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Excitotoxic lesions of the nucleus paragigantocellularis facilitate male sexual behavior but attenuate female sexual behavior in rats

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Abbreviations

aVCS = artificial vaginocervical stimulation

CPP = conditioned place preference

KPBS = potassium phosphate buffered saline

MePD = posterodorsal medial amygdala

MPOA = medial preoptic area

NAcc = nucleus accumbens

NeuN = Neuron-specific nuclear protein

NMDA = N-methyl-D-aspartate

nPGi = nucleus paragigantocellularis

PVN = paraventricular hypothalamic nucleus

SEM = standard error of the mean

vlPAG = ventrolateral periaqueductal gray

VMN = ventromedial hypothalamic nucleus

Abstract

Little is known regarding the descending inhibitory control of genital reflexes such as ejaculation and vaginal contractions. The brainstem nucleus paragigantocellularis (nPGi) projects bilaterally to the lumbosacral motoneuron pools that innervate the genital musculature of both male and female rats. Electrolytic nPGi lesions facilitate ejaculation in males, leading to the hypothesis that the nPGi is the source of descending inhibition to genital reflexes. However, the function of the nPGi in female sexual behavior remains to be elucidated. To this end, male and female rats received bilateral excitotoxic fiber-sparing lesions of the nPGi, and sexual behavior and sexual behavior-induced Fos expression were examined. In males, nPGi lesions facilitated copulation, supporting the hypothesis that the nPGi, and not fibers-of-passage, is the source of descending inhibition of genital reflexes in male rats. nPGi lesions in males did not alter sexual behavior-induced Fos expression in any brain region examined. nPGi-lesioned females spent significantly less time mating with stimulus males and had significantly longer ejaculation-return latencies compared to baseline. These results did not significantly differ from control females, but this trend warranted further analysis of the reinforcing value of sexual behavior. Both lesioned and non-lesioned females formed a conditioned place preference (CPP) for artificial vaginocervical stimulation (aVCS). However, post-reinforcement, nPGi-lesioned females did not differ in the percentage of time in spent in the non-reinforced chamber versus the reinforced chamber, suggesting a weakened CPP for aVCS. nPGi lesions in females reduced sexual behavior-induced Fos expression throughout the hypothalamus and amygdala. Taken together, these results suggest that while nPGi lesions in males facilitate copulation, such lesions in females attenuate several aspects of sexual behavior resulting in a reduction in the rewarding value of copulation that may be mediated by nPGi control of genital reflexes. This work has important implications for the understanding and treatment of sexual dysfunction in people including delayed/premature ejaculation, involuntary vaginal spasms, and pain during intercourse.

Keywords: penis, vagina, sex differences, orgasm, lateral paragigantocellular nucleus, ejaculation, descending inhibition, reticulospinal

Approximately 30-40% of men and women experience some form of sexual dysfunction throughout their lifespan, including erectile dysfunction, premature ejaculation, and delayed ejaculation in males, and dyspareunia and vaginal spasms in females (Laumann et al., 1999, Breiner, 2004). One underlying factor that contributes to all of these dysfunctions is a dysregulation of genital reflexes. 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 bilateral projections to the lower lumbar and upper sacral (L5-S1) spinal cord motor neurons that innervate the bulbospongiosus and ischiocavernosus muscles, which, in combination with the hypothesized ejaculation generating lumbar spinothalamic cells (Truitt and Coolen, 2002), are critical for the expression of ejaculation and erection in male rats (Holmes et al., 1991, Sachs and Liu, 1991, Marson and McKenna, 1996, Marson and Carson 3rd, 1999, Tang et al., 1999, Hermann et al., 2003). 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, electrolytic 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 and McKenna, 1990, Marson et al., 1992) 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 of genital reflexes.

Studies examining the impact of nPGi lesions on the full range of male sexual behavior have exclusively employed fiber-destroying electrolytic lesions (Yells et al., 1992, Liu and Sachs, 1999, Holmes et al., 2002). 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) produces 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 (Murphy and Hoffman, 2001, Marson and Foley, 2004, Marson and Murphy, 2006, Normandin and Murphy, 2008), further suggesting a role for the nPGi in female sexual behavior.

In the present study, excitotoxic lesions of the female nPGi were conducted 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 (Erskine, 1992, Coopersmith et al., 1996, Camacho et al., 2009), 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 et al., 1993, Hoffman and Lyo, 2002) in brain regions associated with reward and sexual behavior.

1. Experimental procedures

1.1 Subjects

Adult male (n=23) and female (n=55) Sprague-Dawley Rats (Rattus Norvegicus; Charles River, Wilmington, MA; 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. The Georgia State Institutional Animal Care and Use Committee approved all experiments, with pain and suffering minimized in accordance with the Committee's policies.

1.2 Ovariectomy and gonadal-steroid replacement

All females were ovariectomized and received injections of β-estradiol-3-benzoate (10ug/0.1ml sesame oil s.c.; Sigma Aldrich, St. Louis, MO) 48 hours before testing and progesterone (500ug/0.1ml sesame oil s.c.; Sigma Aldrich) 4 hours before testing, to induce sexual receptivity (Barfield and Lisk, 1970, Quadagno et al., 1972, McEwen et al., 1987).

1.3 nPGi lesions

All surgeries were performed under aseptic conditions. Animals were anesthetized by inhalation of isoflurane (2-5%; Henry Schein, Melville, NY) 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_2O; Sigma Aldrich; 14 males, 40 females), using a 1µl Hamilton syringe. Control animals received bilateral injection of vehicle (dH_2O; 7 males, 20 females). The coordinates for the nPGi were (in mm): AP -12.0 Bregma, Ml +/-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 minutes before being slowly removed. 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.

1.4 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 recorded. 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 recorded 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.

1.5 Conditioned place preference for aVCS

A separate group of hormone primed females engaged 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 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 ascetic 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; this served as the 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 (Tetel et al., 1993, Meerts and Clark, 2009). 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 ([time spent in reinforced chamber – time spent in non-reinforced chamber] / total time), and a difference score (time spent in non-reinforced chamber – time spent in reinforced chamber). Following the final

CPP test, females received aVCS (as described above). 60 minutes later animals were given a euthanizing dose of SleepAway (0.5ml i.p.; Henry Schein) and perfused as described below. Animals that did not explore both of the CPP chambers were excluded from analysis.

1.6 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, Warrington, PA) 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.

1.7 Immunohistochemistry

Brainstem tissue was sectioned at 25µm in a 1:4 series and processed for neuron-specific nuclear protein (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 (Murphy and Hoffman, 2001, Loyd and Murphy, 2006). 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, Billerica, MA; MAB377; monoclonal, raised in mouse; 1:70,000) or Fos (EMD Chemicals, Darmstadt, DE; 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, 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, Burlingame, CA; 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 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 (Sigma Aldrich), and cover-slipped using Permount (Sigma Aldrich). No NeuN or Fos positive cells were observed when the mouse anti-NeuN or rabbit anti-Fos primary antibodies were omitted.

1.8 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 (or absence) of lesions. Analysis was limited to lesions that were bilaterally localized within the nPGi, defined as a bilateral structure located between Bregma -11.30 to -12.80 (Paxinos and Watson, 2005): caudal to the facial nucleus, rostral to the lateral reticular nucleus, and lateral to the pyramidal tract (see Figure 2). The effectiveness of the lesions was confirmed by reduced neuronal nuclei immunoreactivity within the nPGi (minimum of 75% cell loss) determined densitometrically as previously described (Loyd and Murphy, 2006).

1.9 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 (vlPAG), 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. For each animal, one rostro-caudal section (with respect to Bregma in mm.) for each region was selected, and the number of Fos+ neurons determined: MPOA (-0.72); PVN (-1.56); VMN (-2.92), MePD (-2.92), vlPAG (-7.68), and NAcc (1.92).

1.10 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 $p<0.05$.

2 Results

2.1 Lesion verification

Representative photomicrographs of an NMDA lesion of the nPGi and a vehicle sham lesion with a focal point in the nPGi in a male rat are shown in Figure 1. The smallest and largest of the NMDA lesions included for analysis for both sexes are plotted on serial brainstem

sections in Figure 2. Missed injections outside the nPGi that included regions of spread in some acceptable nPGi lesions did not produce any effects on sexual behavior in any group (data not shown).

2.2 Male sexual behavior

nPGi lesions produced a facilitation of male sexual behavior in several measures related to genital reflex function. The mean number of mounts or intromissions in the sexual behavior test did not change significantly from baseline in either the lesion (n=9) or control (n=8) groups (Figure 3A and 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 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 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 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 3E).

Other measures of male sexual behavior not directly related to genital reflex function, including latency to mate and post-ejaculatory interval, 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 4).

2.3 Female sexual behavior

A total of 14 lesioned and 12 control rats were used in this analysis. As shown in Figure 5, female sexual behavior was largely unaffected by lesions of the nPGi. The mean number of intromissions and ejaculations received did not differ from baseline within either group.

Similarly, the stimulus males mean ejaculation latency did not change from baseline within either group. Mean lordosis rating, mean lordosis quotient, and mean number of hop-darts were unchanged from baseline within either group (Figure 6).

nPGi lesions did result 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 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 7). 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 ($p=0.159$; Figure 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 decreased in nPGi-lesioned females, we next tested whether these animals were still able to form a CPP for aVCS.

2.4 Female CPP for aVCS

A total of 13 lesioned and 8 control animals were used for this analysis. It is important to note that, as a group, animals had no overwhelming preference for either the white or gray compartments at baseline, indicating that at a group level, these compartments had equal subjective "value." Individual animals, however, spent more time in one of the compartments, indicating their preference for that compartment, but such preferences were random for both the control and lesion groups (data not shown). 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).

nPGi lesions attenuated several 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 the control group (preference score: p=0.009; difference score: p=0.010), with a mean preference score above 0.50 post-reinforcement, indicating the formation of a CPP for aVCS (Figure 8A & B). However, while the preference score for the reinforced compartment increased as a function of aVCS in the lesion group (preference score: p=0.006) the mean preference score in the lesion group was below 0.50, indicating that lesioned females still preferred the non-reinforced compartment on average. The percent time spent in the reinforced chamber was significantly increased in the control group (p=0.036; Figure 8C), whereas no such increase in the time spent in the lesion group was observed.

2.5 Sexual behavior- and aVCs-induced Fos expression

In males, there were no significant differences between the lesion and control groups in the mean number of sexual behavior-induced Fos+ cells (Figure 9A) for all regions examined.

By contrast, nPGi lesions in females produced a significant reduction in the number of sexual behavior-induced Fos neurons in the MPOA (p=0.040; Figure 9B), PVN (p=0.001; Figure 10), VMN (p=0.029; Figure 11), and MePD (p=0.012) in females. No significant differences in the mean number of sexual behavior-induced Fos+ cells were observed in the NAcc.

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 9C).

3.0 Discussion

3.1 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 behavior 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 associated with MPOA and the mesolimbic dopamine reward system (Agmo and Fernandez, 1989, Hull et al., 1995, Guevara et al., 2008, Kleitz-Nelson et al., 2010). Therefore, nPGi lesions would not be expected to produce any changes in these measures.

Despite the dramatic effects of nPGi lesions on male copulatory behavior, nPGi lesions did not alter sexual behavior-induced Fos in the areas examined 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 several 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 (cell body and fiber-destroying) nPGi lesions on the full range of male sexual behavior (Yells et al., 1992, Liu and Sachs, 1999). 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 urethrogenital reflex is an appropriate model for studying male sexual behavior, as excitotoxic lesions of the nPGi facilitate the urethrogenital reflex in a

manner similar to our results (Marson and McKenna, 1990, Marson et al., 1992). 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.

3.2 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 these data suggest 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, we hypothesized 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 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). These data, together, suggest 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 suggest 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 stimulation females received during aVCS, while strong enough to produce a CPP, did 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 these 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.

3.3 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. The activity of an nPGi homologue in humans could account for such dysregulation if activity in this region is abnormally regulated. For example, brain regions associated with anxiety in humans and non-human animals, such as the central amygdala (McEwen, 2007), project to the nPGi (Normandin and Murphy, 2008) providing a mechanism whereby psychological stress, often noted in ejaculatory disorders in men (McCabe et al., 2010, Rowland et al., 2010) and in female sexual dysfunction (Clayton and Hamilton, 2010, McCabe et al., 2010), could affect nPGi regulation of genital reflexes. In addition, selective serotonin reuptake inhibitors, commonly prescribed for depression (Arroll et al., 2009, Koenig and Thase, 2009), appear to increase inhibition of ejaculation in men

(Kennedy and Rizvi, 2009, Schweitzer et al., 2009) which could in part be mediated by the serotonergic neurons of the nPGi (Marson and McKenna, 1992) or its targets (2007).

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Figure 1 Photomicrograph of sham lesion and lesion sites

Representative photomicrograph of neuron-specific nuclear protein (NeuN) immunolabeled cells within the nucleus paragigantocellularis (nPGi) following injection of vehicle (left) or Intra-nPGi injection of N-methyl-D-aspartate (right) in male rats. Note the markedly reduced immunoreactivity for NeuN in the nPGi of the lesioned animal. nPGi = nucleus paragigantocellularis, py = pyramidal tract, 4V = 4th ventricle

Figure 2 Diagram of lesion sites

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

Figure 3 Measures of male sexual behaviors related to genital reflex function

Mean number of mounts (A), intromissions (B), ejaculations (C), intromissions per ejaculation (D), and ejaculation latency (in min.; E) from baseline to sexual behavior test for control and nPGi-lesioned male rats. * = $p<0.05$, error bars = standard error of the mean (SEM).

Figure 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 5 Measures of stimulus males' sexual behavior during mating with experimental females

Mean number of mounts received (A), intromissions received (B), ejaculations received (C), or mean ejaculatory latency (D) from baseline to sexual behavior test for control and nPGi-lesioned female rats. * = $p<0.05$, error bars = SEM.

Figure 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 7 Measures of female paced-mating behaviors during sexual behavior

Mean number of chamber changes (A), time spent in the mating chamber (in min.; B), and ejaculation return latency (in min.; C) from baseline to sexual behavior test for control and nPGi-lesioned female rats. * = $p<0.05$, error bars = SEM.

Figure 8 Measures of condition placed preference for aVCS in females

Mean preference score for the reinforced compartment (A), difference score (B), and percent time spent in each chamber post-reinforcement (in min.; C) from baseline to the post- artificial vaginocervical stimulation (aVCS) reinforcement test in control and nPGi-lesioned female rats. * = $p<0.05$, error bars = SEM.

Figure 9 Sexual behavior- and aVCS-induced Fos expression in male and female rats

Mean number of sexual behavior-induced Fos+ cells in male (A) and female (B) control and nPGi-lesioned rats in regions associated with sexual behavior and reward. Mean number of aVCS-induced Fos+ cells in female (C) control and nPGi lesioned rats. NAcc = nucleus accumbens, MPOA = medial preoptic area, PVN = paraventricular hypothalamic nucleus, VMN

= ventromedial hypothalamic nucleus, MePD = posterodorsal medial amygdala, vlPAG = ventrolateral periaqueductal gray, * = $p<0.05$, error bars = SEM.

Figure 10 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 paraventricular hypothalamic nucleus. 3V = third ventricle, PVN = paraventricular hypothalamic nucleus, f = fornix.

Figure 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 ventromedial hypothalamic nucleus. 3V = third ventricle, VMN = ventromedial nucleus, f = fornix.