment 3: Serotonergic lesions of the midbrain source of serotonin to the nucleus paragigantocellularis facilitate sexual behavior in male rats .....</b>	<b>7</b>
<b>2 NUCLEUS PARAGIGANTOCELLULARIS AFFERENTS IN MALE AND FEMALE RATS: ORGANIZATION, GONADAL STEROID RECEPTOR EXPRESSION, AND ACTIVATION DURING SEXUAL BEHAVIOR.....</b>	<b>8</b>
<b>2.1 Introduction .....</b>	<b>8</b>
<b>2.2 Materials and Methods .....</b>	<b>10</b>
<b>2.2.1 Subjects .....</b>	<b>10</b>
<b>2.2.2 Fluorogold injections .....</b>	<b>10</b>
<b>2.2.3 Sexual behavior.....</b>	<b>11</b>
<b>2.2.4 Perfusion fixation.....</b>	<b>11</b>
<b>2.2.5 Immunocytochemistry.....</b>	<b>11</b>
<b>2.2.6 Data analysis and presentation .....</b>	<b>13</b>
<b>2.3 Results.....</b>	<b>15</b>
<b>2.3.1 Injection sites .....</b>	<b>15</b>
<b>2.3.2 General patterns in labeling .....</b>	<b>15</b>

2.3.3	Organization and gonadal steroid receptor expression of nPGi afferents .....	15
2.3.4	Activation of nPGi afferents during sexual behavior .....	19
2.4	Discussion .....	21
2.4.1	Preoptic area .....	21
2.4.2	Amygdala and bed nucleus of the stria terminalis .....	23
2.4.3	Hypothalamus .....	24
2.4.4	Periaqueductal gray .....	25
2.4.5	Superior and inferior colliculus .....	26
2.4.6	The nPGi as a central integrator for arousal .....	27
2.5	Conclusions .....	29
3	<b>EXCITOTOXIC LESIONS OF THE NUCLEUS PARAGIGANTOCELLULARIS FACILITATE MALE SEXUAL BEHAVIOR BUT ATTENUATE FEMALE SEXUAL BEHAVIOR IN RATS .....</b>	<b>45</b>
3.1	Introduction .....	45
3.2	Materials and methods .....	47
3.2.1	Subjects .....	47
3.2.2	Ovariectomy and gonadal-steroid replacement .....	47
3.2.3	nPGi lesions .....	47
3.2.4	Sexual behavior .....	48
3.2.5	Conditioned place preference for aVCS .....	48
3.2.6	Perfusion / fixation / tissue preparation .....	50
3.2.7	Immunohistochemistry .....	50
3.2.8	Lesion analysis .....	51
3.2.9	Sexual behavior- and aVCS-induced Fos expression analysis .....	51
3.2.10	Statistical analysis .....	51
3.3	Results .....	52
3.3.1	Lesion verification .....	52
3.3.2	Male sexual behavior .....	52
3.3.3	Female sexual behavior .....	52
3.3.4	Female CPP for aVCS .....	53
3.3.5	Male sexual behavior-induced Fos expression .....	54
3.3.6	Female sexual behavior-induced Fos expression .....	54
3.3.7	Female aVCS-induced Fos expression .....	54

3.4	Discussion.....	54
3.4.1	Males .....	54
3.4.2	Females .....	56
3.5	Conclusions.....	58
4	<b>SEROTONERGIC LESIONS OF THE MIDBRAIN SOURCE OF SEROTONIN TO THE NUCLEUS PARAGIGANTOCELLULARIS FACILITATE SEXUAL BEHAVIOR IN MALE RATS.....</b>	<b>71</b>
4.1	Introduction .....	71
4.2	Methods and materials.....	73
4.2.1	Subjects .....	73
4.2.2	Ovariectomy and gonadal-steroid replacement in stimulus females .....	73
4.2.3	vIPAG 5-HT lesions .....	73
4.2.4	Sexual behavior tests .....	74
4.2.5	Perfusion / fixation / tissue preparation .....	74
4.2.6	Immunohistochemistry.....	75
4.2.7	Lesion analysis.....	75
4.2.8	Statistical analysis .....	76
4.3	Results.....	76
4.3.1	Lesion Verification .....	76
4.3.2	Sexual Behavior.....	76
4.4	Discussion.....	77
4.5	Conclusions.....	78
5	<b>GENERAL CONCLUSIONS.....</b>	<b>84</b>
	<b>REFERENCES.....</b>	<b>88</b>
	<b>APPENDICES.....</b>	<b>105</b>
	Appendix A – Preliminary data: Manipulation of serotonin at the nucleus paragigantocellularis of male rats produces paradoxical effects on sexual behavior .....	105
	Appendix B – Preliminary data: Trans-synaptic tracing of functional genitosensory pathways in male and female rats .....	109
	Appendix C – Preliminary data: The paraventricular nucleus of the hypothalamus contains oxytocinergic cells integrated into ascending and descending genital circuitry .....	116
	Appendix D – Curriculum Vitae .....	124

### LIST OF TABLES

Table 2.1 Distribution of FG+ cells, and FG+ cells that also contained ER $\alpha$ , AR, or sex behavior-induced Fos following injection of FG into the nPGi .....	30
Table 2.2 Sexual Behavior Measures (mean) in Tracer-injected Male and Female Rats.....	31
Table 3.1 Sexual behavior measures (mean +/- standard error of the mean (SEM)) in control group and lesion group males.....	59
Table 3.2 Sexual behavior measures (mean +/- SEM) in control group and lesion group females.....	59
Table 3.3 Conditioned place preference measures (mean +/- SEM) in control group and lesion group females .....	59
Table 4.1 Sexual behavior measures (mean +/- standard error of the mean) in sham group and lesion group males .....	80
Table B.1 Abundance of H129 labeling in supraspinal sites of male and female rats as a result of genital inoculation .....	113

## LIST OF FIGURES

Figure 2.1 Photomicrograph of Fluorogold injection sites .....	32
Figure 2.2 Distribution of FG+ cells and co-localization with gonadal steroid receptors .....	33
Figure 2.3 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors in the MPO .....	35
Figure 2.4 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors throughout the brain .....	36
Figure 2.5 Photomicrograph of FG+ cells and co-localization with gonadal steroid receptors in the PVN	37
Figure 2.6 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors in the PAG .....	38
Figure 2.7 Distribution of FG+ cells and co-localization with sexual behavior-induced Fos.....	39
Figure 2.8 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos in the MPO ..	41
Figure 2.9 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos throughout the brain .....	41
Figure 2.10 Photomicrograph of FG+ cells and co-localization with sexual behavior-induced Fos in the PeF .....	42
Figure 2.11 Photomicrograph of FG+ cells and co-localization with sexual behavior-induced Fos in the PVN .....	42
Figure 2.12 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos in the PAG..	43
Figure 2.13 Diagram of regions providing dense input to the nPGi .....	44
Figure 3.1 Photomicrograph of sham lesion and lesion sites .....	60
Figure 3.2 Diagram of lesion sites .....	61
Figure 3.3 Measures of male sexual behaviors related to genital reflex function .....	62
Figure 3.4 Measures of male sexual behavior unrelated to genital reflex function.....	63
Figure 3.5 Measures of stimulus males' sexual behavior during mating with experimental females .....	64
Figure 3.6 Measures of female receptive and proceptive sexual behavior.....	65
Figure 3.7 Measures of female paced-mating behaviors during sexual behavior.....	66
Figure 3.8 Measures of condition placed preference for aVCS in females.....	67
Figure 3.9 Sexual behavior- and aVCS-induced Fos expression in male and female rats.....	68
Figure 4.1 Photomicrograph of sham lesion and lesion sites .....	81
Figure A.1 Sexual behaviors of male rats treated with mCPP, SB-242,084, or vehicle.....	108
Figure B.1 Photomicrographs of H129 labeling in the brainstem of a male and a female rat .....	114
Figure B.2 Photomicrographs of H129 labeling in the midbrain of a male and a female rat .....	114
Figure B.3 Photomicrographs of H129 labeling in the thalamus, hypothalamus, and amygdala of a male and a female rat.....	115
Figure B.4 Photomicrographs of H129 labeling in the cerebral cortex of a male rat .....	115
Figure C.1 Photomicrograph of PVN cells labeled with FG or H129 .....	121
Figure C.2 Photomicrograph of PVN cells labeled for FG and oxytocin .....	122
Figure C.3 Photomicrograph of PVN cells labeled for H129 and oxytocin.....	122
Figure C.4 Photomicrograph of PVN cells labeled for FG and oxytocin.....	123
Figure C.5 Photomicrograph of PVN cells labeled for H129 and oxytocin.....	123

## **1 GENERAL INTRODUCTION**

### **1.1 The Study of Genital Reflexes**

The neural circuits underlying genital response to internal and external cues have not been fully elucidated. In both men and women, internally or externally derived stimulation results in increased blood flow and engorgement of genital tissue, and rhythmic contractions of genital muscles often associated with orgasm (Argiolas and Melis, 2003). All of these processes are reflexive and under the control of sympathetic, parasympathetic, and somatic spinal efferents (Giuliano and Clement, 2005; McKenna, 2002; Temel et al., 2005); these efferents are subsequently under both excitatory and inhibitory control from numerous supraspinal sites including the paraventricular hypothalamic nucleus (PVN), nucleus paragigantocellularis (nPGi), and lumbar spinal cord spinothalamic cells (Liu et al., 1997a; Marson and McKenna, 1990; Truitt and Coolen, 2002). Coordination of the various circuits controlling genital reflexes has been studied extensively in men, with recent interest in defining homologous circuits in women. Non-human animal models, in particular the rat, have been particularly informative in elucidating the basic anatomy and physiology of these circuits (Pfaus et al., 2003). From this work, a general understanding of the discrete processes underlying genital reflex control has been established. First, the autonomic nervous system plays the key role in providing the necessary signals for increased blood flow to the genitalia for both men (erection) and women (genital engorgement) as well as providing signals for the production of secretive fluids used for lubrication and seminal transfer (McKenna, 2002; Temel et al., 2005; Yang and Jiang, 2009). Second, the somatic nervous system provides the signals for rhythmic contractions of the genital musculature in both men (ejaculation: expulsion phase) and women (vaginocervical contractions), which are often associated with orgasm (Giuliano and Clement, 2005; Levin, 1998; Yang and Jiang, 2009). This dissertation is concerned with the latter of these processes in which there has been considerable interest in understanding how supraspinal sites impose inhibitory and excitatory control on somatic genital reflexes.

## 1.2 Neural control of Genital Reflexes

### 1.2.1 Anatomy & Physiology

The bulbospongiosus (also referred to as the bulbocavernosus) and ischiocavernosus muscles are the primary genital muscles in mammals providing the rhythmic contractions needed for somatic genital reflexes (Gerstenberg et al., 1990; Meston et al., 2004; Sachs, 1982). They are innervated by the pelvic and pudendal nerves (Pacheco et al., 1989; Pastelin et al., 2008) from the lower lumbar and upper sacral divisions (L5-S1) of the spinal cord (de Araujo et al., 1982; Katagiri et al., 1986; Roppolo et al., 1985). In rats, the spinal motoneuron pools associated with these nerves are referred to as the dorso-medial nucleus (DM; also referred to as the spinal nucleus of the bulbocavernosus) and dorsolateral nucleus (DL) of L5-S1 ventral horn (Collins et al., 1991; Katagiri et al., 1986; McKenna and Nadelhaft, 1986; Peshori et al., 1995; Schroder, 1980). The DM and DL are considered homologues of Onuf's nucleus in humans and rhesus monkeys (Breedlove and Arnold, 1980; Roppolo et al., 1985; Schroder, 1981). Interneurons within the spinal cord appear to connect primary afferent somatosensory information with the DM and DL (Collins et al., 1991; Peshori et al., 1995; Wiedey et al., 2008). Descending projections from other parts of the spinal cord, as well as from supraspinal sites, provide both excitatory and inhibitory drive to these motoneuron pools (Allard et al., 2005; Coolen, 2005).

Supraspinal inhibitory and excitatory modulatory inputs to the DM and DL have been described in rats (Liu et al., 1997a; Marson and McKenna, 1990; Truitt and Coolen, 2002), cats (Kausz, 1990), and birds (Berk and Finkelstein, 1983). These excitatory and inhibitory drives compete at the level of the spinal cord motoneuronal pools to regulate genital reflexes within specific behavioral contexts. A hypothesized ejaculation generator, constituting a central pattern generator for the muscles of ejaculation, has been described in the lumbar spinal cord of rats, which provides input to the DM and DL (Truitt and Coolen, 2002; Truitt et al., 2003). Lesions of these lumbar spinothalamic cells abolish the ability of male rats to ejaculate, while leaving other aspects of sexual behavior intact, suggesting that this region is crit-

ical for ejaculation (Truitt and Coolen, 2002). In addition, the PVN has direct connections to the DM and DL (Wagner and Clemens, 1991). The PVN has been previously implicated in the control of genital reflexes, and is most likely a source of descending excitatory input to the genital musculature (Chen et al., 1997; Eaton et al., 1991). With respect to descending inhibition, the nPGi of the medulla provides dense input to the DM and DL (Tang et al., 1999) and is the hypothesized source of descending inhibition to genital reflexes (Marson and McKenna, 1990).

### **1.2.2 The Nucleus Paragigantocellularis**

Numerous lines of evidence suggest that the nPGi is the primary source of descending inhibition of genital reflexes in male rats. The nPGi sends direct descending projections to the L5-S1 motoneuronal pools that innervate the bulbospongiosus and ischiocavernosus muscles in both male and female rats (Hermann et al., 2003; Marson and Carson 3rd, 1999; Marson and McKenna, 1996; Tang et al., 1999). In humans, the homologous structure is referred to as the nucleus paragigantocellularis lateralis (Zec and Kinney, 2001) and is also believed to be associated with descending inhibition of genital reflexes (Johnson, 2006).

Lesions of the nPGi in rats consistently result in the facilitation of sexual behavior, as indicated by a decrease in mount and intromission frequency, ejaculation latency, and an increase the number of ejaculations to satiety (Yells et al., 1992; Yells et al., 1994). nPGi lesions also decrease the latency to and increase the number of ex copula erections (Marson et al., 1992; Marson and McKenna, 1990). Similarly, electrical stimulation of nPGi neurons produces increased firing latency and decreased amplitude of firing in the DM (Johnson and Hubscher, 1998), consistent with the role of the nPGi as a source of tonic descending inhibitions of genital reflexes.

To date, the role of the nPGi in female sexual behavior has not been directly tested. However, anatomical evidence suggests that the nPGi is important for sexual behavior in females. Retrograde trans-synaptic tracing from rat clitoris (Marson and Murphy, 2006), vagina (Marson and Murphy, 2006),



and cervix (Lee and Erskine, 2000) produce labeling in the nPGi of females. In addition, a number of brain regions associated with sexual behavior project to the nPGi female rats (Marson and Foley, 2004; Marson and Murphy, 2006; Murphy and Hoffman, 2001; Normandin and Murphy, 2008), suggesting a role for the nPGi in female sexual behavior.

### **1.2.3 The Influence of Serotonin on Sexual Behavior**

Many behavioral systems in mammals are modulated by serotonin (5-HT), including mood (Dayan and Huys, 2009) and sexual behavior (Pfaus, 2009). Increased 5-HT levels through systemic administration of selective serotonin reuptake inhibitors (SSRIs) produces delayed ejaculation in male rats (Ahlenius et al., 1980; de Jong et al., 2005a; Vega Matuszcyk et al., 1998) and humans (Hsu and Shen, 1995). Importantly, the effect of 5-HT on sexual behavior is brain region and receptor dependent, producing both facilitation and attenuation of sexual behavior. For example, administration of the 5-HT<sub>1A</sub> agonist 8-hydroxy-2-(di-n-propylamino) tetralin facilitates male sexual behavior when injected into the medial preoptic area of the hypothalamus or nucleus accumbens (de Castilhos et al., 2006; Fernandez-Guasti et al., 1992), while administration of trifluoromethyl-phenyl-piperazine, a 5-HT<sub>1B/C</sub> agonist to the same regions inhibits male sexual behavior (Fernandez-Guasti et al., 1992; Hillegaart et al., 1991). To date, site-specific manipulations of 5-HT have not been used extensively, but are critical for delineating inhibitory and facilitatory circuits modulating sexual behavior.

### **1.2.4 Serotonin and the Nucleus Paragigantocellularis**

Serotonergic cells within the nPGi send direct descending projections to the DM and DL (Azmitia and Gannon, 1986; Marson and McKenna, 1992). The human homologue, the nucleus paragigantocellularis lateralis, also contains serotonergic cells (Azmitia and Gannon, 1986). Application of 5-HT to the spinal targets of the nPGi blocks the urethro-genital reflex (an artificial measure of genital reflexes), an effect reversed by application of a 5-HT antagonist (Marson and McKenna, 1992). In addition, the SSRI dapoxetine reduces DM motoneuron activity, and this effect is reversed by lesions of the nPGi (Clement

et al., 2007). These studies make it clear that 5-HT acts at the level of the spinal cord to inhibit genital reflexes, and that 5-HT from the nPGi is a necessary antecedent for normal inhibition of genital reflexes. It is unclear, however, whether 5-HT may be acting at the level of the nPGi itself.

The nPGi contains receptors for 5-HT including the 5-HT<sub>1A</sub> (Thor et al., 1990; 1992), 5-HT<sub>1c</sub> (Hoffman and Mezey, 1989), 5-HT<sub>2A</sub> (Fay and Kubin, 2000; Fonseca et al., 2001), 5-HT<sub>2C</sub> (Fonseca et al., 2001), and 5-HT<sub>3</sub> (Fonseca et al., 2001) subtypes, although only the 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> subtypes are found in abundance (Fonseca et al., 2001; Thor et al., 1990). The ventrolateral periaqueductal gray (vlPAG) is the primary source of 5-HT to the nPGi (Li et al., 2001; Lu et al., 2010; Underwood et al., 1999; Zeng et al., 1991). To date, the effect of 5-HT neurotransmission at the level of the nPGi has not been studied.

### **1.3 Implications for Human Health**

Sexual dysfunction is a common problem in both men and women. Up to 31% of men and 43% of women will experience sexual dysfunction in their lifetime (Laumann et al., 1999). Such dysfunctions can include loss of sex drive (libido), premature ejaculation, delayed ejaculation, anorgasmia, involuntary vaginal spasms, and dyspareunia (vaginal pain during intercourse; (Breiner, 2004). These dysfunctions have a large impact on fertility and quality of life experiences (Cameron and Tomlin, 2007). More alarmingly, SSRIs used to treat depression and anxiety in people have well-documented sexual dysfunction side effects that include decreased libido, delayed ejaculation, and anorgasmia (Kennedy and Rizvi, 2009; Schweitzer et al., 2009). These sexual dysfunctions all have an underlying dysregulation of genital reflexes in common, with 5-HT a key mediator. By identifying the regions of the brain that regulate nPGi function in males and females, and the role of 5-HT within the nPGi, we can provide new insights and treatment targets for people with sexual dysfunction.

## **1.4 Experimental Rationale**

Our experiments were designed to address three general gaps in knowledge of how the nPGi provides descending inhibition of genital reflexes. First, anatomical tract tracing was used to provide a complete description of brain regions that project to the nPGi in males and females (Experiment 1). Second, excitotoxic lesions were used to delineate the role of the nPGi in male and female sexual behavior (Experiment 2). The final series of experiments examined the role of 5-HT drive from the vIPAG to the nPGi in male sexual behavior using 5-HT specific lesions of the vIPAG (Experiment 3). Together these experiments will provide detailed analysis of nPGi afferents, provide information as to how the nPGi affects sexual behavior in males and females, and determine whether serotonergic drive to the nPGi is important in sexual behavior.

### **1.4.1 Experiment 1: Nucleus paragigantocellularis afferents in male and female rats: organization, gonadal steroid receptor expression, and activation during sexual behavior**

Key to understanding how genital reflexes are regulated, and how this might be disrupted in sexual dysfunction, is knowledge of what regions of the brain can influence nPGi activity. In Experiment 1, we used anatomical tract tracing to delineate the afferents projections to the nPGi in male and female rats. As gonadal steroids are key modulators of sexual behavior (van Dis and van de Poll, 1974; Wrobel and Karasek, 2008), we also examined whether nPGi afferents contained receptors for estrogens and androgens. We also examined whether sites providing afferent drive to the nPGi were active during sexual behavior, using the protein Fos (as a marker of neural activity).

### **1.4.2 Experiment 2: Excitotoxic lesions of the nucleus paragigantocellularis facilitate male sexual behavior but attenuate female sexual behavior in rats**

Previous studies using lesions to examine the role of the nPGi in male sexual behavior used electrolytic (fiber destroying) lesions. Therefore, it is not clear if nPGi neurons and not fibers-of-passage affect genital reflexes. In addition, the role of the nPGi in female sexual behavior is unknown, although

studies have pointed to the likelihood of its importance to female sexual behavior through anatomical tract tracing (Lee and Erskine, 2000; Marson and Murphy, 2006). In Experiment 2, we used excitotoxic lesions of the nPGi in male and female rats to examine the role of nPGi neurons in male and female sexual behavior.

#### **1.4.3 Experiment 3: Serotonergic lesions of the midbrain source of serotonin to the nucleus paragigantocellularis facilitate sexual behavior in male rats**

The nPGi contains receptors for 5-HT (Fonseca et al., 2001; Thor et al., 1990), and serotonergic input to the nPGi is provided by the vIPAG (Li et al., 2001; Lu et al., 2010; Underwood et al., 1999; Zeng et al., 1991). However, the role of 5-HT drive to the nPGi in male sexual behavior is unknown. To this end, in Experiment 3, we produced 5-HT-specific lesions of the vIPAG to the nPGi in male rats and examined its impact on male sexual behavior.

## **2 NUCLEUS PARAGIGANTOCELLULARIS AFFERENTS IN MALE AND FEMALE RATS: ORGANIZATION, GONADAL STEROID RECEPTOR EXPRESSION, AND ACTIVATION DURING SEXUAL BEHAVIOR**

### **2.1 Introduction**

Genital reflexes in males and females subserve critical functions in reproductive biology. Despite this obvious importance, little is known about the supraspinal control of these reflexes. Dysfunctions within these systems contribute to, or are the basis of, sexual dysfunctions that produce profound disruptions not only in fertility, but also in quality of life experiences (Cameron and Tomlin, 2007; Laumann et al., 1999). Across the lifespan, approximately 31% percent of men will experience sexual dysfunction, including an inability to achieve an erection and/or premature or delayed ejaculation (Laumann et al., 1999). Similarly, 43% of women will experience some form of female sexual dysfunction, including involuntary vaginal spasms, painful sensations during penetration, and/or early or delayed orgasm (Laumann et al., 1999). Our understanding of the supraspinal control of genital reflexes is an important contribution to understanding and treating sexual disorders.

Studies in male rats have identified the nucleus paragigantocellularis (nPGi) of the brainstem as the primary source of tonic descending inhibition of erectile and ejaculatory reflexes. Projections from the nPGi terminate onto the spinal motor neurons that innervate the bulbospongiosus and ischiocavernosus muscles (Hermann et al., 2003; Murphy and Marson, 2000; Tang et al., 1999); these muscles are critical to the control of erection and ejaculation (Holmes et al., 1991; McKenna and Nadelhaft, 1986). Bilateral lesions of the nPGi in male rats decrease mount and intromission frequency, ejaculation latency, and increase the number of ejaculations to satiety (Yells et al., 1992; Yells et al., 1994). Lesions of the nPGi also decrease the latency, and increase the number of ex copula erections (Marson et al., 1992). By contrast, electrical stimulation of nPGi neurons results in an increased latency, and decreased

amplitude of firing in motor neurons associated with genital reflexes (Johnson and Hubscher, 1998). Interestingly, nPGi lesions do not alter the number of non-contact erections in males exposed to females behind a wire mesh screen (Liu and Sachs, 1999), suggesting that nPGi modulation of penile reflexes is context dependent. How this context is signaled to the nPGi remains to be elucidated.

The central regulation of the nPGi is poorly understood. In males rats, both the medial preoptic area (Murphy et al., 1999a) and the midbrain periaqueductal gray (Lovick, 1986; Murphy and Hoffman, 2001) send direct projections to the nPGi. Interestingly, MPO projections to the PAG terminate in close apposition to PAG projections to the nPGi, forming an indirect MPO-PAG-nPGi pathway in addition to the direct MPO-nPGi pathway (Murphy and Hoffman, 2001). In females, remarkably little is known regarding the anatomy and physiology of the nPGi. Spinally-projecting nPGi efferents terminate among the motor neurons involved in the urethrogenital reflex (Marson et al., 2003), and trans-synaptic retrograde tracer injection into rat clitoris (Marson, 1995), cervix (Lee and Erskine, 2000), and vagina/clitoris (Marson and Murphy, 2006) results in dense retrograde labeling in the nPGi. Similar to males, direct MPO projections to the nPGi of female rats have been reported (Marson and Foley, 2004) and nPGi cells retrogradely labeled from rat vagina/clitoris with a trans-neuronal tracer were associated with terminals from the MPO and PAG (Marson and Murphy, 2006). Contributions of other central sites of input to the nPGi in females remain to be described.

The nPGi can be conceptualized as the final common output from supraspinal sites involved in sexual behavior to the spinal cord motor neurons controlling genital reflexes. An integration of appropriate external and internal signals related to sexual behavior must occur to determine when it is appropriate for genital reflexes to be produced. The nPGi may be one such area where an integration of signals occurs. This study was conducted to comprehensively characterize nPGi afferents as to their source, expression of gonadal steroid receptors, and activation during sexual behavior in male and fe-

male rats. Possible sex-differences in the anatomical and/or physiological organization of this circuit were also examined.

## **2.2 Materials and Methods**

### **2.2.1 Subjects**

Adult male (n=56) and female (n=88) Sprague-Dawley rats (Zivic Laboratories Inc., Pittsburgh, PA) were used in these experiments. Animals were weight-matched (250-300 g) and housed in same-sex pairs in separate rooms on a 12:12 hour light:dark cycle (lights on at 7:00AM). Animals used in the sexual behavior studies were housed in reverse light cycle (lights off at 7:00AM). Access to food and water was ad libitum throughout the experiment, except during surgery. These studies were performed in strict compliance with the Institutional Animal Care and Use Committee at Georgia State University. All efforts were made to minimize any possible suffering by the animal, and to reduce the number of animals used.

### **2.2.2 Fluorogold injections**

Rats were anesthetized with ketamine/xylazine/acepromazine (50 mg/kg, 3.3 mg/kg, 3.3 mg/kg; i.p.; Henry Schein, Melville, NY) and placed in a stereotaxic frame upon achieving a deep surgical plane of anesthesia. The skull was adjusted so that Bregma and lambda were at the same dorsoventral plane. Glass micropipettes (10-20  $\mu$ m) were filled with the retrograde tracer Fluorogold (FG; 2% soln. w/v in saline; Fluorochrome LLC, Denver, CO) and lowered into the nPGi at the following coordinates (in mm): AP: -2.0 Lambda; ML: -1.0; DV: -8.5. FG was iontophoresed (50% duty cycle, 7.5  $\mu$ A current) for 20 min. Pipettes remained in place for five minutes after injection to prevent backflow of tracer along the pipette tract and to facilitate neuronal uptake. Special care was taken to ensure that all injection protocols were comparable for males and females. Following surgery, the animals were allowed to recover under a heat lamp in clean cages, and returned to their original housing facilities upon recovery from the anesthetic. Animals were sacrificed 10 days after FG injections.

### **2.2.3 Sexual behavior**

A subset of our FG injected intact males (n=44) and females (n=36) rats engaged in three one hour mating bouts with stimulus animals before injection of FG. All females were ovariectomized and hormone-primed (10 µg estradiol in sesame oil 48 hours before mating, 500 µg progesterone in sesame oil) 4 hours before mating. Mating took place in a 60 cm x 30 cm transparent acrylic arena. Experimental males had free access to stimulus females, whereas experimental females engaged in paced-mating with a separator in the arena (with two 4 cm diameter holes through which only females could fit). Following retrograde tracer injections, the rats engaged in three additional one hour mating bouts, and were sacrificed on the last mating bout one hour after producing or receiving (male and females respectively) an ejaculation.

### **2.2.4 Perfusion fixation**

Animals were given a euthanizing dose of sodium pentobarbital (160 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO) and perfused transcardially with 100-200 ml of 0.9% sodium chloride containing 2% sodium nitrite as a vasodilator, followed immediately by 250 ml of 4% paraformaldehyde in 0.1M phosphate buffer containing 2.5% acrolein (Polysciences Inc., Warrington, PA). Following fixation, a final rinse (100-200 ml) with the sodium chloride/sodium nitrate solution was used to remove any residual acrolein from the animal. Immediately following perfusion, the brains were removed and stored at 4°C in 30% sucrose solution until sectioned. The brains were cut into 25 µm coronal sections with a Leica 2000R freezing microtome and stored free-floating in cryoprotectant-antifreeze solution (Watson et al., 1986) at -20°C until immunocytochemical processing was initiated.

### **2.2.5 Immunocytochemistry**

A 1:6 series through the rostrocaudal axis of each brain was processed for FG and estrogen receptor alpha (ER $\alpha$ ), FG and androgen receptor (AR), or FG and the immediate-early gene product Fos as previously described (Loyd and Murphy, 2006; Murphy and Hoffman, 2001). Briefly, sections were re-



moved from the cryoprotectant-antifreeze solution, rinsed extensively in potassium phosphate-buffered saline (pH 7.4), and then reacted for 20 minutes in 1% sodium borohydride to remove excess aldehydes. Sections were then incubated in primary antibody solution directed against either ER $\alpha$ , AR, or Fos in potassium phosphate buffered saline (KPBS) containing 0.1% Triton-X for 1 hour at room temperature followed by 48 hours at 4°C. Cells containing ER $\alpha$  were identified using a polyclonal rabbit anti-ER $\alpha$  antibody (Santa Cruz Biotech Inc., sc-542, Lot: B1505, Santa Cruz, CA) at a concentration of 1:20,000. This rabbit antiserum was prepared against a peptide mapping at the C-terminus of ER $\alpha$  of mouse origin (HSLQTYYPPEAEGFPNTI) corresponding to amino acids 580-599 (manufacturer's technical information), and specificity of this antibody has been confirmed by preabsorbtion with epitope peptide (Quesada et al., 2007). Cells containing AR were identified using the polyclonal rabbit anti-AR antibody (Santa Cruz Biotech Inc., sc-816, Lot: B0204, Santa Cruz, CA) at a concentration of 1:10,000. This rabbit antiserum was prepared against a peptide mapping at the N-terminus of AR of human origin (MEVQLGLGRVYPRPPSKTYRG) corresponding to amino acids 2-21 (manufacturer's technical information), and specificity of this antibody has been confirmed by preabsorbtion with epitope peptide (Creutz and Kritzer, 2004). Cells containing Fos were identified using the polyclonal rabbit anti-Fos antibody (Calbiochem, PC38, Lot: 4191, San Diego, CA) at a concentration of 1:700,000. This rabbit antiserum was prepared against a synthetic peptide (SGFNADYEASSSRC) corresponding to amino acids 4-17 of human c-Fos, and specificity of this antibody has been confirmed by preabsorbtion with epitope peptide (Sun et al., 2007). In Western blots, this antibody recognizes the ~55 kDa c-Fos and ~62 kDa c-Fos proteins, and does not cross-react with the ~39 kDa Jun protein (manufacturer's technical information).

After primary antibody incubation, the tissue was rinsed in KPBS, incubated for 1 hour in biotinylated goat anti-rabbit IgG (Jackson ImmunoResearch Labs Inc., West Grove, PA) at a concentration of 1:600, rinsed in KPBS, followed by a 1 hour incubation in avidin-biotin peroxidase complex (Vector Labs, ABC Elite Kit PK-6100, Burlingame, CA) at a concentration of 1:10. After rinsing in KPBS and sodium ace-

tate (0.175 M; pH 6.5), ER $\alpha$ , AR, and Fos were visualized as a black reaction product using nickel sulfate intensified 3,3'-diaminobenzidine solution containing 0.08% hydrogen peroxide in sodium acetate buffer. The reaction product was terminated after approximately 30 minutes by rinsing in sodium acetate buffer. Tissue was then processed for FG immunoreactivity as above. Cells containing FG were identified using the polyclonal rabbit anti-Fluorogold antibody (Chemicon, AB153, Lot: 25060005, Temecula, CA) at a concentration of 1:30,000. This antibody was raised against the chemical compound hydroxys-tildamidine (Fluorogold; manufacturer's technical information). No cytoplasmic FG+ staining was present in animals in which the tracer failed to be ejected from the electrode. FG was visualized as a brown reaction product using 3,3'-diaminobenzidine containing 0.08% hydrogen peroxide in Tris buffer (pH 7.2). The reaction product was terminated after approximately 30 minutes by rinsing in Tris buffer. Sections were mounted out of saline onto gelatin-subbed slides, air dried overnight, dehydrated in a series of graded alcohols, cleared in Histoclear, and cover-slipped using Histomount.

## **2.2.6 Data analysis and presentation**

A single individual conducted all analyses. Animals were coded to blind the counter to sex and animal number. Injection sites were subjectively graded with respect to their location and spread. Only those animals with injections that corresponded to a focal point within the nPGi were selected for cell counting (see Figure 1). Thirteen males and 15 females had injections that met our strict and conservative criteria. Of these animals, all were used for our retrograde tracing analysis, 6 males and 7 females were used in our retrograde tracer/gonadal-steroid receptor expression analysis, and 5 males and 6 females were used in our retrograde tracer/sexual behavior-induced Fos expression analysis.

Missed injections dorsal or lateral to the nPGi (i.e. into the pyramidal tract) were examined for specificity of retrograde tracing in target injections. All regions containing FG labeling were examined. The MPO and PAG were additionally subdivided into four (Bregma -0.26, -0.30, -0.40, -0.80) and six (Bregma -5.30, -6.04, -6.80, -7.64, -8.30, -8.80) rostrocaudal levels, respectively. Counts for FG+,

ER $_{\alpha}$ /FG+, AR/FG+, or Fos/FG+ cells were determined on the side ipsilateral to the injection site, and FG+ labeling on the side contralateral to the injection site were noted, but not counted. FG+ cells were easily identifiable based on the brown reaction product in the cytoplasm. FG+ cells that contained ER $_{\alpha}$ , AR, or Fos were readily identifiable based on the black reaction product restricted to the nucleus. The minimum distance between any two sections analyzed was 40  $\mu$ m (Bregma -0.26 and -0.30). The majority of retrograde labeling in this area was observed in the MPO, where the maximum diameter of the largest cells can be expected to be approximately 12  $\mu$ m (calculated from (Madeira et al., 1999)); therefore cell counts between these sections (and any two sections) are independent and do not reflect duplicate counting of cells.

Single and dual-labeled cells were plotted and quantified across the rostrocaudal axis of the rat brain based on the atlas of (Paxinos and Watson, 1997). The mean and standard error of the mean (SEM) of FG+, ER $_{\alpha}$ /FG+, AR/FG+, and Fos/FG+ cells for each sex, section, and region of interest were calculated. We use the term “density” within this manuscript as shorthand for the number or proportion of cells observed within a given brain region. The relative density of FG+ labeled cells was defined as sparse when <25 labeled cells were observed and dense when  $\geq$ 25 cells were observed. The relative density of the percent co-localization of FG+ cells with ER $_{\alpha}$ , AR, or Fos was defined as low when <10%, moderate when between 10% and 24%, and high when >25%.

Behavioral measures for our sexual behavior-induced Fos experiments include the mean number of mounts (M), intromissions (I), and ejaculations (E) in males, and the mean number of M/I/E received, lordosis quotient (LQ; lordosis/M+I+E received), and lordosis rating (LR; magnitude of dorsoflexion; 0 = none, 1 = slight, 2 = full) as defined in (Pfaus, 1996).

Statistical comparisons of FG+, ER $_{\alpha}$ /FG+, AR/FG+, and Fos/FG+ cell numbers were made between males and females for regions providing dense input to the nPGi. T-tests were used for mean comparisons and differences were considered significant at the alpha  $\leq$ 0.05 level for planned comparisons of the

MPO and PAG. The family-wise alpha level ( $\leq 0.05$ ) was adjusted to  $\leq 0.0031$  using the Bonferroni method for the sixteen unplanned comparisons.

For data presentation, a representative animal from each experimental group was selected and the distribution of FG+, ER $_{\alpha}$ /FG+, AR/FG+, and Fos/FG+ cells were plotted using a Nikon Drawing Tube attached to a Nikon Optiphot microscope. Plots were scanned, imported to a computer, and finalized using Adobe Illustrator 10. Photomicrographs were generated using a Synsys digital camera attached to a Nikon Eclipse E800 microscope. Images were captured with QCapture and finalized using Adobe Photoshop 7.0. Alterations to the images were strictly limited to enhancement of brightness/contrast.

## **2.3 Results**

### **2.3.1 Injection sites**

Injections were centered within the rostral portion of the nPGi with moderate spread to the pyramidal tract. Subjective blind ratings of injection quality did not differ between the sexes. Figure 1 shows a representative FG injection site within the nPGi of a male and female rat.

### **2.3.2 General patterns in labeling**

Table 1 lists all regions that contained FG+ cells in male and female rats, weighted by density of FG+ cells and co-localization with ER $_{\alpha}$ , AR, or Fos. Regions ipsilateral to the injection site showed a qualitatively higher degree of retrograde labeling than contralateral regions. There were a few notable exceptions: the rostral parvocellular red nucleus (RPC) and rostral portion of the ventral nucleus of the lateral lemniscus in males, and the caudal portion of the RPC and the paralemniscal nucleus (PL) in females had a qualitatively higher degree of retrograde labeling on the side contralateral to the injection site.

### **2.3.3 Organization and gonadal steroid receptor expression of nPGi afferents**

Diencephalon. Representative plots of FG+, ER $_{\alpha}$ /FG+, and AR/FG+ cells in the diencephalic regions are shown in Figure 2. In both males and females, sparse FG labeling was observed in the rostral

MPO (Figure 2A-B). However, within the caudal half of MPO (Figure 2C-D), the number of FG+ cells increased significantly in males [ $p=0.014$ ; Figure 3A]. FG labeling in the caudal MPO in males formed a discrete cell group that was not consistently observed in females (Figure 2D). The proportion of FG+ cells that contained  $ER_{\alpha}$  varied for males and females along the rostrocaudal axis of MPO (Figure 3B). In the rostral pole of MPO, 33% of FG+ cells in females also contained  $ER_{\alpha}$ , whereas only 9% of FG+ cells contained  $ER_{\alpha}$  in males (Figure 3B). For the androgen receptor, at most rostrocaudal levels, males generally had a higher percent co-localization than females (mean 37% AR/FG+ cells versus 18% in females), and males differed from females significantly at two rostrocaudal levels [Bregma -0.26,  $p=0.024$  and Bregma -0.40,  $p=0.037$ ; Figure 3C]. The lateral preoptic area was sparsely labeled for FG in both sexes.

In the bed nucleus of the stria terminalis (BNST; Figure 2A-D), retrograde labeling was primarily restricted to the caudal regions (Figure 2C-D) in both males and females (Figure 4A).  $ER_{\alpha}$  and AR co-localization with FG+ caudal BNST cells was comparable in both sexes (approximately 30%; Figure 4B). With respect to the androgen receptor, 43% of FG+ caudal BNST cells contained AR in males, and 26% co-localized in females (Figure 4C).

Within the amygdala (Figure 2E-F), the medial amygdala exhibited sparse FG+ labeling in both sexes. In contrast, dense FG+ labeling was present in the central amygdala (CeM) in males, with less labeling observed in females (Figure 4A).  $ER_{\alpha}$  co-localization with FG+ CeM cells was low in males (9%), and moderate in females (17%; Figure 4B). For AR, higher levels of co-localization in the CeM was observed in both sexes (between 40-50%; Figure 4C).

Dense FG+ labeling in the hypothalamus (Figure 2E-F) was observed in the paraventricular nucleus (PVN) and posterior hypothalamus (PH) of both sexes, and the perifornical nucleus (PeF) in females (Figure 4A). Figure 5 shows an example of FG labeled cells in the PVN for males and females. These regions exhibited low levels of  $ER_{\alpha}$  (Figure 4B) or AR (Figure 4C and 5) co-localization, with the exception of the PeF in males where 23% of FG+ cells contained AR (Figure 4C). Sparse FG+ labeling was present in

the anterior hypothalamus, lateral hypothalamus, retrochiasmatic area, dorsomedial hypothalamus, ventromedial hypothalamus, arcuate nucleus, tuber cinereum area, and the magnocellular nucleus of the lateral hypothalamus in both sexes.

In the thalamus (Figure 2E-G) dense FG+ labeling was observed in the precommissural nucleus (PrC) in both sexes and the parafascicular thalamic nucleus (PF) in females (Figure 4A). ER $\alpha$  co-localization with FG+ PrC cells was moderate in males (10%) and low in females (3%; Figure 4B). By contrast, AR co-localization with FG+ PrC cells was higher (males, 38%; females, 11%; Figure 4C). In the PF, while ER $\alpha$  was present, there were no ER $\alpha$ /FG+ cells observed. However, AR/FG+ cells were observed in males (28%) and females (12%; Figure 4C) in this same region. Sparse FG+ labeling was observed in the rostral interstitial nucleus of the medial longitudinal fasciculus, prerubral field, parvocellular subparafascicular nucleus, subincertal nucleus, and zona incerta in both sexes.

Mesencephalon. Representative plots of FG+, ER $\alpha$ /FG+, and AR/FG+ labeling in the mesencephalic regions are shown in Figure 2. In the midbrain (Figure 2G-M), dense FG+ labeling was observed in the deep mesencephalic nucleus (DpMe) and intercollicular nucleus (InCo) in both sexes (Figure 4A), with low to moderate levels of ER $\alpha$  co-localization (Figure 4B). A greater percentage of AR/FG+ cells were observed in males within the DpMe (23% versus 5% in females (Figure 4C). Sparse FG+ labeling was observed in the oculomotor nucleus, Edinger-Wetsphal nucleus, nucleus of the posterior commissure, magnocellular nucleus of the posterior commissure, interstitial nucleus of the medial longitudinal fasciculus, RPC, retrorubral field, posterior intralaminar thalamic nucleus, peripeduncular nucleus, lateral substantia nigra, reticular substantia nigra, parabrachial pigmented nucleus, dorsal substantia nigra compacta, nucleus of the optic tract, posterior limitans thalamic nucleus, subbrachial nucleus, and the ventral tegmental area (VTA).

In both sexes, dense FG+ labeling was observed throughout the rostrocaudal axis of the PAG (Figure 2H-M). In the dorsomedial region (dmPAG), females consistently had a greater number of FG+

cells at all rostrocaudal levels [Bregma -5.30,  $p=0.009$ , Bregma -6.80,  $p=0.009$ , Bregma -7.64,  $p=0.001$  and Bregma -8.30,  $p=0.029$ ; Figure 6A]. Similar sex differences (females > males) were observed in the lateral [IPAG; Bregma -8.30,  $p=0.022$ ] and ventrolateral regions of the PAG (Figure 6A). For the dmPAG and IPAG, the percentage of FG+ cells that also contained ER $\alpha$  increased moving caudally through the PAG in both males and females. Interestingly, while females had a greater number of FG+ cells throughout the rostrocaudal axis of PAG, co-localization with ER $\alpha$  or AR was generally higher in males (Figure 6B and 6C). Sparse FG+ labeling was observed in the dorsolateral PAG.

In the superior colliculus (Figure 2H-K) dense FG+ labeling was observed in the deep gray layer (DpG) in both sexes, and the rostral superior colliculus (rSC) in females (Figure 4A). Females had a greater number of FG+ cells in both the DpG and rSC. Little ER $\alpha$  expression is present within the superior colliculus, and consequently steroid receptor co-localization with superior collicular FG+ cells was low or absent (Figure 4B). Despite the low amount of AR present in this region, AR/FG+ co-localization was higher in males than females in the DpG and rSC (Figure 4C). Sparse FG+ labeling was observed in the optic nerve layer of the superior colliculus and intermediate gray layer of the superior colliculus.

Dense FG+ labeling was present in the inferior colliculus (Figure 2H-M) of both sexes. In the external cortex of the inferior colliculus (ECIC) the number of FG+ cells was significantly greater in females [ $p=0.002$ ; Figure 4A]. Little ER $\alpha$  and AR expression is present within the inferior colliculus in either sex, and consequently low levels ER $\alpha$  or AR co-localization with FG+ cells in the ECIC were observed in both sexes (Figures 4B and 4C). Sparse labeling was observed in the brachium of the inferior colliculus in both sexes.

*Metencephalon.* Representative plots of FG+, ER $\alpha$ /FG+, and AR/FG+ labeling in the metencephalic regions are shown in Figure 2. Dense pontine (Figure 2K-M) FG+ labeling was observed in the region of the motor root of the trigeminal nerve, possibly encompassing a caudal portion of the PL, in both sexes (Figure 4A). Little ER $\alpha$  and AR expression is present within the pons, and consequently stero-

id receptor co-localization with pontine FG+ cells was low or absent (Figures 4B and 4C). Sparse FG+ labeling was observed in the retrorubral nucleus, pedunclopontine tegmental nucleus, cuneiform nucleus, dorsal tegmental bundle, parabrachial nucleus, laterodorsal tegmental nucleus, oral pontine reticular nucleus, motor trigeminal nucleus, ventrolateral tegmental area, and the olivary nuclei.

*Myelencephalon.* Representative plots of FG+, ER $\alpha$ /FG+, and AR/FG+ labeling in the myelencephalic regions are shown in Figure 2. Dense medullary (Figure 2N-O) FG+ labeling was observed in the lateral reticular nucleus in both sexes and interpolar spinal trigeminal nucleus in females (Figure 4A). Little ER $\alpha$  and AR expression is present within the myelencephalon and consequently steroid receptor co-localization with medullary FG+ cells was absent (Figure 4B and 4C). Sparse FG+ labeling was observed in the locus coeruleus, nucleus of the solitary tract, cuneate nucleus, intermediate reticular nucleus, and the parvocellular reticular nucleus.

### **2.3.4 Activation of nPGi afferents during sexual behavior**

Several sexual behavior measures were quantified for the last mating bout (Table 2). These values are consistent with previous studies examining male and female sexual behavior (Pfaus, 1996).

*Diencephalon.* Representative plots of Fos/FG+ labeling in the diencephalic regions are shown in Figure 7. In the preoptic area (Figure 7A-D), the proportion of sex-induced Fos present in FG+ cells in males and females differed along the rostrocaudal axis. In the rostral MPO, females had a higher percentage of co-localization than males (24% versus 13%, respectively; Fig 8). By contrast, in the caudal MPO males had a higher percentage of Fos/FG+ cells than females (Figure 8). At mid levels of the MPO, 43% of FG+ cells contained Fos in males, in comparison to 20% in females [ $p=0.035$ ]. Significant differences were also noted in the caudal MPO (32% Fos/FG+ cells in males, versus 15% in females [ $p=0.042$ ]). In males, FG+ cells that co-localized with Fos formed a discrete group of cells that was rarely observed in females.



In the BNST (Figure 7A-D), while high levels of Fos was observed in both males and females, very few were present in FG+ cells (Figure 9). Similarly, in the CeM (Figure 7E-F), while moderate amounts of Fos were present in males and females, low levels of co-localization were observed in both sexes (Figure 9).

In the hypothalamus (Figure 7E-F), males and females differed in the proportion of sex-induced Fos in FG+ cells. For example, in the PeF, females had a higher level of co-localization than males (44% versus 18% in males, Figures 9 and 10). Moderate amounts of Fos/FG+ cells were observed in both males and females in the PVN (Figures 9 and 11) and PH (Figure 9).

In the thalamus (Figure 7E-G) moderate levels of Fos/FG+ co-localization were present in the PrC for males and females. In the PF, females had a greater percentage of Fos/FG+ co-localization than males (16% versus 2%; Figure 9).

Mesencephalon. Representative plots of Fos/FG+ labeling in the mesencephalic regions are shown in Figure 7. In the midbrain (Figure 7G-M), moderate levels of Fos/FG co-localization were observed in the DpMe and InCo in both sexes (Figure 9). Interestingly, while the PAG (Figure 7H-M) contained high levels of both Fos and FG, low levels of co-localization (on average 13%) were observed in both males and females (Figure 12). This was true even though females had significantly higher levels of retrogradely labeled cells (Figure 6A).

High levels of Fos were only present in the outermost layers of the superior and inferior colliculus of both sexes, where retrograde labeling was not observed. Consequently, low to moderate levels of Fos/FG co-localization were observed in the superior and inferior colliculus (Figure 7H-M).

Metencephalon and Myelencephalon. Representative plots of Fos/FG+ labeling in the metencephalic regions are shown in Figure 7K-M and for the myelencephalon in Figure 7N-O. Overall, sex-induced Fos was generally low or absent in both sexes.

## 2.4 Discussion

In this study, we characterized nPGi afferents as to their location, gonadal steroid receptor expression, and activation during sexual behavior. The general anatomical organization of nPGi afferents was similar between the sexes in that there were no regions projecting to the nPGi in only one sex. However, qualitative and quantitative sex differences were observed within specific subregions of the brain (see Figure 13). ER $\alpha$  expression in nPGi afferents was highly variable between the sexes with no consistent pattern overall. Conversely, AR expression in nPGi afferents was almost exclusively higher in males than females. Activation of nPGi afferents during sex was observed throughout the brain in almost all regions that provide dense input to the nPGi, and subregion-specific sex differences in nPGi afferent activation during sex were observed. Sex differences in FG co-localization with ER $\alpha$ , AR, or sex-induced Fos may be reflective of sex differences in basal expression of these proteins in the regions examined. Nevertheless, any sex differences noted in cells projecting to the nPGi have implications for how males and females may sex-specifically regulate genital reflexes. Those regions known to be important to sexual behavior, or that exhibited large sex differences in organization or activity, are discussed below.

### 2.4.1 Preoptic area

Our analysis revealed that male rats have significantly more nPGi afferents from the caudal MPO than females, and that these projections contain significantly more AR and sex-induced Fos than females. We also observed that females had more nPGi afferents from the rostral MPO than males, and these projections contained more ER $\alpha$  and sex-induced Fos.

The rostrocaudal difference between the sexes in the relative number of nPGi afferents from the MPO, their gonadal steroid receptor expression, and their activation during sex may represent a fundamental principle in the control of sexual behavior, at least with regards to genital reflexes. Other studies have underscored the importance of this rostrocaudal MPO distinction with regard to sexual

behavior. In male rats, lesions restricted to the caudal MPO disrupt sexual behavior (Van De Poll and Van Dis, 1979), while lesions restricted to the rostral MPO do not effect non-contact erections (a genital reflex) although other aspects of sexual behavior (including ejaculation) are impaired (Liu et al., 1997b). In female rats, Fos immunoreactivity by mounts or intromissions alone is induced in ER $\alpha$  expressing rostral, but not caudal preoptic cells (Greco et al., 2003). Our findings are in agreement with this work where nPGi afferents from the rostral MPO have a female-bias in number, ER $\alpha$  expression, and sex-induced Fos. By contrast, nPGi afferents from the caudal MPO have a male-bias in number, AR expression, and sex-induced Fos.

A subregion of the caudal MPO in male rats, in the dorsolateral portion of the MPO, near the striohypothalamic nucleus may be critical to the control of genital reflexes in males, but not females, as retrograde labeling in this group of cells is consistently observed in males but not females. Sex-induced Fos was co-localized to a high degree in this region in males. The striohypothalamic nucleus is known to receive projections from the amygdala (Perez-Clausell et al., 1989), and the retrograde labeling we observed may be part of this complex. It is conceivable that projections from the striohypothalamic nucleus inhibit nPGi function upon activation of relevant pheromonal signals processed by the amygdala. Support for this idea comes from work in hamsters where the magnocellular preoptic nucleus, which may be a homologue to the region described here, has been described as part of a BNST-amygdala network that transduces pheromonal signals to sexual behavior motor output (Swann et al., 2003; Wood and Newman, 1995). Furthermore, in male rats, MPO lesions that also encompassed this region produce deficits in the percent of males that mounted, intromitted, and ejaculated, during a sexual behavior test (Liu et al., 1997b). Work in quail also supports the importance of this subregion of the MPO in male sexual behavior. Although a clear homologue to the quail medial preoptic nucleus (POM) has not been established, the behavioral effects and anatomical connectivity (Balthazart and Ball, 2007; Carere et al., 2007) are similar to the region we describe here. In fact, the dorsolateral portion of the POM ap-

pears to be critical for the consummatory aspects of male copulatory behavior in quail (Balthazart et al., 1998). Taken together, the evidence is mounting that this subregion of the MPO is the critical component to male consummatory sexual behavior across species.

In males, the MPO can also affect the nPGi through a relay in the PAG (Murphy et al., 1999a). It is not clear how the projections from the MPO to the nPGi and the MPO to PAG to nPGi work together. We found that MPO efferents to the nPGi were active during sexual behavior, but that PAG afferents to the nPGi were not, despite the large contribution of this region in input to the nPGi. It is possible that the MPO has a pro-sexual effect by inhibiting the nPGi directly, and also by inhibiting PAG cells that normally enhance nPGi activity.

#### **2.4.2 Amygdala and bed nucleus of the stria terminalis**

We observed a greater number of nPGi afferents from the caudal BNST in males than females. These afferents expressed AR to a higher degree in males than females, but expressed ER $\alpha$  similarly in both sexes, and neither sex expressed sex-induced Fos to a large degree. The BNST of rodents is part of a highly interconnected network with the amygdala and MPO where the contextual relevancy of pheromones is determined (Fiber et al., 1993; Kollack-Walker and Newman, 1997; Wood and Newman, 1995). BNST efferents to the nPGi may signal this relevancy, modulating nPGi activity and thereby providing the nPGi external contextual information (i.e. sexual odor vs. food odor). Although we have shown that the caudal portion of the BNST provides dense input to the nPGi, the majority of these afferents did not express Fos following sexual behavior. These regions may preferentially signal appetitive aspects of sexual behavior to the nPGi, which our sexual behavior model would not have necessarily been able to detect, or alternatively, were inhibited by sexual behavior and therefore would not be expected to show Fos immunoreactivity.

The number of cells projecting from the CeM to the nPGi, their steroid receptor expression, and sex-behavior induced Fos in these cells were similar between the sexes. CeM efferents to the nPGi may

be active in other behavioral contexts. For example, the CeM is known to be involved in the processing of stressful stimuli (McEwen, 2007), and CeM-nPGi projections have previously been hypothesized to regulate behavioral defense responses in cats (Hopkins and Holstege, 1978). It is possible that this region, when active during stressful situations, inhibits genital reflexes by enhancing nPGi activity. Interestingly, the amygdala in men is inhibited during ejaculation, as measured by functional magnetic resonance imaging (Holstege et al., 2003), suggesting that its activity may be part of an inhibitory network. The CeM also appears to be part of a genitosensory network. Recent work in our lab using an antero-grade trans-neuronal tracer injected into the genitals of male and female rats has found dense antero-grade labeling in the central amygdala (Normandin and Murphy, 2007), providing a functional pathway for the modulation of nPGi activity by the CeM through the integration of genitosensory information.

### **2.4.3 Hypothalamus**

The PVN provides dense projections to the nPGi, which show little expression of ER $\alpha$  or AR, and are highly active following sexual activity in both sexes. The robust input to the nPGi from the PVN in, and its associated activity during sexual behavior in, indicates that this region may be critical in both sexes in the control of genital reflexes in both sexes. The PVN has been previously implicated in sexual behavior. Oxytocin fibers from the PVN are found in the spinal cord regions mediating genital reflexes in male rats (Tang et al., 1998), and there is evidence that the PVN can directly affect motoneuron pools associated with sexual behavior (Perez et al., 2005; Wagner and Clemens, 1991). PVN efferents to the nPGi may inhibit nPGi activity to reduce the overall inhibitory tone to genital reflexes, while at the same time producing direct excitation of spinal motor neurons. Indeed, PVN activity has been associated with both erectile and ejaculatory behavior in male rats (Chen et al., 1997), and both males and females show an increase in Fos immunoreactivity in the PVN following mating (Flanagan et al., 1993; Rowe and Erskine, 1993; Witt and Insel, 1994).

The PeF projections to the nPGi were highly active in females but not males, indicating that this region may be important for sex-specific the control of genital reflexes. This neuropeptide rich region, containing orexin, dynorphin, and neuropeptide W (Nambu et al., 1999; Niimi and Murao, 2005; Zardetto-Smith et al., 1988) has previously been associated with feeding behavior (Sweet et al., 1999), as well as arousal (Suntsova et al., 2007; Uschakov et al., 2006) and suggests that this nucleus may be part of a general motivational circuit. Interestingly, the PeF has also been implicated as part of the dopaminergic system of rats, as it projects heavily to dopaminergic neurons of the VTA (Fadel and Deutch, 2002), suggesting a role of the PeF in reward/reinforcement systems. In fact, at least in males, systemic orexin antagonists impair copulation (Muschamp et al., 2007). Furthermore, orexin-A administration increases the firing rate of VTA cells, and tyrosine hydroxylase-positive VTA cells also express mating-induced Fos and are in close apposition to orexinergic fibers (Muschamp et al., 2007). Presumably, PeF activity would also inhibit nPGi function, enabling pro-sexual behavior, and our data suggests that PeF signaling to the nPGi may be more important in females.

#### **2.4.4 Periaqueductal gray**

Throughout the rostrocaudal extent of the PAG, there were significantly more retrogradely labeled cells in females in comparison to males. Co-localization with ER $\alpha$  was comparable between the sexes while AR expression in these cells was greater in males than females at all levels examined. Sex differences in PAG output to the brainstem have been previously reported (Loyd and Murphy, 2006), and suggest an overarching principle of PAG-brainstem organization whereby females in general have a larger number of output neurons utilized in a specific circuit than males. Given the large population of gonadal steroid receptors localized within the PAG (Murphy et al., 1999b), these observed sex differences could be due to circulating gonadal steroids during a critical period or alternatively, represent plasticity within the female PAG due to changes in gonadal steroid levels across the estrous cycle (Griffiths and Lovick, 2005).

In males, PAG neurons that project to the nPGi are preferentially localized within regions receiving input from the MPO (Murphy and Hoffman, 2001). For example, there are no projections from the MPO to the dorsolateral PAG; similarly, no PAG-nPGi output neurons are located there, suggesting there is an MPO-PAG-nPGi circuit. In addition to receiving input from the MPO, PAG-nPGi output neurons also receive direct input from the lumbosacral spinal cord (unpublished observations), thereby forming a unique spinal-supraspinal-spinal circuit. Interestingly, while sexual behavior induced extensive Fos within the PAG of both males and females, very little Fos was observed in PAG-nPGi output neurons, suggesting that these neurons were not directly excited during sexual behavior. Rather, the finding of little Fos expression in PAG-nPGi output neurons suggests that these neurons may actually have been inhibited during sex; given that nPGi-spinal cord pathway must be disinhibited for genital reflexes to occur, it is intriguing to consider the possibility that PAG output to the nPGi is the source of disinhibition. The PAG receives chemosensory and neuroendocrine input, as well as sensory information pertaining to the exterior environment. Together, this suggests that the PAG may function as the primary 'decision maker' as to whether the intrinsic and extrinsic environment is appropriate for mating to occur. If the conditions are appropriate for mating, inhibitory drive from the PAG to the nPGi is initiated and the tonic inhibition over nPGi-spinal cord is removed. In addition, excitatory drive to the nPGi from the MPO is initiated (reflected in the high percentage of Fos in MPO-nPGi neurons) and mating occurs. Obviously, this is highly speculative and requires additional testing to evaluate these hypotheses.

#### **2.4.5 Superior and inferior colliculus**

Females had a greater number of collicular cells projecting to the nPGi than males; moderate amounts of sex-induced Fos were observed. The superior colliculus is classically described as part of the circuit producing saccades in mammalian systems. However, recent evidence suggests that the superior colliculus may also be described as a source of attentional modulation for goal directed behavior that is independent of specific motor output (Krauzlis et al., 2004). Stimulation of the deep layers of the supe-

rior colliculus, the same region where the majority of afferent contributions to the nPGi are located, results in increased attentiveness in rats (Schenberg et al., 2005). In addition, we found that the PrC of the thalamus projects heavily to the nPGi, and this region is known to be connected to areas governing attention, including the superior colliculus (Canteras and Goto, 1999). The activity of superior collicular/PrC projections to the nPGi may modulate nPGi function based on specific goal directed behaviors, such as mate pursuit and proceptive sexual behaviors.

Like the superior colliculus, females had a greater number of inferior collicular cells projecting to the nPGi than males; moderate amounts of sex-induced Fos were observed. The inferior colliculus is a part of the auditory system (Brozoski et al., 2007; Sun et al., 2007), and inferior collicular input to the nPGi may function to integrate auditory signals into sexual behavior output. Both male and female rats produce ultrasonic vocalizations during sexual behavior (McGinnis and Vakulenko, 2003), and detection of these signals has the potential to modulate sexual behavior in rodents (Floody et al., 1998; Floody and Lisk, 1987; McGinnis and Vakulenko, 2003). The relative difference between male and female rats in inferior collicular projections to the nPGi may indicate a difference in the importance of these ultrasonic vocalizations to the animals behavioral output. Indeed females show higher levels of activity in the mid-brain when listening to simulated mating-like ultrasonic vocalizations (Floody and Lisk, 1987).

#### **2.4.6 The nPGi as a central integrator for arousal**

While we have focused on the role of the nPGi in sexual behavior, the nPGi has been implicated in a variety of seemingly disparate systems including blood pressure (Lovick, 1992), respiration (Saether et al., 1987), nociception (Lanteri-Minet et al., 1994), reinforcement (Fathi-Moghaddam et al., 2006), audition (Kandler and Herbert, 1991) *and* sexual behavior (Yells et al., 1992). Collectively, these behavioral domains all contain an arousal component, and the nPGi may be one brainstem region that integrates a multitude of inputs modulating arousal in these behavioral systems (Van Bockstaele and Aston-Jones, 1995).



Anatomical data support this hypothesized “integrator” role of the nPGi. Descending projections from the nPGi to spinal cell groups outside of those devoted to the somatic component of genital reflexes have been noted, including the intermediolateral cell column and the sacral parasympathetic nucleus (Hermann et al., 2003; Holstege et al., 1979). In addition, the nPGi projects to the LC in rats (Aston-Jones et al., 1986; Luppi et al., 1995), and this nPGi-LC pathway has been implicated in a number of arousal-dependant functions (Chen and Engberg, 1989; Clark and Proudfit, 1991; Clayton and Williams, 2000). The widespread afferents to the nPGi observed in the present study, in conjunction with nPGi output to sympathetic and parasympathetic neurons, and a supraspinal noradrenergic center, are one obvious way the nPGi can be seen as a stimulus integrator for arousal modulation in rats. The nPGi receives extensive input from the PAG, a region known to regulate “fight or flight” responses (Carrive et al., 1987; Misslin, 2003), lending further support to this idea. The regulation of arousal by the nPGi may be sexually dimorphic, at least with respect to the information generated by the PAG, as we observed a greater number of nPGi afferents from the PAG in females.

In addition to these regions classically associated with arousal, the auditory system inputs to the nPGi from the cochlear nucleus and inferior colliculus may represent an integration of important auditory cues for generating changes in arousal as well. The nPGi receives input from the cochlear nucleus (Bellintani-Guardia et al., 1996), an auditory processing center, and we observed significant input from the inferior colliculus in both sexes. Lesions of the inferior colliculus produce an increase in startle responses to an auditory stimuli (Leitner and Cohen, 1985), suggesting a loss of behavioral inhibition that could be mediated by the nPGi. Pontine regions are known to produce inhibition of the startle reflex (Fendt et al., 2001), and we have observed that many of the regions associated with this regulatory role (PPTg, LDTg, SNR) project to the nPGi. As females had a greater number of cells in the inferior colliculus that projected to the nPGi than males, this suggests that the regulation of arousal by auditory stimulation may be sexually dimorphic.

## 2.5 Conclusions

The results of this study indicate that both male and female rats have extensive projections to the nPGi throughout the brain. Some of these nPGi-projecting regions, such as the PAG, superior colliculus, and inferior colliculus, show a significant female-biased sex difference in the relative number of cells projecting to the nPGi. In the MPO, however, we observed a significant male-biased sex-difference in the relative number of cells projecting to the nPGi. Throughout the brain nPGi afferents contained receptors for the gonadal steroids, suggesting that changes in hormone levels during sexual behavior, or across the estrous cycle, may modulate the excitability of nPGi afferents. ER $\alpha$  is expressed in many nPGi afferents in both sexes almost equally with few exceptions. AR is also expressed in nPGi afferents of both sexes, with a clear male-bias in many regions, significantly so in the PAG. Many of these nPGi afferents are activated during sexual behavior, though a minority do so to a large degree.

Our characterization of the anatomical organization, gonadal steroid receptor expression, and activity during sexual behavior of nPGi afferents is another step in understanding the control of genital reflexes. Our analysis provides a new insight into the regulation of genital reflexes by supraspinal sites. The number and variety of nPGi afferents suggest that the nPGi itself is a major integrator of signals relevant to the production of genital reflexes. This integration is likely to be context dependant as brain regions implicated in sexual behavior, stress, arousal, audition and attention provide input to the nPGi. Our analysis reveals that this integration is sexually dimorphic, possibly reflecting a bias in the importance of particular signals sent to the nPGi for appropriate genital reflex function. This information advances the understanding of the basic principles underlying human sexual dysfunction as it pertains to the supraspinal regulation of genital reflexes, and provides targets for further study.

**Table 2.1 Distribution of FG+ cells, and FG+ cells that also contained ER $\alpha$ , AR, or sex behavior-induced Fos following injection of FG into the nPGi**

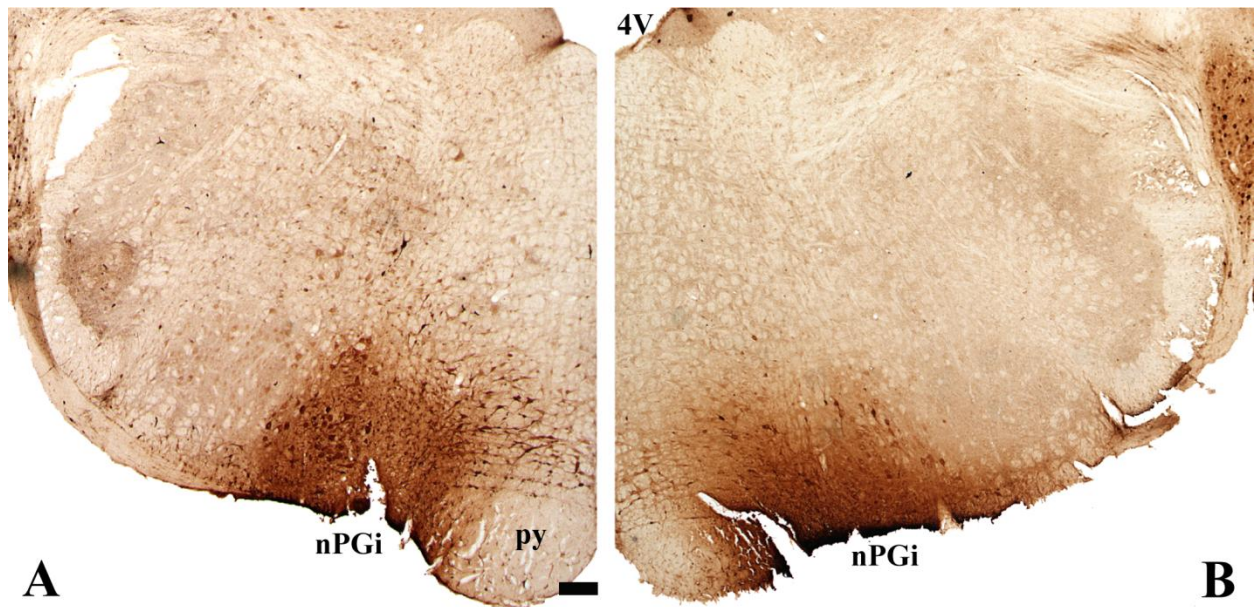
	Region	Area	Male				Female			
			FG	ER $\alpha$ /FG	AR/FG	Fos/FG	FG	ER $\alpha$ /FG	AR/FG	Fos/FG
Diencephalon	Preoptic Area	MPO Rostral	+	++	+++	++	+	++	++	++
		MPO Caudal	++	+++	+++	+++	+	++	++	++
		LPO	+	++	+++	+	+	++	++	++
		BNST	+	+++	+++	+	+	+++	++	++
	Amygdala	MeA	+	++	++	++	+	+	++	+
		CeM	++	+	+++	+	+	++	+++	+
	Hypothalamus	PVN	++	+	+	++	+++	+	++	++
		AH	+	+	++	+	+	+	+	++
		LH	+	+	++	++	+	++	++	+++
		Rch	+	+	++	+	+	+++	+++	++
		PH	++	++	+	++	++	+	+	+++
		DM	+	-	++	++	+	++	+	+
		VMH	+	++	++	+	+	+++	+++	+++
		Arch	+	++	++	+++	+	++	+	++
		PeF	+	+	++	++	++	+	+	+++
		TC	+	++	+++	+	+	++	+++	+++
		MCLH	+	++	+++	+++	+	+	++	++
	Thalamus	PrC	++	++	+++	++	++	+	++	++
		PF	+	-	+++	+	++	-	++	++
		RI	+	-	+	-	+	-	+	++
		PR	+	-	+++	++	+	-	+	+
		SPFPC	+	-	-	+	+	-	-	+
		SubI	+	++	+++	++	+	+	+	++
		ZI	+	-	++	+	+	-	+	+
		3	+	+	+	++	+	+	-	+
Mesencephalon	Midbrain	EW	+	-	-	++	+	-	-	-
		PCom	+	-	+++	++	+	+	+	+
		MCPC	+	-	-	+	+	-	-	+
		IMLF	+	-	-	+	+	-	-	++
		DpMe	++	+	++	++	++	+	+	++
		RPC	+	-	-	++	+	-	-	-
		RMC	+	-	-	-	+	-	-	+
		RRF	+	-	++	++	+	-	+	+++
		PIL	+	-	-	+	+	-	++	+++
		PP	+	++	+	-	+	++	+	++
		SNL	+	+	++	++	+	-	+	+
		SNR	+	-	-	++	+	-	-	+
		PBP/SNCD	+	+	++	+	+	-	+	+
		InCo	++	++	+++	++	++	+	+	++
		OT	+	-	-	+++	+	-	+	+++
		PLi	+	-	+++	+++	+	-	-	+
		SubB	+	+	++	+	+	-	+	+
		VTA	+	-	-	+	+	+	-	-

Table 2.1 Continued

		Region	Area	Male				Female			
				FG	ERα/FG	AR/FG	Fos/FG	FG	ERα/FG	AR/FG	Fos/FG
Mesencephalon	PAG	dmPAG	+++	+	+++	++	+++	+	++	++	
		dIPAG	+	-	+++	+	+	-	++	+	
		IPAG	+++	+	+++	++	+++	++	++	++	
		vIPAG	++	+	++	++	+++	++	++	++	
	Superior Colliculus	rSC	+	-	+++	+	++	-	+	++	
		Op	+	-	-	-	+	+	-	++	
		InG	+	-	++	+	+	-	-	+	
		DpG	++	+	++	++	+++	+	+	+	
	Inferior Colliculus	BIC	+	+	+	+++	+	-	+	++	
		ECIC	++	+	+	+	+++	+	+	+	
Metencephalon	Pons	RR	+	-	-	-	+	-	-	-	
		PPTg	+	+	++	++	+	-	-	+	
		CnF	+	+	+	++	+	-	+	+	
		dtg	+	-	+	+	+	-	-	-	
		PB	+	-	+	+	+	-	+	+	
		LDTg	+	-	+	+	+	-	-	+	
		m5	++	-	-	+	+++	-	+	+	
		PL	+	-	-	+	+	-	+	+	
		PnO	+	-	-	+	+	-	-	+	
		Mo5	+	-	+	-	+	-	-	+	
		VLTg	+	-	+	-	+	-	-	-	
		O	+	-	+	++	+	-	-	++	
		Myelencephalon	Medulla	LC	+	-	+	-	+	-	+
Sol	+			-	+	-	+	-	+	-	
Cu	+			-	-	+	+	-	-	++	
Gl	+			-	-	-	+	-	-	+	
IRt	+			-	-	-	+	-	-	-	
PCRt	+			-	+	+	+	-	-	-	
LRt	++			-	-	+	++	-	-	++	
Sp5l	+			-	-	+	++	-	-	+	

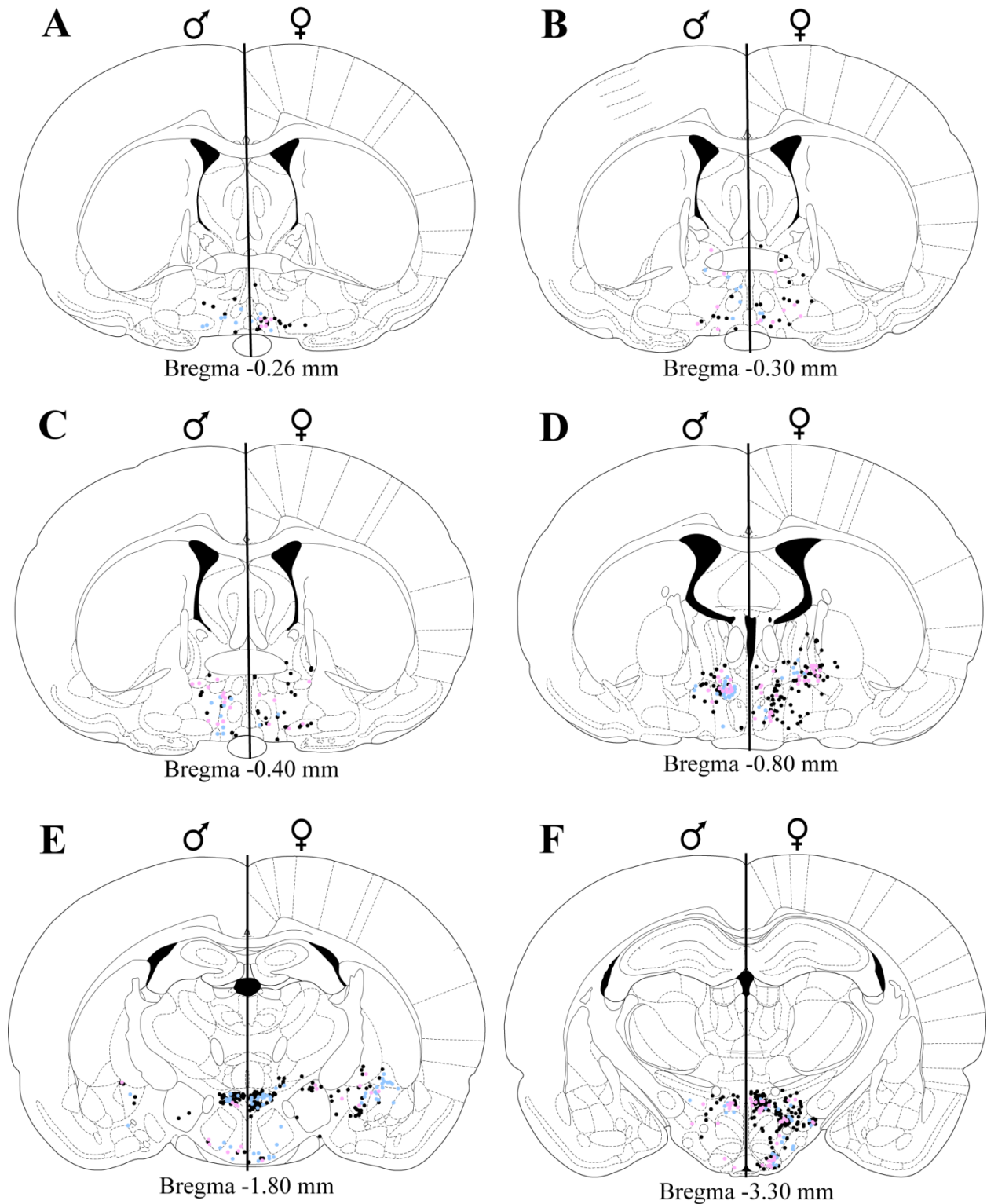
Table 2.2 Sexual Behavior Measures (mean) in Tracer-injected Male and Female Rats

Males			Females				
Mounts	Intromissions	Ejaculations	Mounts	Intromissions	Ejaculations	LQ	LR
14.2	43.8	4.8	24.7	33.7	3.7	0.99	1.95



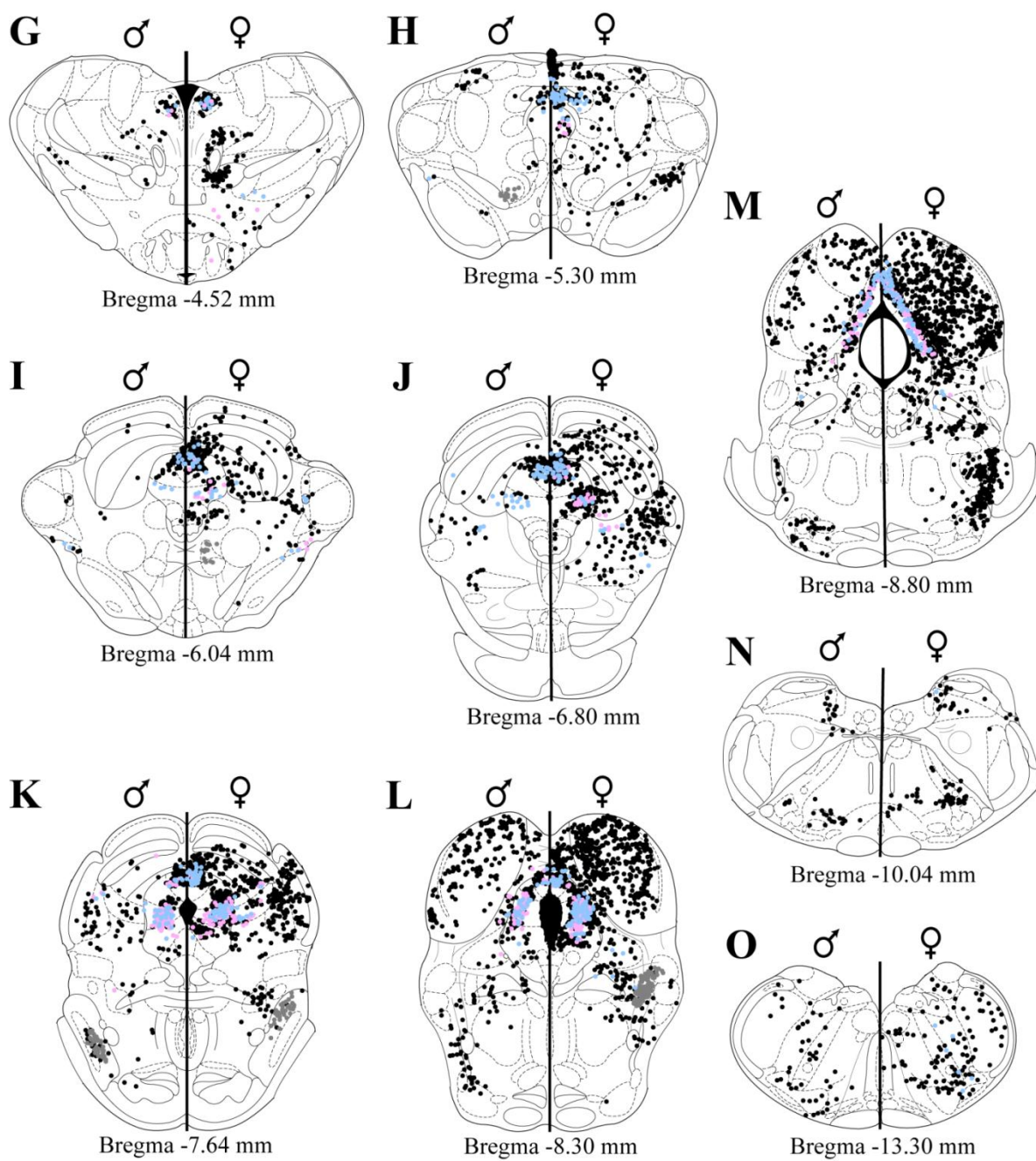
**Figure 2.1 Photomicrograph of Fluorogold injection sites**

Photomicrograph depicting a typical Fluorogold (FG) injection in the nucleus paragigantocellularis (nPGi) of the male (A) and female (B) rat. Note that FG injections are centered in the nPGi with little spread to surrounding regions. 4V=4<sup>th</sup> ventricle, py=pyramidal tract. Scale bar = 200  $\mu$ m in A (applies to A, B).



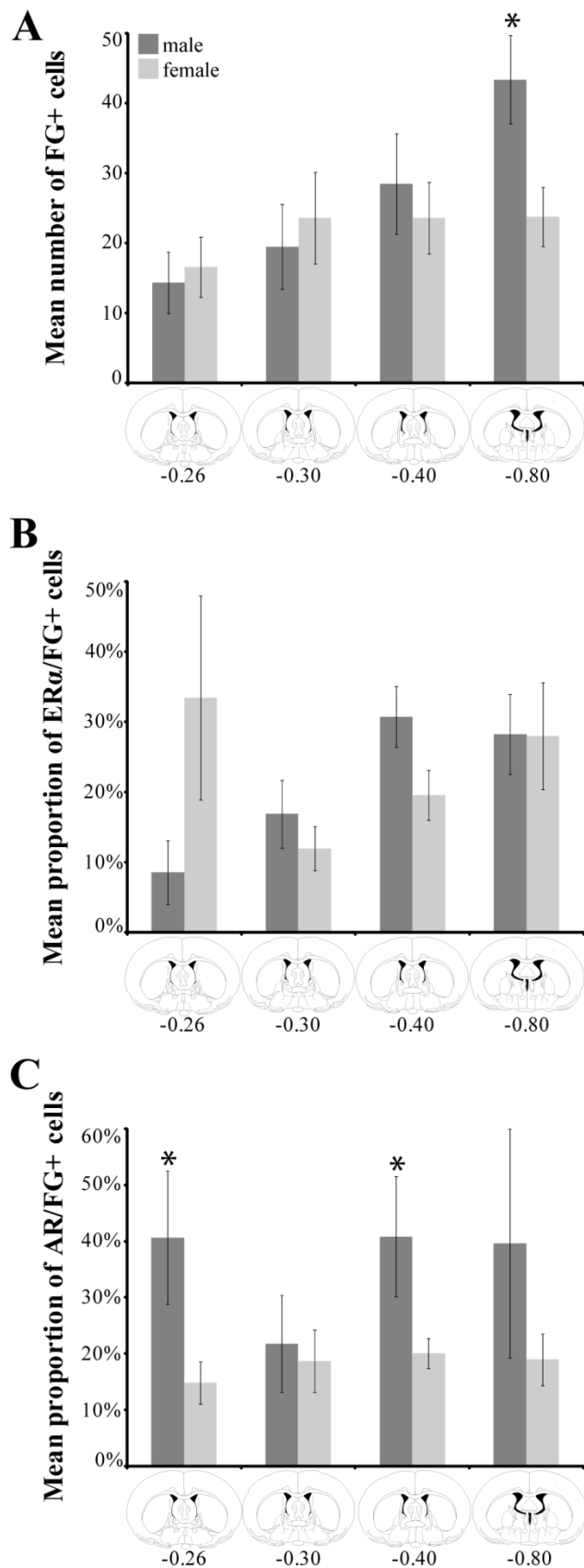
**Figure 2.2 Distribution of FG+ cells and co-localization with gonadal steroid receptors**

Plots of FG+ cells retrogradely labeled from the nPGi (black: ipsilateral to injection site, gray: solely contralateral to injection site). Also plotted are FG+ cells containing estrogen receptor alpha (ER $\alpha$ ; pink), or androgen receptor (AR; blue) of a representative male (left side) and female (right side) rat in all sections examined, relative to Bregma.



**Figure 2.2 Continued**

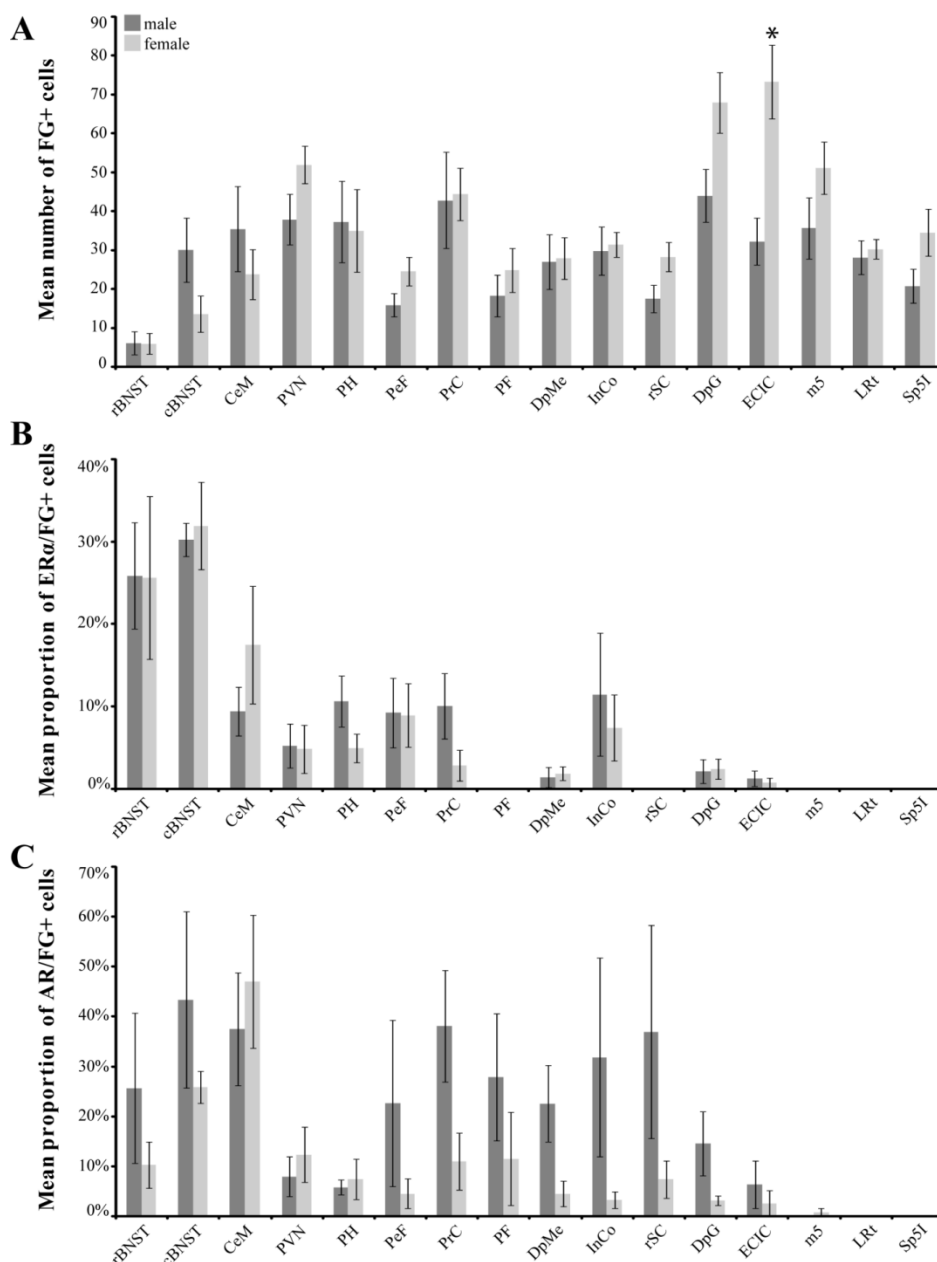




**Figure 2.3 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors in the MPO**

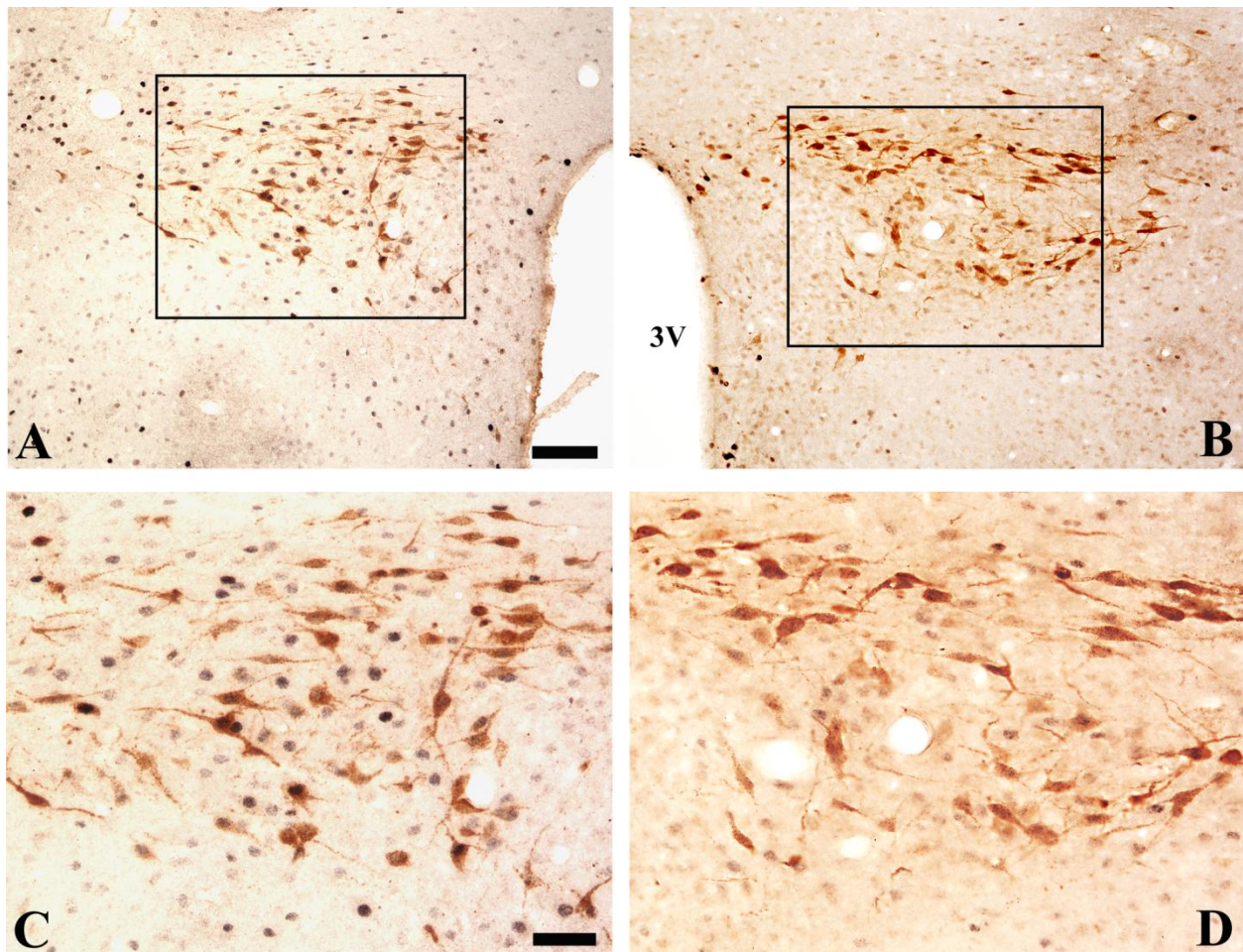
Mean number ( $\pm$  standard error of the mean (SEM)) of FG+ cells retrogradely labeled from the nPGi for four rostrocaudal levels of the medial preoptic area (MPO; A). The mean percentage ( $\pm$  SEM) of FG+ cells that also contained ER $\alpha$  (B) or AR (C) is also shown. Numbers below atlas images are millimeters relative to Bregma. Note that females have a higher number of FG+ cells in the rostral MPO, whereas males have a higher number of FG+ cells in the caudal MPO. Also note that females have a higher proportion of ER $\alpha$ /FG+ in the rostral-most level, whereas males have a higher proportion of ER $\alpha$ /FG+ cells in a caudal level. A higher proportion of AR/FG+ cells at all levels of the MPO were observed in males. \* = significant male/female difference,  $p \leq 0.05$ ; MPO=medial preoptic area.



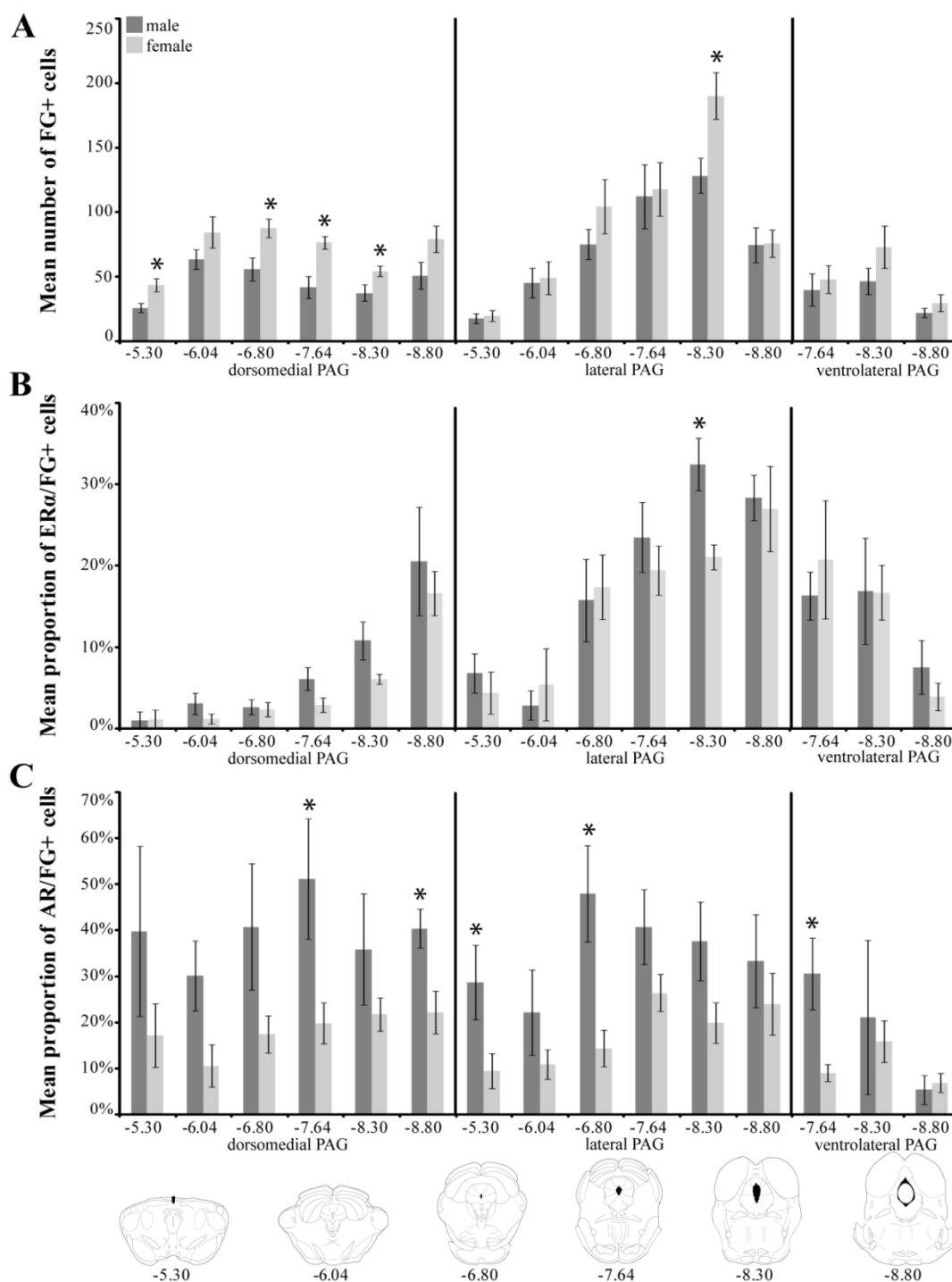


**Figure 2.4 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors throughout the brain**

Mean number ( $\pm$  SEM) of FG+ cells retrogradely labeled from the nPGi in all regions with where dense labeling was observed (A). Mean percentage ( $\pm$  SEM) of FG+ cells that also contained ER $\alpha$  (B) or AR (C) are also shown. Note the regional specificity in sex differences in FG+ cells. Also note the similarity between the sexes in the proportion of ER $\alpha$ /FG+ cells, but the male-bias at almost all levels with respect to the proportion of AR/FG+ cells. \* = significant male/female difference,  $p \leq 0.0031$ ; r/cBNST= rostral/caudal bed nucleus of the stria terminalis, CeM=central amygdala, PVN=paraventricular nucleus, PH=posterior hypothalamus, PeF=perifornical nucleus, PrC=precommissural nucleus, PF=parafascicular thalamic nucleus, DpMe=deep mesencephalic nucleus, InCo=intercollicular nucleus, rSC=rostral superior colliculus, DpG=deep gray layer of the superior colliculus, ECIC=external cortex of the inferior colliculus, m5=motor root of the trigeminal nerve, LRT=lateral reticular nucleus, Sp5I=interpolateral spinal trigeminal nucleus

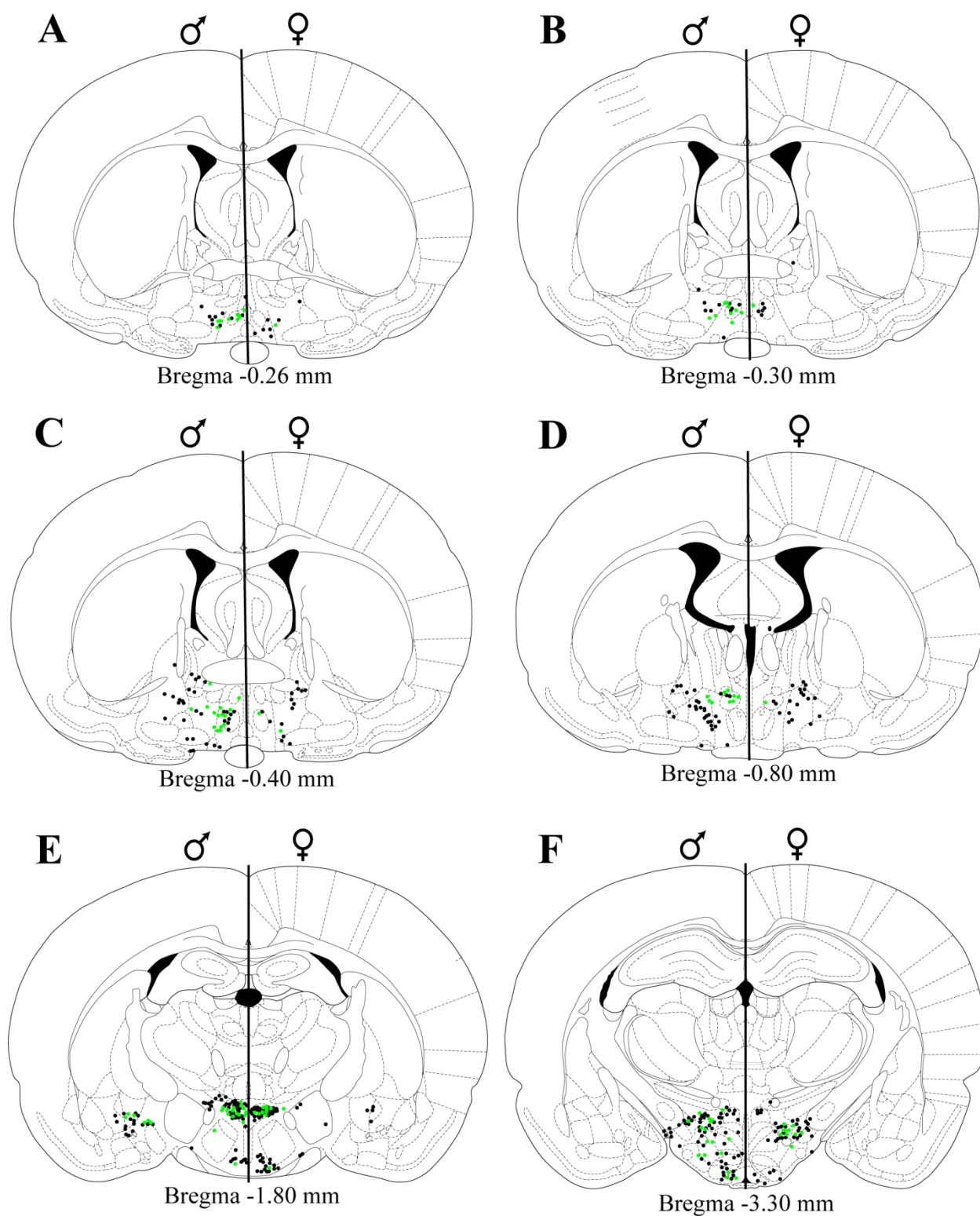


**Figure 2.5 Photomicrograph of FG+ cells and co-localization with gonadal steroid receptors in the PVN**  
 Photomicrograph of a representative section of the paraventricular nucleus (PVN) in a male (A and C) and female (B and D) rat. C and D are higher magnifications of the area indicated by the box. Neurons stained brown contain FG retrogradely labeled from the nPGi, nuclei stained black contain AR and neurons stained brown with nuclei stained black contain both FG and AR. Note that both males and females have a similar high density of retrogradely labeled cells, and that some of these cells contain AR. Scale bars = 100  $\mu$ m in A (applies to A, B); 50  $\mu$ m in C (applies to C, D).



**Figure 2.6 Graphs of FG+ cell counts and co-localization with gonadal steroid receptors in the PAG**

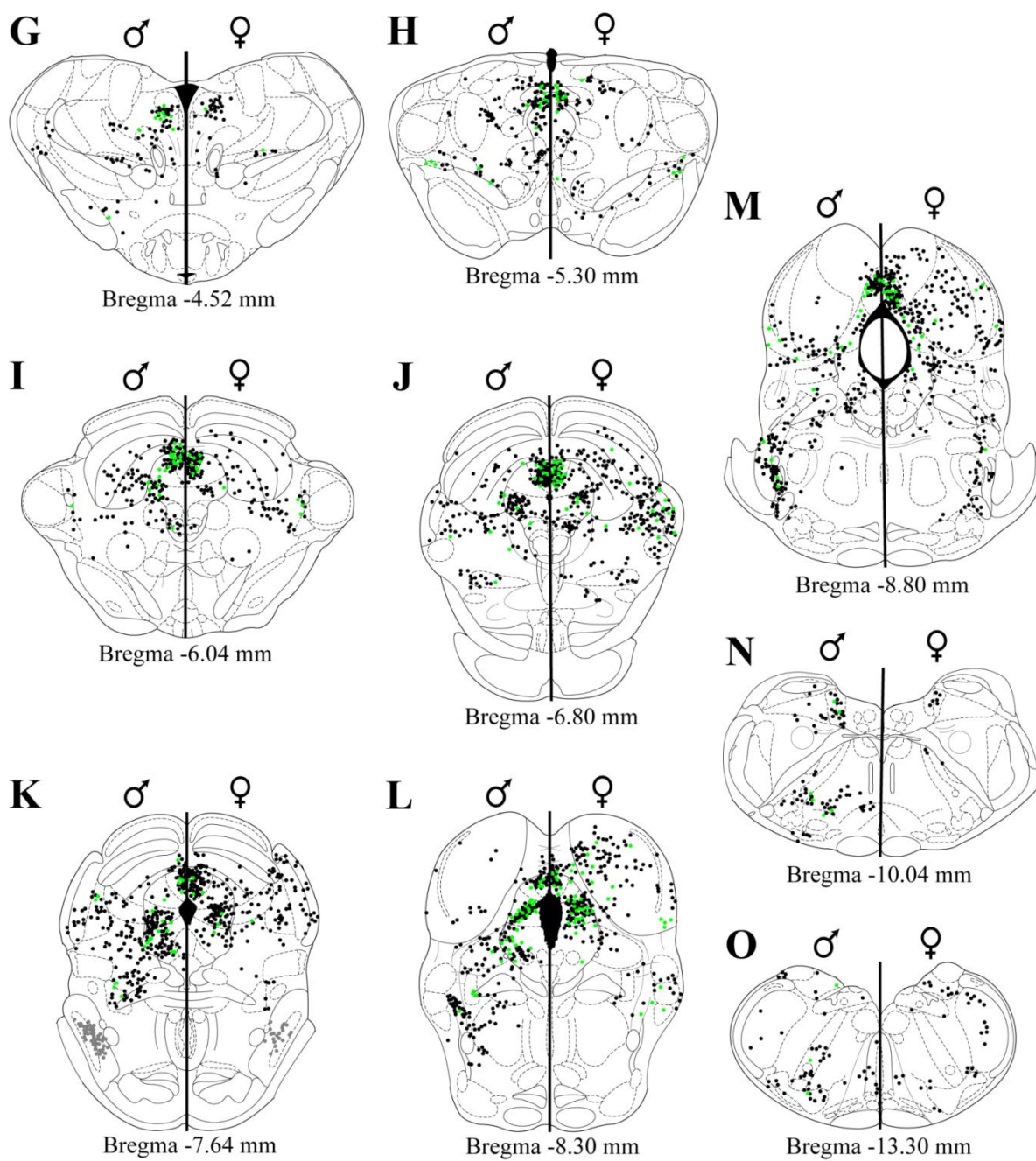
Mean number ( $\pm$  SEM) of FG+ cells retrogradely labeled from the nPGi for six rostrocaudal levels of the periaqueductal gray (PAG; A). The mean percentage ( $\pm$  SEM) of FG+ cells that also contained ER $\alpha$  (B) or AR (C) is also shown. Numbers below atlas images are millimeters relative to Bregma. Note that females have a higher number of FG labeled cells throughout the PAG, most notably in the dorsomedial subdivision of the PAG, and that females differed from males significantly at four rostrocaudal levels. Also note that males have a higher proportion of ER $\alpha$ /FG+ or AR/FG+ cells at almost all levels. \* = significant male/female difference,  $p \leq 0.05$



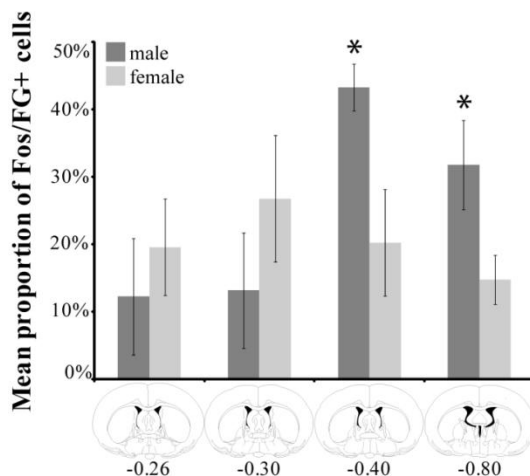
**Figure 2.7 Distribution of FG+ cells and co-localization with sexual behavior-induced Fos**

Plots of FG+ cells retrogradely labeled from the nPGi (black: ipsilateral to injection site; gray: solely contralateral to injection site), and FG+ cells also containing Fos (green) of representative male (left sides) and female (right side) rat in all sections examined.

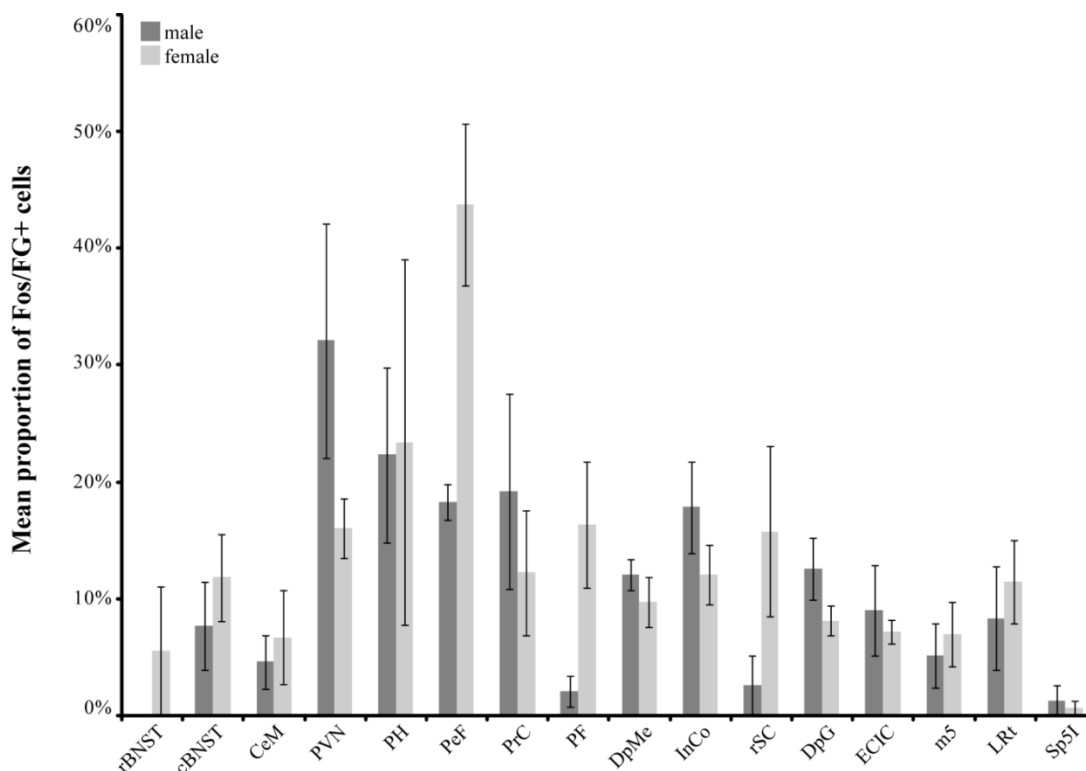




**Figure 2.7 Continued**

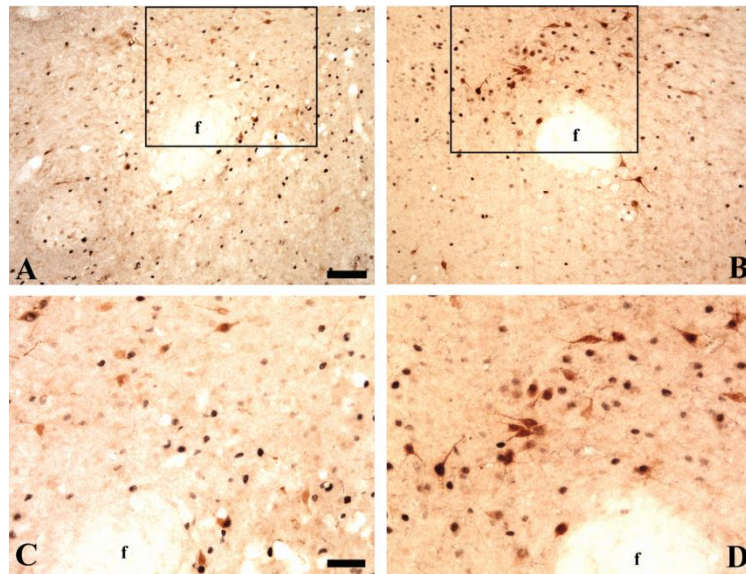


**Figure 2.8 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos in the MPO**  
 Mean proportion ( $\pm$  SEM) of FG+ cells retrogradely labeled from the nPGi that also contain sex-induced Fos for four rostrocaudal levels of the MPO. Numbers below atlas images are millimeters relative to Bregma. Note that females have a higher proportion of Fos/FG+ cells in rostral MPO levels, and that males have a higher proportion of Fos/FG+ cells in caudal MPO levels. \* = significant male/female difference,  $p \leq 0.05$ .



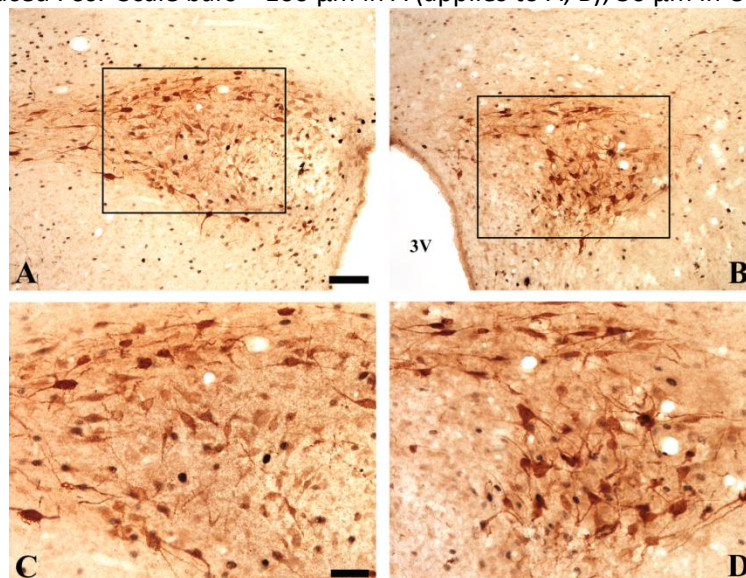
**Figure 2.9 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos throughout the brain**

Mean proportion ( $\pm$  SEM) of FG+ cells that also contain sex-induced Fos in all regions with dense input to the nPGi. Note the regional specificity in sex differences in the proportion of Fos/FG+, and that only a few regions show high sex-induced Fos in projections to the nPGi. No differences reached significance at the  $\alpha \leq 0.0031$  level.



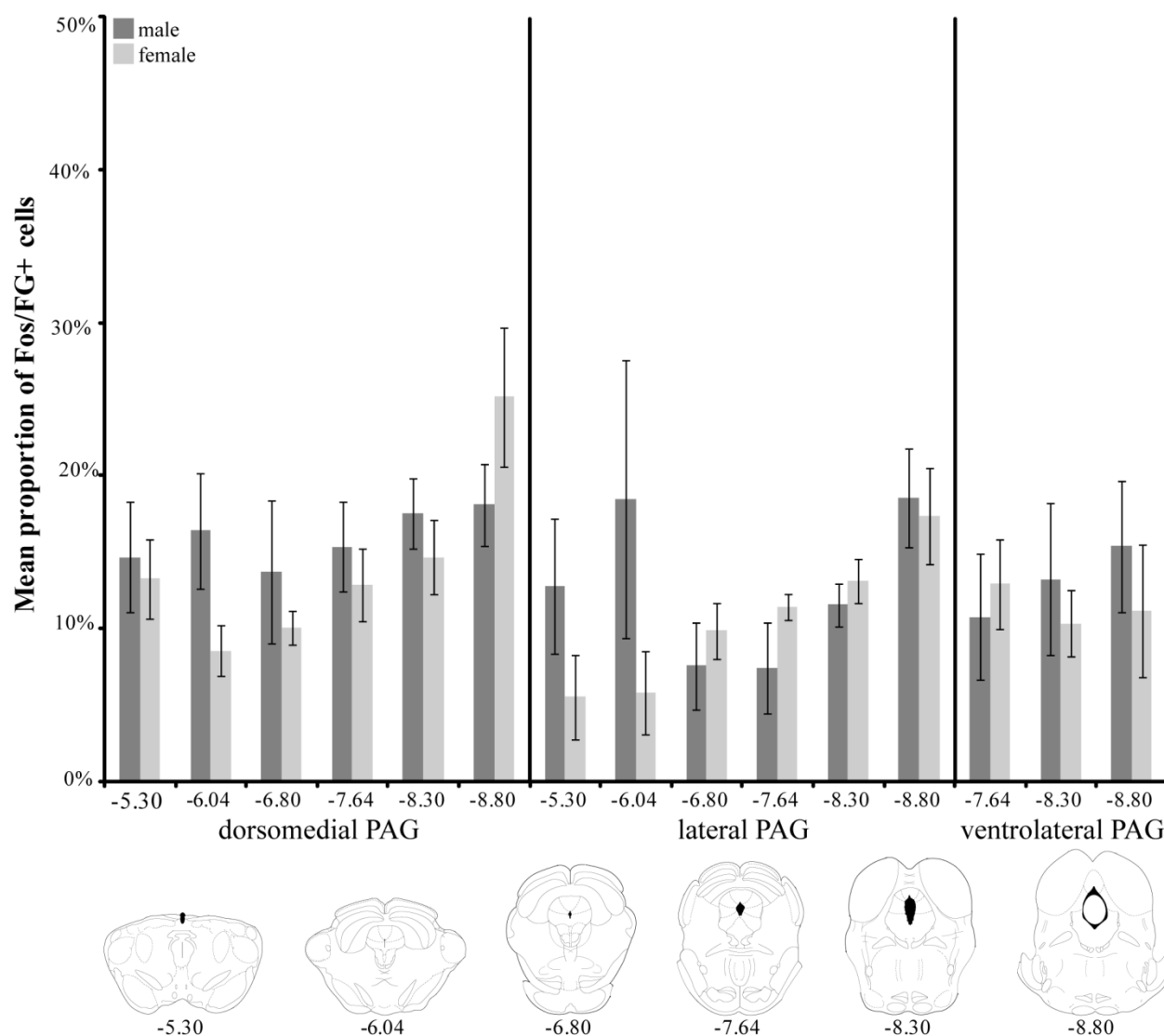
**Figure 2.10 Photomicrograph of FG+ cells and co-localization with sexual behavior-induced Fos in the PeF**

Photomicrograph of a representative section of the perifornical nucleus (PeF) of the hypothalamus in a male (A and C) and female (B and D) rat. C and D are higher magnifications of the area indicated by the box. Neurons stained brown contain FG retrogradely labeled from the nPGi, nuclei stained black contain sex-induced Fos, and neurons stained brown with nuclei stained black are retrogradely labeled FG cells that also contain sex-induced Fos. Note that females have a higher proportion of FG labeled cells that also contain sex-induced Fos. Scale bars = 100  $\mu$ m in A (applies to A, B); 50  $\mu$ m in C (applies to C, D).



**Figure 2.11 Photomicrograph of FG+ cells and co-localization with sexual behavior-induced Fos in the PVN**

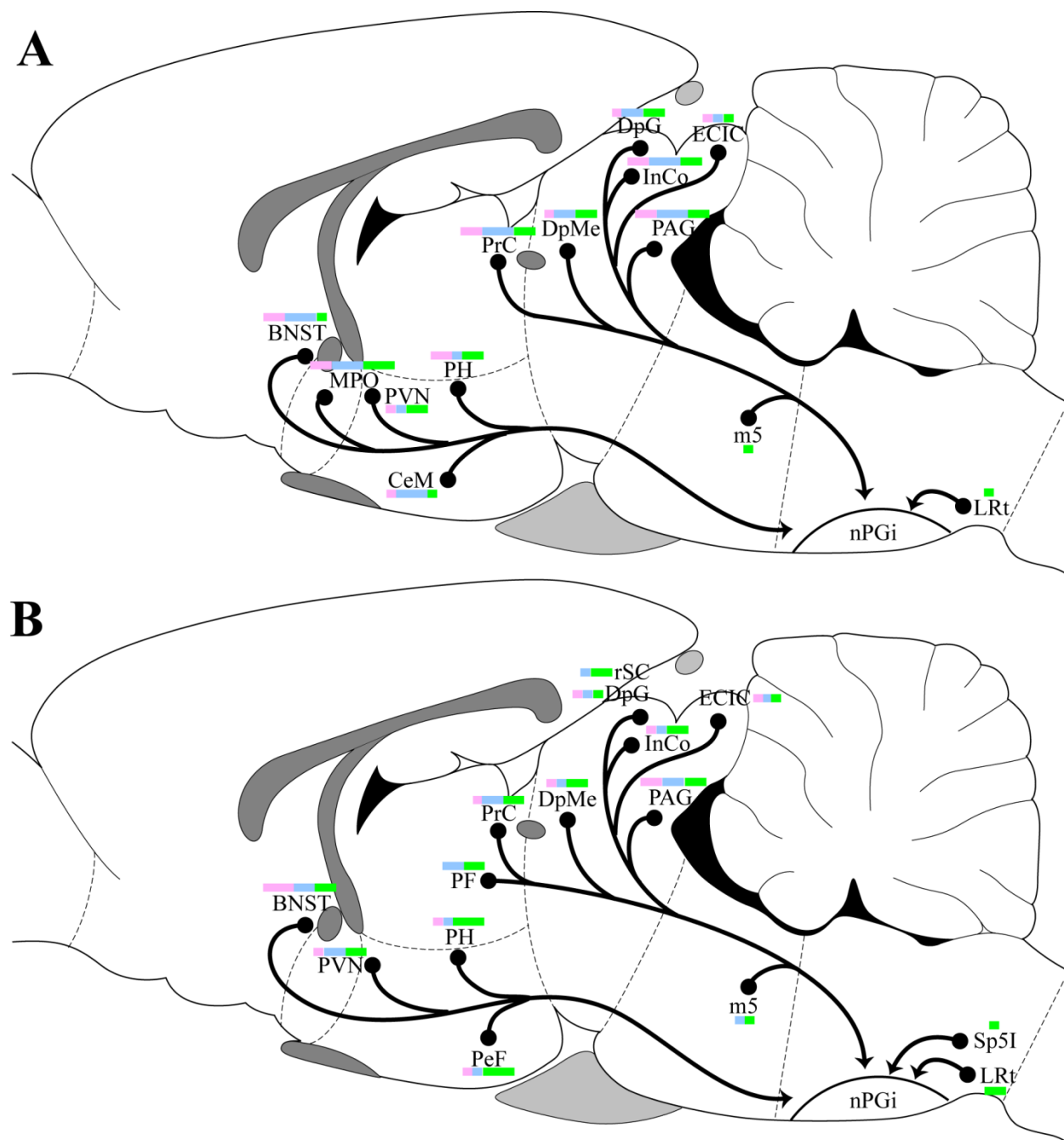
Photomicrograph of a representative section of the PVN in a male (A and C) and female (B and D) rat. C and D are higher magnifications of the area indicated by the box. Neurons stained brown contain FG retrogradely labeled from the nPGi, nuclei stained black contain sex-induced Fos, and neurons stained brown with nuclei stained black contain both FG and Fos. Note that both males and females have a similar high density of retrogradely labeled cells, and that many of these cells contain sex-induced Fos. Scale bars = 100  $\mu$ m in A (applies to A, B); 50  $\mu$ m in C (applies to C, D).



**Figure 2.12 Graph of FG+ cell counts and co-localization with sexual behavior-induced Fos in the PAG**

Mean proportion ( $\pm$  SEM) of FG+ cells retrogradely labeled from the nPGi that also contain sex-induced Fos for six rostrocaudal levels of the PAG. Numbers below atlas images are millimeters relative to Bregma. Male, dark gray; female, light gray. Note that in both sexes the proportion of Fos/FG+ cells is quite low. No differences reached significance at the  $\alpha \leq 0.05$  level.





**Figure 2.13 Diagram of regions providing dense input to the nPGi**

Summary diagram of regions that provide dense input to the nPGi in male (A) and female (B) rats. The color and length of bars adjacent to labels represent the relative density of FG labeled cells that also contain ER $\alpha$  (pink), AR (blue), or sex-induced Fos (green) in those regions.

### **3        EXCITOTOXIC LESIONS OF THE NUCLEUS PARAGIGANTOCELLULARIS FACILITATE MALE SEXUAL BEHAVIOR BUT ATTENUATE FEMALE SEXUAL BEHAVIOR IN RATS**

#### **3.1     Introduction**

Approximately 30% of men and 40% of women have some form of sexual dysfunction, including erectile dysfunction, premature ejaculation, and delayed ejaculation in males, and dyspareunia and vaginal spasms in females (Breiner, 2004; Laumann et al., 1999). One underlying factor that contributes to all of these disorders is a dysregulation of genital reflexes including ejaculation and vaginal contractions. As these reflexes are modulated supraspinally, a clear understanding of the contribution of the different brain sites to genital reflex control is clearly warranted.

The nucleus paragigantocellularis (nPGi) of rostroventrolateral medulla has been hypothesized to be the source of tonic descending inhibition of genital reflexes in male rats (Marson and McKenna, 1990). The nPGi sends projections to the lower lumbar and upper sacral (L5-S1) spinal cord motor neurons that innervate the bulbospongiosus and ischiocavernosus muscles, which are critical for the expression of ejaculation in male rats (Hermann et al., 2003; Marson and Carson 3rd, 1999; Marson and McKenna, 1996; Tang et al., 1999). In humans, the homologous structure is referred to as the nucleus paragigantocellularis lateralis (Zec and Kinney, 2001) and, as in the rat, it is also hypothesized to be associated with descending inhibition of genital reflexes (Johnson, 2006).

In male rats, lesions of the nPGi result in the facilitation of sexual behavior (Yells et al., 1992; Yells et al., 1994) as well as an increase in the number of ex copula erections (Marson et al., 1992; Marson and McKenna, 1990) in an artificial model of genital reflexes. Similarly, electrical stimulation of the nPGi produces increased firing latency and decreased amplitude of firing in the spinal motor neurons associated with genital reflexes (Johnson and Hubscher, 1998), consistent with the role of the nPGi as the source of descending inhibition to genital reflexes..

Studies examining the impact of nPGi lesions on the full range of male sexual behavior have exclusively employed fiber-destroying electrolytic lesions (Holmes et al., 2002; Liu and Sachs, 1999; Yells et al., 1992). Therefore, the contribution of fibers-of-passage in the region of the nPGi to the control of genital reflexes cannot be excluded. In the present study, we used excitotoxic lesions of the nPGi to confirm that nPGi neurons, and not fibers-of-passage, are the primary source of descending inhibition of male sexual behavior. We hypothesized that disruption of descending input from nPGi neurons would facilitate sexual behavior in male rats.

To date, the role of the nPGi in female sexual behavior has not been directly tested. Retrograde trans-synaptic tracing from the rat clitoris (Marson and Murphy, 2006), vagina (Marson and Murphy, 2006), and cervix (Lee and Erskine, 2000) produce labeling in the nPGi at time points consistent with a direct monosynaptic projection from the nPGi to the motoneurons controlling the genital musculature. In addition, a number of brain regions associated with sexual behavior send direct projections to the nPGi in females (Marson and Foley, 2004; Marson and Murphy, 2006; Murphy and Hoffman, 2001; Normandin and Murphy, 2008), further suggesting a role for the nPGi in female sexual behavior.

In the present study, we also used excitotoxic lesions of the female nPGi to determine its impact on sexual behavior. Unlike male rats, where genital reflexes such as ejaculation can be measured directly, there is no discrete measure of female “ejaculation.” However, paced-mating behaviors are dependent on genitosensory feedback (Camacho et al., 2009; Coopersmith et al., 1996; Erskine, 1992) which may be altered by a disruption of genital reflexes. As a secondary measure, we also examined the impact of nPGi lesions on the formation of a conditioned place preference (CPP) for artificial vaginocervical stimulation (aVCS). We hypothesized that disruption of genital reflexes through the nPGi would block the formation of a CPP for aVCS in females. Lastly, we explored how excitotoxic lesions of the nPGi might alter sexual behavior- and aVCS-induced expression of the immediate-early gene product Fos, a

marker of neural activity (Hoffman and Lyo, 2002; Hoffman et al., 1993) in brain regions associated with reward and sexual behavior.

### **3.2 Materials and methods**

#### **3.2.1 Subjects**

23 male and 55 female Sprague-Dawley Rats (*Rattus Norvegicus*; Charles River; 275-375g) were same-sex double-housed in a temperature controlled vivarium in reverse light (lights on 7:00pm, off 7:00am) with ad libitum access to food and water. All experiments were approved by the Georgia State Institutional Animal Care and Use Committee, with pain and suffering minimized in accordance with the Committee's policies.

#### **3.2.2 Ovariectomy and gonadal-steroid replacement**

All females were ovariectomized and received injections of  $\beta$ -Estradiol-3-Benzate (10ug/0.1ml sesame oil s.c.; Sigma Aldrich) 48 hours before testing and Progesterone (500ug/0.1ml sesame oil s.c.; Fluka) 4 hours before testing, to induce sexual receptivity (Barfield and Lisk, 1970; McEwen et al., 1987; Quadagno et al., 1972).

#### **3.2.3 nPGi lesions**

All surgeries were performed under aseptic conditions. Animals were anesthetized by inhalation of Isoflurane (2-5%; Henry Schein) and placed in a stereotaxic frame. The skull was leveled such that Bregma and Lambda were at the same dorsoventral plane. Excitotoxic lesions were produced by bilateral injection of N-methyl-D-aspartate (NMDA; 20mg/ml in dH<sub>2</sub>O; Sigma Aldrich; 14 males, 40 females), using a 1 $\mu$ l Hamilton syringe. Control animals received bilateral injection of vehicle (dH<sub>2</sub>O; 7 males, 20 females). The coordinates for the nPGi were (in mm): AP -12.0 Bregma, ML  $\pm$ 1.25, DV -8.5. NMDA or vehicle (150nl/side) was injected slowly over 2 minutes and the syringe was left in place for 10 min. before being slowly removed. Animals received Buprenorphine (0.1mg/kg s.c.; Henry Schein) for pain re-

lief, and Baytril (5mg/kg i.m.; Henry Schein) as a prophylactic antibiotic. Animals recovered in clean heated cages before being returned to the housing facility.

#### **3.2.4 Sexual behavior**

All sexual behavior tests were conducted in acrylic aquariums (61cm x 30.5cm x 30.5cm). Males engaged in non-paced mating with a non-experimental stimulus female as previously described (Normandin and Murphy, 2008). Female sexual behavior was conducted using a paced mating arena in which a divider containing two 4cm diameter holes through which only the females could pass was placed in the last 1/3 of the arena. Animals were acclimated to the arena prior to the initiation of the experiment for 10 minutes on two consecutive days. Three and six days later, animals engaged in 1-hour mating bouts with stimulus animals to gain sexual experience. A third mating bout on day nine served as the baseline (pre-lesion) measure. Experimental animals then underwent lesion (or sham lesion) surgery as described above. Following a seven-day recovery, a final mating bout served as the post-lesion measure. All sexual behavior bouts were recorded and the number of mounts, intromissions, and ejaculations, latency to begin mating, ejaculation latency, and post-ejaculatory interval was scored. In addition, the lordosis rating (LR; 0, 1, or 2), lordosis quotient (LQ; mean number of lordosis/total number of copulatory events), number of chamber changes, hops/darts, time spent in the mating chamber, and ejaculation-return latency was scored for females. One hour after ejaculation (or reception of an ejaculation in females), animals were euthanized with SleepAway (0.5ml i.p.; Henry Schein) and brains processed for sexual-behavior induced Fos. Only animals with normal sexual behavior (males: at least one ejaculation during the baseline test; females: lordosis reflex and the reception of at least one ejaculation during the baseline test) were used in the nPGi lesion studies.

#### **3.2.5 Conditioned place preference for aVCS**

A separate group of hormone primed females engaging in two 1-hour mating bouts with stimulus males to obtain sexual experience prior to receiving nPGi lesions (or sham). Seven days after surgery

females were tested for the formation of a CPP for aVCS tests using the protocol outlined by Meerts and Clark (2009). The CPP apparatus consisted of three acrylic chambers: an opaque white chamber (46cm x 61cm x 41cm), unscented, lighted, with bedding; a transparent neutral connecting chamber (23cm x 41cm x 41cm); and an opaque gray chamber (46cm x 61cm x 41cm), scented with 2% glacial acetic acid, dark, without bedding. Females were placed in the neutral chamber of the CPP apparatus and the time spent in each of the chambers was recorded for 30 minutes to serve as a baseline preference test. aVCS was administered using a lubricated plunger from a 1ml syringe that was inserted into the vaginal canal up to the cervix for 2 seconds, every 30 seconds, for 15 total stimulations (Meerts and Clark, 2009; Tetel et al., 1993). This semi-natural temporal sequence has been shown to produce a CPP (Meerts and Clark, 2009). The aVCS reinforcement schedule was as follows: alternating every three days, females would receive either aVCS in a mating arena and then be placed in their non-preferred CPP chamber (i.e. the reinforced chamber) for 30 min., or no aVCS and placed in their preferred chamber (i.e. the non-reinforced chamber) for 30 min. Equal time (1.5 hours total) was spent in reinforced and non-reinforced chambers with aVCS only being paired with the reinforced chamber. Three days after the aVCS reinforcement schedule the females were again placed in the neutral chamber of the CPP apparatus and the time spent in each of the chambers was recorded for 30 minutes to serve as a test of reinforcement. Total time in the reinforced and non-reinforced chambers in the baseline and post-reinforcement test were calculated, as well as a preference score for the reinforced chamber ( $[\text{time spent in reinforced chamber} - \text{time spent in non-reinforced chamber}] / \text{total time}$ ), and a difference score ( $\text{time spent in non-reinforced chamber} - \text{time spent in reinforced chamber}$ ). Following the final CPP test, females received aVCS (as above), were euthanized by SleepAway (0.5ml i.p.; Henry Schein) 60 minutes later, and the brains were processed for aVCS-induced Fos expression. Animals that did not explore both of the CPP chambers were excluded from analysis.

### **3.2.6 Perfusion / fixation / tissue preparation**

After receiving a euthanizing dose of SleepAway (0.5ml i.p.; Henry Schein) animals were transcardially perfused with 250 ml of 0.9% sodium chloride/2% sodium nitrite, followed 300 ml of 4% paraformaldehyde 2.5% acrolein (Polysciences) in 0.1 M phosphate buffer then 150ml of the sodium chloride/sodium nitrite solution. Following perfusion/fixation, brains were removed and stored at 4°C in 30% sucrose solution until sectioned. Brains were cut into 25µm coronal sections in a 1:6 series from the rostrum to the brainstem, and a 1:4 series from brainstem to spinal cord, with a Leica 2000R freezing microtome and stored free-floating in cryoprotectant-antifreeze solution (Watson et al., 1986) at -20°C.

### **3.2.7 Immunohistochemistry**

Brainstem tissue was sectioned at 25µm in a 1:4 series and processed for NeuN for lesion verification. Tissue rostral to the brainstem was cut in a 1:6 series and processed for sexual behavior- or aVCS-induced Fos expression as previously described (Lloyd and Murphy, 2006; Murphy and Hoffman, 2001). Briefly, sections were removed from the cryoprotectant solution, rinsed extensively in potassium phosphate buffered saline (KPBS; pH 7.4), and then reacted for 20 minutes in 1% sodium borohydride to remove excess aldehydes. Sections were then incubated in primary antibody solution directed against either NeuN (Millipore, MAB377; monoclonal, raised in mouse; 1:70,000) or Fos (Calbiochem, PC38; polyclonal, raised in rabbit; 1:20,000) in KPBS containing 0.1% Triton-X for 1 hour at room temperature followed by 48 hours at 4°C. After primary antibody incubation, tissue was rinsed in KPBS, incubated for 1 hour in biotinylated goat-anti mouse (mouse anti-NeuN primary antibody) or goat anti-rabbit (rabbit anti-Fos primary antibody) IgG (Jackson ImmunoResearch Labs Inc.) at a concentration of 1:600, rinsed in KPBS, followed by a 1 hour incubation in avidin-biotin peroxidase complex (Vector Labs, ABC Elite Kit PK-6100) at a concentration of 1:10. After rinsing in KPBS and sodium acetate (0.175 M; pH 6.5), NeuN and Fos were visualized as a black reaction product using nickel sulfate intensified 3,3'-diaminobenzidine solution containing 0.08% hydrogen peroxide in sodium acetate buffer. The reaction product was termi-

nated after approximately 15 minutes by rinsing in sodium acetate buffer. Sections were mounted out of saline onto gelatin-subbed slides, air dried overnight, dehydrated in a series of graded alcohols, cleared in HistoClear, and cover-slipped using Permount.

### **3.2.8 Lesion analysis**

NMDA and sham lesion sites were verified by microscopic analysis of nPGi-containing brainstem sections stained immunohistochemically for NeuN (as above). Sections were examined by light-microscopy and any neural destruction was plotted based on the atlas of Paxinos and Watson (2005), to determine the extent of- or absence of lesions.

### **3.2.9 Sexual behavior- and aVCS-induced Fos expression analysis**

Fos expression was used as a measure of neural activity induced by sexual behavior in males and females and aVCS in females. The number of Fos+ cells was counted in regions previously associated with sexual behavior and/or reward, including the medial preoptic area (MPOA), paraventricular hypothalamic nucleus (PVN), ventromedial hypothalamic nucleus (VMN), posterodorsal medial amygdala (MePD), ventrolateral periaqueductal gray (vIPAG), and nucleus accumbens (NAcc). Sections immunohistochemically stained for Fos (as above) were examined by light microscopy and the number of cells expressing Fos, noted as dark nuclei, were counted manually by a rater blind to experimental condition. One rostro-caudal section (with respect to Bregma in mm.) for each region was examined, and the number of Fos+ neurons determined: MPOA (-0.72); PVN (-1.56); VMN (-2.92), MePD (-2.92), vIPAG (-7.68), and NAcc (1.92).

### **3.2.10 Statistical analysis**

Planned comparisons of means, both between (lesion vs. control) and within groups (pre- and post-lesion), were conducted using independent and dependent t-tests. Percentile data in the CPP tests were analyzed with nonparametric Mann-Whitney tests. All statistical comparisons were made with the alpha value set at  $p < 0.05$ .



### 3.3 Results

#### 3.3.1 Lesion verification

Representative photomicrographs of an NMDA lesion of the nPGi and a vehicle sham lesion with a focal point in the nPGi are shown in Figure 3.1. The smallest and largest of the NMDA lesions included for analysis are plotted on serial brainstem sections in Figure 3.2.

#### 3.3.2 Male sexual behavior

nPGi lesions produced a facilitation of male sexual behavior in many measures related to genital reflex functions. Table 3.1 summarizes the results of all male sexual behavior for the lesion (n=9) and control (n=8) groups. The mean number of mounts or intromissions in the sexual behavior test did not change significantly from baseline in either the lesion or control groups (Figure 3.3A & B). The mean number of ejaculations significantly increased from baseline within the lesion group ( $p=0.004$ ) but did not change within the control group ( $p=0.451$ ; Figure 3.3C). In addition, the mean number of ejaculations in the sexual behavior test was significantly greater in the lesion group than in the control group ( $p=0.017$ ; Figure 3.3C). There was a concomitant decrease in the mean number of intromissions required for ejaculation from baseline within the lesion group ( $p=0.011$ ) but not the control group ( $p=0.886$ ; Figure 3.3D). There was also a decrease in the mean ejaculation latency from baseline within the lesion group ( $p=0.038$ ) but not within the control group ( $p=0.731$ ; Figure 3.3E).

Other measures of male sexual behavior not directly related to genital reflex function were unaltered by nPGi lesions. Both the mean latency to begin mating and the mean post-ejaculatory interval did not differ from baseline in either the group. (Figure 3.4A, B).

#### 3.3.3 Female sexual behavior

Table 3.2 summarizes the results of all female sexual behavior in the lesion (n=14) and control (n=12) groups. Female sexual behavior was largely unaffected by lesions of the nPGi in measures of the stimulus males' behavior, as well as in most measures of the females' behavior. The mean number of

intromissions and ejaculations received did not differ from baseline within either group (Figure 3.5B, C). The stimulus males mean ejaculation latency also did not change from baseline within either group (Figure 3.5D). Mean lordosis rating and the mean lordosis quotient were unchanged from baseline within either group (Figure 3.6A, B). In addition, the mean number of hop-darts and the mean number of chamber changes did not change from baseline in either group (Figure 3.6C, 3.7A).

nPGi lesions resulted in the attenuation of some behaviors. The mean number of mounts increased from baseline within the control group ( $p=0.046$ ) but did not change within the lesion group ( $p=0.252$ ; Figure 3.5A). Similarly, there was an increase in the mean time spent in the mating chamber from baseline within the control group ( $p=0.041$ ) but not within the lesion group ( $p=0.394$ ; Figure 3.7B). In parallel, there was an increase in the mean ejaculation-return latency from baseline within the lesion group ( $p=0.037$ ), but not within the control group ( $p=0.159$ ), although the lesion and control groups did differ in mean ejaculation-return latency baselines ( $p=0.020$ ; Figure 3.7C). Together these results suggested that while nPGi lesions did not alter female sexual behavior directly, the quality of the mating experience was altered for the lesion females. Therefore, to test the hypothesis that the rewarding aspect of sexual behavior was altered in nPGi lesioned females, we next tested whether these animals were still able to form a CPP for aVCS.

### **3.3.4 Female CPP for aVCS**

Table 3.3 summarizes the results of all CPP tests for the lesion ( $n=13$ ) and control ( $n=8$ ) groups. Within both the lesion and control groups animals preferred (i.e. spent more time in) either the white or gray compartments. Henceforth the preferred compartment is referred to as the “non-reinforced” compartment, and the non-preferred compartment is referred to as the “reinforced” compartment, regardless of their quality (i.e. white or gray). The preference scores for the reinforced compartments for animals preferring either the white or gray compartment did not differ for either the lesion or control group indicating that animals had no overwhelming preference for just one kind of compartment.

nPGi lesions attenuated some aspects of CPP formation for aVCS. Post-reinforcement, there was a significant increase in the mean preference score, and a significant decrease in the mean difference score in both the lesion group (preference score:  $p=0.006$ ; difference score:  $p=0.015$ ), and control group (preference score:  $p=0.009$ ; difference score:  $p=0.010$ ), indicating the formation of a CPP for aVCS (Figure 3.8A, B). However, the percent time spent in the reinforced chamber after reinforcement did not change in the lesion group ( $p=0.096$ ) but was significantly increased in the control group ( $p=0.036$ ; Figure 3.8C), indicating that the CPP formed in the lesion group was attenuated or of a lesser quality in comparison to the control group.

### **3.3.5 Male sexual behavior-induced Fos expression**

There were no significant differences between the lesion and control groups in the mean number of sexual behavior-induced Fos+ cells (Figure 3.9A) in males.

### **3.3.6 Female sexual behavior-induced Fos expression**

nPGi lesions produced a significant reduction in the number of sexual behavior-induced Fos-expressing neurons in the MPOA ( $p=0.040$ ), PVN ( $p=0.001$ ; Figure 3.11), VMN ( $p=0.029$ ; Figure 3.12), and MePD ( $p=0.012$ ; Figure 3.9B) in females. No significant differences in the mean number of sexual behavior-induced Fos+ cells were observed in the NAcc.

### **3.3.7 Female aVCS-induced Fos expression**

No significant differences in the mean number of aVCS-induced Fos+ cells were found between the lesion and control groups for all regions examined (Figure 3.9C).

## **3.4 Discussion**

### **3.4.1 Males**

As hypothesized, excitotoxic lesions of the nPGi facilitated sexual behavior in males. We observed a significant increase in the mean number of ejaculations, as well as a significant decrease in the number of intromissions required for ejaculation within the nPGi lesion group. Facilitation of sexual be-

havior as a result of excitotoxic nPGi lesions is further supported by a significant decrease in ejaculation latency within the lesion group. The mean latency to begin mating and the post-ejaculatory interval were not altered by nPGi lesions, suggesting that the nPGi is not involved in the expression of these behaviors. Indeed, these behaviors are thought to be under the control of upstream sites (Phillips-Farfan and Fernandez-Guasti, 2009), particularly those sites associated with the mesolimbic dopamine reward system (Agmo and Fernandez, 1989; Guevara et al., 2008; Hull et al., 1995; Kleitz-Nelson et al., 2010) and nPGi lesions would not be expected to produce any changes in these measures.

Despite the dramatic effects of nPGi lesions on behavior, nPGi lesions did not alter sexual behavior-induced Fos in male rats. This suggests that the feedback from the reduction in ejaculation latency and the increase in the number of ejaculations observed in our nPGi lesioned males does not alter activity in regions associated with sexual behavior. This further suggests that the regions examined do not alter their activity as a function of feedback during sexual behavior, but rather provide input that is under the control of intrinsic drives (e.g. circulating hormones). Indeed, transections of the pelvic nerve do not alter mating-induced Fos-expression in the MPOA, VMN, or MePD indicating that some mechanism, other than genitosensory feedback, is responsible for activity associated with sexual behavior in these regions (Wersinger et al., 1993).

The results of the present study are in line with previous studies examining the impact of electrolytic (fiber-destroying) nPGi lesions on the full range of male sexual behavior (Liu and Sachs, 1999; Yells et al., 1992). Specifically, these studies reported a decrease in ejaculation latency, as well as an increase in the number of ejaculations as a result of nPGi lesions. Our results confirm that it is indeed nPGi neurons, and not fibers-of-passage, that provide the tonic descending inhibitory control over genital reflexes in males. These results also confirm that the artificially induced urethro-genital reflex is an appropriate model for studying male sexual behavior, as excitotoxic lesions of the nPGi facilitate the urethro-genital reflex in a similar manner to our results (Marson et al., 1992; Marson and McKenna,

1990). Interestingly we did not see a statistically significant reduction in the mean number of mounts or intromissions as a result of excitotoxic nPGi lesions as others have reported (Yells et al., 1992), suggesting that fibers-of-passage (and not nPGi neurons) might be responsible for the observed change.

### **3.4.2 Females**

Excitotoxic lesions of the nPGi altered unique aspects of paced-mating behavior. In particular, nPGi lesioned animals did not increase the number of mounts received, or increase the amount of time spent in the mating arena, as is typically observed in females following successive mating experiences (Nofrey et al., 2008). Similarly, ejaculation-return latencies were significantly longer in nPGi lesioned animals. Other measures of the stimulus males' sexual behavior, such as the mean number of intromissions, ejaculations, and the mean ejaculation latency did not change in either group. In addition, nPGi lesions had no effect on proceptive (mean number of hops/darts) and receptive (mean lordosis rating and lordosis quotient) behaviors. Taken together this data suggests that excitotoxic nPGi lesions in females attenuate only paced-mating behaviors.

With the combination of the lack of increase in time spent mating, and the increase in the ejaculation-return latency, it appears that the lesioned females might find some aspects of sexual behavior either aversive or unrewarding. To address this possibility, we conducted the CPP for aVCS experiment. Our analysis revealed that while our lesion group formed a CPP, the strength of the CPP was limited compared to controls. Specifically, while there was a statistically significant increase in the mean preference score from baseline to post-reinforcement within the lesion group, the mean value of the preference score was still below 0.50, indicating that lesioned animals still preferred the non-reinforced compartment. In addition, the mean percentage of time spent in the reinforced chamber was significantly greater within the control group, but not within the lesion group (indeed the means are in opposing directions). This data, together, suggests that excitotoxic lesions of the nPGi attenuate CPP formation for aVCS.

In contrast to males, nPGi lesions reduced the number of Fos+ cells in a number of brain regions including the MPOA, PVN, VMN, and MePD. As mentioned above, while transections of the pelvic nerve do not alter mating-induced Fos-expression in the MPOA, VMN, or MePD in male rats, such transections attenuate mating-induced Fos in these regions in female rats (Wersinger et al., 1993). This indicates that genitosensory feedback is important for the modulation of activity in these regions.

Our nPGi lesion data suggests that the quality of the mating experience for the female might be altered given that these females spend less time with the male and had longer ejaculation-return latencies. We suggest that the quality of the mating, as a result of disruption of supraspinal modulation of genital reflexes and suboptimal genitosensory input, is reduced for these females, and that the reduction in sexual behavior-induced Fos that we observed results from such dysregulation, much like the attenuation of mating-induced Fos as a result of pelvic nerve transection. However, we do not observe any changes in aVCS-induced Fos expression in our lesioned females. Therefore, it is possible that the stimulus females received during aVCS, while strong enough to produce a CPP, does not constitute an ethologically valid stimulus with regards to activity in the brain regions we examined.

The effects of nPGi lesions in females, taken together, are admittedly subtle. Given the anatomical connections between upstream sites to the nPGi, and from the nPGi to the spinal cord, one interpretation of this data is that during mating, normal muscular contractions within the vagina are disrupted by nPGi lesions. Such dysregulation of genital reflexes would produce either an increase in vaginal muscular tone that would be aversive, or a decrease in tone that would not provide appropriate reinforcing feedback to the female. It is also possible that other behavioral systems override any perceived aversion or lack of stimulation. These females were given a standard receptivity-priming regimen of estradiol and progesterone. Such doses maximize mating behavior in females and this state in which motivational drive for mating is high might overcome any perceived aversion or lack of stimulation. A direct examination of vaginal muscular tone after nPGi lesions is warranted to help address this issue.

### 3.5 Conclusions

The nPGi provides a relay between upstream sites regulating sexual behavior and the spinal cord motor neurons responsible for genital reflexes. Excitotoxic lesions of the nPGi facilitated male sexual behavior in measures directly related to genital reflex function in a manner consistent with previous reports, confirming that nPGi neurons, and not fibers-of-passage, are the locus for descending inhibition of genital reflexes. Excitotoxic lesions of the nPGi in females attenuated paced-mating behaviors, but not other aspects of female sexual behavior, as well as sexual behavior-induced Fos-expression in the hypothalamus and amygdala. These lesions also weakened the formation of a CPP for aVCS, indicating that dysregulation of vaginal reflexes impacts the quality of the mating experience in females. The common occurrence of sexual dysfunctions in humans often includes an underlying dysregulation of genital reflexes. The activity of an nPGi homologue in humans could account for such dysregulation if activity in this region is abnormal.

**Table 3.1 Sexual behavior measures (mean +/- standard error of the mean (SEM)) in control group and lesion group males**

	Sexual Behavior							
	Mounts		Intromissions		Ejaculations		Intromissions pre Ejaculation	
	Baseline	Test	Baseline	Test	Baseline	Test	Baseline	Test
Control	56.25 +/- 12.91	29.50 +/- 10.02	48.88 +/- 7.60	38.00 +/- 5.38	4.38 +/- 0.42	3.88 +/- 0.48	11.68 +/- 1.98	12.17 +/- 3.42
Lesion	35.33 +/- 5.02	32.56 +/- 6.05	41.89 +/- 1.90	35.11 +/- 3.99	4.22 +/- 0.31	5.33 +/- 0.27 * †	10.33 +/- 0.82	6.55 +/- 0.70 *

	Ejaculation Latency		Post-Ejaculatory Interval		Latency to Mating	
	Baseline	Test	Baseline	Test	Baseline	Test
Control	5.99 +/- 0.95	5.49 +/- 0.92	7.53 +/- 0.43	7.62 +/- 0.40	6.32 +/- 1.19	16.72 +/- 7.67
Lesion	5.49 +/- 1.03	3.80 +/- 0.35 *	7.07 +/- 0.37	7.56 +/- 0.54	21.73 +/- 7.01	14.70 +/- 5.89

\* = p<0.05 within-group comparison, † = p<0.05 between-group comparison

**Table 3.2 Sexual behavior measures (mean +/- SEM) in control group and lesion group females**

	Stimulus Male Sexual Behavior							
	Mounts Received		Intromissions Received		Ejaculations Received		Ejaculation Latency	
	Baseline	Test	Baseline	Test	Baseline	Test	Baseline	Test
Control	20.67 +/- 5.63	33.67 +/- 6.97 *	26.75 +/- 3.08	31.92 +/- 3.11	3.33 +/- 0.28	3.67 +/- 0.36	7.23 +/- 0.87	7.16 +/- 1.15
Lesion	22.43 +/- 5.66	32.00 +/- 6.31	23.86 +/- 2.50	30.71 +/- 2.89	3.00 +/- 0.21	3.79 +/- 0.28	8.56 +/- 0.58	7.63 +/- 0.82

	Proceptive and Receptive Behaviors					
	Lordosis Rating		Lordosis Quotient		Hops & Darts	
	Baseline	Test	Baseline	Test	Baseline	Test
Control	1.64 +/- 0.09	1.58 +/- 0.10	0.99 +/- 0.01	1.00 +/- 0.00	49.67 +/- 13.21	57.75 +/- 13.83
Lesion	1.78 +/- 0.04	1.75 +/- 0.05	1.00 +/- 0.00	1.00 +/- 0.00	59.86 +/- 11.50	69.71 +/- 12.91

	Paced-Mating Behaviors					
	Chamber Changes		Time Mating		Ejaculation-Return	
	Baseline	Test	Baseline	Test	Baseline	Test
Control	73.33 +/- 10.30	58.50 +/- 10.96	18.01 +/- 2.02	25.85 +/- 3.49 *	4.73 +/- 0.70	3.88 +/- 0.43
Lesion	75.86 +/- 9.52	74.00 +/- 8.76	22.85 +/- 3.84	20.65 +/- 3.06	2.82 +/- 0.39 †	4.08 +/- 0.48 *

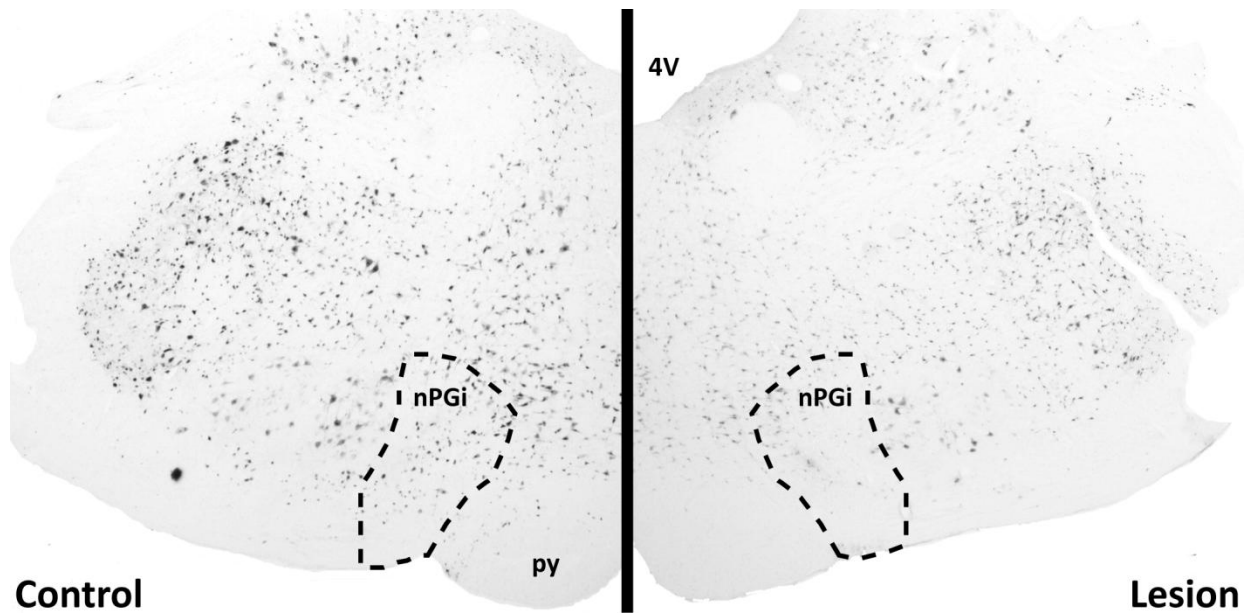
\* = p<0.05 within-group comparison, † = p<0.05 between-group comparison

**Table 3.3 Conditioned place preference measures (mean +/- SEM) in control group and lesion group females**

	Conditioned Place Preference for aVCS Behavior					
	Preference Score		Difference Score		% Time Post-Reinforcement	
	Baseline	Test	Baseline	Test	Non-Reinforced	Reinforced
Control	0.28 +/- .07	0.57 +/- 0.04 *	258.88 +/- 77.35	-106 +/- 69.67 *	44.59 +/- 3.99	55.41 +/- 3.99 *
Lesion	0.32 +/- 0.03	0.46 +/- 0.04 *	376.31 +/- 66.85	69.00 +/- 108.39 *	52.93 +/- 4.40	47.07 +/- 4.40

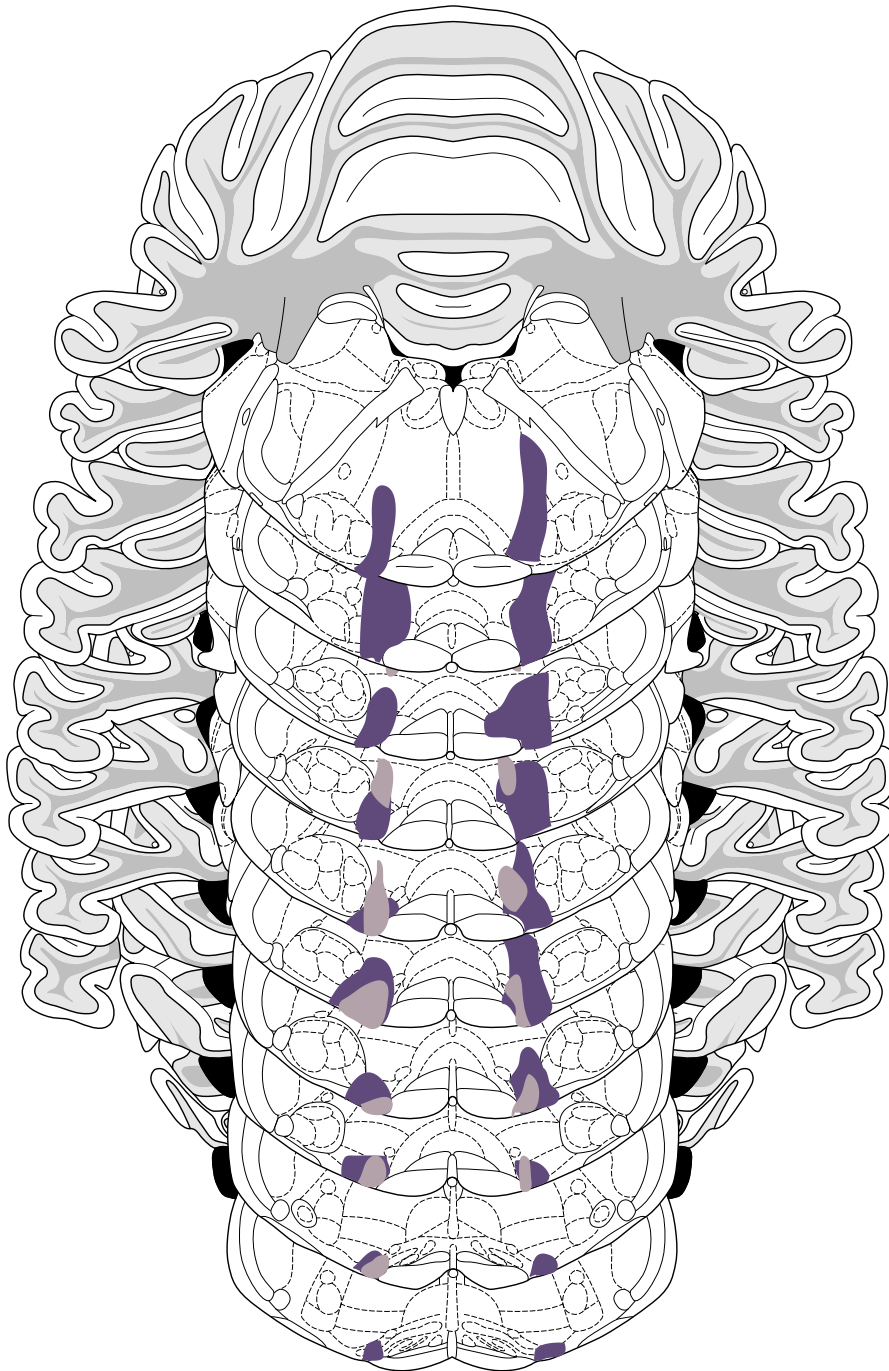
\* = p<0.05 within-group comparison, † = p<0.05 between-group comparison





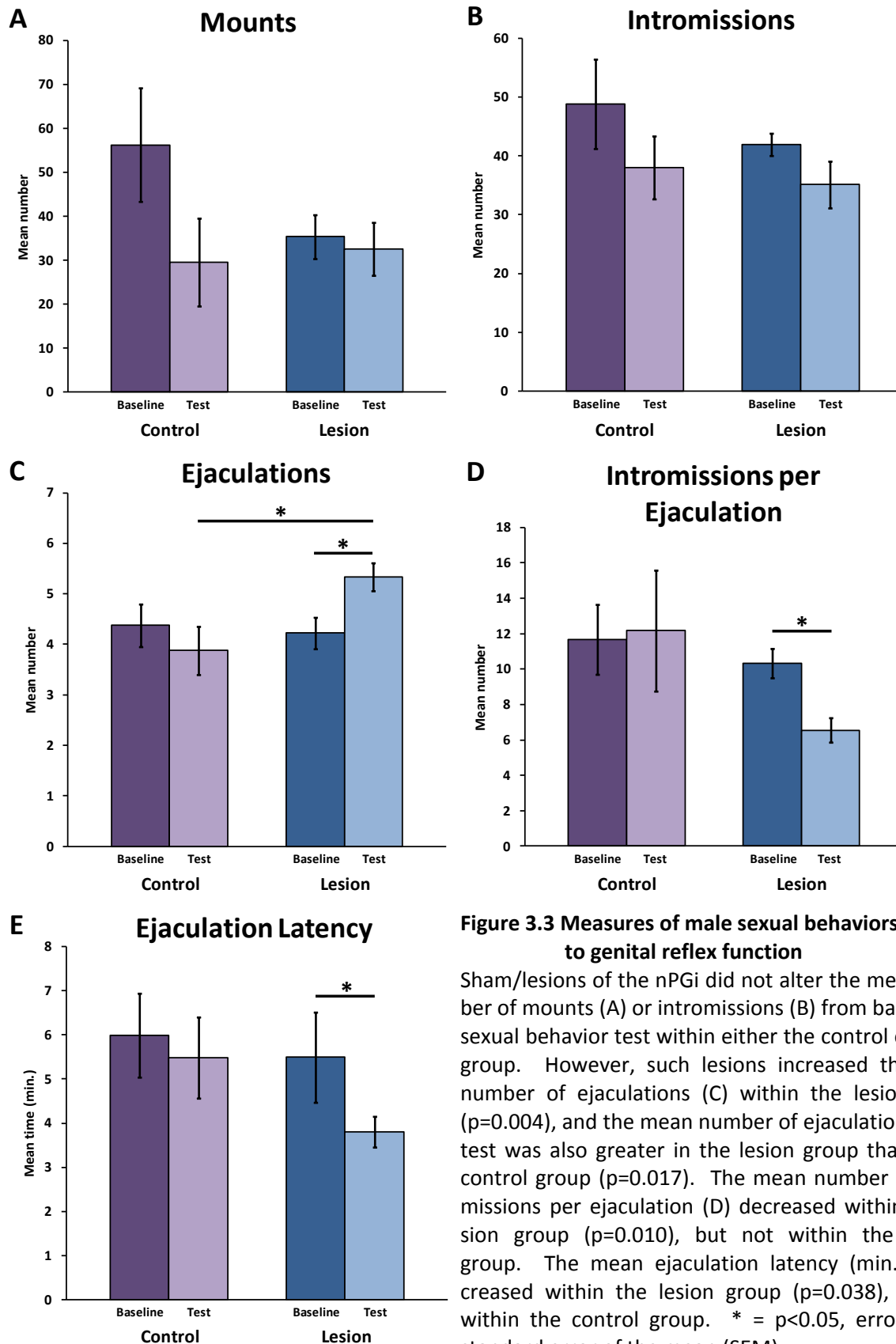
**Figure 3.1 Photomicrograph of sham lesion and lesion sites**

Photomicrograph of Neuronal Nuclei (NeuN) immunolabeled cells of the brainstem. Intra-nucleus paragigantocellularis (nPGi) injection of vehicle (left) preserved NeuN immunoreactivity in the nPGi. Intra-nPGi injection of N-methyl-D-aspartate (right) markedly reduced immunoreactivity for NeuN in the nPGi. nPGi=nucleus paragigantocellularis, py=pyramidal tract, 4V=4<sup>th</sup> ventricle



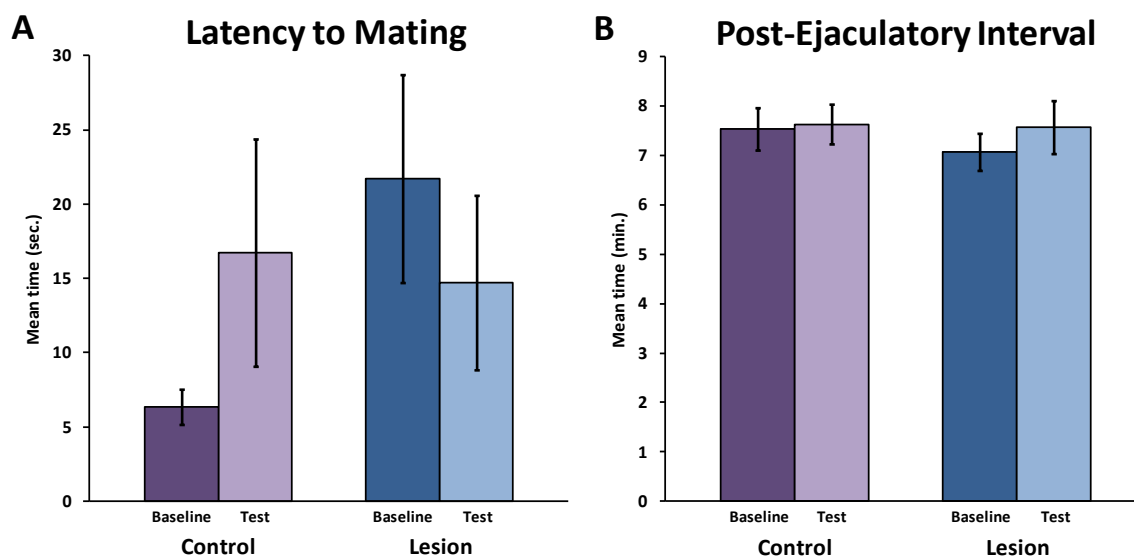
**Figure 3.2 Diagram of lesion sites**

Diagram of the extent of the largest lesion (dark purple) and smallest lesion (light purple) in animals considered to have appropriate nPGi lesions and who were subsequently used in behavioral analysis. Modified from Paxinos and Watson (2005).



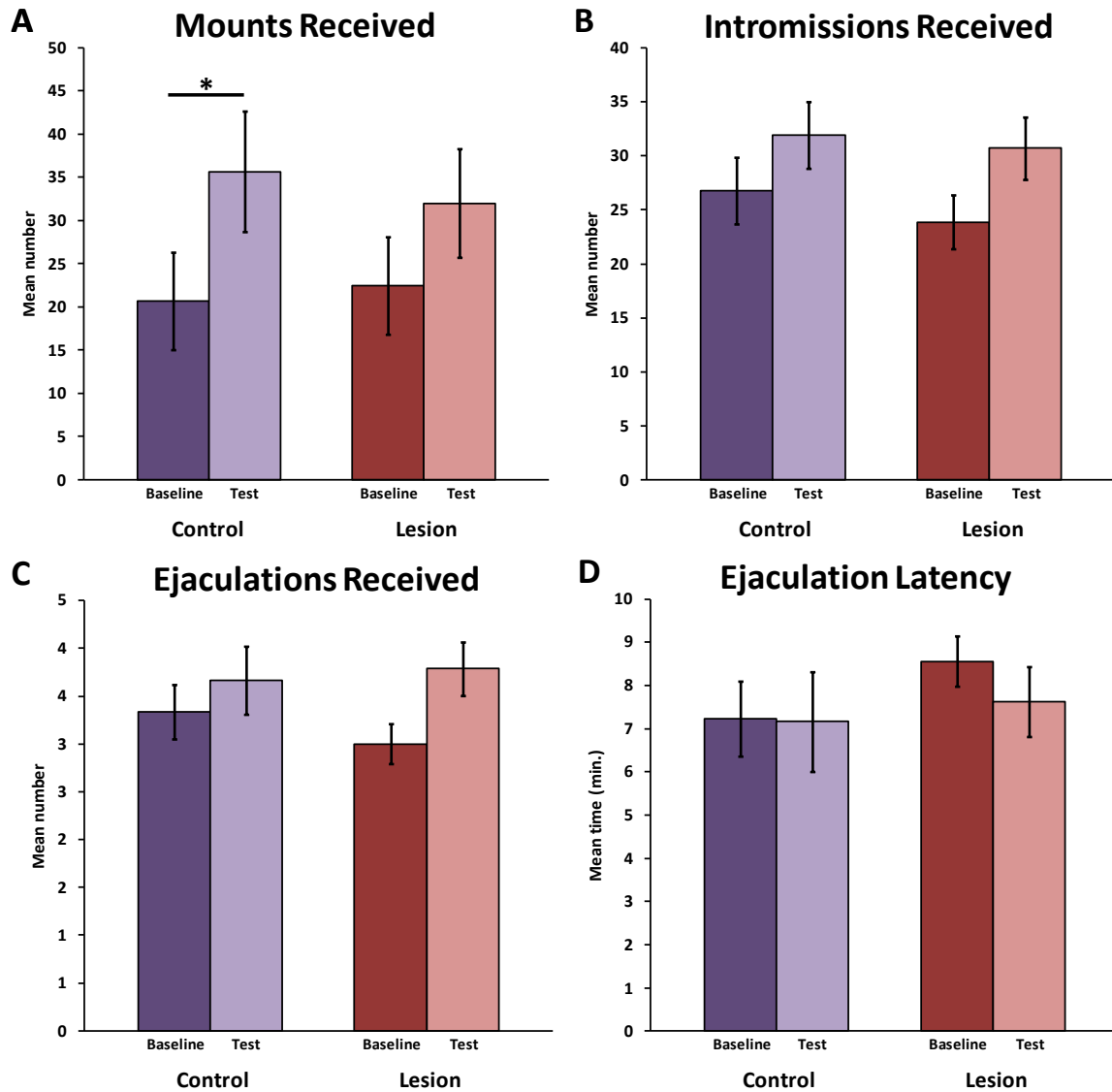
**Figure 3.3 Measures of male sexual behaviors related to genital reflex function**

Sham/lesions of the nPGi did not alter the mean number of mounts (A) or intromissions (B) from baseline to sexual behavior test within either the control or lesion group. However, such lesions increased the mean number of ejaculations (C) within the lesion group ( $p=0.004$ ), and the mean number of ejaculations in the test was also greater in the lesion group than in the control group ( $p=0.017$ ). The mean number of intromissions per ejaculation (D) decreased within the lesion group ( $p=0.010$ ), but not within the control group. The mean ejaculation latency (min.; E) decreased within the lesion group ( $p=0.038$ ), but not within the control group. \* =  $p<0.05$ , error bars = standard error of the mean (SEM).

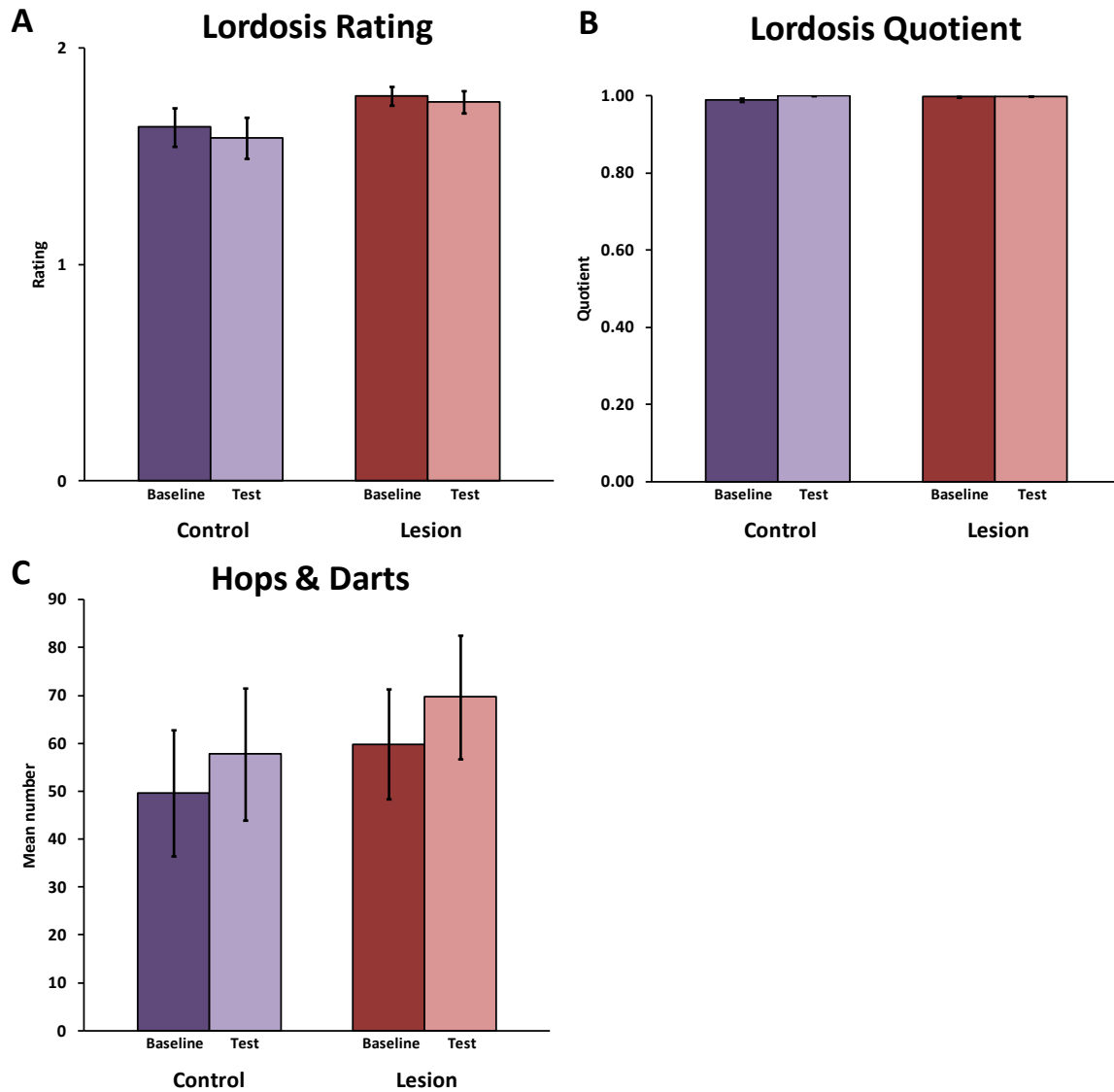


**Figure 3.4 Measures of male sexual behavior unrelated to genital reflex function**

Sham/lesions of the nPGi did not alter the latency to mating (A) or post-ejaculatory interval (B) from baseline to sexual behavior test within either the control or lesion group. Error bars = SEM.

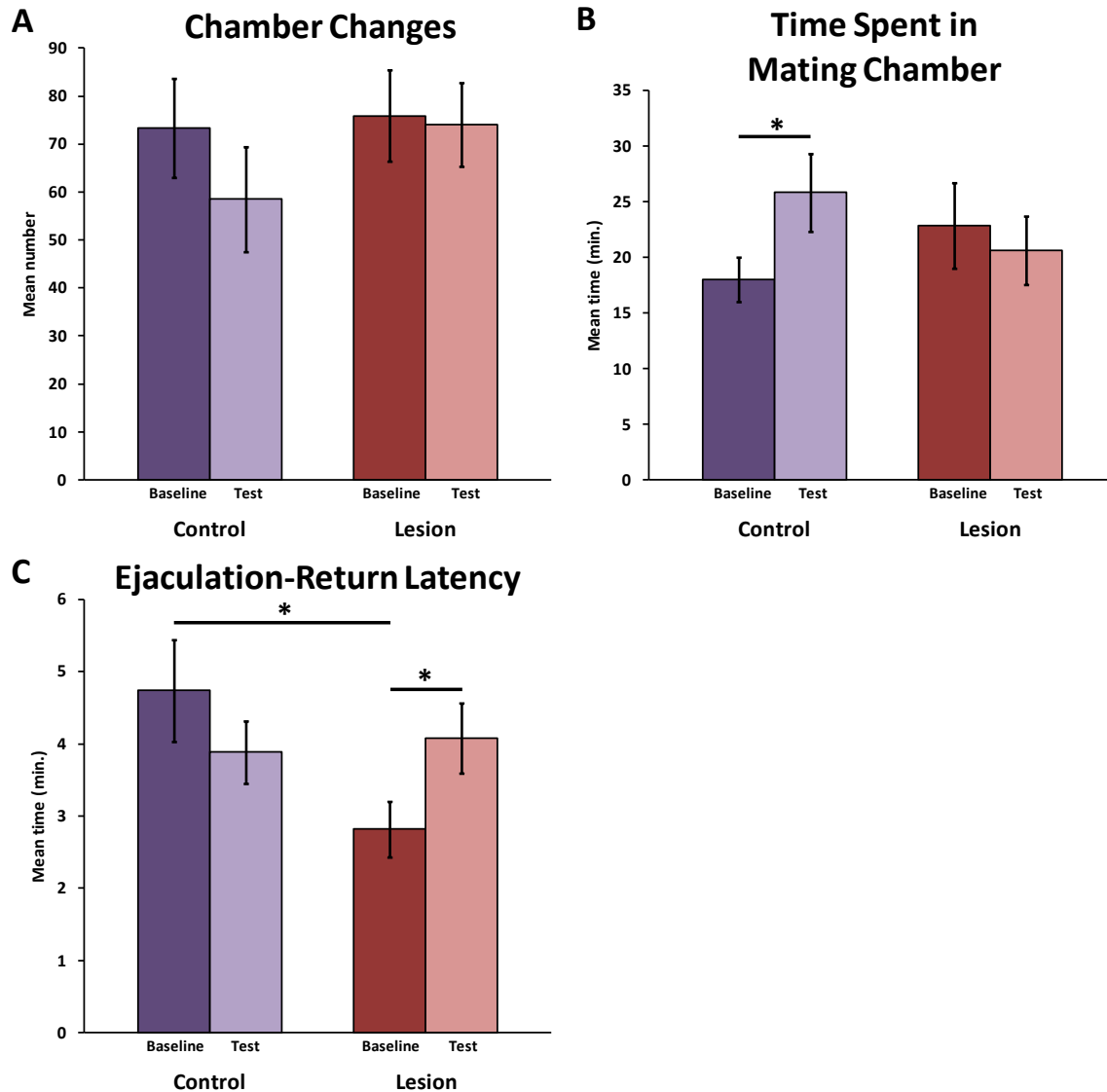


**Figure 3.5 Measures of stimulus males' sexual behavior during mating with experimental females**  
 Sham/lesions of the nPGi did not alter the mean number of mounts received (A), intromissions received (B), ejaculations received (C), or mean ejaculatory latency (D) from baseline to sexual behavior test with- in either the control or lesion group. \* =  $p < 0.05$ , error bars = SEM.



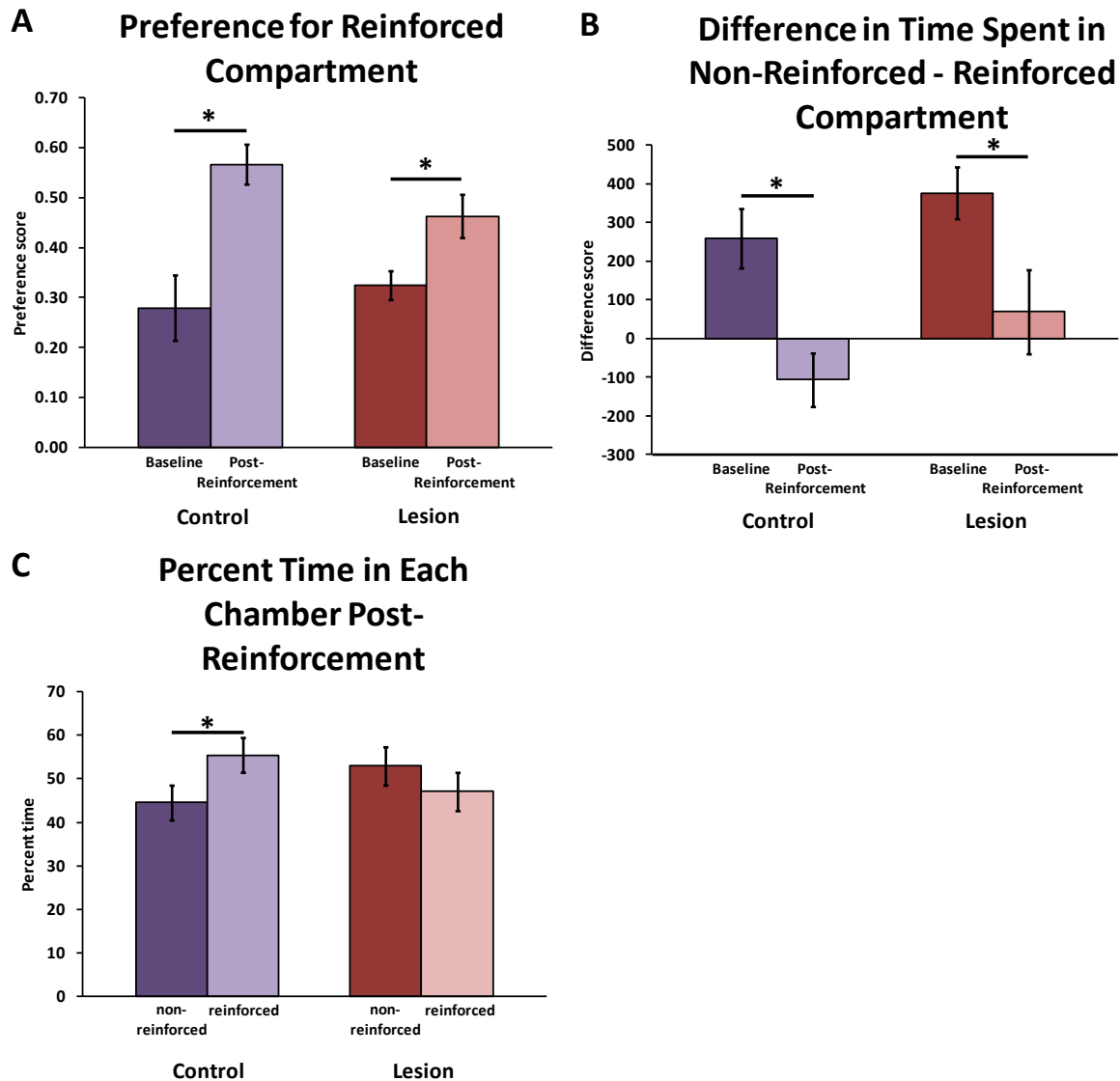
**Figure 3.6 Measures of female receptive and proceptive sexual behavior**

Sham/lesions of the nPGi did not alter the lordosis rating (A), lordosis quotient (B), or the mean number hops and darts (C) from baseline to sexual behavior test within either the control or lesion group. Error bars = SEM.



**Figure 3.7 Measures of female paced-mating behaviors during sexual behavior**

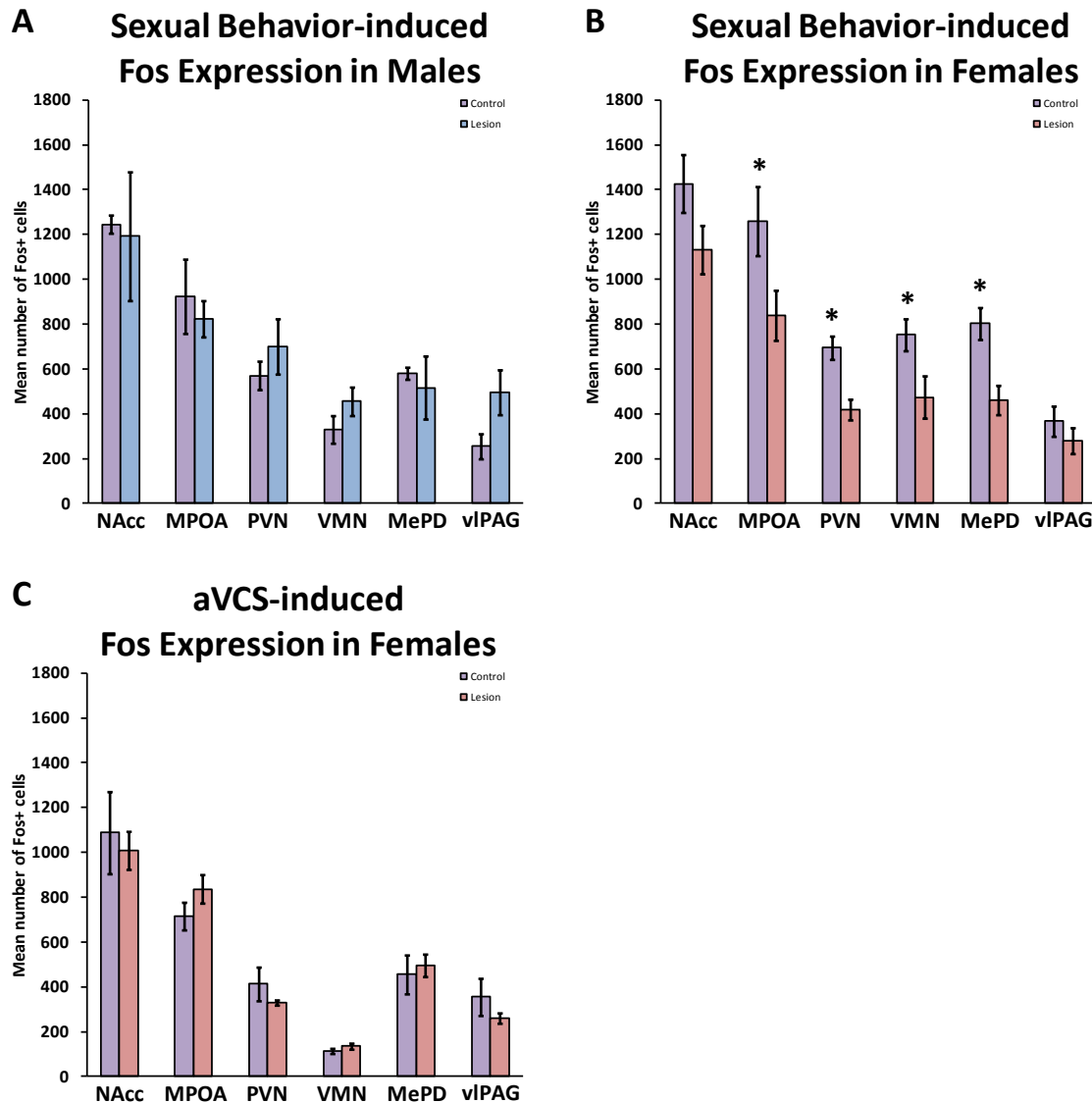
Sham/lesions of the nPGi did not alter the number of chamber changes (A) from baseline to sexual behavior test within either the control or lesion groups. The time spent in the mating chamber (B) increased within the control group ( $p=0.041$ ) but not within the lesion group. The ejaculation return latency (C) increased within the lesion group ( $p=0.037$ ) but not within the control group, although the ejaculation return latency at baseline was higher in the control group than in the lesion group ( $p=0.020$ ). \* =  $p<0.05$ , error bars = SEM.



**Figure 3.8 Measures of condition placed preference for aVCS in females**

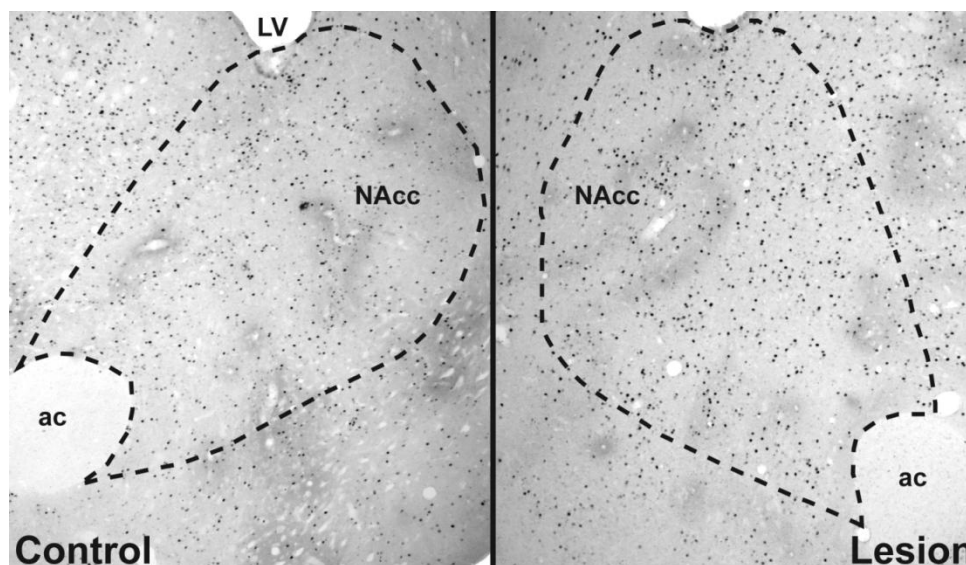
Both the control and lesion groups had a significant increase in the preference score for the reinforced compartment (A) from baseline to the post-reinforcement test (control:  $p=0.009$ , lesion:  $p=0.006$ ). There was also a significant decrease in the difference score (B) within both the control and lesion groups (control:  $p=0.010$ , lesion:  $p=0.015$ ). The percent time spent in each chamber post-reinforcement (C) was higher in the reinforced compartment than the non-reinforced compartment within the control group ( $p=0.036$ ) but not within the lesion group. \* =  $p<0.05$ , error bars = SEM.





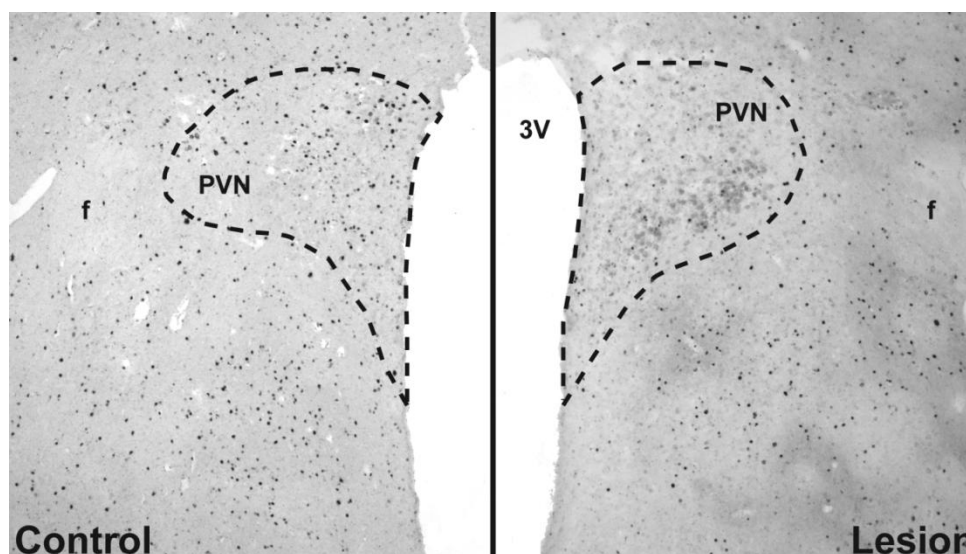
**Figure 3.9 Sexual behavior- and aVCS-induced Fos expression in male and female rats**

There were no significant differences in sexual behavior-induced Fos expression in the brain regions examined in male rats (A) as a result of nPGi lesions. In female rats, nPGi lesions produced a significant reduction in sexual behavior-induced Fos expression (B) in the medial preoptic area (MPOA;  $p=0.040$ ), paraventricular hypothalamic nucleus (PVN;  $p=0.001$ ), ventromedial hypothalamic nucleus (VMN;  $p=0.029$ ), and posterodorsal medial amygdala (MePD;  $p=0.012$ ). Artificial vaginocervical stimulation (aVCS)-induced Fos expression in female rats within the brain regions examined did not differ as a function of nPGi lesions (C). NAcc=nucleus accumbens, vIPAG=ventrolateral periaqueductal gray, \* =  $p<0.05$ , error bars = SEM.



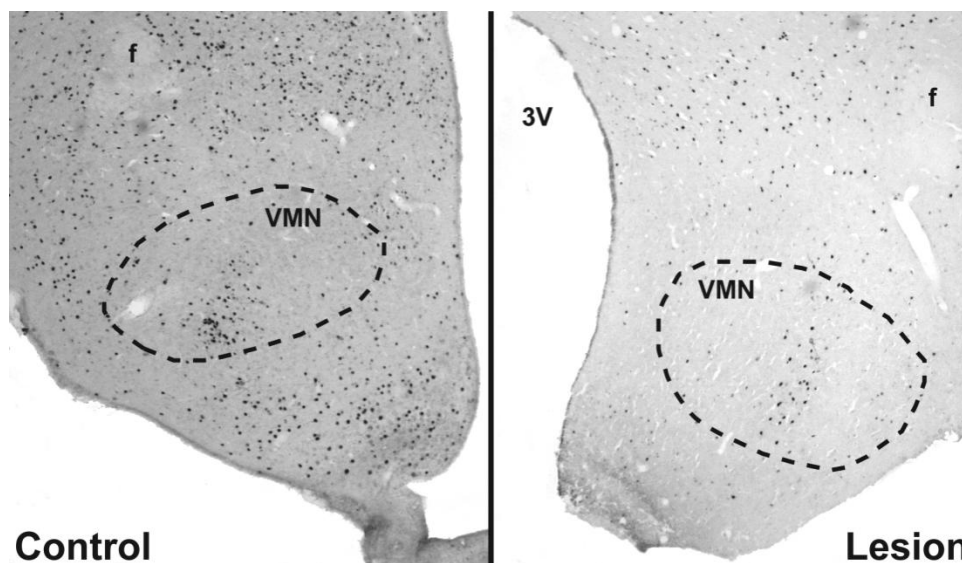
**Figure 3.10 Photomicrographs of sexual behavior-induced Fos immunoreactivity in the nucleus accumbens of a control and lesion female rat**

Photomicrographs of sexual behavior-induced Fos immunoreactivity in the nucleus accumbens (outlined) of a control (left) and lesion (right) female rat reveal no difference in the number of Fos+ cells as a result nPGi lesions. LV=lateral ventricle, NAcc=nucleus accumbens, ac=anterior commissure.



**Figure 3.11 Photomicrographs of sexual behavior-induced Fos immunoreactivity in the paraventricular hypothalamic nucleus of a control and lesion female rat**

Photomicrographs of sexual behavior-induced Fos immunoreactivity in the paraventricular nucleus (outlined) of a control (left) and lesion (right) female rat reveal that nPGi lesions reduce Fos immunoreactivity in the PVN. 3V=third ventricle, PVN=paraventricular nucleus, f=fornix.



**Figure 3.11 Photomicrographs of sexual behavior-induced Fos immunoreactivity in the ventromedial hypothalamic nucleus of a control and lesion female rat**

Photomicrographs of sexual behavior-induced Fos immunoreactivity in the ventromedial nucleus (outlined) of a control (left) and lesion (right) female rat reveal that nPGi lesions reduce Fos immunoreactivity in the VMN. 3V=third ventricle, VMN=ventromedial nucleus, f=fornix.

## **4 SEROTONERGIC LESIONS OF THE MIDBRAIN SOURCE OF SEROTONIN TO THE NUCLEUS PARAGIGANTOCELLULARIS FACILITATE SEXUAL BEHAVIOR IN MALE RATS**

### **4.1 Introduction**

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of drugs for individuals with the clinical diagnosis of depression (Arroll et al., 2009; Koenig and Thase, 2009). SSRIs increase synaptic availability of serotonin (5-HT) by blocking reuptake its reuptake at the axon terminal (Hiemke and Hartter, 2000; Richelson, 1994). A well-documented side effect of SSRI treatment is sexual dysfunction (Kennedy and Rizvi, 2009; Schweitzer et al., 2009), with symptoms of decreased libido, premature ejaculation, delayed ejaculation, and anorgasmia most commonly reported.

The interaction between 5-HT and the neural circuitry underlying mammalian sexual behavior is not completely understood, although experiments in the male rat have suggested that serotonin can either inhibit or facilitate sexual behavior, depending on the brain site of administration and they type of receptor targeted (Bitran and Hull, 1987; de Jong et al., 2006). For example, systemic administration of 5-HT or 5-hydroxytryptophan (a precursor to 5-HT synthesis) to male rats increases ejaculation latency (Ahlenius and Larsson, 1991; Gonzales et al., 1982), whereas the 5-HT synthesis inhibitor p-chlorophenylalanine decreases ejaculation latency (McIntosh and Barfield, 1984). In addition, general increases in 5-HT levels through chronic systemic administration of an SSRI produce delayed ejaculation in male rats (Ahlenius et al., 1980; de Jong et al., 2005a; Mos et al., 1999; Vega Matuszczyk et al., 1998) much like in humans (Hsu and Shen, 1995). In these studies, 5-HT was administered or manipulated systematically; therefore the specific brain region(s) mediating the effects of 5-HT on sexual behavior are not known.

The nucleus paragigantocellularis (nPGi) of the rostroventrolateral medulla in rats, and the homologous structure in humans, the nucleus paragigantocellularis lateralis (Zec and Kinney, 2001), is the hypothesized site of descending inhibition of genital reflexes in both rats (Marson and McKenna, 1990)

and humans (Johnson, 2006). The nPGi receives projections from upstream sites related to sexual behavior, including the paraventricular nucleus of the hypothalamus, medial preoptic area, and periaqueductal gray (Murphy and Hoffman, 2001; Murphy et al., 1999a; Normandin and Murphy, 2008) and projects to the spinal motoneurons (pudendal motoneurons) innervating the bulbospongiosus and ischiocavernosus muscles (Hermann et al., 2003; Marson and Carson 3rd, 1999; Marson and McKenna, 1996; Tang et al., 1999), which are critical for ejaculation in male rats (Miura et al., 2001; Pescatori et al., 1993) and humans (Hsu et al., 2004; Shafik et al., 2009).

The rat nPGi and the human homologue contain 5-HT immunoreactive cells (Azmitia and Gannon, 1986; Marson and McKenna, 1992) and application of 5-HT to the spinal targets of the nPGi in male rats blocks the urethro-genital reflex (Marson and McKenna, 1992). Similarly, Clément, et al. (2007) found that in male rats, systemic administration of the SSRI dapoxetine reduced fictive ejaculatory reflexes, which was reversed by lesions of the nPGi. In addition, lesions of the nPGi in male rats block the inhibitory effects of systemic fluoxetine (an SSRI) on sexual behavior (Yells et al., 1994). These studies make it clear that 5-HT acts at the level of the spinal cord to inhibit genital reflexes, and that 5-HT from the nPGi is a necessary antecedent for normal inhibition of genital reflexes. It is unclear, however, whether 5-HT may be acting at the level of the nPGi to produce the observed effects.

The nPGi, contains receptors for 5-HT including the 5-HT<sub>1A</sub> (Thor et al., 1990; 1992), 5-HT<sub>1C</sub> (Hoffman and Mezey, 1989), 5-HT<sub>2A</sub> (Fay and Kubin, 2000; Fonseca et al., 2001), 5-HT<sub>2C</sub> (Fonseca et al., 2001), and 5-HT<sub>3</sub> (Fonseca et al., 2001) subtypes, although only the 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> subtypes are found in abundance (Fonseca et al., 2001; Thor et al., 1990). The primary source of 5-HT to the nPGi is the ventrolateral periaqueductal gray (vLPAG). vLPAG neurons project directly to the nPGi (Normandin and Murphy, 2008) and these cells co-localize with 5-HT (Bago et al., 2002), and sexual behavior-induced Fos (Normandin and Murphy, 2008), suggesting a role for the serotonergic vLPAG-nPGi pathway in male sexual behavior.

The present studies were designed to test the hypothesis that the effects of SSRIs on male sexual behavior are mediated via the nPGi. Our working hypothesis is that removal of the source of 5-HT to the nPGi would alter the expression of genital reflexes but not other aspects of sexual behavior.

## **4.2 Methods and materials**

### **4.2.1 Subjects**

Thirty male Sprague-Dawley Rats (*Rattus Norvegicus*; Charles River; 275-375g) were same-sex double-housed in a temperature-controlled vivarium in reverse light (lights on 7:00pm, off 7:00am) with ad libitum access to food and water. All experiments were approved by the Georgia State Institutional Animal Care and Use Committee, with pain and suffering minimized in accordance with the Committee's policies.

### **4.2.2 Ovariectomy and gonadal-steroid replacement in stimulus females**

All females were ovariectomized and received injections of  $\beta$ -Estradiol-3-Benzate (10ug/0.1ml sesame oil s.c.; Sigma Aldrich) 48 hours before testing and Progesterone (500ug/0.1ml sesame oil s.c.; Fluka) 4 hours before testing, to induce sexual receptivity (Barfield and Lisk, 1970; McEwen et al., 1987; Quadagno et al., 1972).

### **4.2.3 vIPAG 5-HT lesions**

All surgeries were performed under aseptic conditions. Male rats (n=30) were anesthetized by inhalation of Isoflurane (2-5%; Henry Schein) and placed in a stereotaxic frame. The skull was leveled such that Bregma and Lambda were at the same dorsoventral plane, and a small hole was drilled above our region of interest. Thirty minutes prior to the introduction of the serotonergic neurotoxin, desipramine hydrochloride (25mg/kg in 0.9% Saline i.p.; Sigma) was administered to block uptake of the 5-HT neurotoxin by noradrenergic transporters on noradrenergic cells (Bjorklund et al., 1975). vIPAG serotonergic-specific lesions were produced by bilateral injection of 5,7-dihydroxytryptamine creatine sulfate (5,7-DHT; 3mg/ml in 0.9% Saline 0.1% Ascorbic Acid; Fluka; n=22 males) using a 1 $\mu$ l Hamilton syringe.

Sham animals received equivolume injections of vehicle (0.9% Saline 0.1% Ascorbic Acid; n=8 males). The coordinates for the vIPAG were (in mm): AP-8.5 Bregma, ML +/-0.75, DV-5.75. 5,7-DHT or vehicle (300nl/side) was slowly injected over 2 minutes and the syringe was left in place for 10 min. before being slowly removed. A small amount of bone wax was placed in the burr-hole and the animals' skin was wound-clipped. Animals received Buprenorphine (0.1mg/kg s.c.; Henry Schein) for pain relief and Baytril (5mg/kg i.m.; Henry Schein) as a prophylactic antibiotic. Animals recovered in clean heated cages before being returned to the housing facility.

#### **4.2.4 Sexual behavior tests**

Sexual behavior tests were conducted in acrylic aquariums (61cm x 30.5cm x 30.5cm). Animals were acclimated to the arena prior to the initiation of the experiment for 10 min. on two consecutive days. Three and six days later, males engaged in 1-hour mating bouts with stimulus females to gain sexual experience. A third mating bout on day nine served as the baseline (pre-lesion) measure. Experimental animals then underwent lesion (or sham lesion) surgery as described above. Following a seven-day recovery, a final mating bout served as the post-lesion measure. All sexual behavior bouts were recorded and the number of mounts, intromissions, and ejaculations, as well as the latency to begin mating, ejaculation latency, and post-ejaculatory interval was scored.

#### **4.2.5 Perfusion / fixation / tissue preparation**

After animals were given a euthanizing dose of SleepAway (0.5ml i.p.; Henry Schein) they were transcardially perfused with 250 ml of 0.9% sodium chloride/2% sodium nitrite, followed 300 ml of 4% paraformaldehyde 2.5% acrolein (Polysciences) in 0.1 M phosphate buffer then 150ml of the sodium chloride/sodium nitrite solution. Following perfusion/fixation, brains were removed and stored at 4°C in 30% sucrose solution until sectioned. Brains were cut into 25µm coronal sections in a 1:4 series through the PAG with a Leica 2000R freezing microtome and stored free-floating in cryoprotectant-antifreeze solution (Watson et al., 1986) at -20°C.

#### **4.2.6 Immunohistochemistry**

Midbrain tissue sectioned at 25 $\mu$ m in a 1:4 series was processed for NeuN or 5-HTP for lesion verification as previously described (Loyd and Murphy, 2006; Murphy and Hoffman, 2001). Briefly, sections were removed from the cryoprotectant solution, rinsed extensively in potassium phosphate buffered saline (KPBS; pH 7.4), and then reacted for 20 minutes in 1% sodium borohydride to remove excess aldehydes. Sections were then incubated in primary antibody solution directed against either NeuN (Millipore, MAB377; monoclonal, raised in mouse; 1:70,000) or 5-HTP (Immunostar, 24446; polyclonal, raised in rabbit; 1:1,000) in KPBS containing 0.1% Triton-X for 1 hour at room temperature followed by 48 hours at 4°C. After primary antibody incubation, tissue was rinsed in KPBS, incubated for 1 hour in biotinylated goat-anti mouse (mouse anti-NeuN primary antibody) or goat anti-rabbit (rabbit anti-5-HTP primary antibody) IgG (Jackson ImmunoResearch) at a concentration of 1:600, rinsed in KPBS, followed by a 1-hour incubation in avidin-biotin peroxidase complex (Vector Labs, ABC Elite Kit PK-6100) at a concentration of 1:10. After rinsing in KPBS and sodium acetate (0.175 M; pH 6.5), NeuN and 5-HTP were visualized as a black reaction product using nickel sulfate intensified 3,3'-diaminobenzidine solution containing 0.08% hydrogen peroxide in sodium acetate buffer. The reaction product was terminated after approximately 15 minutes by rinsing in sodium acetate buffer. Sections were mounted out of saline onto gelatin-subbed slides, air dried overnight, dehydrated in a series of graded alcohols, cleared in HistoClear, and cover-slipped using Permount.

#### **4.2.7 Lesion analysis**

5,7-DHT lesion and vehicle sham lesion sites were verified by microscopic analysis of VIPAG-containing brain sections immunohistochemically stained for NeuN or 5-HTP. Sections were examined by light-microscopy and the position of the bottom of the injection track was noted in the NeuN stained sections, and the absence or presence of 5-HTP was noted in 5-HTP stained sections. Only those injec-



tions centered in the vIPAG and with an absence of 5-HTP immunoreactivity in the vIPAG were considered for analysis.

#### **4.2.8 Statistical analysis**

Planned comparisons of mean sexual behavior within the lesion or sham groups were analyzed with two-tailed paired t-tests. Between group (lesion vs. sham) comparisons of means of sexual behavior were two-tailed two independent sample t-tests. All statistical comparisons were made with the alpha value set at 0.05.

### **4.3 Results**

#### **4.3.1 Lesion Verification**

An example of 5-HTP immunoreactivity in a lesioned animal and sham-lesioned animal is provided in Figure 4.1.

#### **4.3.2 Sexual Behavior**

Table 4.1 summarizes the results of all baseline and sexual behavior tests for the lesion (n=9) and sham-lesion (n=7) groups.

Serotonergic lesions of the vIPAG did not affect behavior not directly related to genital reflex function. For example, there was no change from baseline in the mean latency to initiate copulation, or post-ejaculatory interval within either group (Figure 4.2). By contrast, serotonergic lesions of the vIPAG facilitated male sexual behavior in many measures related to genital reflex functions. While there was no change from baseline in the mean number of mounts or intromissions within either group (Figure 4.2A & B), there was an increase from baseline in the mean number of ejaculations within the lesion group ( $p=0.005$ ) but not the sham group ( $p=1.000$ ). In addition, the mean number of ejaculations in the sexual behavior test was significantly greater in the lesion group than in the sham group ( $p=0.039$ , Figure 4.3C). There was a decrease from baseline in the mean number of intromissions required for ejaculation within both groups (lesion:  $p=0.004$ ; sham:  $p=0.039$ ; Figure 4.2D). There was a trend approaching

statistical significance for a decrease from baseline in the mean ejaculation latency within the lesion group ( $p=0.054$ ; Figure 4.2E), and this trend is supported by the significantly shorter mean ejaculation latency in the sexual behavior test in the lesion group versus the sham group ( $p=0.026$ ; Figure 4.2E).

#### 4.4 Discussion

Overall, serotonergic lesions of the vIPAG in male rats produced a facilitation of sexual behavior. In particular, 5-HT lesions of the vIPAG significantly increased the number of ejaculations, and there was a trend for a decrease in the mean latency to ejaculation. 5-HT vIPAG lesioned animals also had significantly shorter ejaculation latencies. There were no differences in the mean latency to mate, or in the post-ejaculatory interval for either group indicating that the effect of serotonergic vIPAG lesions is limited to those behaviors directly associated with genital reflexes.

The facilitation of sexual behavior we observed was specific to those measures associated with genital reflex function, and mirrors the effects seen with lesions of the nPGi (Liu and Sachs, 1999; Yells et al., 1992). Given the anatomical connections between vIPAG 5-HT cells and the nPGi (Bago et al., 2002; Normandin and Murphy, 2008; Underwood et al., 1999), as well as the concordance of effects in both vIPAG 5-HT lesions and nPGi lesions, it is likely that the effects observed here are dependent on vIPAG 5-HT neurotransmission to the nPGi. In the context of a normally behaving animal, one would expect that 5-HT from the vIPAG serves to enhance nPGi activity, thereby increasing descending inhibition of genital reflexes. This serotonergic signal from the vIPAG would be particularly active in behavioral contexts where sexual behavior would not be appropriate (e.g. a “fight-or-flight” response) and likely “de-activated” when mating conditions were optimal (e.g. availability of a sexually receptive conspecific in the absence of a predator). This serotonergic vIPAG-nPGi pathway may effectively act as a gating mechanism for descending inhibition of genital reflexes.

Regulation of nPGi function (and thereby genital reflexes) through vIPAG is an interesting prospect as midbrain periaqueductal gray (PAG) cells are known to be involved in the coordination of cardi-

ovascular responses to anxiety and stress (Johnson et al., 2004; Moraes et al., 2008; Murphy et al., 1995), nociception (Haghighparast and Ahmad-Molaei, 2009; Loyd et al., 2007), as well as social behaviors (Lonstein and Stern, 1998; Pavesi et al., 2007), and could therefore signal the current behavioral context to the nPGi. With respect to sexual behavior, we have previously found that the medial preoptic area (MPOA) of the hypothalamus, a region critical in the expression of male sexual behavior (Arendash and Gorski, 1983; Malsbury, 1971) including genital reflexes (Giuliano et al., 1996; Marson and McKenna, 1994), provides input to the nPGi both directly (Normandin and Murphy, 2008) and through a PAG relay (Murphy and Hoffman, 2001) which may also provide necessary input to the nPGi regarding sexual “tone.” Indeed electrolytic lesions of the PAG, including the vIPAG, blocks normal MPOA-elicited bulbospongiosus contractions (Marson, 2004), suggesting that the MPOA-vIPAG-nPGi circuit is required for the elicitation of genital reflexes.

The medially adjacent dorsal raphe nucleus (DR) also contains serotonergic neurons that may have been affected by our lesions. However, results from previous work suggest that our observed findings are primarily the result of vIPAG lesions, and not due to the spread of the toxin into the DR. For example, in male rats, lesions of the DR, or application of 5-HT or 5-HT agonists to the DR have no effect on male ejaculatory behavior (Albinsson et al., 1996; Fernandez-Guasti et al., 1992; Hillegaart, 1991), although one study has reported that 5-HT DR manipulations shortened both the latency to ejaculate and the post-ejaculatory interval (McIntosh and Barfield, 1984). Interestingly, lesions of the DR also have been found to facilitate lordosis in male rats treated with estradiol (Kakeyama and Yamanouchi, 1992), leading to speculation that the DR is not involved in male typical sexual behavior per se, but rather inhibits female-typical behavior in male rats.

#### **4.5 Conclusions**

Serotonergic lesions of the vIPAG in male rats facilitated genital reflex function in a manner similar to nPGi lesions. This result lends evidence to the functional serotonergic connectivity between the

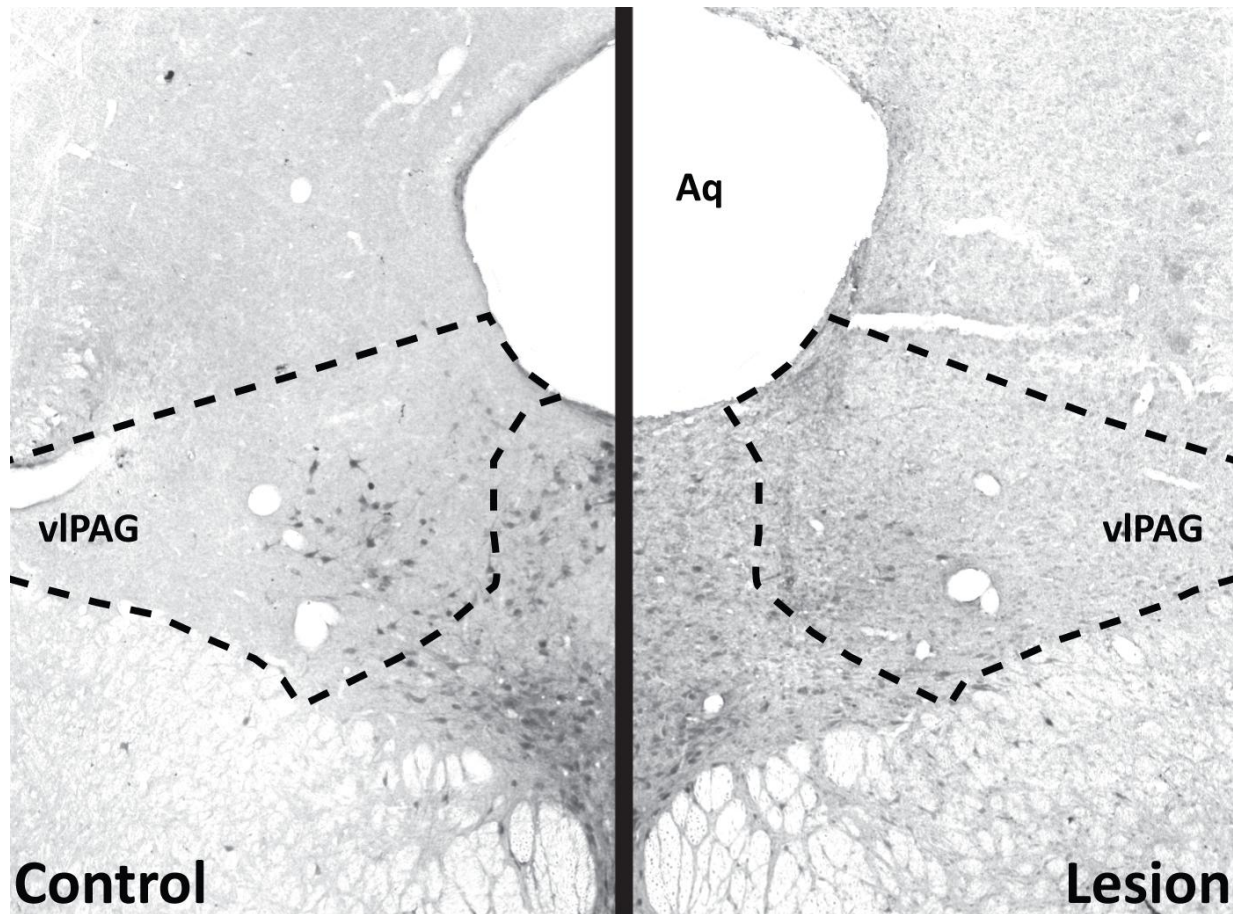
vIPAG and nPGi. This work has implications for the occurrence of SSRI-induced delayed ejaculation in human males, and in particular, suggests that these drugs may be producing an effect by increasing serotonergic neurotransmission within the vIPAG-nPGi pathway. Limiting serotonergic drugs for mood disorders in humans to receptors and loci that influence mood regulation systems, but not sexual behavior systems is an emerging area of research (Baldwin et al., 2006; Breuer et al., 2008; Kennedy and Rizvi, 2010). While it has been known that SSRIs can inhibit genital reflexes in male rats at the level of the spinal cord (Marson and McKenna, 1992), our work implies that this could also occur through the nPGi via vIPAG 5-HT input. Thus, in developing treatments for depression and anxiety, clinicians must consider both the spinal and supraspinal targets of serotonergic drugs in this genital reflex circuitry. In addition, SSRIs are often prescribed to men experiencing premature ejaculation (Hatzimouratidis et al., 2010; Hellstrom, 2009). A global increase in 5-HT is neither warranted nor desired in these patients, as other side effects may be incurred (Haddad and Dursun, 2008; Hellstrom, 2009) as a result of treatment. Targeting the serotonergic vIPAG pathway to the nPGi with appropriately specific drugs may be one way to circumvent global treatment of these patients with SSRIs. However, it remains to be elucidated which 5-HT receptor subtypes on nPGi cells are responsible for modulating nPGi activity.

**Table 4.1 Sexual behavior measures (mean +/- standard error of the mean) in sham group and lesion group males**

	Sexual Behavior							
	Mounts		Intromissions		Ejaculations		Intromissions pre Ejaculation	
	Baseline	Test	Baseline	Test	Baseline	Test	Baseline	Test
Sham	61.43 +/- 12.71	38.57 +/- 10.04	59.71 +/- 7.39	41.29 +/- 5.20	4.14 +/- 0.40	4.14 +/- 0.51	15.04 +/- 2.48	10.74 +/- 1.55 *
Lesion	44.11 +/- 14.03	21.00 +/- 2.68	45.22 +/- 5.64	46.56 +/- 4.31	3.56 +/- 0.56	5.56 +/- 0.38 * †	14.35 +/- 1.69	5.68 +/- 0.98 *

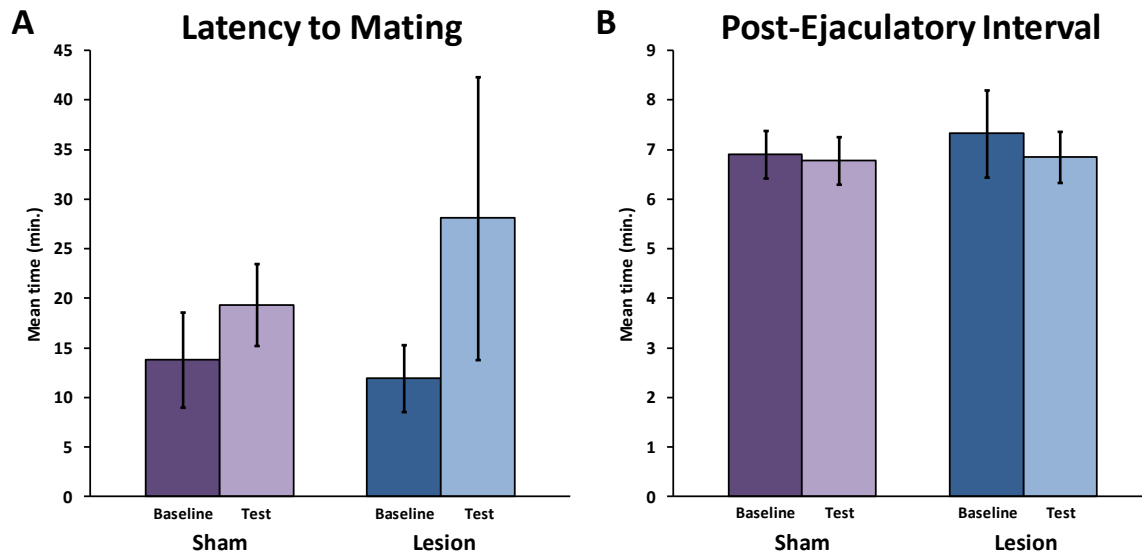
	Sexual Behavior					
	Ejaculation Latency		Post-Ejaculatory Interval		Latency to Mating	
	Baseline	Test	Baseline	Test	Baseline	Test
Sham	7.37 +/- 1.33	5.95 +/- 0.77	6.90 +/- 0.47	6.78 +/- 0.47	12.82 +/- 4.83	19.36 +/- 4.11
Lesion	8.92 +/- 2.53	3.83 +/- 0.46 †	7.32 +/- 0.87	6.85 +/- 0.51	11.93 +/- 3.39	28.08 +/- 14.29

\* = p<0.05 within-group comparison, † = p<0.05 between-group comparison



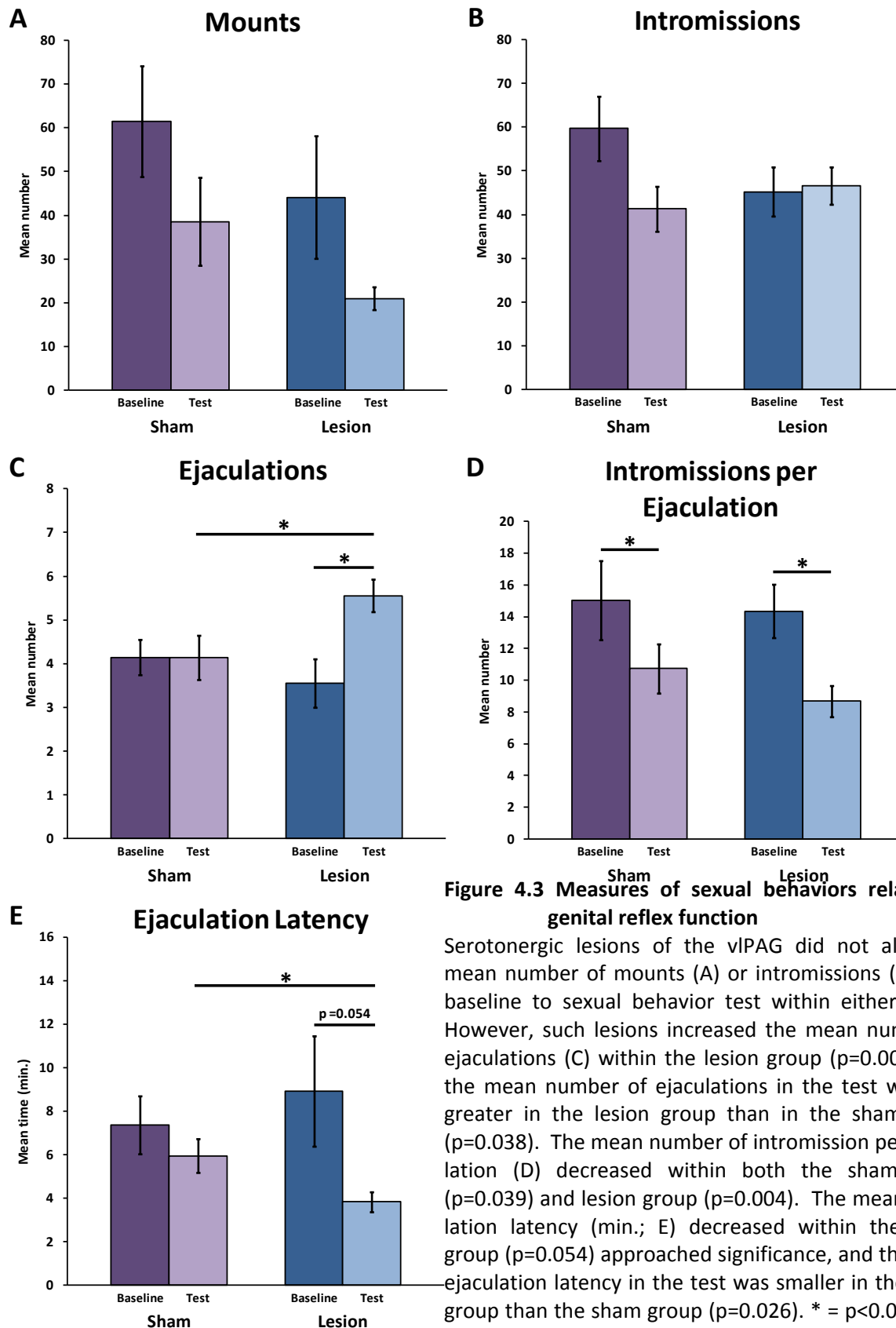
**Figure 4.1 Photomicrograph of sham lesion and lesion sites**

Photomicrograph of 5-hydroxytryptophan (5-HTP) immunolabeled cells of the ventrolateral periaqueductal gray (vIPAG) and dorsal raphe. Intra-vIPAG injection of vehicle (left) preserved 5-HTP immunoreactivity in the vIPAG. Intra-vIPAG injection of 5,7-dihydroxytryptamine (right) markedly reduced immunoreactivity for 5-HTP in the vIPAG. Aq=cerebral aqueduct.



**Figure 4.2 Measures of sexual behavior unrelated to genital reflex function**

Serotonergic lesions of the vPAG did not alter the latency to mating (A) or post-ejaculatory interval (B) from baseline to sexual behavior test within either the sham or lesion group. Error bars = standard error of the mean (SEM).





## 5 GENERAL CONCLUSIONS

The general aim of this dissertation was to gain more information regarding how the nucleus paraventricularis (nPGi) provides descending inhibition of genital reflexes in male and female rats. To this end, Experiment 1 produced a comprehensive description of nPGi afferents in both sexes with respect to distribution, number, co-localization with gonadal steroids, co-localization with sexual behavior-induced Fos expression, and any sex differences therein. Experiment 2 revealed that the nPGi is an important regulator of ejaculatory reflexes in males, and regulates the quality of the mating experience in females. Lastly, Experiment 3 provided evidence that the source of serotonin (5-HT) to the nPGi influences how the nPGi regulates genital reflexes in male rats.

Several regions provide input to the nPGi in male and female rats as evidenced by our retrograde tract tracing. Within this group of nPGi afferents we found that only a minority provide robust input to the nPGi and co-localized with sexual behavior-induced Fos expression, including the medial preoptic area (MPOA), paraventricular nucleus of the hypothalamus (PVN), perifornical nucleus (PeF), and periaqueductal gray (PAG). Furthermore, we found sexual dimorphisms in the number of cells, percentage of co-localization with sexual behavior-induced Fos, and co-localization with estrogen receptor alpha ( $ER_{\alpha}$ ) or androgen receptor (AR) within some regions. There were a greater number of nPGi-projecting caudal MPOA cells in males than in females and these cells co-localized with Fos to a greater degree in males than in females. There were also a greater number of nPGi-projecting cells that co-localized with AR, but not  $ER_{\alpha}$ , in the MPOA of the male than in that of the female. Both sexes had a large percentage of nPGi-projecting PVN and PeF cells that co-localized with sexual behavior-induced Fos. Within the PAG, there were a greater number of nPGi-projecting cells in the dorsomedial subdivision in females than in males, but no such difference in other PAG subregions.

The information gained from this work provides insight into how nPGi activity (and thereby genital reflexes) might be regulated in each sex. Certainly, the large number and high degree of co-

localization of nPGi-projecting MPO cells in male rats belies its importance to the regulation of genital reflexes in males, but this region may play a different role in females. We know that lesions of the MPOA produce profound deficits in male sexual behavior (Arendash and Gorski, 1983), and this could, in part, be occurring through an MPO-nPGi pathway. Our work suggests that the regulation of genital reflexes by the MPOA in females occurs through a separate MPOA-nPGi pathway than in males. Conversely, the greater number of nPGi-projecting dorsomedial PAG cells in females indicates that this region might be important for the regulation of genital reflexes in females. PAG cells in females could be signaling an arousal state to the nPGi that responds to “fight-or-flight” cues. Both the PVN and PeF seem to be important in both sexes. The PVN contains the so-called “social neuropeptides” oxytocin and vasopressin (Argiolas, 1999) which could be modulating sexual responses through the nPGi. The PeF contains the neuropeptide orexin (Nambu et al., 1999) which has been linked to the expression of motivated behaviors (Sweet et al., 1999). The information that PeF provides to the nPGi may be a critical component of nPGi regulation as orexin appears to be important for male rat sexual behavior (Muschamp et al., 2007). All of the regions described can be modulated by gonadal steroids as evidenced by co-localization with gonadal steroid receptors. In both males and females, the hormonal milieu is therefore critical in expression of genital reflexes through the nPGi. The sexually-dimorphic projections to the nPGi that we describe also provide targets for understanding how such networks may be dysregulated in people with sexual dysfunction, and this is likely different between men and women.

When the nPGi was lesioned while sparing fibers-of-passage, males had more ejaculations per mating bout, and these ejaculations occurred faster and with lesser stimulation. In females, nPGi lesions altered their pace of mating, where the females spent less time in contact with stimulus males, and sexual behavior-induced Fos immunoreactivity was decreased in the MPOA, PVN, ventromedial nucleus of the hypothalamus, and posterodorsal medial amygdala. In addition, females with lesions of the nPGi had attenuated conditioned place preference for artificial vaginocervical stimulation.

From this lesion work, we can conclude that in male rats nPGi neurons, and not fibers-of passage, are responsible for descending inhibition of genital reflexes. Extrapolating from this observation, individual differences in the function of the nPGi could possibly account for observed individual differences in ejaculatory performance in male rats (Pattij et al., 2005). In men, the nPGi could be one site in which delayed or premature ejaculation is produced because of dysregulation of nPGi activity. In females, lesions of the nPGi produced more subtle effects in sexual behavior. The quality of mating appears to be altered for females with nPGi lesions given that they spend less time mating with stimulus males, and formed weaker CPP to vaginocervical stimulation. It is possible that without a functional nPGi, vaginocervical muscular tone could provide aversive or unrewarding feedback during sex, which could account for the observed effects. This work also has implications, at least in rats, in fertility. Vaginocervical feedback is a necessary component for the induction of a hormonal state supportive of pregnancy in rats (Castro-Vasquez and Carreno, 1981). Differences in vaginal muscular tone through individual differences in nPGi function could produce differences in fertility. In women, involuntary vagina spasms, and dyspareunia might also occur because of problems with nPGi regulation.

By removing the source of 5-HT to the nPGi with 5-HT-specific lesions of the ventrolateral PAG (vlPAG) in male rats we were able to facilitate sexual behavior in a manner similar to our lesions of the nPGi in male rats. The number of ejaculations per mating bout was greater in males with vlPAG 5-HT lesions and a reduction in ejaculation latency approached significance.

This work gives us insight into how 5-HT affects the regulation of genital reflexes. 5-HT from the vlPAG to the nPGi presumably provides enhancement of the inhibition of genital reflexes through the nPGi. When disrupted, this enhancement of inhibition is removed, producing facilitation of genital reflexes. This provides another location whereby 5-HT acts to inhibit genital reflexes, exclusive of the spinal cord, as has already been reported (Marson and McKenna, 1992). This work is particularly important with regard to people experiencing sexual dysfunction while taking selective serotonin reuptake inhibi-

tors (SSRIs) for depression and anxiety. The increases in 5-HT as a result of SSRI treatment could be altering nPGi function, and thereby sexual functioning in these individuals.

Taken together this work provides evidence that the nPGi is a critical regulator of genital reflexes in male and female rats. The sites that regulate the nPGi are sexually dimorphic, are active during sexual behavior, and contain receptors for gonadal steroids. The nPGi regulates genital reflexes in males and females differently, and 5-HT is an important modulator of nPGi function. How the brain integrates information to produce sexual behavior is informed by this work, and this work provides important insights into how sexual dysfunction may be occurring in people.

## REFERENCES

- Ackerman AE, Lange GM, Clemens LG. 1997. Effects of paraventricular lesions on sex behavior and seminal emission in male rats. *Physiol Behav* 63(1):49-53.
- Agmo A, Fernandez H. 1989. Dopamine and sexual behavior in the male rat: a reevaluation. *J Neural Transm* 77(1):21-37.
- Ahlenius S, Larsson K. 1991. Opposite effects of 5-methoxy-N,N-di-methyl-tryptamine and 5-hydroxytryptophan on male rat sexual behavior. *Pharmacol Biochem Behav* 38(1):201-205.
- Ahlenius S, Larsson K, Svensson L. 1980. Further evidence for an inhibitory role of central 5-HT in male rat sexual behavior. *Psychopharmacology (Berl)* 68(3):217-220.
- Ahlenius S, Larsson K, Svensson L, Hjorth S, Carlsson A, Lindberg P, Wikstrom H, Sanchez D, Arvidsson LE, Hackzell U, Nilsson JL. 1981. Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. *Pharmacol Biochem Behav* 15(5):785-792.
- Albinsson A, Andersson G, Andersson K, Vega-Matuszczyk J, Larsson K. 1996. The effects of lesions in the mesencephalic raphe systems on male rat sexual behavior and locomotor activity. *Behav Brain Res* 80(1-2):57-63.
- Allard J, Truitt WA, McKenna KE, Coolen LM. 2005. Spinal cord control of ejaculation. *World J Urol* 23(2):119-126.
- Arendash GW, Gorski RA. 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 Bull* 10(1):147-154.
- Argiolas A. 1999. Neuropeptides and sexual behaviour. *Neurosci Biobehav Rev* 23(8):1127-1142.
- Argiolas A, Melis MR. 2003. The neurophysiology of the sexual cycle. *J Endocrinol Invest* 26(3 Suppl):20-22.
- Arroll B, Elley CR, Fishman T, Goodyear-Smith FA, Kenealy T, Blashki G, Kerse N, Macgillivray S. 2009. Antidepressants versus placebo for depression in primary care. *Cochrane Database Syst Rev*(3):CD007954.
- Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT. 1986. The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science* 234(4777):734-737.
- Azmitia EC, Gannon PJ. 1986. The primate serotonergic system: a review of human and animal studies and a report on *Macaca fascicularis*. *Adv Neurol* 43:407-468.
- Bago M, Marson L, Dean C. 2002. Serotonergic projections to the rostroventrolateral medulla from midbrain and raphe nuclei. *Brain Res* 945(2):249-258.
- Baldwin D, Bridgman K, Buis C. 2006. Resolution of sexual dysfunction during double-blind treatment of major depression with reboxetine or paroxetine. *J Psychopharmacol* 20(1):91-96.

- Balthazart J, Absil P, Gerard M, Appeltants D, Ball GF. 1998. Appetitive and consummatory male sexual behavior in Japanese quail are differentially regulated by subregions of the preoptic medial nucleus. *J Neurosci* 18(16):6512-6527.
- Balthazart J, Ball GF. 2007. Topography in the preoptic region: Differential regulation of appetitive and consummatory male sexual behaviors. *Front Neuroendocrinol*.
- Barfield MA, Lisk RD. 1970. Advancement of behavioral estrus by subcutaneous injection of progesterone in the 4-day cyclic rat. *Endocrinology* 87(5):1096-1098.
- Barnett EM, Evans GD, Sun N, Perlman S, Cassell MD. 1995. Anterograde tracing of trigeminal afferent pathways from the murine tooth pulp to cortex using herpes simplex virus type 1. *J Neurosci* 15(4):2972-2984.
- Baskerville TA, Douglas AJ. 2008. Interactions between dopamine and oxytocin in the control of sexual behaviour. *Prog Brain Res* 170:277-290.
- Bellintani-Guardia B, Schweizer M, Herbert H. 1996. Analysis of projections from the cochlear nucleus to the lateral paragigantocellular reticular nucleus in the rat. *Cell Tissue Res* 283(3):493-505.
- Berk ML, Finkelstein JA. 1983. Long descending projections of the hypothalamus in the pigeon, *Columba livia*. *J Comp Neurol* 220(2):127-136.
- Bitran D, Hull EM. 1987. Pharmacological analysis of male rat sexual behavior. *Neurosci Biobehav Rev* 11(4):365-389.
- Bjorklund A, Baumgarten HG, Rensch A. 1975. 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. *J Neurochem* 24(4):833-835.
- Breedlove SM, Arnold AP. 1980. Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. *Science* 210(4469):564-566.
- Breiner SJ. 2004. Male sexual dysfunction. *JAMA* 292(22):2722; author reply 2722-2723.
- Breuer ME, Chan JS, Oosting RS, Groenink L, Korte SM, Campbell U, Schreiber R, Hanania T, Snoeren EM, Waldinger M, Olivier B. 2008. The triple monoaminergic reuptake inhibitor DOV 216,303 has antidepressant effects in the rat olfactory bulbectomy model and lacks sexual side effects. *Eur Neuropsychopharmacol* 18(12):908-916.
- Brozoski TJ, Ciobanu L, Bauer CA. 2007. Central neural activity in rats with tinnitus evaluated with manganese-enhanced magnetic resonance imaging (MEMRI). *Hearing research* 228(1-2):168-179.
- Camacho FJ, Garcia-Horsman P, Paredes RG. 2009. Hormonal and testing conditions for the induction of conditioned place preference by paced mating. *Horm Behav* 56(4):410-415.
- Cameron A, Tomlin M. 2007. The effect of male erectile dysfunction on the psychosocial, relationship, and sexual characteristics of heterosexual women in the United States. *J Sex Marital Ther* 33(2):135-149.
- Canteras NS, Goto M. 1999. Connections of the precommissural nucleus. *J Comp Neurol* 408(1):23-45.

- Cantor JM, Binik YM, Pfaus JG. 1999. Chronic fluoxetine inhibits sexual behavior in the male rat: reversal with oxytocin. *Psychopharmacology (Berl)* 144(4):355-362.
- Carere C, Ball GF, Balthazart J. 2007. Sex differences in projections from preoptic area aromatase cells to the periaqueductal gray in Japanese quail. *J Comp Neurol* 500(5):894-907.
- Carmichael MS, Humbert R, Dixen J, Palmisano G, Greenleaf W, Davidson JM. 1987. Plasma oxytocin increases in the human sexual response. *J Clin Endocrinol Metab* 64(1):27-31.
- Carrive P, Dampney RA, Bandler R. 1987. Excitation of neurones in a restricted portion of the midbrain periaqueductal grey elicits both behavioural and cardiovascular components of the defence reaction in the unanaesthetised decerebrate cat. *Neurosci Lett* 81(3):273-278.
- Castro-Vasquez A, Carreno NB. 1981. A study of the sensory requirements for eliciting the pseudopregnancy response. *Brain Res* 230(1-2):205-220.
- Chen KK, Chan SH, Chang LS, Chan JY. 1997. Participation of paraventricular nucleus of hypothalamus in central regulation of penile erection in the rat. *J Urol* 158(1):238-244.
- Chen Z, Engberg G. 1989. The rat nucleus paragigantocellularis as a relay station to mediate peripherally induced central effects of nicotine. *Neurosci Lett* 101(1):67-71.
- Clark FM, Proudfit HK. 1991. Projections of neurons in the ventromedial medulla to pontine catecholamine cell groups involved in the modulation of nociception. *Brain Res* 540(1-2):105-115.
- Clayton EC, Williams CL. 2000. Posttraining inactivation of excitatory afferent input to the locus coeruleus impairs retention in an inhibitory avoidance learning task. *Neurobiol Learn Mem* 73(2):127-140.
- Clement P, Bernabe J, Gengo P, Denys P, Laurin M, Alexandre L, Giuliano F. 2007. Supraspinal site of action for the inhibition of ejaculatory reflex by dapoxetine. *Eur Urol* 51(3):825-832.
- Collins WF, 3rd, Erichsen JT, Rose RD. 1991. Pudendal motor and premotor neurons in the male rat: a WGA transneuronal study. *J Comp Neurol* 308(1):28-41.
- Consiglio AR, Lucion AB. 1996. Lesion of hypothalamic paraventricular nucleus and maternal aggressive behavior in female rats. *Physiol Behav* 59(4-5):591-596.
- Coolen LM. 2005. Neural control of ejaculation. *J Comp Neurol* 493(1):39-45.
- Coolen LM, Veening JG, Petersen DW, Shipley MT. 2003. Parvocellular subparafascicular thalamic nucleus in the rat: anatomical and functional compartmentalization. *J Comp Neurol* 463(2):117-131.
- Coopersmith C, Candurra C, Erskine MS. 1996. Effects of paced mating and intromissive stimulation on feminine sexual behavior and estrus termination in the cycling rat. *J Comp Psychol* 110(2):176-186.
- Creutz LM, Kritzer MF. 2004. Mesostriatal and mesolimbic projections of midbrain neurons immunoreactive for estrogen receptor beta or androgen receptors in rats. *J Comp Neurol* 476(4):348-362.

- Dail WG, Manzanares K, Moll MA, Minorsky N. 1985. The hypogastric nerve innervates a population of penile neurons in the pelvic plexus. *Neuroscience* 16(4):1041-1046.
- Dayan P, Huys QJ. 2009. Serotonin in affective control. *Annu Rev Neurosci* 32:95-126.
- de Araujo CG, Schmidt RA, Tanagho EA. 1982. Neural pathways to lower urinary tract identified by retrograde axonal transport of horseradish peroxidase. *Urology* 19(3):290-295.
- de Castilhos J, Marcuzzo S, Forti CD, Frey RM, Stein D, Achaval M, Rasia-Filho AA. 2006. Further studies on the rat posterodorsal medial amygdala: dendritic spine density and effect of 8-OH-DPAT microinjection on male sexual behavior. *Brain Res Bull* 69(2):131-139.
- de Geest K, Thiery M, Piron-Possuyt G, Vanden Driessche R. 1985. Plasma oxytocin in human pregnancy and parturition. *J Perinat Med* 13(1):3-13.
- de Jong TR, Pattij T, Veening JG, Dederen PJ, Waldinger MD, Cools AR, Olivier B. 2005a. Citalopram combined with WAY 100635 inhibits ejaculation and ejaculation-related Fos immunoreactivity. *Eur J Pharmacol* 509(1):49-59.
- de Jong TR, Pattij T, Veening JG, Dederen PJ, Waldinger MD, Cools AR, Olivier B. 2005b. Effects of chronic paroxetine pretreatment on (+/-)-8-hydroxy-2-(di-n-propyl-amino)tetralin induced c-fos expression following sexual behavior. *Neuroscience* 134(4):1351-1361.
- de Jong TR, Veening JG, Waldinger MD, Cools AR, Olivier B. 2006. Serotonin and the neurobiology of the ejaculatory threshold. *Neurosci Biobehav Rev* 30(7):893-907.
- Donaldson ZR, Young LJ. 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* 322(5903):900-904.
- Eaton RC, Markowski VP, Lumley LA, Thompson JT, Moses J, Hull EM. 1991. D2 receptors in the paraventricular nucleus regulate genital responses and copulation in male rats. *Pharmacol Biochem Behav* 39(1):177-181.
- Erskine MS. 1992. Pelvic and pudendal nerves influence the display of paced mating behavior in response to estrogen and progesterone in the female rat. *Behav Neurosci* 106(4):690-697.
- Fadel J, Deutch AY. 2002. Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* 111(2):379-387.
- Fathi-Moghaddam H, Kesmati M, Kargar HM. 2006. The effect of paraventricular lateral lesion on conditioned place preference (CPP) in presence or absence of alpha2 adrenergic agonist (clonidine) in male rats. *Acta Physiol Hung* 93(1):33-40.
- Fay R, Kubin L. 2000. Pontomedullary distribution of 5-HT2A receptor-like protein in the rat. *J Comp Neurol* 418(3):323-345.
- Fendt M, Li L, Yeomans JS. 2001. Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology* 156(2-3):216-224.
- Fernandez-Guasti A, Escalante AL, Ahlenius S, Hillegaart V, Larsson K. 1992. Stimulation of 5-HT1A and 5-HT1B receptors in brain regions and its effects on male rat sexual behaviour. *Eur J Pharmacol* 210(2):121-129.



- Fiber JM, Adames P, Swann JM. 1993. Pheromones induce c-fos in limbic areas regulating male hamster mating behavior. *Neuroreport* 4(7):871-874.
- Flanagan LM, Pfau JG, Pfaff DW, McEwen BS. 1993. Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. *Neuroendocrinology* 58(3):352-358.
- Floody OR, Cooper TT, Albers HE. 1998. Injection of oxytocin into the medial preoptic-anterior hypothalamus increases ultrasound production by female hamsters. *Peptides* 19(5):833-839.
- Floody OR, Lisk RD. 1987. Effects of sex and reproductive state on acoustic responsiveness in hamsters. *Brain research bulletin* 18(2):235-244.
- Fonseca MI, Ni YG, Dunning DD, Miledi R. 2001. Distribution of serotonin 2A, 2C and 3 receptor mRNA in spinal cord and medulla oblongata. *Brain Res Mol Brain Res* 89(1-2):11-19.
- Freund-Mercier MJ, Moos F, Poulain DA, Richard P, Rodriguez F, Theodosis DT, Vincent JD. 1988. Role of central oxytocin in the control of the milk ejection reflex. *Brain Res Bull* 20(6):737-741.
- Gardier AM, Malagie I, Trillat AC, Jacquot C, Artigas F. 1996. Role of 5-HT<sub>1A</sub> autoreceptors in the mechanism of action of serotonergic antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam Clin Pharmacol* 10(1):16-27.
- Garner JA, LaVail JH. 1999. Differential anterograde transport of HSV type 1 viral strains in the murine optic pathway. *J Neurovirol* 5(2):140-150.
- Gerhardt CC, van Heerikhuizen H. 1997. Functional characteristics of heterologously expressed 5-HT receptors. *Eur J Pharmacol* 334(1):1-23.
- Gerstenberg TC, Levin RJ, Wagner G. 1990. Erection and ejaculation in man. Assessment of the electromyographic activity of the bulbocavernosus and ischiocavernosus muscles. *Br J Urol* 65(4):395-402.
- Giuliano F, Clement P. 2005. Neuroanatomy and physiology of ejaculation. *Annu Rev Sex Res* 16:190-216.
- Giuliano F, Rampin O, Brown K, Courtois F, Benoit G, Jardin A. 1996. Stimulation of the medial preoptic area of the hypothalamus in the rat elicits increases in intracavernous pressure. *Neurosci Lett* 209(1):1-4.
- Gobrogge KL, Liu Y, Young LJ, Wang Z. 2009. Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *Proc Natl Acad Sci U S A* 106(45):19144-19149.
- Gonzales G, Mendoza L, Ruiz J, Torrejon J. 1982. A demonstration that 5-hydroxytryptamine administered peripherally can affect sexual behavior in male rats. *Life Sci* 31(24):2775-2781.
- Greco B, Blasberg ME, Kosinski EC, Blaustein JD. 2003. Response of ER $\alpha$ -IR and ER $\beta$ -IR cells in the forebrain of female rats to mating stimuli. *Hormones and behavior* 43(4):444-453.
- Griffiths JL, Lovick TA. 2005. GABAergic neurones in the rat periaqueductal grey matter express  $\alpha$ 4,  $\beta$ 1 and  $\delta$  GABA<sub>A</sub> receptor subunits: plasticity of expression during the estrous cycle. *Neuroscience* 136(2):457-466.

- Guevara MA, Martinez-Pelayo M, Arteaga Silva M, Bonilla-Jaime H, Hernandez-Gonzalez M. 2008. Electrophysiological correlates of the mesoaccumbens system during male rat sexual behaviour. *Physiol Behav* 95(4):545-552.
- Haddad PM, Dursun SM. 2008. Neurological complications of psychiatric drugs: clinical features and management. *Hum Psychopharmacol* 23 Suppl 1:15-26.
- Haghpourast A, Ahmad-Molaei L. 2009. Effects of electrolytic lesion of dorsolateral periaqueductal gray on analgesic response of morphine microinjected into the nucleus cuneiformis in rat. *Neurosci Lett* 451(2):165-169.
- Hammock EA, Young LJ. 2006. Oxytocin, vasopressin and pair bonding: implications for autism. *Philos Trans R Soc Lond B Biol Sci* 361(1476):2187-2198.
- Hatzimouratidis K, Amar E, Eardley I, Giuliano F, Hatzichristou D, Montorsi F, Vardi Y, Wespes E. 2010. Guidelines on Male Sexual Dysfunction: Erectile Dysfunction and Premature Ejaculation. *Eur Urol*.
- Hawthorn J, Ang VT, Jenkins JS. 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(1):51-57.
- Heinrichs M, von Dawans B, Domes G. 2009. Oxytocin, vasopressin, and human social behavior. *Front Neuroendocrinol* 30(4):548-557.
- Hellstrom WJ. 2009. Emerging treatments for premature ejaculation: focus on dapoxetine. *Neuropsychiatr Dis Treat* 5:37-46.
- Hermann GE, Holmes GM, Rogers RC, Beattie MS, Bresnahan JC. 2003. Descending spinal projections from the rostral gigantocellular reticular nuclei complex. *J Comp Neurol* 455(2):210-221.
- Hiemke C, Hartter S. 2000. Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacol Ther* 85(1):11-28.
- Hillegaart V. 1991. Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on core temperature in the rat. *Psychopharmacology (Berl)* 103(3):291-296.
- Hillegaart V, Ahlenius S, Larsson K. 1991. Region-selective inhibition of male rat sexual behavior and motor performance by localized forebrain 5-HT injections: a comparison with effects produced by 8-OH-DPAT. *Behav Brain Res* 42(2):169-180.
- Hoffman BJ, Mezey E. 1989. Distribution of serotonin 5-HT<sub>1C</sub> receptor mRNA in adult rat brain. *FEBS Lett* 247(2):453-462.
- Hoffman GE, Lyo D. 2002. Anatomical markers of activity in neuroendocrine systems: are we all 'fos-ed out'? *J Neuroendocrinol* 14(4):259-268.
- Hoffman GE, Smith MS, Verbalis JG. 1993. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Front Neuroendocrinol* 14(3):173-213.
- Holmes GM, Chapple WD, Leipheimer RE, Sachs BD. 1991. Electromyographic analysis of male rat perineal muscles during copulation and reflexive erections. *Physiol Behav* 49(6):1235-1246.

- Holmes GM, Hermann GE, Rogers RC, Bresnahan JC, Beattie MS. 2002. Dissociation of the effects of nucleus raphe obscurus or rostral ventrolateral medulla lesions on eliminatory and sexual reflexes. *Physiol Behav* 75(1-2):49-55.
- Holstege G. 1992. The emotional motor system. *Eur J Morphol* 30(1):67-79.
- Holstege G, Georgiadis JR, Paans AM, Meiners LC, van der Graaf FH, Reinders AA. 2003. Brain activation during human male ejaculation. *J Neurosci* 23(27):9185-9193.
- Holstege G, Kuypers HG, Boer RC. 1979. Anatomical evidence for direct brain stem projections to the somatic motoneuronal cell groups and autonomic preganglionic cell groups in cat spinal cord. *Brain Res* 171(2):329-333.
- Hopkins DA, Holstege G. 1978. Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp Brain Res* 32(4):529-547.
- Hsu GL, Hsieh CH, Wen HS, Hsu WL, Wu CH, Fong TH, Chen SC, Tseng GF. 2004. Anatomy of the human penis: the relationship of the architecture between skeletal and smooth muscles. *J Androl* 25(3):426-431.
- Hsu JH, Shen WW. 1995. Male sexual side effects associated with antidepressants: a descriptive clinical study of 32 patients. *Int J Psychiatry Med* 25(2):191-201.
- Hu SH, Wei N, Wang QD, Yan LQ, Wei EQ, Zhang MM, Hu JB, Huang ML, Zhou WH, Xu Y. 2008. Patterns of brain activation during visually evoked sexual arousal differ between homosexual and heterosexual men. *AJNR Am J Neuroradiol* 29(10):1890-1896.
- Hull EM, Du J, Lorrain DS, Matuszewich L. 1995. Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. *J Neurosci* 15(11):7465-7471.
- Ivell R, Balvers M, Rust W, Bathgate R, Einspanier A. 1997. Oxytocin and male reproductive function. *Adv Exp Med Biol* 424:253-264.
- Jard S, Butlen D, Cantau B, Guillon G, Marie J, Roy C. 1984. The mechanisms of action of antidiuretic hormone. *Adv Nephrol Necker Hosp* 13:163-177.
- Johnson PL, Lightman SL, Lowry CA. 2004. A functional subset of serotonergic neurons in the rat ventrolateral periaqueductal gray implicated in the inhibition of sympathoexcitation and panic. *Ann N Y Acad Sci* 1018:58-64.
- Johnson RD. 2006. Descending pathways modulating the spinal circuitry for ejaculation: effects of chronic spinal cord injury. *Prog Brain Res* 152:415-426.
- Johnson RD, Hubscher CH. 1998. Brainstem microstimulation differentially inhibits pudendal motoneuron reflex inputs. *Neuroreport* 9(2):341-345.
- Takeyama M, Yamanouchi K. 1992. Lordosis in male rats: the facilitatory effect of mesencephalic dorsal raphe nucleus lesion. *Physiol Behav* 51(1):181-184.
- Kandler K, Herbert H. 1991. Auditory projections from the cochlear nucleus to pontine and mesencephalic reticular nuclei in the rat. *Brain Res* 562(2):230-242.

- Karama S, Lecours AR, Leroux JM, Bourgouin P, Beaudoin G, Joubert S, Beauregard M. 2002. Areas of brain activation in males and females during viewing of erotic film excerpts. *Hum Brain Mapp* 16(1):1-13.
- Katagiri T, Gibson SJ, Su HC, Polak JM. 1986. Composition and central projections of the pudendal nerve in the rat investigated by combined peptide immunocytochemistry and retrograde fluorescent labelling. *Brain Res* 372(2):313-322.
- Kausz M. 1990. Distribution of hypothalamic neurons projecting to the thoracic and sacral spinal segments in the cat. *J Hirnforsch* 31(6):697-703.
- Kennedy SH, Rizvi S. 2009. Sexual dysfunction, depression, and the impact of antidepressants. *J Clin Psychopharmacol* 29(2):157-164.
- Kennedy SH, Rizvi SJ. 2010. Agomelatine in the Treatment of Major Depressive Disorder: Potential for Clinical Effectiveness. *CNS Drugs*.
- Kleitz-Nelson HK, Dominguez JM, Cornil CA, Ball GF. 2010. Is sexual motivational state linked to dopamine release in the medial preoptic area? *Behav Neurosci* 124(2):300-304.
- Koenig AM, Thase ME. 2009. First-line pharmacotherapies for depression - what is the best choice? *Pol Arch Med Wewn* 119(7-8):478-486.
- Kollack-Walker S, Newman SW. 1997. Mating-induced expression of c-fos in the male Syrian hamster brain: role of experience, pheromones, and ejaculations. *Journal of neurobiology* 32(5):481-501.
- Krauzlis RJ, Liston D, Carello CD. 2004. Target selection and the superior colliculus: goals, choices and hypotheses. *Vision research* 44(12):1445-1451.
- Lanteri-Minet M, Weil-Fugazza J, de Pommery J, Menetrey D. 1994. Hindbrain structures involved in pain processing as revealed by the expression of c-Fos and other immediate early gene proteins. *Neuroscience* 58(2):287-298.
- Laumann EO, Paik A, Rosen RC. 1999. Sexual dysfunction in the United States: prevalence and predictors. *JAMA* 281(6):537-544.
- Lee JW, Erskine MS. 2000. Pseudorabies virus tracing of neural pathways between the uterine cervix and CNS: effects of survival time, estrogen treatment, rhizotomy, and pelvic nerve transection. *J Comp Neurol* 418(4):484-503.
- Leitner DS, Cohen ME. 1985. Role of the inferior colliculus in the inhibition of acoustic startle in the rat. *Physiol Behav* 34(1):65-70.
- Levin RJ. 1998. Sex and the human female reproductive tract--what really happens during and after coitus. *Int J Impot Res* 10 Suppl 1:S14-21.
- Li YQ, Kaneko T, Mizuno N. 2001. Collateral projections of nucleus raphe dorsalis neurones to the caudate-putamen and region around the nucleus raphe magnus and nucleus reticularis gigantocellularis pars alpha in the rat. *Neurosci Lett* 299(1-2):33-36.
- Liu Y, Curtis JT, Wang Z. 2001. Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (*Microtus ochrogaster*). *Behav Neurosci* 115(4):910-919.

- Liu Y, Wang ZX. 2003. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience* 121(3):537-544.
- Liu YC, Sachs BD. 1999. Erectile function in male rats after lesions in the lateral paragigantocellular nucleus. *Neurosci Lett* 262(3):203-206.
- Liu YC, Salamone JD, Sachs BD. 1997a. Impaired sexual response after lesions of the paraventricular nucleus of the hypothalamus in male rats. *Behav Neurosci* 111(6):1361-1367.
- Liu YC, Salamone JD, Sachs BD. 1997b. Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. *J Neurosci* 17(13):5245-5253.
- Lonstein JS, Stern JM. 1998. Site and behavioral specificity of periaqueductal gray lesions on postpartum sexual, maternal, and aggressive behaviors in rats. *Brain Res* 804(1):21-35.
- Lovick TA. 1986. Projections from brainstem nuclei to the nucleus paragigantocellularis lateralis in the cat. *J Auton Nerv Syst* 16(1):1-11.
- Lovick TA. 1992. Midbrain influences on ventrolateral medullo-spinal neurones in the rat. *Exp Brain Res* 90(1):147-152.
- Loyd DR, Morgan MM, Murphy AZ. 2007. Morphine preferentially activates the periaqueductal gray-rostral ventromedial medullary pathway in the male rat: a potential mechanism for sex differences in antinociception. *Neuroscience* 147(2):456-468.
- Loyd DR, Murphy AZ. 2006. Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: a potential circuit mediating the sexually dimorphic actions of morphine. *J Comp Neurol* 496(5):723-738.
- Lu N, Han M, Yang ZL, Wang YQ, Wu GC, Zhang YQ. 2010. Nociceptin/Orphanin FQ in PAG modulates the release of amino acids, serotonin and norepinephrine in the rostral ventromedial medulla and spinal cord in rats. *Pain* 148(3):414-425.
- Luppi PH, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M. 1995. Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. *Neuroscience* 65(1):119-160.
- Macdonald K, Macdonald TM. 2010. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. *Harv Rev Psychiatry* 18(1):1-21.
- Madeira MD, Leal S, Paula-Barbosa MM. 1999. Stereological evaluation and Golgi study of the sexual dimorphisms in the volume, cell numbers, and cell size in the medial preoptic nucleus of the rat. *Journal of neurocytology* 28(2):131-148.
- Malsbury CW. 1971. Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area. *Physiol Behav* 7(6):797-805.
- Marson L. 1995. Central nervous system neurons identified after injection of pseudorabies virus into the rat clitoris. *Neurosci Lett* 190(1):41-44.

- Marson L. 2004. Lesions of the periaqueductal gray block the medial preoptic area-induced activation of the urethro-genital reflex in male rats. *Neurosci Lett* 367(3):278-282.
- Marson L, Cai R, Makhanova N. 2003. Identification of spinal neurons involved in the urethro-genital reflex in the female rat. *J Comp Neurol* 462(4):355-370.
- Marson L, Carson 3rd CC. 1999. Central Nervous System Innervation of the Penis, Prostate, and Perineal Muscles: A Transneuronal Tracing Study. *Mol Urol* 3(2):43-50.
- Marson L, Foley KA. 2004. Identification of neural pathways involved in genital reflexes in the female: a combined anterograde and retrograde tracing study. *Neuroscience* 127(3):723-736.
- Marson L, List MS, McKenna KE. 1992. Lesions of the nucleus paragigantocellularis alter ex copula penile reflexes. *Brain Res* 592(1-2):187-192.
- Marson L, McKenna KE. 1990. The identification of a brainstem site controlling spinal sexual reflexes in male rats. *Brain Res* 515(1-2):303-308.
- Marson L, McKenna KE. 1992. A role for 5-hydroxytryptamine in descending inhibition of spinal sexual reflexes. *Exp Brain Res* 88(2):313-320.
- Marson L, McKenna KE. 1994. Stimulation of the hypothalamus initiates the urethro-genital reflex in male rats. *Brain Res* 638(1-2):103-108.
- Marson L, McKenna KE. 1996. CNS cell groups involved in the control of the ischiocavernosus and bulbospongiosus muscles: a transneuronal tracing study using pseudorabies virus. *J Comp Neurol* 374(2):161-179.
- Marson L, Murphy AZ. 2006. Identification of neural circuits involved in female genital responses in the rat: a dual virus and anterograde tracing study. *American journal of physiology* 291(2):R419-428.
- Martin-Alguacil N, Schober JM, Sengelaub DR, Pfaff DW, Shelley DN. 2008. Clitoral sexual arousal: neuronal tracing study from the clitoris through the spinal tracts. *J Urol* 180(4):1241-1248.
- McCormick DA, Pape HC, Williamson A. 1991. Actions of norepinephrine in the cerebral cortex and thalamus: implications for function of the central noradrenergic system. *Prog Brain Res* 88:293-305.
- McEwen BS. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiological reviews* 87(3):873-904.
- McEwen BS, Jones KJ, Pfaff DW. 1987. Hormonal control of sexual behavior in the female rat: molecular, cellular and neurochemical studies. *Biol Reprod* 36(1):37-45.
- McGinnis MY, Vakulenko M. 2003. Characterization of 50-kHz ultrasonic vocalizations in male and female rats. *Physiol Behav* 80(1):81-88.
- McIntosh TK, Barfield RJ. 1984. Brain monoaminergic control of male reproductive behavior. I. Serotonin and the post-ejaculatory refractory period. *Behav Brain Res* 12(3):255-265.
- McKenna KE. 2002. The neurophysiology of female sexual function. *World J Urol* 20(2):93-100.

- McKenna KE, Nadelhaft I. 1986. The organization of the pudendal nerve in the male and female rat. *J Comp Neurol* 248(4):532-549.
- Meerts SH, Clark AS. 2009. Artificial vaginocervical stimulation induces a conditioned place preference in female rats. *Horm Behav* 55(1):128-132.
- Meston CM, Levin RJ, Sipski ML, Hull EM, Heiman JR. 2004. Women's orgasm. *Annu Rev Sex Res* 15:173-257.
- Misslin R. 2003. The defense system of fear: behavior and neurocircuitry. *Neurophysiol Clin* 33(2):55-66.
- Miura T, Kondo Y, Akimoto M, Sakuma Y. 2001. Electromyography of male rat perineal musculature during copulatory behavior. *Urol Int* 67(3):240-245.
- Moraes CL, Bertoglio LJ, Carobrez AP. 2008. Interplay between glutamate and serotonin within the dorsal periaqueductal gray modulates anxiety-related behavior of rats exposed to the elevated plus-maze. *Behav Brain Res* 194(2):181-186.
- Mos J, Mollet I, Tolboom JT, Waldinger MD, Olivier B. 1999. A comparison of the effects of different serotonin reuptake blockers on sexual behaviour of the male rat. *Eur Neuropsychopharmacol* 9(1-2):123-135.
- Murphy AZ, Ennis M, Rizvi TA, Behbehani MM, Shipley MT. 1995. Fos expression induced by changes in arterial pressure is localized in distinct, longitudinally organized columns of neurons in the rat midbrain periaqueductal gray. *J Comp Neurol* 360(2):286-300.
- Murphy AZ, Hoffman GE. 2001. Distribution of gonadal steroid receptor-containing neurons in the preoptic-periaqueductal gray-brainstem pathway: a potential circuit for the initiation of male sexual behavior. *J Comp Neurol* 438(2):191-212.
- Murphy AZ, Marson L. 2000. Identification of neural circuits underlying male reproductive behavior: combined viral and traditional tract tracing studies. Annual Meeting of the Society for Neuroscience. New Orleans, LA.
- Murphy AZ, Rizvi TA, Ennis M, Shipley MT. 1999a. The organization of preoptic-medullary circuits in the male rat: evidence for interconnectivity of neural structures involved in reproductive behavior, antinociception and cardiovascular regulation. *Neuroscience* 91(3):1103-1116.
- Murphy AZ, Shupnik MA, Hoffman GE. 1999b. Androgen and estrogen (alpha) receptor distribution in the periaqueductal gray of the male Rat. *Hormones and behavior* 36(2):98-108.
- Muschamp JW, Dominguez JM, Sato SM, Shen RY, Hull EM. 2007. A role for hypocretin (orexin) in male sexual behavior. *J Neurosci* 27(11):2837-2845.
- Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K. 1999. Distribution of orexin neurons in the adult rat brain. *Brain Res* 827(1-2):243-260.
- Niimi M, Murao K. 2005. Neuropeptide W as a stress mediator in the hypothalamus. *Endocrine* 27(1):51-54.
- Nilaver G, Zimmerman EA, Wilkins J, Michaels J, Hoffman D, Silverman AJ. 1980. Magnocellular hypothalamic projections to the lower brain stem and spinal cord of the rat.

- Immunocytochemical evidence for predominance of the oxytocin-neurophysin system compared to the vasopressin-neurophysin system. *Neuroendocrinology* 30(3):150-158.
- Nistico G, Nappi G. 1993. Locus coeruleus, an integrative station involved in the control of several vital functions. *Funct Neurol* 8(1):5-25.
- Nofrey B, Rocha B, Lopez HH, Ettenberg A. 2008. The effects of sexual experience and estrus on male-seeking motivated behavior in the female rat. *Physiol Behav* 95(3):533-538.
- Normandin JJ, Murphy AZ. 2007. Feeling good in the neighborhood: trans-synaptic tracing of genitosensory afferents. Annual Meeting of the Society for Behavioral Neuroendocrinology. Pacific Grove, CA.
- Normandin JJ, Murphy AZ. 2008. Nucleus paragigantocellularis afferents in male and female rats: organization, gonadal steroid receptor expression, and activation during sexual behavior. *J Comp Neurol* 508(5):771-794.
- Nunez R, Gross GH, Sachs BD. 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(4):417-429.
- Pacheco P, Martinez-Gomez M, Whipple B, Beyer C, Komisaruk BR. 1989. Somato-motor components of the pelvic and pudendal nerves of the female rat. *Brain Res* 490(1):85-94.
- Pastelin CF, Zempoalteca R, Pacheco P, Downie JW, Cruz Y. 2008. Sensory and somatomotor components of the "sensory branch" of the pudendal nerve in the male rat. *Brain Res* 1222:149-155.
- Pattij T, de Jong TR, Uitterdijk A, Waldinger MD, Veening JG, Cools AR, van der Graaf PH, Olivier B. 2005. Individual differences in male rat ejaculatory behaviour: searching for models to study ejaculation disorders. *Eur J Neurosci* 22(3):724-734.
- Pavesi E, Enck MC, De Toledo CA, Terenzi MG. 2007. Disruption of maternal behaviour by acute conspecific interaction induces selective activation of the lateral periaqueductal grey. *Eur J Neurosci* 26(7):2055-2065.
- Paxinos G, Watson C. 1997. *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press.
- Paxinos G, Watson C. 2005. *The rat brain in stereotaxic coordinates*. Amsterdam ; Boston: Elsevier Academic Press. 1 v. (unpaged) p.
- Perez-Clausell J, Frederickson CJ, Danscher G. 1989. Amygdaloid efferents through the stria terminalis in the rat give origin to zinc-containing boutons. *J Comp Neurol* 290(2):201-212.
- Perez CA, Concha A, Hernandez ME, Manzo J. 2005. Influence of the paraventricular nucleus and oxytocin on the retrograde stain of pubococcygeus muscle motoneurons in male rats. *Brain Res* 1041(1):11-18.
- Pescatori ES, Calabro A, Artibani W, Pagano F, Triban C, Italiano G. 1993. Electrical stimulation of the dorsal nerve of the penis evokes reflex tonic erections of the penile body and reflex ejaculatory responses in the spinal rat. *J Urol* 149(3):627-632.



- Peshori KR, Erichsen JT, Collins WF, 3rd. 1995. Differences in the connectivity of rat pudendal motor nuclei as revealed by retrograde transneuronal transport of wheat germ agglutinin. *J Comp Neurol* 353(1):119-128.
- Peters LC, Kristal MB, Komisaruk BR. 1987. Sensory innervation of the external and internal genitalia of the female rat. *Brain Res* 408(1-2):199-204.
- Pfaus JG. 1996. Frank A. Beach award. Homologies of animal and human sexual behaviors. *Hormones and behavior* 30(3):187-200.
- Pfaus JG. 2009. Pathways of sexual desire. *J Sex Med* 6(6):1506-1533.
- Pfaus JG, Kippin TE, Coria-Avila G. 2003. What can animal models tell us about human sexual response? *Annu Rev Sex Res* 14:1-63.
- Pfaus JG, Manitt C, Coopersmith CB. 2006. Effects of pelvic, pudendal, or hypogastric nerve cuts on Fos induction in the rat brain following vaginocervical stimulation. *Physiol Behav* 89(5):627-636.
- Phelps SM, Campbell P, Zheng DJ, Ophir AG. 2010. Beating the boojum: comparative approaches to the neurobiology of social behavior. *Neuropharmacology* 58(1):17-28.
- Phillips-Farfan BV, Fernandez-Guasti A. 2009. Endocrine, neural and pharmacological aspects of sexual satiety in male rats. *Neurosci Biobehav Rev* 33(3):442-455.
- Purinton PT, Fletcher TF, Bradley WE. 1976. Innervation of pelvic viscera in the rat. Evoked potentials in nerves to bladder and penis (clitoris). *Invest Urol* 14(1):28-32.
- Quadagno DM, McCullough J, Langan R. 1972. The effect of varying amounts of exogenous estradiol benzoate on estrous behavior in the rat. *Horm Behav* 3(3):175-179.
- Quesada A, Romeo HE, Micevych P. 2007. Distribution and localization patterns of estrogen receptor-beta and insulin-like growth factor-1 receptors in neurons and glial cells of the female rat substantia nigra: localization of ERbeta and IGF-1R in substantia nigra. *J Comp Neurol* 503(1):198-208.
- Richelson E. 1994. The pharmacology of antidepressants at the synapse: focus on newer compounds. *J Clin Psychiatry* 55 Suppl A:34-39; discussion 40-31, 98-100.
- Rinaman L, Schwartz G. 2004. Anterograde transneuronal viral tracing of central viscerosensory pathways in rats. *J Neurosci* 24(11):2782-2786.
- Romero L, Bel N, Casanovas JM, Artigas F. 1996. Two actions are better than one: avoiding self-inhibition of serotonergic neurones enhances the effects of serotonin uptake inhibitors. *Int Clin Psychopharmacol* 11 Suppl 4:1-8.
- Roppolo JR, Nadelhaft I, de Groat WC. 1985. The organization of pudendal motoneurons and primary afferent projections in the spinal cord of the rhesus monkey revealed by horseradish peroxidase. *J Comp Neurol* 234(4):475-488.
- Ross HE, Cole CD, Smith Y, Neumann ID, Landgraf R, Murphy AZ, Young LJ. 2009. Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. *Neuroscience* 162(4):892-903.

- Ross HE, Young LJ. 2009. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front Neuroendocrinol* 30(4):534-547.
- Rowe DW, Erskine MS. 1993. c-Fos proto-oncogene activity induced by mating in the preoptic area, hypothalamus and amygdala in the female rat: role of afferent input via the pelvic nerve. *Brain Res* 621(1):25-34.
- Sachs BD. 1982. Role of striated penile muscles in penile reflexes, copulation, and induction of pregnancy in the rat. *J Reprod Fertil* 66(2):433-443.
- Saether K, Hilaire G, Monteau R. 1987. Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Res* 419(1-2):87-96.
- Schenberg LC, Pova RM, Costa AL, Caldellas AV, Tufik S, Bittencourt AS. 2005. Functional specializations within the tectum defense systems of the rat. *Neuroscience and biobehavioral reviews* 29(8):1279-1298.
- Schroder HD. 1980. Organization of the motoneurons innervating the pelvic muscles of the male rat. *J Comp Neurol* 192(3):567-587.
- Schroder HD. 1981. Onuf's nucleus X: a morphological study of a human spinal nucleus. *Anat Embryol (Berl)* 162(4):443-453.
- Schweitzer I, Maguire K, Ng C. 2009. Sexual side-effects of contemporary antidepressants: review. *Aust N Z J Psychiatry* 43(9):795-808.
- Shafik A, Shafik AA, El Sibai O, Shafik IA. 2009. Electromyographic study of ejaculatory mechanism. *Int J Androl* 32(3):212-217.
- Sofroniew MV. 1980. Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. *J Histochem Cytochem* 28(5):475-478.
- Song CK, Schwartz GJ, Bartness TJ. 2009. Anterograde transneuronal viral tract tracing reveals central sensory circuits from white adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 296(3):R501-511.
- Stoneham MD, Everitt BJ, Hansen S, Lightman SL, Todd K. 1985. Oxytocin and sexual behaviour in the male rat and rabbit. *J Endocrinol* 107(1):97-106.
- Sun X, Xia Q, Lai CH, Shum DK, Chan YS, He J. 2007. Corticofugal modulation of acoustically induced Fos expression in the rat auditory pathway. *J Comp Neurol* 501(4):509-525.
- Suntsova N, Guzman-Marin R, Kumar S, Alam MN, Szymusiak R, McGinty D. 2007. The median preoptic nucleus reciprocally modulates activity of arousal-related and sleep-related neurons in the perifornical lateral hypothalamus. *J Neurosci* 27(7):1616-1630.
- Swaab DF, Pool CW, Nijveldt F. 1975. Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. *J Neural Transm* 36(3-4):195-215.
- Swann JM, Wang J, Govek EK. 2003. The MPN mag: introducing a critical area mediating pheromonal and hormonal regulation of male sexual behavior. *Annals of the New York Academy of Sciences* 1007:199-210.

- Swanson LW, Sawchenko PE. 1980. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology* 31(6):410-417.
- Sweet DC, Levine AS, Billington CJ, Kotz CM. 1999. Feeding response to central orexins. *Brain Res* 821(2):535-538.
- Tang Y, Rampin O, Calas A, Facchinetti P, Giuliano F. 1998. Oxytocinergic and serotonergic innervation of identified lumbosacral nuclei controlling penile erection in the male rat. *Neuroscience* 82(1):241-254.
- Tang Y, Rampin O, Giuliano F, Ugolini G. 1999. Spinal and brain circuits to motoneurons of the bulbospongiosus muscle: retrograde transneuronal tracing with rabies virus. *J Comp Neurol* 414(2):167-192.
- Temel Y, Hafizi S, Beuls E, Visser-Vandewalle V. 2005. The supraspinal network in the control of erection. *Expert Opin Ther Targets* 9(5):941-954.
- Tetel MJ, Getzinger MJ, Blaustein JD. 1993. Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. *J Neuroendocrinol* 5(4):397-404.
- Thor KB, Blitz-Siebert A, Helke CJ. 1990. Discrete localization of high-density 5-HT<sub>1A</sub> binding sites in the midline raphe and parapyramidal region of the ventral medulla oblongata of the rat. *Neurosci Lett* 108(3):249-254.
- Thor KB, Blitz-Siebert A, Helke CJ. 1992. Autoradiographic localization of 5HT<sub>1</sub> binding sites in the medulla oblongata of the rat. *Synapse* 10(3):185-205.
- Truitt WA, Coolen LM. 2002. Identification of a potential ejaculation generator in the spinal cord. *Science* 297(5586):1566-1569.
- Truitt WA, Shipley MT, Veening JG, Coolen LM. 2003. Activation of a subset of lumbar spinothalamic neurons after copulatory behavior in male but not female rats. *J Neurosci* 23(1):325-331.
- Underwood MD, Arango V, Bakalian MJ, Ruggiero DA, Mann JJ. 1999. Dorsal raphe nucleus serotonergic neurons innervate the rostral ventrolateral medulla in rat. *Brain Res* 824(1):45-55.
- Uschakov A, Gong H, McGinty D, Szymusiak R. 2006. Sleep-active neurons in the preoptic area project to the hypothalamic paraventricular nucleus and perifornical lateral hypothalamus. *The European journal of neuroscience* 23(12):3284-3296.
- Van Bockstaele EJ, Aston-Jones G. 1995. Integration in the ventral medulla and coordination of sympathetic, pain and arousal functions. *Clin Exp Hypertens* 17(1-2):153-165.
- Van De Poll NE, Van Dis H. 1979. The effect of medial preoptic--anterior hypothalamic lesions on bisexual behavior of the male rat. *Brain research bulletin* 4(4):505-511.
- van Dis H, van de Poll NE. 1974. Sexual differentiation of behavior in rat. *Prog Brain Res* 41:321-329.
- Veenema AH, Neumann ID. 2008. Central vasopressin and oxytocin release: regulation of complex social behaviours. *Prog Brain Res* 170:261-276.

- Vega Matuszcyk J, Larsson K, Eriksson E. 1998. The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. *Pharmacol Biochem Behav* 60(2):527-532.
- Wagner CK, Clemens LG. 1991. Projections of the paraventricular nucleus of the hypothalamus to the sexually dimorphic lumbosacral region of the spinal cord. *Brain Res* 539(2):254-262.
- Walter M, Bermpohl F, Mouras H, Schiltz K, Tempelmann C, Rotte M, Heinze HJ, Bogerts B, Northoff G. 2008. Distinguishing specific sexual and general emotional effects in fMRI-subcortical and cortical arousal during erotic picture viewing. *Neuroimage* 40(4):1482-1494.
- Watson NV, Gorzalka BB. 1991. DOI-induced inhibition of copulatory behavior in male rats: reversal by 5-HT<sub>2</sub> antagonists. *Pharmacol Biochem Behav* 39(3):605-612.
- Watson RE, Jr., Wiegand SJ, Clough RW, Hoffman GE. 1986. Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology. *Peptides* 7(1):155-159.
- Wersinger SR, Baum MJ, Erskine MS. 1993. Mating-induced FOS-like immunoreactivity in the rat forebrain: a sex comparison and a dimorphic effect of pelvic nerve transection. *J Neuroendocrinol* 5(5):557-568.
- Wiedey J, Alexander MS, Marson L. 2008. Spinal neurons activated in response to pudendal or pelvic nerve stimulation in female rats. *Brain Res* 1197:106-114.
- Witt DM, Insel TR. 1994. Increased Fos expression in oxytocin neurons following masculine sexual behavior. *J Neuroendocrinol* 6(1):13-18.
- Wood RI, Newman SW. 1995. Integration of chemosensory and hormonal cues is essential for mating in the male Syrian hamster. *J Neurosci* 15(11):7261-7269.
- Wrobel B, Karasek M. 2008. Human sexuality and sex steroids. *Neuro Endocrinol Lett* 29(1):3-10.
- Yang CC, Jiang X. 2009. Clinical autonomic neurophysiology and the male sexual response: an overview. *J Sex Med* 6 Suppl 3:221-228.
- Yells DP, Hendricks SE, Prendergast MA. 1992. Lesions of the nucleus paragigantocellularis: effects on mating behavior in male rats. *Brain Res* 596(1-2):73-79.
- Yells DP, Prendergast MA, Hendricks SE, Nakamura M. 1994. Fluoxetine-induced inhibition of male rat copulatory behavior: modification by lesions of the nucleus paragigantocellularis. *Pharmacol Biochem Behav* 49(1):121-127.
- Young LJ, Murphy Young AZ, Hammock EA. 2005. Anatomy and neurochemistry of the pair bond. *J Comp Neurol* 493(1):51-57.
- Zardetto-Smith AM, Moga MM, Magnuson DJ, Gray TS. 1988. Lateral hypothalamic dynorphinergic efferents to the amygdala and brainstem in the rat. *Peptides* 9(5):1121-1127.
- Zec N, Kinney HC. 2001. Anatomic relationships of the human nucleus paragigantocellularis lateralis: a Dil labeling study. *Auton Neurosci* 89(1-2):110-124.
- Zemanick MC, Strick PL, Dix RD. 1991. Direction of transneuronal transport of herpes simplex virus 1 in the primate motor system is strain-dependent. *Proc Natl Acad Sci U S A* 88(18):8048-8051.

Zeng SL, Li YQ, Rao ZR, Shi JW. 1991. Projections from serotonin- and substance P-like immunoreactive neurons in the midbrain periaqueductal gray onto the nucleus reticularis gigantocellularis pars alpha in the rat. *Neurosci Lett* 131(2):205-209.

## APPENDICES

### **Appendix A – Preliminary data: Manipulation of serotonin at the nucleus paragigantocellularis of male rats produces paradoxical effects on sexual behavior**

Serotonin (5-HT) inhibits sexual activity in men and rodents when 5-HT levels are systemically increased (Ahlenius and Larsson, 1991; Ahlenius et al., 1980; de Jong et al., 2005a; Gonzales et al., 1982; Kennedy and Rizvi, 2009; Schweitzer et al., 2009; Vega Matuszyk et al., 1998). Site-specific and pharmacological manipulation of 5-HT in rats has revealed that the locus of serotonergic activity, and which 5-HT receptors are activated, are important in determining whether 5-HT acts as a facilitator (Ahlenius et al., 1981; de Castilhos et al., 2006; de Jong et al., 2005b; Fernandez-Guasti et al., 1992) or inhibitor (Fernandez-Guasti et al., 1992; Hillegaart et al., 1991; Watson and Gorzalka, 1991) of sexual behavior. The nucleus paragigantocellularis (nPGi) of the rat rostroventrolateral medulla provides tonic descending inhibition of genital reflexes (Holmes et al., 2002; Marson et al., 1992; Marson and McKenna, 1990; Yells et al., 1992; Yells et al., 1994) that appears to be under serotonergic control. Genital reflexes inhibited by systemic increases in 5-HT can be reversed by nPGi lesions (Clement et al., 2007; Yells et al., 1994). The nPGi contains serotonergic cells (Marson and McKenna, 1992), and when 5-HT is applied to the spinal targets of the nPGi, genital reflexes are inhibited (Marson and McKenna, 1992). In addition, when the source of 5-HT to the nPGi is removed, genital reflexes are disinhibited (Chapter 4; unpublished results). Given that the source of 5-HT to the nPGi appears to be important for nPGi function, and that the nPGi contains a high density of 5-HT<sub>2C</sub> receptors (Fonseca et al., 2001; Thor et al., 1990), we asked whether manipulation of 5-HT at the level of the nPGi would modulate genital reflexes in male rats.

15 male Sprague-Dawley rats (*Rattus Norvegicus*; Charles River Labs; 275-375g) were implanted with bilateral cannula directed at the nPGi (in mm): DV-9.5, ML+/-1.25, AP-12.0 Bregma. After recovery, males engaged in sexual behavior with stimulus females for 4 1-hour mating bouts over 2 weeks. On the

third mating bout, baseline sexual behaviors (number of mounts, intromissions, and ejaculations, as well as the latency to begin mating, ejaculation latency, and post-ejaculatory interval) were scored. On the fourth mating bout, immediately before mating, males received *meta*-chlorophenylpiperazine (n=6, mCPP; a broad 5-HT agonist; 20mg/ml in dH<sub>2</sub>O), SB-242,084 (n=3; a 5-HT<sub>2C</sub> antagonist; 20mg/ml in dH<sub>2</sub>O), or vehicle (n=6; dH<sub>2</sub>O) injected (500nl/side) slowly through implanted cannula, and sexual behavior was scored. Planned comparisons of mean sexual behaviors within groups were analyzed with two-tailed paired t-tests. Between group comparisons of mean sexual behaviors were two-tailed two independent sample t-tests. All statistical comparisons were made with the alpha value set at p<0.05.

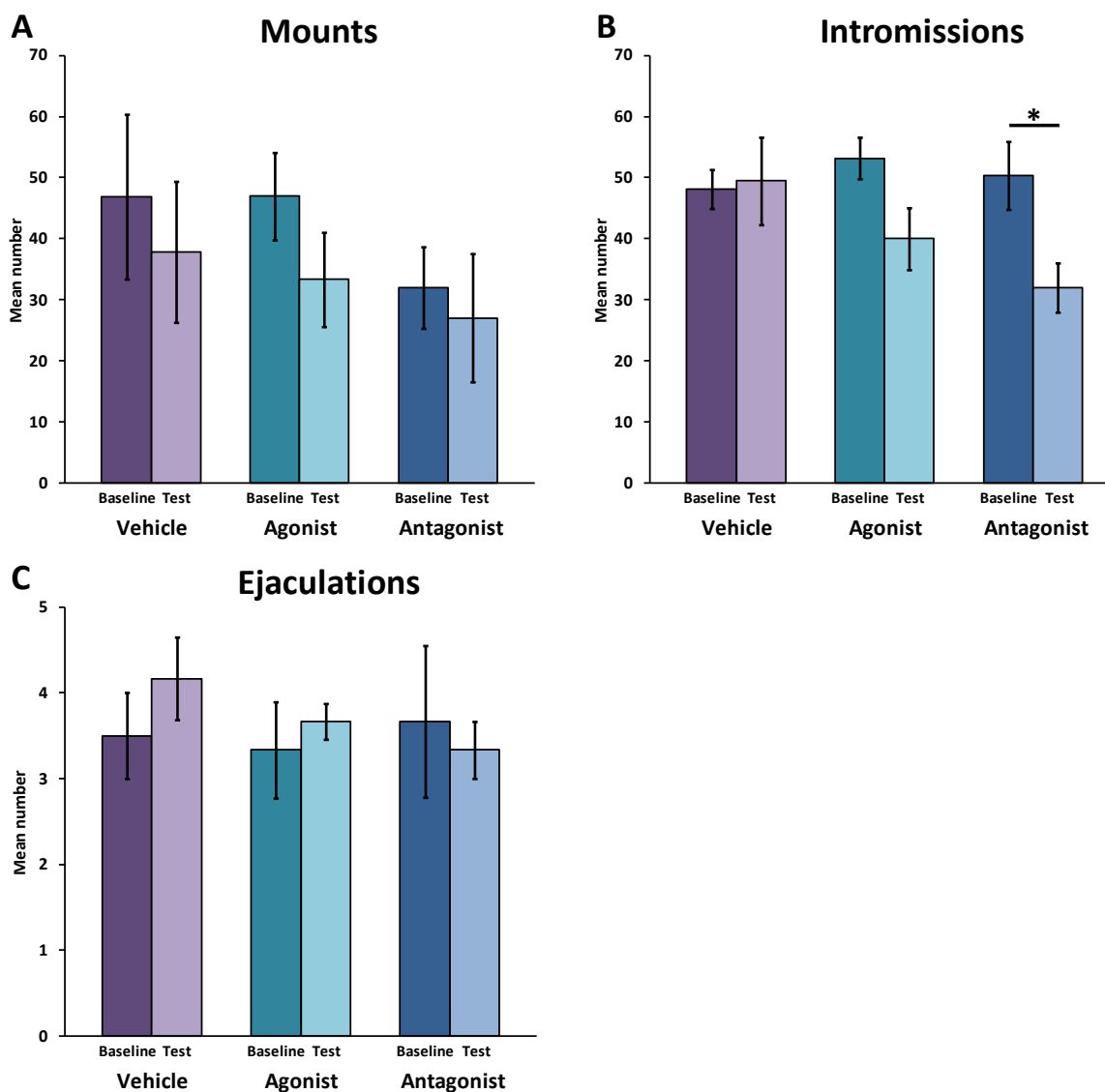
In all comparisons of sexual behavior (number of mounts, intromissions, and ejaculations, as well as the latency to begin mating, ejaculation latency, and post-ejaculatory interval), there were no statistically significant differences between groups. In addition, the only within group difference observed was a reduction in the number of intromissions during the mating bout from baseline to test in the SB-242,084 (antagonist) group (Figure A.1).

It is important to note that in this preliminary data that statistical power is weakened by the small numbers of animals used in each group. This is particularly important in regards to the SB-242,084 group where only 3 animals were used. It appears from our data that broad activation of 5-HT receptors at the level of the nPGi by the agonist mCPP has no effect on sexual behavior in male rats. This is surprising given that removal of the source of 5-HT to the nPGi disinhibits genital reflexes (Chapter 4; unpublished results), and that the nPGi contains receptors for 5-HT (Fonseca et al., 2001; Thor et al., 1990). It is possible that within the nPGi, the activation of multiple 5-HT receptor families with our methods have opposing effects on nPGi function, resulting in no net change. For example, activation of 5-HT<sub>1A</sub> autoreceptors are known to result in a decrease in 5-HT release (Gardier et al., 1996; Romero et al., 1996), and these receptors are found in the nPGi (Fonseca et al., 2001; Thor et al., 1990). Given that 5-HT appears to act to enhance genital reflex inhibition through the nPGi (Chapter 4; unpublished results),

we would expect a facilitation of genital reflexes by activation of 5-HT<sub>1A</sub> receptors. However, mCPP would also activate 5-HT<sub>2C</sub> receptors, also found in the nPGi (Fonseca et al., 2001; Thor et al., 1990), producing excitation (Gerhardt and van Heerikhuizen, 1997) that would be expected to inhibit genital reflexes, resulting in no net change in nPGi function. This notion is supported by our (albeit limited) evidence that antagonism of 5-HT<sub>2C</sub> receptors (by SB-242,084) produced a significant reduction in the number of intromissions in the mating bout, a marker of increased copulatory efficiency.

In conclusion we found limited evidence for the specificity of 5-HT<sub>2C</sub> in the regulation of the nPGi. 5-HT<sub>2C</sub> antagonism at the nPGi appears to facilitate copulatory efficiency, while broad based 5-HT agonists have no net effect on sexual behavior. A larger number of animals and further manipulation of 5-HT receptor subtypes is needed to fully understand 5-HT effects on sexual behavior at the level of the nPGi.





**Figure A.1 Sexual behaviors of male rats treated with mCPP, SB-242,084, or vehicle**

Manipulation of serotonin pharmacology at the level of the nPGi did not alter the number of mounts (A) or ejaculations (C) in any group. However, treatment with the 5-HT<sub>2C</sub> antagonist SB-242,084 reduced the number of intromissions from baseline to test ( $p=0.010$ ). \* =  $p<0.05$ , error bars = standard error of the mean.

## **Appendix B – Preliminary data: Trans-synaptic tracing of functional genitosensory pathways in male and female rats**

Despite decades of research on sexual behavior circuits in mammalian models, a complete description of neural targets receiving genitosensory information is unavailable. The rat model has been studied most extensively with regard to genitosensory circuits. The somatosensory afferents from the genitalia travel along the sensory branches of the pelvic, pudendal, and hypogastric nerves (Dail et al., 1985; Nunez et al., 1986; Peters et al., 1987; Purinton et al., 1976) terminating within the dorsal horn of the lumbosacral spinal cord (Martin-Alguacil et al., 2008; Nunez et al., 1986), with collaterals traveling to supraspinal sites. Specific locations receiving genitosensory information have been described. Important sites within the spinal cord (lumbar spinothalamic cells) and thalamus (medial parvocellular subparafascicular nucleus (mSPFpc)) that receive genitosensory information, and that are important for the regulation of sexual response, have been described (Coolen et al., 2003; Truitt and Coolen, 2002). In addition, in female rats, transections of the pelvic nerve reduced Fos expression induced by vaginocervical stimulation or mating in medial preoptic area, bed nucleus of the stria terminalis, ventromedial hypothalamus, and medial amygdala (Pfaus et al., 2006; Rowe and Erskine, 1993), indicating that pelvic genitosensory afferents in female rats are important in regulating those regions.

In order to provide a full map of the functional genitosensory pathway we utilized a novel tract tracing agent, the anterograde trans-synaptic viral tracer herpes simplex virus 1 strain 129 (H129). The H129 virus has been found to infect neurons in the region where it is injected, replicate in infected cells, travel along axons in an anterograde fashion, and release from axon terminals to synaptically connected neurons, continuing this process indefinitely (Garner and LaVail, 1999; Zemanick et al., 1991). The H129 virus has been used to study pain (Barnett et al., 1995), viscerosensory (Rinaman and Schwartz, 2004), and adipose sensory (Song et al., 2009) circuits. We injected the penis of male rats (n=2), as well as the vagina of a female rat (n=1; Sprague-Dawley, *Rattus Norvegicus*; Charles River; 275-375g) with H129

(6x1  $\mu$ l, 1.2x10<sup>10</sup> pfu) along the right side of the respective genitalia. Six days later, animals were euthanized (SleepAway; 0.5ml i.p.; Henry Schein) and transcardially perfused first with a rinsing agent (0.9% sodium chloride/2% sodium nitrite), then fixative (4% paraformaldehyde 2.5% acrolein). Brains were removed, sectioned (25 $\mu$ M), and immunohistochemically stained for H129 (rabbit anti-HSV-1, 1:300,000; Dako) using a peroxidase method with diaminobenzadine as the chromagen, then counterstained with cresyl violet.

Table B.1 provides a summary of H129 labeling in supraspinal sites in both males and females. In males, we confirmed that genitosensory afferents project to the lumbosacral spinal cord. Within the brainstem, we found extensive H129 labeling with the rostroventrolateral medulla (RVLM; Figure B.1), in addition to labeling in more dorsal regions of the brainstem including the locus coeruleus (LC). Midbrain H129 labeling included the ventral tegmentum, lateral periaqueductal gray (IPAG), ventrolateral periaqueductal gray (vlPAG), cuneiform nucleus (Figure B.2), and magnocellular red nucleus (RMC). Thalamic H129 labeling was observed in the posteromedial ventral nucleus (Figure B.3) and subicular nucleus. Within the hypothalamus, we observed prominent labeling within the paraventricular nucleus (PVN; Figure B.3) and perifornical nucleus (PeF), and the bed nucleus of the stria terminalis. The central amygdala was also robustly labeled (Figure B.3). Cortical labeling was restricted to pyramidal cells in somatomotor cortex, the insular cortex, and granular cells of the ventral orbital cortex (VO; Figure B.4).

H129 labeling in females was observed in the lumbosacral spinal cord, as in males. Within the brainstem, unlike males, H129 labeling was restricted to the RVLM (Figure B.1) and LC. Midbrain H129 was also more restricted than in males with minor labeling observed in the IPAG (Figure B.2) and more robust labeling in the RMC. Thalamic H129 labeling was curiously absent in stark contrast to males (Figure B.3). Within the hypothalamus, we observed robust labeling in the PVN as in males (Figure B.3), and minor labeling in the PeF in contrast to males. Cortical H129 labeling was observed in pyramidal cells of somatomotor cortex.

The pattern of labeling we observed as a result of trans-synaptic tracing of genitosensory afferents in male and female rats includes some interesting findings and implications. With respect to sex differences within our functional genitosensory maps, we find that the number of brain regions receiving genitosensory information in males is much greater than in females. This was evident throughout the brain where functional genitosensory maps in males included more regions with H129 infection in the brainstem, midbrain, thalamus, hypothalamus, amygdala, and cerebral cortex. These results imply that responses to genitosensory stimulus could be very different for males versus females. Indeed, well-established phenomenon regarding neuroendocrine changes in rats as a result of sexual behavior exist. For example, vaginocervical stimulation or mating in female rats produces pregnancy-promoting changes to neuroendocrine function with the establishment of pseudopregnancy (Castro-Vasquez and Carreno, 1981).

A number of brain areas receiving genitosensory information, as evidenced by our genitosensory maps, have been implicated in sexual behavior. In the brainstem, the nPGi is an established inhibitor of genital reflexes in male rats (Marson and McKenna, 1990). The genitosensory input to the nPGi that we detail here (as part of the RVLM) in both sexes could be a modulator of nPGi activity. Likewise, the LC receives genitosensory input in both sexes, which could modulate general arousal through noradrenergic LC activity (McCormick et al., 1991; Nistico and Nappi, 1993). The periaqueductal gray (PAG) is a known integrator of cardiovascular, nociceptive and sexual behavior (Holstege, 1992; Murphy et al., 1999a). The genitosensory inputs to the PAG in males, though not found in females, might be one important source of information for PAG function. In both sexes, the PVN receives an abundance of genitosensory information. The PVN has been implicated in a variety of sociosexual behaviors (Ackerman et al., 1997; Consiglio and Lucion, 1996; Veenema and Neumann, 2008) and is the main source of the neuropeptide oxytocin to the central nervous system (Hawthorn et al., 1985). It has long been known that oxytocin blood levels increase after sexual behavior in rats (Ivell et al., 1997) and humans (Carmichael et

al., 1987). Interestingly, oxytocin has been implicated in mating-induced pair-bonding in voles (Young et al., 2005), and genitosensory may be directly modulating oxytocin release. One striking result was the robust genitosensory input to the VO in males but not females. The VO has been shown to be active in a number of functional magnetic resonance imaging sexual tasks in men (Holstege et al., 2003; Hu et al., 2008; Karama et al., 2002; Walter et al., 2008) and might represent a sexual-behavior specific percept, or modulator of executive function, in both rats and humans.

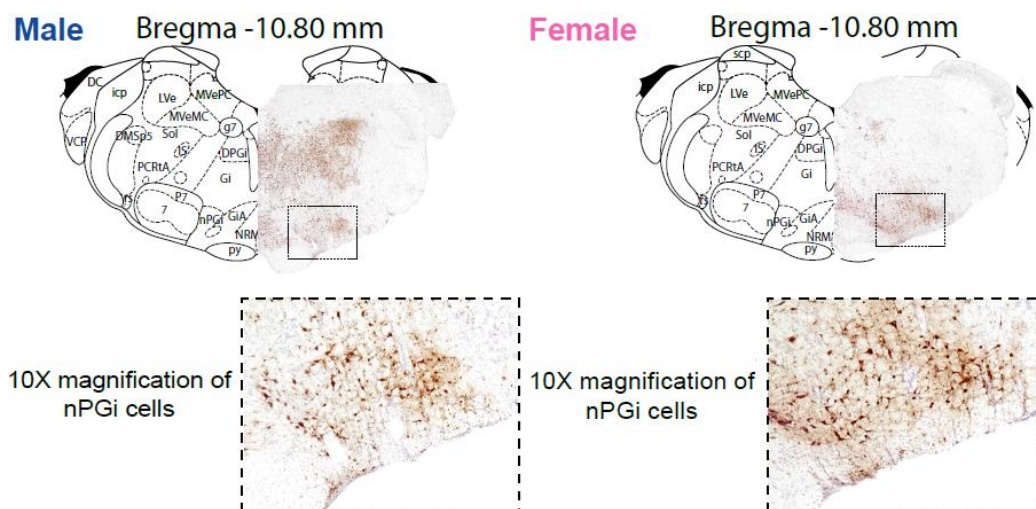
In conclusion, we found that genitosensory inputs to supraspinal sites are distributed throughout the brain of male and female rats, though in a sexually dimorphic manner. Many of the sites receiving genitosensory information have been previously implicated in sexual behavior, but this anatomical evidence suggests a direct role for modulation of activity in these regions that heretofore has not been established.

**Table B.1 Abundance of H129 labeling in supraspinal sites of male and female rats as a result of genital inoculation**

Region	Male	Female
VO	+++	-
AIC	++	-
M1	+	+
S1	+	-
BNST	+++	-
CeM	+++	-
VP	++	-
MPG	++	-
LPG	++	-
PVN	+++	+++
LH	+++	+
MCLH	-	+
PeF	+++	++
Sub	+++	-
ZI	+++	-
VPM	++	-
VA	+++	-
SPFPC	++	-
SNR	++	-
PF	+	+

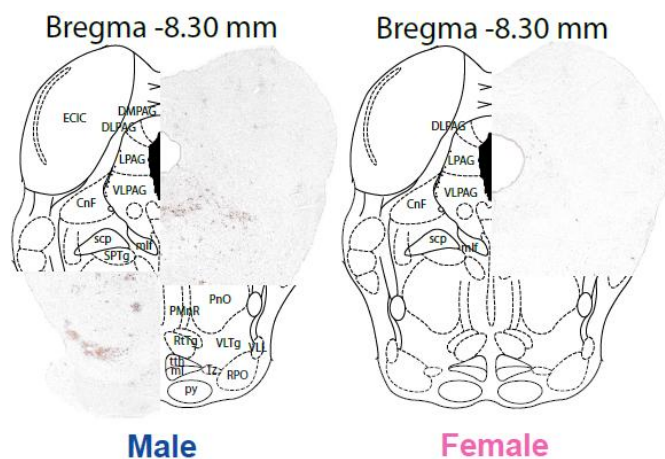
Region	Male	Female
LPAG	+	+
RPC	+++	+++
RMC	+++	+++
IMLF	++	+
PL	+	+
DpMe	+	-
DpG	++	-
CnF	+	-
VLTg	++	-
PB	++	-
NRM	+++	+++
DMTg	++	-
LC	+++	+++
Mo5	-	++
A5	-	++
nPGi	+++	+++
PnC	++	-
Sol	+++	-
PCRt	++	-

VO = ventral orbital cortex, AIC = anterior insular cortex, M1 = primary motor cortex, S1 = primary somatosensory cortex, BNST = bed nucleus of the stria terminalis, CeM = central amygdala, VP = ventral posterior thalamic nucleus, MPG = medial globus pallidus, LPG = lateral globus pallidus, PVN = paraventricular hypothalamic nucleus, LH = lateral hypothalamus, MCLH = magnocellular nucleus of the lateral hypothalamus, PeF = perifornical nucleus, Sub = subicular nucleus, ZI = zona incerta, VPM = posteromedial ventral nucleus of the thalamus, VA = ventral anterior thalamic nucleus, SPFPC = parvocellular subparafascicular nucleus, SNR = substantia nigra, PF = paraflocculus, LPAG = lateral periaqueductal gray, RPC = parvocellular red nucleus, RMC = magnocellular red nucleus, IMLF = interstitial nucleus of the medial longitudinal fasciculus, PL = paralemniscal nucleus, DpMe = deep mesencephalic nucleus, DpG = deep gray layer of the superior colliculus, CnF = cuneiform nucleus, VLTg = ventrolateral tegmental area, PB = parabrachial nucleus, NRM = nucleus raphe magnus, DMTg = dorsomedial tegmental area, LC = locus coeruleus, Mo5 = motor trigeminal nucleus, A5 = A5 noradrenergic cell group, nPGi = nucleus paragigantocellularis, PnC = caudal pontine reticular nucleus, Sol = solitary nucleus, PCRt = parvocellular reticular nucleus.



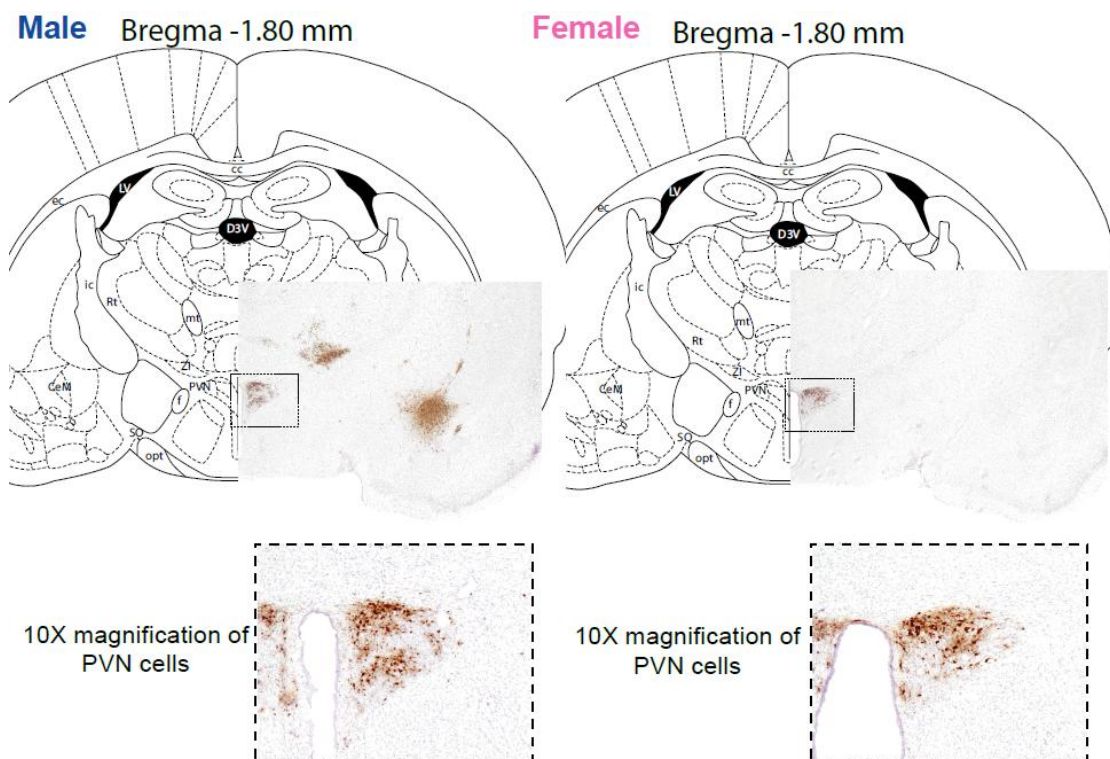
**Figure B.1 Photomicrographs of H129 labeling in the brainstem of a male and a female rat**

Photomicrographs overlaid on rat brain atlas images (Paxinos and Watson, 2005) of H129 labeling in the brainstem of a male and female rat as a result of genital inoculation show robust labeling within the rostroventrolateral medulla in both sexes, and more dorsal brainstem regions in males. Note the discrete labeling within the nPGi in both sexes. nPGi=nucleus paragigantocellularis.



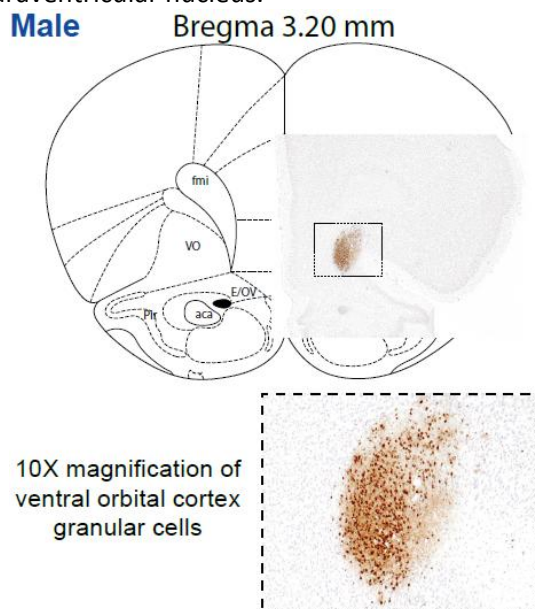
**Figure B.2 Photomicrographs of H129 labeling in the midbrain of a male and a female rat**

Photomicrographs overlaid on rat brain atlas images of H129 labeling in the midbrain of a male and female rat as a result of genital inoculation show labeling within the ventrolateral periaqueductal gray in a male but not a female rat.



**Figure B.3 Photomicrographs of H129 labeling in the thalamus, hypothalamus, and amygdala of a male and a female rat**

Photomicrographs overlaid on rat brain atlas images of H129 labeling in the thalamus, hypothalamus, and amygdala of a male and female rat as a result of genital inoculation show robust labeling in the thalamus and central amygdala in a male but not a female rat. Note that both sexes show robust H129 labeling in the PVN. PVN = paraventricular nucleus.



**Figure B.4 Photomicrographs of H129 labeling in the cerebral cortex of a male rat**

Photomicrographs overlaid on rat brain atlas images of H129 labeling in the cerebral cortex of a male rat as a result of genital inoculation show discrete labeling with ventral orbital granular cells.



**Appendix C – Preliminary data: The paraventricular nucleus of the hypothalamus contains oxytocinergic cells integrated into ascending and descending genital circuitry**

The neuropeptides and hormones oxytocin (OT) and vasopressin (VP) have both peripheral and central actions in mammals, including humans. Both neuropeptides are found in the paraventricular nucleus of the hypothalamus (PVN) with projections to the posterior pituitary, as well as central brain regions (Nilaver et al., 1980; Sofroniew, 1980; Swaab et al., 1975; Swanson and Sawchenko, 1980). OT has long been known to be important in the initiation of vaginal muscle contractions during birth (de Geest et al., 1985), as well as for its facilitatory role in milk ejection during breast feeding (Freund-Mercier et al., 1988). VP (also known as antidiuretic hormone) plays a key role in water retention with its effects on the kidney (Jard et al., 1984). In addition to these peripheral actions, there has been increasing interest in recent years of the central role of OT and VP in the brain. OT has been linked to the control of social behavior in mammals (Donaldson and Young, 2008; Phelps et al., 2010; Ross and Young, 2009), with special focus on the role of OT in the formation of pair-bonds (Hammock and Young, 2006; Macdonald and Macdonald, 2010), as well as its role in sexual behavior (Baskerville and Douglas, 2008). VP has also been linked to social behavior (Donaldson and Young, 2008; Heinrichs et al., 2009), including pair-bonding (Hammock and Young, 2006), as well as aggression (Gobrogge et al., 2009).

With respect to the role of these neuropeptides in sociosexual behavior, one key link in our understanding is missing. How does sexual behavior result in OT and VP neurotransmission? We have previously shown (Appendix A; unpublished results) that genitosensory information reaches the PVN using a trans-synaptic viral tracer to map the functional anatomical pathway of genitosensory information from the genitalia of male and female rats. Given that, in both humans and rats, OT is released into the blood as a result of sexual behavior (Carmichael et al., 1987; Stoneham et al., 1985), it is possible that PVN cells receiving genitosensory information in male and female contain OT and VP. In addition, we have shown that the PVN provides robust projections (that are active during sexual behavior) to the

nucleus paragigantocellularis (nPGi) of the rat rostroventrolateral medulla (Normandin and Murphy, 2008); a tonic inhibitor of genital reflexes in male and female rats (Marson and McKenna, 1990). This places the PVN in a unique functional anatomical position to release OT and/or VP as a result of sexual behavior to regulate both sociosexual behavior and genital reflexes.

To explore the possibility that the PVN cells receiving genitosensory information or that project to the nPGi contain OT or VP male (n=2) and female (n=1) rats (Sprague-Dawley, *Rattus Norvegicus*; Charles River; 275-375g) were inoculated with the anterograde trans-synaptic viral tracer herpes simplex virus 1 strain 129 (H129; see previous section for a detailed description of this tracer) by injection of the virus into the penis and vagina (6x1  $\mu$ l,  $1.2 \times 10^{10}$  pfu) along the right side of the respective genitalia. Six days later, animals were euthanized (SleepAway; 0.5ml i.p.; Henry Schein) and transcardially perfused first with a rinsing agent (0.9% sodium chloride/2% sodium nitrite), then fixative (4% paraformaldehyde 2.5% acrolein). Another group of animals (males, n=2; females, n=2) received unilateral injections of the retrograde tracer Fluorogold (FG; 2% soln. w/v in saline; Fluorochrome) into the nPGi at the following coordinates (in mm): AP: -12.0 Bregma; ML: -1.0; DV: -8.5, by iontophoresis. 10 days later, animals were euthanized and perfused/fixed as above. Brains were removed, sectioned (25 $\mu$ M), and immunohistochemically stained for H129 (rabbit anti-HSV-1, 1:300,000; Dako) or FG (rabbit anti-FG, 1:30,000; Chemicon) with a fluorescent-labeled secondary antibody (Cy2 goat anti-rabbit; Jackson ImmunoResearch) and OT (mouse anti-oxytocin, 1:100,000; Millipore) or VP (guinea pig anti-vasopressin, 1:40,000; Peninsula) with a fluorescent-labeled secondary antibody (Texas Red giant anti-mouse or guinea pig; Jackson ImmunoResearch).

The pattern of labeling of PVN cells that project to the nPGi or receive genitosensory information was remarkably similar in both sexes (Figure C.1). In both sexes, the number of FG and H129 labeled cells in the PVN increased moving from rostral to caudal sections, and appeared to be the same cells groups. These cells were a mix of parvocellular and magnocellular cells, though the majority appear

to be parvocellular. In the more caudal sections of the PVN, some FG labeled cells co-localized with OT labeled cells in both sexes, though the majority did not (Figure C.2), and such co-localization occurred in mostly parvocellular and some magnocellular cells. H129 labeled cells in the caudal portion of the PVN co-localized with OT (Figure C.3) in magnocellular cells, and these cells appear to co-localize with OT to a greater degree in females. Neither FG labeled nor H129 labeled cells in the PVN co-localized with VP in either sex (Figures C.4, C.5).

We found that the patterns of labeling of PVN cells that project to the nPGi and of PVN cells receiving genitosensory information was remarkably similar for both sexes. PVN cells projecting to the nPGi and PVN cells receiving genitosensory information are both located in discrete cell groups mainly comprised of parvocellular PVN cells, though some appear to be magnocellular. The number of PVN cells projecting to the nPGi and PVN cells receiving genitosensory information increased as we move caudally through the PVN. Although we cannot definitively say that the same PVN cells both project to the nPGi and receive genitosensory information, because our tracing was conducted in separate animals, the pattern of labeling of the two circuits is so similar throughout the PVN that it is likely such labeling represents the same cells. This intriguing prospect would place the PVN as a primary integrator of genitosensory input and sexual behavioral output.

OT co-localized with PVN cells projecting to the nPGi, and with PVN cells receiving genitosensory information in both sexes. Such co-localization was primarily observed in the caudal portion of the PVN in parvocellular cells and some magnocellular cells. Such co-localization implies that OT could be a modulator of genital reflexes through the nPGi. Inhibition of male rat sexual behavior by the selective serotonin reuptake inhibitor fluoxetine can be reversed by administration of OT (Cantor et al., 1999). It is possible that such an effect could be mediated by the circuit we have delineated here. Indeed, OT fibers are present in the nPGi in both sexes (unpublished observation), and PVN OT cells also project to the spinal cord motor neurons involved in genital reflexes in male rats (Tang et al., 1998).

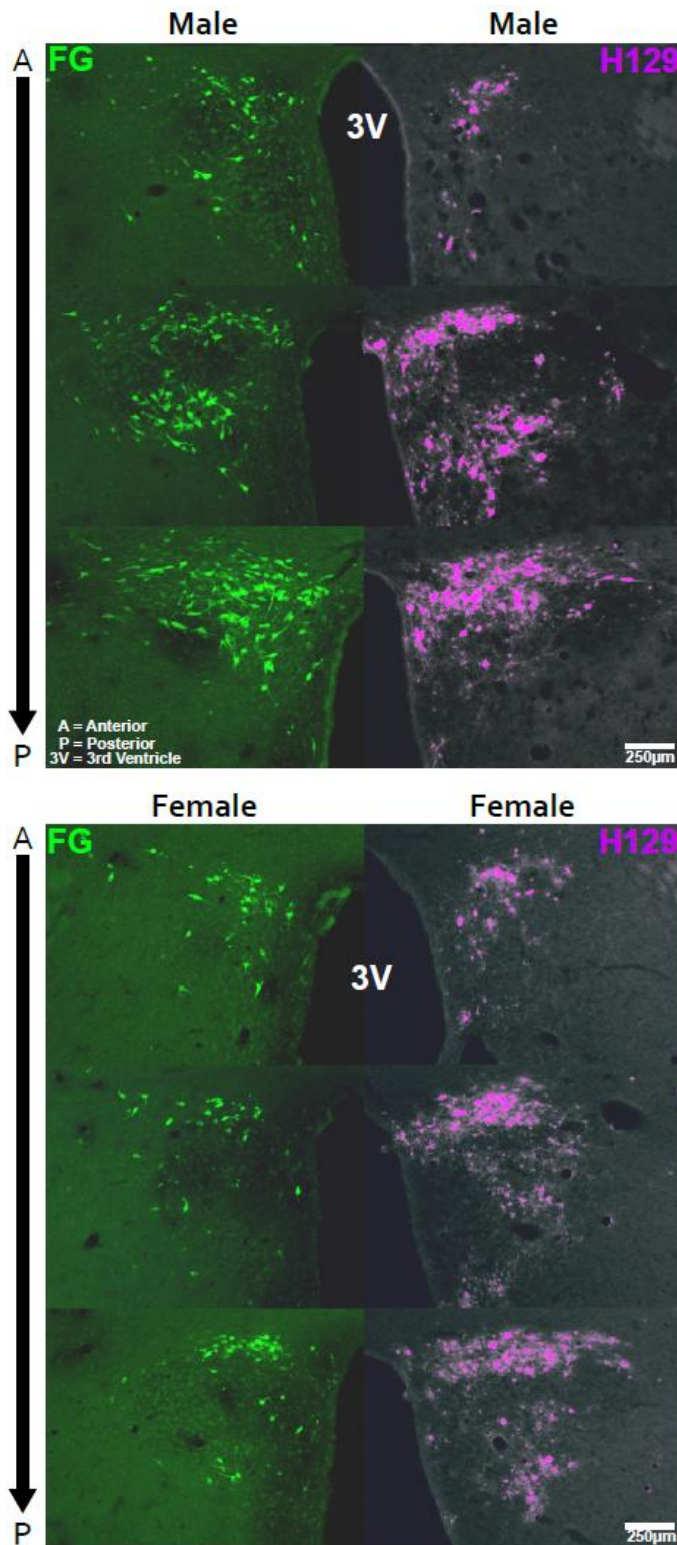
OT also co-localized with PVN cells receiving genitosensory information. In contrast to OT co-localization with PVN cells projecting to the nPGi, OT co-localization with PVN cells receiving genitosensory information was primarily magnocellular. The difference in the localization of our tracers within different subsets of PVN cells implies separate functional connectivity. It is possible that the magnocellular OT cells that receive genitosensory information further project to forebrain regions involved in social behavior, whereas the parvocellular OT cells projecting to the nPGi participate in sexual behavioral output as described above. Supporting this idea, Ross et al. (2009) found that magnocellular OT cells project to the nucleus accumbens in voles. OT in the accumbens is critical for the expression of mating-induced pair-bonding in female voles (Liu and Wang, 2003). Magnocellular oxytocinergic PVN cells that receive genitosensory information may therefore represent the functional anatomical link between mating and pair-bonding.

Contrary to our OT co-localization results, neither PVN cells projecting to the nPGi, nor PVN cells receiving genitosensory information contained VP. With respect to sexual behavior output through the nPGi circuit this result is not surprising, given that VP fibers are not found in the nPGi (unpublished observations), and to do no evidence of vasopressinergic modulation of genital reflexes has been described. However, VP is an important mediator of mating-induced pair-bonding in male voles (Liu et al., 2001) and one might expect that PVN cells receiving genitosensory information might contain VP, which we did not observe. This implies that there is a mediator between genitosensation and VP release.

The majority of PVN cells projecting to the nPGi and PVN cells receiving genitosensory information did not contain OT. This is an important finding suggesting that these circuits, with respect to the PVN, also have other neurochemical components the need to be characterized.

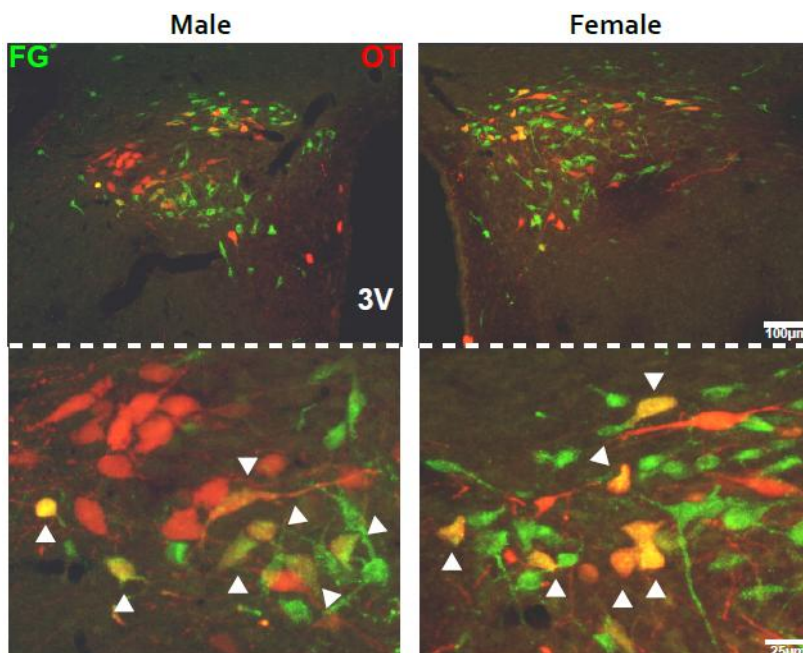
In conclusion, we found that the distribution of cells in the PVN projecting to the nPGi or receiving genitosensory information is quite similar in both sexes. Despite this similarity, oxytocinergic cells within these two pools appear to be distinct. PVN cells projecting to the nPGi that contained OT in both

sexes appear to be predominantly parvocellular cells. In contrast, PVN cells receiving genitosensory information that contain OT appear to be magnocellular, and may be greater in number in females than in males. VP does not appear to be contained in PVN cells projecting to the nPGi or in PVN cells receiving genitosensory information. This data provides the functional neuroanatomical substrate for genitosensory modulation of OT release, and implies that OT can modulate genital reflexes through the nPGi. Taken together, our results add to the growing evidence that OT is an important modulator of sociosexual behavior in both sexes.



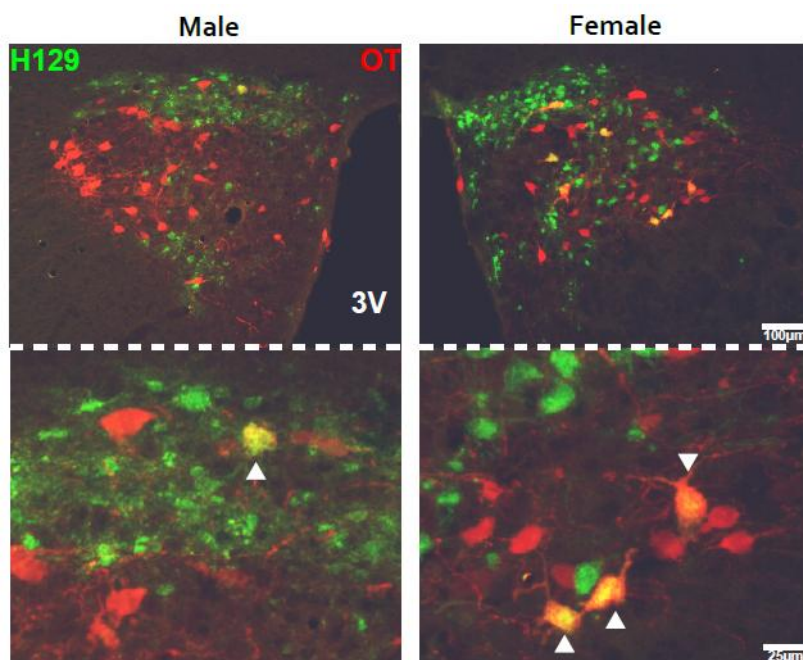
**Figure C.1 Photomicrograph of PVN cells labeled with FG or H129**

Photomicrograph of the paraventricular nucleus of the hypothalamus (PVN) indicates that the number of FG+ cells (green, left side) increased moving caudally through the PVN in a male (top) and a female (bottom) rat. The number of H129+ cells (pseudocolor purple, right side) also increased moving caudally through the PVN in a male (top) and a female (bottom) rat. Note the concordance of labeling between those cells projecting to the nPGi (FG+) and those receiving genitosensory information (H129+). 3V=third ventricle, FG=Fluorogold, H129=herpes simplex virus type 1.



**Figure C.2 Photomicrograph of PVN cells labeled for FG and oxytocin**

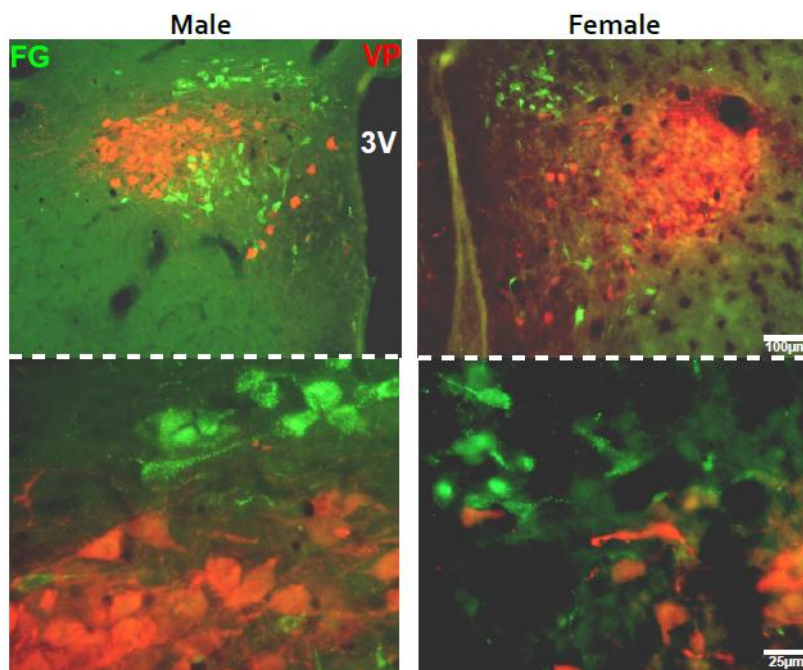
Photomicrographs of the PVN at a lower magnification (top) and higher magnification (bottom) show that FG+ cells (green) co-localized with oxytocin cells (red) in a male (left) and a female (right) rat. Arrows indicate co-localization. 3V=third ventricle, FG=Fluorogold, OT=oxytocin.



**Figure C.3 Photomicrograph of PVN cells labeled for H129 and oxytocin**

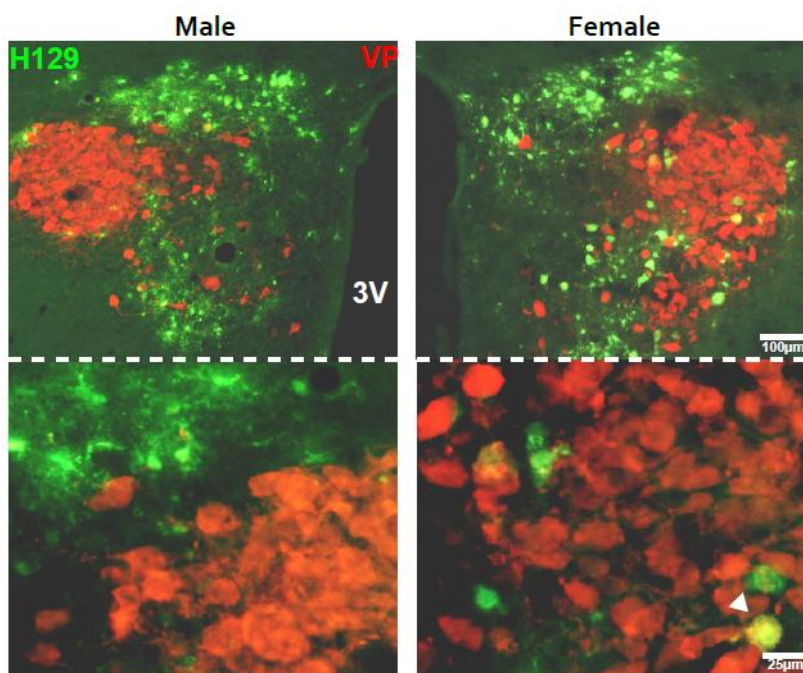
Photomicrographs of the PVN at a lower magnification (top) and higher magnification (bottom) show that H129+ cells (green) co-localized with oxytocin cells (red) in a male (left) and a female (right) rat. Arrows indicate co-localization. 3V=third ventricle, H129=herpes simplex virus strain 129, OT=oxytocin.





**Figure C.4 Photomicrograph of PVN cells labeled for FG and oxytocin**

Photomicrographs of the PVN at a lower magnification (top) and higher magnification (bottom) show that FG+ cells (green) do not co-localize with vasopressin cells (red) in a male (left) and a female (right) rat. 3V=third ventricle, FG=Fluorogold, VP=vasopressin.



**Figure C.5 Photomicrograph of PVN cells labeled for H129 and oxytocin**

Photomicrographs of the PVN at a lower magnification (top) and higher magnification (bottom) show that H129+ cells (green) do not co-localize with vasopressin cells (red) in a male (left) and a female (right) rat. Arrows indicate co-localization. 3V=third ventricle, H129=herpes simplex virus strain 129, VP=vasopressin.



**Appendix D – Curriculum Vitae****CURRICULUM VITAE****Joseph J. Normandin**

American Citizen

Born 5/30/1978, Methuen, Massachusetts, USA

**Home:**

110 Marshall Street

Methuen, MA 01844, USA

Phone: (978) 655-1146

e-mail: jjn78@comcast.net

**Work:**

Netherlands Institute for Neuroscience

Meibergdreef 47

1105 BA, Amsterdam, The Netherlands

Mobile: +31 (0)6 24 91 96 84

e-mail: j.normandin@nin.knaw.nl

**EDUCATION AND EXPERIENCE***Position:* Postdoctoral Fellow*Advisor:* Julie Bakker, Ph.D.*Institution:* Netherlands Institute for Neuroscience, Amsterdam, The Netherlands  
(2010-current)*Degree:* Doctor of Philosophy*Advisor:* Anne Z. Murphy, Ph.D.*Major:* Biological Sciences – Neurobiology and Behavior*Institution:* Georgia State University, Department of Biology, Atlanta, GA  
(2004-2010)*Degree:* Bachelor of Arts*Advisor:* Mary S. Erskine, Ph.D.*Major:* Biology – Specialization in Neuroscience*Institution:* Boston University, Department of Biology, Boston, MA  
(1996-2001)*Position:* Visiting Scientist*Sponsor:* Jacques Balthazart, Ph.D.*Institution:* University of Liege, Center for Cellular and Molecular Neurobiology, Liege, Belgium  
(Summer 2008)*Position:* Research Technician*Sponsor:* Nikos Makris, M.D., Ph.D.*Institution:* Massachusetts General Hospital, Center for Morphometric Analysis, Boston, MA  
(2001-2004)*Position:* Research Assistant*Sponsor:* Mary S. Erskine, Ph.D.*Institution:* Boston University, Department of Biology, Boston, MA  
(2000-2001)*Position:* Research Assistant

*Sponsor:* Richard C. Pillard, M.D

*Institution:* Boston University School of Medicine, Department of Psychiatry, Boston, MA (1996-1998)

### **AWARDS & HONORS**

- Society for Behavioral Neuroscience Travel Award (2008)
- Georgia State University Center for Neuromics Travel Award (2007)
- Atlanta Chapter of the Society for Neuroscience Poster Award (2006)

### **FELLOWSHIP & GRANT AWARDS**

- Georgia State University William M. Suttles Fellow (2008)
- Georgia State University Dissertation Grant (2008)
- Center for Behavioral Neuroscience Venture Grant (PI's A.Z. Murphy & A. Clancy; 2006-2009)
- Center for Behavioral Neuroscience Graduate Scholar (2004-2010)
- Boston University Undergraduate Research Opportunity Program Summer Research Award and Funded Research Opportunity Grant (1998)

### **PROFESSIONAL SERVICE**

- Georgia State University Neuroscience Institute Graduate Education Committee (2008-2009)
- Georgia State University Neurobiology and Behavior Graduate Student Association Co-Chair (2007-2008)
- Center for Behavioral Neuroscience Graduate Student Association Steering Committee (2006-2007)

### **PROFESSIONAL MEMBERSHIPS**

- Society for Neuroscience
- Society for Behavioral Neuroendocrinology
- Organization for the Study of Sex Differences
- American Association for the Advancement of Science

### **INSTRUCTION**

- Mentor, Georgia State University Undergraduates Hau Phuc Bui, Hila Eichenbaum, and Vincent Laufer as part of their work in the lab of Anne Z. Murphy (2008-2010)
- Invited Lecturer, Fundamentals of Neurobiology, "Sex and the Brain" (Georgia State University BIOL 4102/6102; Fall 2007)
- Invited Lecturer, Biology Seminar, "Nature's Chastity Belt: Anatomy and Physiology of the Nucleus Paragigantocellularis" (Georgia State University BIOL 4970; Fall 2007)
- Teaching Assistant, Introduction to Biology for Non-Majors Laboratory (Georgia State University BIOL 1104; Fall 2005, Spring 2006, Spring 2008, Spring 2009)

### **PUBLIC EDUCATION**

- Chamblee High School, Invited Speaker, "Sex Hormones and the Brain," Chamblee, GA (2005)
- Brain Awareness Month, Speaker, GA (2004-2009)
- Zoo Atlanta Brain Expo, Volunteer, Atlanta, GA (2004-2008)
- YouthPride, Invited Speaker, "Neuroscience Comes Out: The Biology of Sexual Orientation and Gender Identity," Decatur, GA (2004)

### **RESEARCH INTERESTS**

- Neural control of sexual behavior
- Neural basis of sex, gender, and sexual orientation

## PUBLICATIONS

**J.J. Normandin**, A.Z. Murphy. Serotonergic lesions of the midbrain source of serotonin to the nucleus paragigantocellularis facilitate sexual behavior in male rats. *In preparation*.

**J.J. Normandin**, A.Z. Murphy. Excitotoxic lesions of the nucleus paragigantocellularis facilitate male sexual behavior but attenuate female sexual behavior in rats. *In preparation*.

**J.J. Normandin**, A.Z. Murphy. Nucleus paragigantocellularis afferents in male and female rats: organization, gonadal steroid sensitivity, and activation during sexual behavior. *Journal of Comparative Neurology*, 508(5):771-94, 2008.

M.R. Herbert, D.A. Ziegler, N. Makris, P.A. Filipek, **J.J. Normandin**, H.A. Sanders, D.N. Kennedy, V. S. Caviness Jr. Localization of white matter volume increase in autism and developmental language disorder. *Annals of Neurology*, 56(3):453, 2004.

N. Makris, D.N. Pandya, **J.J. Normandin**, G.M. Papadimitriou, S.L. Rauch, V.S. Caviness Jr., D.N. Kennedy. Quantitative DT-MRI investigations of the human cingulum bundle. *CNS Spectrums*, 7(7):522-528, 2001.

## ORAL PRESENTATIONS

Center for the Integrative Study of Animal Behavior, Indiana University, "Nature's Chastity Belt: Anatomy and Physiology of Nucleus Paragigantocellularis Afferents," Bloomington, IN (2008)

Southeast Nerve Net Annual Meeting, "Nature's Chastity Belt: Anatomy and Physiology of Nucleus Paragigantocellularis Afferents," Atlanta, GA (2008)

Keck Center for Behavioral Biology Symposium, North Carolina State University, "Nature's Chastity Belt: Anatomy and Physiology of the Nucleus Paragigantocellularis," Raleigh, NC (2008)

## POSTER PRESENTATIONS

**J.J. Normandin**, A.Z. Murphy. Excitotoxic lesions of the nucleus paragigantocellularis facilitate sexual behavior in male but not female rats. Society for Neuroscience, Chicago, IL, 2009.

**J.J. Normandin**, A.Z. Murphy. Excitotoxic lesions of the nucleus paragigantocellularis facilitate sexual behavior in male but not female rats. Society for Behavioral Neuroendocrinology, East Lansing, MI, 2009.

**J.J. Normandin**, H.P. Bui, A.Z. Murphy. Paraventricular Oxytocin Cells Co-localize with Ascending and Descending Genital Reflex Circuitry. Society for Neuroscience, Washington, D.C., 2008.

**J.J. Normandin**, H.P. Bui, A.Z. Murphy. Paraventricular Oxytocin Cells Co-localize with Ascending and Descending Genital Reflex Circuitry. Society for Behavioral Neuroendocrinology, Groningen, Netherlands, 2008.

**J.J. Normandin**, A.Z. Murphy. Feeling Good in the Neighborhood: Trans-Synaptic Tracing of Genitosen-sory Afferents in Male and Female Rats. Society for Neuroscience, San Diego, CA, 2007.

**J.J. Normandin**, A.Z. Murphy. Feeling Good in the Neighborhood: Trans-Synaptic Tracing of Genitosensory Afferents in Male and Female Rats. Society for Behavioral Neuroendocrinology, Pacific Grove, CA, 2007.

**J.J. Normandin**, A.Z. Murphy. Sexually Dimorphic Anatomy and Activation of Nucleus Paragigantocellularis Afferents During Reproductive Behavior. Society for Neuroscience, Atlanta, GA, 2006.

**J.J. Normandin**, A.Z. Murphy. Sexually Dimorphic Anatomy and Activation of Nucleus Paragigantocellularis Afferents During Reproductive Behavior. Society for Behavioral Neuroendocrinology, Pittsburgh, PA, 2006.

**J.J. Normandin**, A.Z. Murphy. Sex-differences in nPGi inputs: role in sexual behavior. Morehouse School of Medicine Women's Health Symposium, Atlanta, GA, 2006.

**J.J. Normandin**, A.Z. Murphy. Sex-differences in the neural regulation of the nucleus paragigantocellularis: potential role in reproductive behavior. Southeast Nerve Net, Atlanta, GA, 2005.

**J.J. Normandin**, A.Z. Murphy. Sex-differences in the neural regulation of the nucleus paragigantocellularis: potential role in reproductive behavior. Society for Neuroscience, Washington, D.C., 2005.

D.N.Kennedy, J.R.Kaiser, M.D.Albaugh, **J.J.Normandin**, G.M.Papadimitriou, J.S.Jackson, O.C.Davis, S.M.Hodge, S.N.Tulloch, D.L.Boriol, C.Haselgrove, R.Piennar, L.Tang, J.D.Howard, V.S.Caviness Jr., N.Makris, E.H.Yeterian. Capturing connectational information: an MRI-based anatomical reference system. Society for Neuroscience, Washington, D.C., 2005

**J.J. Normandin**, A.Z. Murphy. Sex-differences in the neural regulation of the nucleus paragigantocellularis: potential role in reproductive behavior. International Behavioral Development Symposium, Minot, North Dakota, 2005.

**J.J. Normandin**, A.Z. Murphy. Sex-differences in the neural regulation of the nucleus paragigantocellularis: potential role in reproductive behavior. Society for Behavioral Neuroendocrinology, Austin, Texas, 2005.

N.Makris, D.N.Kennedy, M.D.Albaugh, **J.J.Normandin**, G.M.Papadimitriou, J.D.Howard, S.M.Hodge, J.R.Kaiser, L.Tang, S.N.Tulloch, M.E.Dieterich, C.Haselgrove, V.S.Caviness, E.H.Yeterian. Matrices of human cerebral corticocortical, corticothalamic, and corticostriatal connections. Society for Neuroscience, San Diego, California, 2004.

J.D. Schaechter, N. Makris, C.I. Moore, B.R. Rosen, B.D. Connell, **J.J. Normandin**, R.M. Dijkhuizen. Reorganization of somatosensory cortex and motor recovery after stroke. Society for Neuroscience, New Orleans, Louisiana, 2003.

N. Makris, **J.J. Normandin**, G.M. Papadimitriou, M.D. Albaugh, S. McInerney, V.S. Caviness Jr., D.N. Kennedy. Quantitative DT-MRI analysis of the human superior longitudinal fascicle in stroke. Society for Neuroscience, New Orleans, Louisiana, 2003.

N. Makris, S.M. Hodge, M.D. Albaugh, G.M. Papadimitriou, M.E. Dieterich, **J.J. Normandin**, V.S. Caviness Jr., D.N. Kennedy. Pontine consequences of cerebral stroke as revealed by MRI-based morphometry. Organization for Human Brain Mapping, New York, New York, 2003.

M.R. Herbert, D.A. Ziegler, N. Makris, **J.J. Normandin**, H.A. Sanders, D.N. Kennedy, V.S. Caviness Jr. White matter in autism and developmental language disorder: localization of volumetric increase with a novel parcellation method. Society for Neuroscience, New Orleans, Louisiana, 2003.

N. Makris, A. van der Kouwe, **J.J. Normandin**, A. Dale, L. Wald, R.J. Killiany, T.L. Moore, G.M. Papadimitriou, S. McInerney, H.A. Sanders, D.N. Kennedy, M.B. Moss, V.S. Caviness Jr., D.L. Rosene. Age-related white matter analysis in the rhesus monkey brain using MRI. Society for Neuroscience, Orlando, Florida, 2002.

M.R. Herbert, D.A. Ziegler, N. Makris, H.A. Sanders, **J.J. Normandin**, C. Deutsch, D.N. Kennedy, V.S. Caviness Jr. White matter increases in autism are largely in superficial radiate regions. International Meeting for Autism Research, Orlando, Florida, 2002.

**J.J. Normandin**, N. Cameron, M.S. Erskine. Effects of propranolol and diethyldithiocarbamate on female sexual behavior and induction of pregnancy/pseudopregnancy. Symposium on Developmental Neuroendocrinology, Amherst, Massachusetts, 2001.