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Lesions of the posterior bed nucleus of the stria terminalis eliminate opposite-sex odor preference and delay copulation in male Syrian hamsters: role of odor volatility and sexual experience

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Abstract

In Syrian hamsters (*Mesocricetus auratus*), the expression of reproductive behavior requires the perception of social odors. The behavioral response to these odors is mediated by a network of ventral forebrain nuclei, including the posterior bed nucleus of the stria terminalis (pBNST). Previous studies have tested the role of pBNST in reproductive behavior, but the use of large, fiber-damaging lesions in these studies make it difficult to attribute post-lesion deficits to pBNST specifically. Thus, the current study used discrete, excitotoxic lesions of pBNST to test the role of pBNST in opposite-sex odor preference and copulatory behavior in both sexually-naïve and sexually-experienced males. Lesions of pBNST decreased sexually-naïve males' investigation of volatile female odors, resulting in an elimination of opposite-sex odor preference. This elimination of preference was not due to a sensory deficit, as males with pBNST lesions were able to discriminate between odors. When, however, subjects were given sexual experience prior to pBNST lesions, their preference for volatile opposite-sex odors remained intact post-lesion. Similarly, when sexually-naïve or sexually-experienced subjects were allowed to contact the social odors during the preference test, lesions of pBNST decreased males' investigation of female odors, but did not eliminate preference for opposite-sex odors, regardless of sexual experience. Finally, lesions of pBNST delayed the copulatory sequence in sexually-naïve, but not sexually-experienced, males such that they took longer to mount, intromit, ejaculate, and display long intromissions. Together, these results demonstrate that pBNST plays a unique and critical role in both appetitive and consummatory aspects of male reproductive behaviors.

Keywords

Olfaction; Reproduction; Pheromone; Extended Amygdala

INTRODUCTION

In many rodent species, including Syrian hamsters, male reproductive behavior depends critically on the perception of odor cues from conspecifics (Johnston 1990). Volatile odor cues are processed primarily by the main olfactory system (MOS), whereas non-volatile cues are processed primarily by the accessory olfactory system (AOS) (Breer 2003). Together, these two systems have been shown to mediate both appetitive and consummatory aspects of reproductive behavior in Syrian hamsters, including males' attraction to vaginal

secretions (Powers et al. 1979) and copulatory behavior (Murphy and Schneider 1970; Powers and Winans 1975).

The behavioral response to these social odors is mediated by a network of ventral forebrain nuclei that receive chemosensory information and are sensitive to steroid hormones (Wood and Coolen 1997). In particular, several lines of evidence suggest the posterior bed nucleus of the stria terminalis (pBNST) may regulate males' attraction to opposite-sex odors. First, pBNST receives social odor information, both directly from the accessory olfactory bulbs (Davis et al. 1978) and indirectly via the corticomedial amygdala (Scalia and Winans 1975; Gomez and Newman 1992). Specifically, pBNST is densely interconnected with the medial amygdala (MA) (Wood and Swann 2005), such that main and accessory olfactory information that converges on MA can terminate at pBNST or continue to the medial preoptic area (MPOA) (Gomez and Newman 1992). In addition to receiving social odor information, pBNST also processes steroid hormone cues that are required for reproductive behavior (Wood et al. 1992; Li et al. 1993; Wood and Newman 1993). Evidence from lesion studies suggests pBNST may be involved in generating the motivation to investigate opposite-sex odors. For example, lesions including pBNST disrupt the copulatory sequence (Valcourt and Sachs 1979; Claro et al. 1995; Liu et al. 1997; Finn and Yahr 2005) and decrease the frequency of penile erections in response to remote odor cues from females in rats (Liu et al. 1997). In male hamsters, large electrolytic lesions including pBNST reduce investigation of female odors when presented alone or during copulation, and also eliminate copulation in some males (Powers et al. 1987).

Although previous studies have implicated pBNST in the regulation of male reproductive behavior, the use of large and/or non-fiber-sparing lesions makes it difficult to attribute these effects to pBNST itself, as BNST is a heterogeneous structure that is bordered by multiple fiber tracts that relay information to other nuclei critical for reproductive behavior, such as MPOA (Wood and Swann 2005). The interpretation of these studies is further complicated by variability in the manner of stimulus presentation and differences in the sexual experience of the subjects. Thus, the following experiments used site-specific, excitotoxic lesions of pBNST to comprehensively test the role of this region in generating the appropriate behavioral response to volatile and non-volatile social odors in both sexually-naïve and sexually-experienced males. We hypothesized pBNST primarily mediates males' attraction to female odors rather than copulatory behavior. We therefore predicted lesions of pBNST would eliminate males' preference for female odors and decrease investigation of females during mating, but leave non-chemoinvestigatory aspects of copulation intact.

MATERIALS AND METHODS

Experimental Design

The goal of the following experiments was to test the role of pBNST in social odor investigation and copulatory behavior in male hamsters. Exposure to female odors causes an increase in circulating testosterone levels in male hamsters (Macrides et al. 1974; Pfeiffer and Johnston 1992) and it is possible that lesions of pBNST may interfere with this surge. Thus, in order to equalize steroid hormone levels across experimental groups, all subjects were gonadectomized and maintained on physiological levels of exogenous testosterone for the duration of the experiment. Following bilateral, excitotoxic lesions of pBNST or sham lesion surgeries, subjects underwent a series of behavioral tests: first, subjects were tested for their preference to investigate female odors over male odors (*Odor Preference*). To determine if any effects of pBNST lesions on odor investigation depend on the volatility of the odor cues being processed, Odor Preference tests were conducted under conditions that either prevented contact with the odor sources (*Non-contact*; volatile odors only) or allowed

contact with the odor sources (*Contact*; volatile and non-volatile odors). Second, in order to determine if a lack of preference in these tests was due to an inability to discriminate between odor stimuli, a separate group of subjects were tested for their ability to discriminate between social and non-social odor sources using a habituation-dishabituation task (*Odor Discrimination*). Lastly, to determine if lesions of pBNST disrupt male copulatory behavior, subjects' response to a receptive stimulus female (*Copulatory Behavior Test*) was assessed. As converging lines of evidence suggest that previous sexual experience can alter neural and behavioral responses to social odors (Dewsbury 1969; Fewell and Meredith 2002; Kelliher and Baum 2002; Meisel and Mullins 2006), we tested the effects of pBNST lesions on both sexually-naïve and sexually-experienced males' behavior.

Animals

Experimental subjects were adult (3 to 6 months old) male Syrian hamsters (*Mesocricetus auratus*) purchased from Charles River Laboratories (Wilmington, MA, USA). A separate group of unrelated adult male and female hamsters served as odor stimulus donors for behavior tests. A third group of ovariectomized and hormone-primed adult female hamsters were used as stimulus females for copulatory behavior tests. Experimental subjects and copulatory stimulus females were single-housed, whereas odor donors were group-housed (three to four animals per cage), in solid-bottom Plexiglas cages (36 cm × 30 cm × 16 cm). All subjects were maintained on a reversed 14 hour light/ 10 hour dark photoperiod, and food and water were available *ad libitum*. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications NO. 80-23; revised 1996) and approved by the Georgia State University Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Sexual Experience

Following gonadectomy (see below), experimental subjects were randomly assigned to one of two experimental conditions: sexually-experienced (EXP; $n = 40$) or sexually-naïve (NVE; $n = 36$). EXP males were given weekly sexual experiences for three consecutive weeks. Subjects were placed into a clear, Plexiglas testing area (50 cm × 25 cm × 30 cm) for five minutes prior to the addition of a receptive stimulus female. An angled mirror was placed below the testing arena to provide a view of the ventral surface of the animals. Encounters lasted for 30 minutes, or until the animals engaged in aggressive behavior, at which point the stimulus female was removed. The second and third encounters were video-recorded and the male's behavior was later scored using the Observer for Windows, version 5.0 (Noldus Information Technology, Wageningen, The Netherlands). Males who failed to copulate on at least two of the three encounters ($n = 7$) were eliminated from the study. To control for possible effects of experimenter handling on future behavior, NVE males were transported to the same behavioral testing room and placed into an empty cage for 30 minutes once a week for three consecutive weeks.

Surgery

All surgeries were performed under 2% isoflurane gas anesthesia vaporized in 100% oxygen (gonadectomy) or a 70:30% oxygen/nitrous oxide mixture (stereotaxic surgery). To minimize post-operative pain, ketoprofen (5 mg/kg subcutaneously, Henry Schein, Melville, NY, USA) was administered intra-operatively.

Gonadectomy and Hormone Implant—One to two weeks prior to lesion surgery, subjects' testes were bilaterally removed via a bilateral midline abdominal incision and cauterization of the ductus deferens and blood vessels. Silastic capsules (i.d. 1.57 mm, o.d.

2.41 mm, Dow Corning, Midland, MI, USA) packed with 20 mm length of crystalline testosterone (Sigma, St. Louis, MO, USA) were implanted subcutaneously between the scapulae immediately following gonadectomy. Vicryl suture (size 4-0, Ethicon, Somerville, NJ, USA) and wound clips were used to close the smooth muscle and skin incisions, respectively.

Stimulus females for copulatory experiences and behavior testing were ovariectomized at least two weeks prior to use. Following bilateral flank incisions, the ovaries were removed via cauterization of the uterine horn and blood vessels. Silastic capsules (i.d. 1.57 mm, o.d. 2.41 mm, Dow Corning, Midland, MI, USA) packed with 5 mm length of crystalline estradiol (Sigma, St. Louis, MO, USA) were implanted subcutaneously between the scapulae immediately following gonadectomy. Vicryl suture (size 4-0, Ethicon, Somerville, NJ, USA) and wound clips were used to close the smooth muscle and skin incisions, respectively. To induce behavioral receptivity, stimulus females were injected subcutaneously with 0.15 ml of progesterone dissolved in sesame oil (2.5 mg/ml, Sigma, St. Louis, MO, USA) 4 hours prior to copulatory behavior tests.

Excitotoxic Lesion—NVE and EXP subjects were randomly assigned to either a pBNST lesion (pBNST-X; NVE $n = 26$, EXP $n = 25$) or a sham lesion surgery (SHAM; NVE $n = 8$, EXP $n = 10$) group. Anesthetized subjects were secured in the stereotaxic apparatus such that their skull was level in the anterior-posterior (A-P) and medial-lateral (M-L) planes. Following a midline scalp incision, the skin and temporal muscles were retracted to expose the skull and a hand-operated drill was used to expose dura. All A-P and M-L measurements were taken in mm relative to bregma and all dorsal-ventral (D-V) measurements were taken in mm relative to dura. Excitotoxic lesions were made by lowering a microinjection syringe (701R 10 μ l syringe, Hamilton, Reno, NV, USA) under stereotaxic control into pBNST and injecting N-methyl-D-aspartic acid (NMDA, 20 mg/ml; 20 nl per injection, Sigma, St. Louis, MO, USA) into two bilateral sites targeting the posterointermediate BNST (BNST_{pi}; A-P: -1.85 , M-L: ± 1.65 , D-V: -5.8) and posteromedial BNST (BNST_{pm}; A-P: -1.85 , M-L: ± 1.05 , D-V: -5.9), respectively. To minimize the flow of excitotoxin up the syringe tract, the syringe was left in place for 10 minutes after each injection.

Sham surgeries were identical to lesion surgeries with two exceptions: 1) the microinjection syringe was lowered to 1 mm above the target injection site and 2) no excitotoxin was infused into the target injection sites. After all surgeries, skull holes were sealed using bone wax and incisions were closed with wound clips. Subjects were allowed to recover for at least two weeks prior to behavioral testing.

Behavioral Testing

All behavior testing took place during the first six hours of the dark phase and under light illumination.

Odor Stimuli—Social odor stimuli used for Odor Preference and Odor Discrimination tests were collected from cages of group-housed, same-sexed odor donors that had not been changed for at least four days prior to odor collection. Each social odor stimulus consisted of soiled cotton bedding (2 Nestlets, 12 g, ANCARE, Bellmore, NY, USA), soiled corncob litter (50 ml, Bed-o-cob, The Andersons, Maumee, OH, USA) and one damp cotton gauze pad that was used to wipe the inner walls of the cage. In addition, a damp gauze pad was used to wipe two of the cage resident's anogenital region and bilateral flank glands 10 times each. For female odor stimuli, vaginal secretion was also collected onto a gauze pad by gently palpating the vaginal area of one female with a disposable probe, and was added to each odor stimulus. Clean odor stimuli consisted of clean cotton bedding (2 Nestlets), clean

corncob litter (50 ml), and clean cotton gauze pads (2). For non-social odor discrimination tests, odor stimuli consisted of 0.10 g (three beads) of an artificial, multi-component odorant (baby powder or strawberry, International Flavors & Fragrances, Inc., NY, USA) mixed with clean odor stimuli.

For Contact tests, additional odors were collected directly onto glass microscope slides (25 mm × 75 mm × 1 mm) by rubbing a clean slide along an odor donor's flank and anogenital regions. All odor slides contained samples from two individual odor donors (collected separately onto each end of the slide). For female odor slides, a sample of vaginal secretion was also collected onto the same slide. All odor stimuli were stored in airtight containers at 4°C until 20 minutes before use. Odor stimuli older than one month were discarded and no subject was tested with the same odor stimulus more than once.

Odor Preference—A three-choice test was used to measure odor preference. Subjects were placed into a glass aquarium (50 cm × 25 cm × 30 cm) with opaque walls. Three acrylic odor presentation boxes (8 cm × 8 cm × 8 cm) were affixed to one of the short walls of the aquarium such that only the front and top surfaces of each box were accessible. Each odor presentation box had 7-mm holes drilled along the front surface that allowed volatile odors to pass, but prevented contact with the odor sources. During testing, a single odor stimulus (see above) was placed into each of the three odor presentation boxes. Additionally, a line bisecting the available floor space was drawn parallel to the short walls so that general activity levels (as measured by total number of line crosses) could be assessed during the test. The top of the aquarium was secured with a clear Plexiglas top to allow for overhead video recording of the subject's behavior. All surfaces of the aquarium and odor containers were thoroughly cleaned with 70% alcohol and allowed to dry between subjects.

Subjects were tested in a series of three tests in the 3-choice apparatus, each separated by 24 hours: Clean, Non-Contact preference, and Contact preference. At the beginning of each test, a subject was placed into the testing arena and then allowed ten minutes to freely explore the apparatus. For all tests, investigation of the odor stimulus was coded when the subject made contact with, or directed its nose within 1 cm of, the perforated front surface of the odor container and/or odor slide. For Clean tests, clean odor stimuli were placed into each of the three odor containers. These tests were used to acclimate the subjects to the testing arena, as well as to obtain baseline levels of activity in the absence of social odor stimuli. For subsequent preference tests, female and male odor stimuli were placed into each of the two outer odor containers, and clean odor stimuli were placed into the center odor container. The side on which each social odor was placed (left or right) was alternated between consecutive subjects. Non-Contact and Contact tests were identical except that during Contact tests, a single odor slide matching the type of odor stimulus in that container (female, male, clean) was secured to the center of the front surface of each odor presentation box.

Video recordings of all tests were digitized onto a computer and scored using the Observer for Windows, version 5.0 (Noldus Information Technology, Wageningen, The Netherlands). All observers were blind to the condition of the subject, and different observers reached at least a 90% inter-observer reliability score prior to coding behavior.

Odor Discrimination—In order to determine if the lack of preference for non-contact odors observed in NVE subjects (see Results) was due to an inability to discriminate between odor stimuli, a separate group of NVE males (pBNST-X $n = 12$, SHAM $n = 8$) were tested for their ability to discriminate between volatile odors using a habituation-dishabituation task. The habituation-dishabituation task involves repeated presentations of the same odor source followed by a test presentation of a novel odor source. A decrease in

investigation during the repeated presentations indicates a perception of the odors as being the same or familiar. An increase in investigation of the novel odor compared to the last presentation of the habituated odor indicates an ability to discriminate between the two odors (Johnston 1993; Baum and Keverne 2002). The testing sequence consisted of four, 3-minute presentations of repeated odors (habituation) followed by a fifth, 3-minute presentation of a novel odor (dishabituation). Five-minute inter-trial intervals separated each odor presentation. Odor stimuli were presented in modified 50 ml polypropylene collection tubes, with 0.5-cm holes drilled 1 cm apart along the surface of the tube. Wire mesh lined the inner surface of the odor container to prevent contact with the odor stimulus. Odor containers were placed into the center of subjects' home cage and investigation was measured using a stopwatch. Odor containers were cleaned with 70% alcohol and allowed to dry between subjects.

Under these testing parameters, male hamsters consistently display a lack of habituation to repeated presentations of female odors (Maras and Petrulis 2006) and so all subjects were tested using male odors as the habituation stimuli and female odors as the dishabituation stimuli for social odor discrimination tests. Subjects were presented with different male odor sources on each of the habituation trials so that subjects were habituated to the sexual identity of the repeated odor, rather than to the individual identity of odor donors. In addition, in order to determine if lesion-induced deficits were specific to social odors, rather than a general deficit in odor processing, subjects were tested for their discrimination of two complex, artificial odors (strawberry and baby powder; see above).

Copulatory Behavior—Copulatory behavior test procedures were identical to those used to provide sexual experience to EXP males (see above). Tests were video-recorded and later scored using Observer for Windows, version 5.0 (Noldus Information Technology, Wageningen, The Netherlands). The total number and latencies (from onset of test) of several behavioral measures were scored: mounts (M), intromissions (I), ejaculations (E), and long intromissions (LI). LI are distinguished from I in that males do not quickly dismount the female following vaginal penetration, but instead display a repetitive thrusting pattern (Bunnell et al. 1977). Importantly, the expression of LI is associated with the onset of sexual satiety in Syrian hamsters (Bunnell et al. 1977; Parfitt and Newman 1998). In addition, the total durations of time the male spent investigating the female's anogenital region (AGI), investigating the female's head or body region (HBI), and self-grooming (SG) were also scored. Finally, several derived measures of copulatory behavior were also analyzed: post-ejaculatory interval (PEI; latency to display a mount or intromission after each ejaculation, the number of intromissions to reach each ejaculation (I-E), and mounting efficiency (ME; the total number of intromissions divided by the total number of mounts + intromissions).

Histology and Lesion Verification

Following the last behavioral test, subjects were injected with an overdose of sodium pentobarbital (100 mg/kg; Sleep Away, Ft. Dodge, IA, USA) and transcardially perfused with 200 ml of 0.1M phosphate-buffered saline (PBS, pH 7.4) followed by 200 ml of paraformaldehyde (4%). Brains were post-fixed in paraformaldehyde (4%) overnight and then cryoprotected for 48 hours in 30% sucrose in PBS solution. Coronal sections (30- μ m) of brain tissue were sectioned using a cryostat (-20°C) and stored in cryoprotectant until immunohistochemical localization of Neuronal Nuclei protein (NeuN, see below). Additionally, in order to further delineate lesion damage from fiber tracts (which are not readily detected by NeuN), every third section was mounted onto glass slides using a 1% gelatin mounting solution and stained for Nissl material with cresyl violet (Simga, St. Louis, MO, USA). Nissl- and NeuN-stained sections were examined under a light microscope for

the location and extent of lesion damage as compared with published hamster neuroanatomical plates (Morin and Wood 2001), and the minimum and maximum extents of lesion damage were traced onto anatomical plates using Adobe Illustrator CS 11.0 software.

Immunohistochemistry

Free-floating sections were removed from cryoprotectant, rinsed thoroughly in PBS, and then incubated in a monoclonal antibody against NeuN (1:30,000, Millipore MAB377, Billerica, MA, USA) in PBS with 0.4% Triton-X-100 for 48 hours at 4°C. After rinsing in PBS, sections were incubated in biotinylated secondary antibody (Rabbit Anti-Mouse, 1:600, Jackson ImmunoResearch Laboratories 315-065-003, West Grove, PA, USA) in PBS with 0.4% Triton-X-100 (Sigma, St. Louis, MO, USA) for one hour at room temperature, rinsed in PBS, then incubated in an avidin-biotin complex (1:200, Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA) for one hour at room temperature. Sections were then rinsed in PBS and reacted in a nickel-enhanced 3, 3'-diaminobenzidine tetrahydrochloride (DAB) solution (2 mg DAB plus 250 mg Nickel (II) Sulfate with 8.3 µl 3% H₂O₂ per 10ml of 175 mM sodium acetate, Sigma, St. Louis, MO, USA) to yield a blue-black reaction product. After 15 minutes, sections were rinsed in PBS to stop the chromagen reaction.

Blood Collection and Radioimmunoassay

Blood samples were collected from the inferior vena cava after anesthesia and immediately prior to perfusion and stored in vacutainer collection tubes (4-mL draw, red/gray, VWR, West Chester, PA, USA) on ice until centrifugation. Samples were centrifuged at 2500 revolutions per minute at 4°C for 20 minutes and serum was stored in 200-µl aliquots at -20°C until assay. Testosterone levels were measured by radioimmunoassay kits from Diagnostics System Laboratories (DSL 4000, Beckman Coulter, Brea, CA, USA) with a sensitivity range of 0.05 to 22.92 ng/mL and an interassay variance of 6%, previously validated for hamster serum (Cooper et al. 2000). The mean testosterone levels (ng/nl) for experimental subjects were: NVE pBNST-X = $4.25 \pm .58$; NVE SHAM = $3.027 \pm .36$; EXP pBNST-X = $3.78 \pm .70$; EXP SHAM = $4.193 \pm .66$. There was no difference in testosterone levels between lesion groups for NVE or EXP males (NVE $t_{18} = 0.51$, $P = 0.61$; EXP, $t_{18} = 1.69$, $P = 0.11$).

Data Analysis

All data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows and significance was determined as $p < .05$. To establish investigatory preferences for each type of 3-choice test (Clean, Non-Contact Preference, Contact Preference), 2 (Experimental group: pBNST-X, SHAM) $\times$ 3 (Odor) mixed-design ANOVAs were performed. Significant interactions were explored using simple effects analysis and pair-wise comparisons with Bonferroni alpha adjustments. Furthermore, separate one-way ANOVAs were used to compare the levels of investigation of each stimulus directly across experimental groups. To identify differences in general motor activity, additional one-way ANOVAs were used to compare the total number of midline crosses across experimental groups for the Clean test.

For the habituation-dishabituation data, data were split by experimental group, and paired t -tests (2-tailed, with Bonferroni alpha adjustments, $\alpha_{FW} = .05$) were used to detect both (1) a habituation to the repeated presentations of odors (odor 1 vs. odor 4) and (2) a dishabituation to the presentation of the test odor (odor 4 vs. Test).

Many copulatory behavior tests were terminated before 30 minutes because the male and female engaged in aggressive behavior. Thus, only the first 20 minutes of each copulatory test were analyzed to eliminate variability caused by differences in test duration. Group

differences in all copulatory measures were detected using one-way ANOVAs. Additionally, separate 2×2 (lesion group $\times$ ejaculatory series) ANOVAs were used to detect changes in post-ejaculatory intervals or the number of intromissions to reach each ejaculation in the copulatory tests.

RESULTS

Lesion Reconstruction

Subjects were included in the pBNST-X lesion group (NVE $n = 12$; EXP $n = 12$) only if they had extensive bilateral damage to pBNST, defined as at least 60% bilateral damage to BNSTpi and BNSTpm in at least two stereotaxic planes of section (Figure 1a; (Morin and Wood 2001)). All subjects included in the pBNST-X lesion group sustained significant damage to the largest part of pBNST (Bregma 0.2 mm), including BNSTpi and BNSTpm, as well as the posterolateral BNST (BNSTpl; Figure 1c). Most subjects ($n = 21$) also sustained significant damage to more rostral and anterior (Bregma 0.5 mm) parts of BNSTpi and BNSTpm. Ten subjects had bilateral lesion damage to more caudal and posterior pBNST (Bregma 0.1 mm); of these subjects, half had bilateral damage of both BNSTpi and BNSTpm and half had bilateral damage to BNSTpm only. No subjects included in the pBNST-X group sustained damage to the most caudal and ventral level of pBNST (Bregma -0.3 mm).

Subjects were excluded from the pBNST-X group if their lesions extended significantly outside of pBNST or failed to damage a significant portion of pBNST. Specifically, one group of subjects had partial (less than 60% damage in two stereotaxic planes of section), bilateral damage to pBNST (NVE $n = 3$; EXP $n = 3$) and another separate group of subjects had primarily unilateral damage to pBNST (NVE $n = 6$; EXP $n = 1$). Analyses indicated that these subjects' behavior did not differ significantly from SHAM subjects' behavior in Odor Preference or Copulatory Behavior tests (see Results). Only needle tracts were visible in most SHAM males (Figure 1b; NVE $n = 8$; EXP $n = 8$); three SHAM males also had unilateral cortical damage. These subjects did not differ in behavior from SHAM subjects without cortical damage and were retained in the analysis.

Some subjects included in the pBNST-X group sustained minimal or unilateral damage to other adjacent nuclei, defined as less than 20% damage at only one stereotaxic plane of section of the nucleus (Morin and Wood 2001). This included nuclei within the anterior BNST, such as the anterointermediate BNST ($n = 8$), anteromedial BNST ($n = 6$), and anteroventral BNST ($n = 10$). Outside of the BNST, partial or unilateral damage was sustained to sub-cortical nuclei including the ventral lateral septum ($n = 6$), paraventricular hypothalamic nucleus ($n = 8$), and globus pallidus ($n = 4$). Finally, partial or unilateral damage also occurred in thalamic nuclei, such as the anterodorsal thalamic nucleus ($n = 3$), anteroventral thalamic nucleus ($n = 1$), ventrolateral anteroventral thalamic nucleus ($n = 1$), reuniens thalamic nucleus ($n = 2$), and reticular thalamic nucleus ($n = 3$). Two males also sustained unilateral damage to overlying cortex. There was no difference in behavior across subjects with unilateral, bilateral, or no damage to any of the adjacent nuclei.

Behavioral Measures

Odor Preference

Clean Test: In the Clean test, both NVE and EXP subjects investigated the three stimulus containers equally (NVE $F_{2,23} = 3.37$, $P = 0.14$; EXP $F_{2,35} = 2.69$, $P = 0.23$). There were also no differences in the total number of midline crosses (NVE $t_{18} = 1.48$, $P = 0.15$; EXP $t_{18} = 1.35$, $P = 0.47$) or total duration of investigation of the three odor containers (NVE $t_{18} = .641$, $P = 0.08$; EXP $t_{18} = .861$, $P = 0.13$) between pBNST-X and SHAM males.

Non-Contact Preference Test: Lesions of pBNST decreased NVE males' investigation of volatile female odors, resulting in an elimination of opposite-sex odor preference. There was a significant interaction between lesion group and the duration of investigation of the three odor stimuli ($F_{2,54} = 3.51$, $P = 0.03$). Whereas SHAM males investigated female odors longer than male odors ($t_7 = 5.00$, $P < 0.01$) and investigated both female ($t_7 = 6.31$, $P < 0.01$) and male ($t_7 = 2.65$, $P = 0.03$) odors more than clean odors, pBNST-X males spent equivalent amounts of time investigating male and female odors ($t_{11} = 0.53$, $P = 0.60$), and did not investigate either female ($t_{11} = 1.51$, $P = 0.16$) or male ($t_{11} = 2.07$, $P = 0.06$) odors more than clean odors. In addition, pBNST-X males spent significantly less time investigating female odors ($F_{1,19} = 8.51$, $P < 0.01$), and significantly more time investigating clean odors ($F_{1,19} = 4.79$, $P < 0.01$), than did SHAM males (Fig 2a).

In contrast to NVE subjects, lesions of pBNST did not decrease EXP males' investigation of volatile female odors. In EXP males, there was a significant main effect of odor stimulus ($F_{2,53} = 21.652$, $P < 0.01$), but no interaction between lesion group and the duration of investigation of the three odor stimuli. Both SHAM and pBNST-X males spent more time investigating female odors than male odors (SHAM $t_7 = 2.64$, $P = 0.03$; pBNST-X $t_{11} = 2.24$, $P = 0.04$) and investigated female odors more than clean odors (SHAM $t_7 = 2.79$, $P = 0.03$; pBNST-X $t_{11} = 5.04$, $P < 0.01$). pBNST-X males also spent significantly more time investigating male odors than clean odors ($t_{11} = 5.71$, $P < 0.01$), although this was not the case for SHAM males ($t_7 = 1.371$, $P = 0.21$; Fig 2b).

In both NVE and EXP males, neither unilateral nor partial, bilateral lesions of pBNST disrupted males' preference to investigate opposite-sex odors in the Non-Contact test (Table 1a). Although the small sample sizes of these groups precluded them from statistical analysis, examination of the means confirmed that, like SHAM males, males with incomplete damage to pBNST investigated female odors more than they did male or clean odors.

Contact Preference Test: Lesions of pBNST decreased both NVE and EXP males' investigation of female odors in the Contact test, but did not eliminate opposite-sex odor preference. In NVE males, there was a significant interaction between lesion group and the duration of investigation of the three odor stimuli ($F_{2,54} = 6.43$, $P = 0.03$). Whereas SHAM males spent significantly more time investigating female odors than male odors ($t_7 = 3.86$, $P = 0.01$), and investigated both female ($t_7 = 4.28$, $P < 0.01$) and male ($t_7 = 2.40$, $P = 0.04$) odors more than clean odors, pBNST-X males spent more time investigating female odors than male odors ($t_{11} = 2.49$, $P = 0.03$) or clean odors ($t_{11} = 2.99$, $P = 0.01$), but did not spend more time investigating male odors than clean odors ($t_{11} = 2.05$, $P = 0.06$). In addition, pBNST-X males spent significantly less time investigating female odors ($F_{1,19} = 5.28$, $P = 0.03$), and significantly more time investigating clean odors ($F_{1,19} = 5.55$, $P = 0.03$), than did SHAM males (Figure 3a).

In EXP males, there was a significant interaction between lesion group and the duration of investigation of the three odor stimuli ($F_{2,53} = 8.71$, $P < 0.01$). Both SHAM and pBNST-X males spent significantly more time investigating female odors than male odors (SHAM $t_7 = 9.91$, $P < 0.01$; pBNST-X $t_{11} = 6.55$, $P < 0.01$) and spent more time investigating both female (SHAM $t_7 = 11.37$, $P < 0.01$; pBNST-X $t_{11} = 8.20$, $P < 0.01$) and male (SHAM $t_7 = 3.33$, $P = 0.01$; pBNST-X $t_{11} = 3.21$, $P < 0.01$) odors than clean odors. pBNST-X males, however, spent significantly less time investigating female odors than did SHAM males ($F_{1,19} = 12.21$, $P < .01$; Figure 3b).

In both NVE and EXP males, neither unilateral nor partial, bilateral lesions of pBNST disrupted males' preference to investigate opposite-sex odors in the Contact test (Table 1b).

Although the small sample sizes of these groups precluded them from statistical analysis, examination of the means confirmed that, like SHAM males, males with incomplete damage to pBNST investigated female odors more than they did male or clean odors.

Odor Discrimination

Social Odors: NVE pBNST-X and SHAM males habituated to repeated presentations of different male odors, as indicated by decreased investigation of the male odor on the fourth trial compared to the first trial (pBNST-X $t_{13} = 2.99$, $P < 0.01$; SHAM $t_8 = 3.61$, $P < 0.01$). Importantly, both lesion groups also dishabituated to a novel female odor, as indicated by an increased investigation of the female odor compared to the last presentation of the habituated male odor (pBNST-X $t_{13} = 4.62$, $P < 0.01$; SHAM $t_8 = 5.21$, $P < 0.01$; Figure 4a).

Non-Social Odors: Both lesion groups habituated to repeated presentations of the non-social odors, as indicated by a decreased investigation on the fourth trial compared to the first trial (pBNST-X $t_{11} = 4.12$, $P < 0.01$; SHAM $t_8 = 3.16$, $P < 0.01$). Importantly, both experimental groups also dishabituated to a novel non-social odor, as indicated by an increased investigation of the test odor compared to the last presentation of the habituated odor (pBNST-X $t_{11} = 3.05$, $P < 0.01$; SHAM $t_8 = 3.028$, $P < 0.01$; Figure 4b).

Copulatory Behavior

All NVE and EXP males ejaculated and all except two pBNST-X males reached sexual satiety, as indicated by the expression of long intromissions. In NVE males, however, lesions of pBNST disrupted several aspects of copulatory behavior.

In NVE subjects, the total number of mounts, intromissions, ejaculations, and long intromissions did not differ between pBNST-X and SHAM males (Table 2a; all $P > 0.05$). However, pBNST-X males took significantly longer than SHAM males did to mount ($F_{1,19} = 5.29$, $P = 0.03$), intromit ($F_{1,19} = 6.12$, $P = 0.02$), ejaculate ($F_{1,19} = 11.47$, $P = 0.04$), and display long intromissions ($F_{1,17} = 4.42$, $P = 0.05$; Figure 5). pBNST-X males also had a significantly longer latency to investigate the females' anogenital region ($F_{1,19} = 8.01$, $P = 0.01$), although the total duration of anogenital investigation, head-body investigation, or self-grooming did not differ between lesion groups (Table 2b; all $P > 0.05$). These increased latencies were not simply due to a delay in the initiation of mating, as the latency to display mounts, intromissions, ejaculations, and long intromissions remained significantly longer in pBNST-X males compared to SHAM males when data was normalized for the initiation of anogenital investigation (data not shown). NVE pBNST-X males did not differ from SHAM males in post-ejaculatory interval, intromissions-to-ejaculation, or mount efficiency (Table 2c; all $P > 0.05$).

In EXP subjects, lesions of pBNST did not disrupt any measure of copulatory behavior. pBNST-X males did not differ from SHAM males in their latencies to mount ($F_{1,16} = 0.01$, $P = 0.95$), intromit ($F_{1,16} = 0.51$, $P = 0.49$), ejaculate ($F_{1,16} = 0.55$, $P = 0.46$), or display long intromissions ($F_{1,15} = 2.06$, $P = 0.18$; Figure 5), nor did they differ in the total number (Table 2a) or durations (Table 2b) of any of the behaviors measured (all $P > 0.05$). There was, however, a non-significant trend to spend less time investigating the anogenital region in pBNST-X males than SHAM males ($F_{1,16} = 3.56$, $P = 0.07$). EXP pBNST-X males also did not differ from SHAM males in any of the derived measures of sexual behavior (Table 2c; all $P > 0.05$).

In both NVE and EXP males, neither unilateral nor partial, bilateral lesions of pBNST disrupted any measure of copulatory behavior. Although the small sample sizes of these

groups precluded them from statistical analysis, examination of the means confirmed that males with incomplete damage to pBNST did not differ from SHAM males in their total number (Table 3a) or latencies to display (Table 3b) any mating event.

DISCUSSION

These results demonstrate for the first time that pBNST specifically mediates both appetitive and consummatory aspects of male reproductive behavior. Unlike previous studies in which lesions extended significantly outside of pBNST and/or were created with fiber-damaging techniques, the current study demonstrates that discrete, excitotoxic lesions of pBNST decreased males' investigation of volatile female odors, resulting in an elimination of opposite-sex odor preference and supporting our prediction that pBNST mediates males' attraction to female odors. This lack of preference was not due to an inability to discriminate between volatile odors, as pBNST-X males performed at levels comparable to SHAM males on social and non-social odor discrimination tasks. When, however, subjects were given sexual experience prior to pBNST lesions, their preference for volatile opposite-sex odors remained intact post-lesion. Similarly, when sexually-naïve or sexually-experienced subjects were allowed to contact the social odors during the preference test, thus providing access to both volatile and non-volatile social odors, pBNST lesions did not eliminate preference for opposite-sex odors. Lesions of pBNST did, however, decrease males' investigation of female odors in this test, regardless of sexual experience. Finally, lesions of pBNST delayed the copulatory sequence in sexually-naïve, but not sexually-experienced, male hamsters such that they took longer to mount, intromit, ejaculate, and display long intromissions compared to SHAM males. These results suggest that, contrary to our prediction, pBNST also mediates non-chemoinvestigatory aspects of copulatory behavior in sexually-naïve males.

Role of pBNST in Opposite-Sex Odor Preference

The finding that lesions of pBNST decreased males' investigation of volatile and non-volatile female odors is consistent with data from previous lesion studies that suggest that pBNST may mediate males' attraction to female odors. Specifically, in male rats, radiofrequency lesions including pBNST decrease non-contact erections in response to volatile cues from estrous females (Liu et al. 1997). Similarly, in male hamsters, large, electrolytic lesions including pBNST reduce investigation of female hamster vaginal secretion (Powers et al. 1987). Importantly, our results suggest that the chemoinvestigatory deficits observed in these previous studies can be attributed to pBNST damage specifically, rather than damage to other parts of BNST or to MPOA deafferentation. Interestingly, lesions of pBNST did not decrease investigation of female odors during the social odor discrimination test. It is therefore possible that the novelty of a placing a foreign object in a subject's home cage during this task may override any deficits in attraction to female odors (Johnston 1981).

Role of Odor Volatility—The current results suggest that the importance of pBNST for the appropriate investigation of female odors depends on the volatility of the odor cues available. When only the volatile components of odors are present, as would be the case when animals are detecting odors from a distance, the odors are processed primarily by the main olfactory system (MOS) (Meredith 1991; Sanchez-Andrade and Kendrick 2009). pBNST does not receive any direct projections from the main olfactory bulbs, so MOS information must be transmitted to pBNST indirectly from other corticomedial amygdala structures, such as MA (Scalia and Winans 1975). The elimination of preference for volatile, opposite-sex odors by pBNST lesions suggests that interactions between the MOS and pBNST are critical for the appropriate investigation of volatile social odors. In contrast, non-volatile components of odors, such as those that would be available when animals are in

direct contact with conspecifics or their odors, are processed primarily by the accessory olfactory system (AOS) (Keverne 2004; Keller et al. 2009). pBNST receives both a small, direct projection from the accessory olfactory bulbs and indirect AOS information from MA and other corticomedial amygdala structures (Scalia and Winans 1975), making it well-situated for regulating investigation of non-volatile social odors. Furthermore, pBNST sends projections back to AOB in mice (Fan and Luo 2009), providing an additional substrate for the regulation of non-volatile odor investigation. Our finding that lesions of pBNST decreased investigation of female odors when contact was allowed suggests that AOS connections with pBNST modulate levels of attraction to non-volatile female odors. However, lesions of pBNST did not eliminate preference for non-volatile opposite-sex odors, suggesting that other connections, such as those between MA and MPOA, are sufficient to maintain preference under these conditions.

Role of Sexual Experience—Our results demonstrate that prior sexual experience can compensate for pBNST lesion-induced deficits in the appropriate investigation of volatile odors, but does not rescue behavioral deficits when odors can be contacted. This suggests that sexual experience can change the type of sensory information required for appropriate behavioral responses, a phenomenon that has been demonstrated in several species and contexts. For example, removal of the vomeronasal organ (VNO) eliminates mating in sexually-naïve, but not sexually-experienced, male hamsters (Meredith 1986). Similarly, combined lesions of the MOS and AOS decrease male hamsters investigation of female vaginal secretion and eliminate the characteristic surge in plasma testosterone in response to a female (or her odors) in sexually-naïve, but not sexually-experienced male hamsters (Pfeiffer and Johnston 1994). One neural mechanism by which sexual experience may alter how volatile social odor information is processed is experience-dependent changes in synaptic strength. Given its position in the social behavior circuit (Wood 1997), pBNST may function in sexually-naïve animals to increase the strength of odor signals relayed from MA to MPOA in order to ensure an appropriate behavioral output. Sexual experience may increase synaptic connectivity/strength between MA and MPOA such that pBNST is no longer required for the appropriate behavioral response to social odors (Hosokawa and Chiba 2005).

Role of pBNST in Copulatory Behavior

Although nearly all subjects with pBNST lesions copulated until sexual satiety, lesions of pBNST increased the latencies to several mating events in sexually-naïve males, thus replicating and extending a previous report of copulatory delays in sexually-naïve male rats with BNSTpm lesions (Claro et al. 1995). In the current study, these copulatory delays were not due to a general delay in the initiation of copulation (that would delay all subsequent mating events), as latencies to mount, intromit, ejaculate, and the onset of long intromissions remained significantly longer than those for SHAM males even after the data was corrected for the initiation of anogenital investigation. These deficits also cannot be attributed to a deficit in chemosensory investigation during copulation, as the total duration of anogenital investigation did not differ between pBNST-X and SHAM males and male hamsters do not require odor stimulation once copulation has been initiated (Devor and Murphy 1973). It is therefore likely that the post-lesion copulatory deficits in this study are due to a general decrease in sexual motivation or arousal, consistent with our finding that pBNST lesions decrease the motivation to investigate female odors.

In contrast to sexually-naïve males, sexually-experienced males with lesions of pBNST did not differ from SHAM males on any measure of copulatory behavior. This directly challenges previous reports that large, electrolytic lesions of BNST reduce the duration of anogenital investigation during copulation and eliminate copulation in a subset of sexually-

experienced male hamsters (Powers et al. 1987). Similarly, our results run counter to studies in sexually-experienced male rats, in which BNST lesions increase the number of mounts, intromissions (Claro et al. 1995), I-E, and PEI (Valcourt and Sachs 1979), and decrease the total number of intromissions and ejaculations (Liu et al. 1997). The differences between our results and those of previous studies may be explained by our use of excitotoxic lesions compared to the use of large and/or fiber-damaging lesions by previous investigators. That is, the copulatory deficits in previous studies were not due to damage to BNST itself, but resulted from disruption of social odor information transfer through the stria terminalis, the major fiber pathway between MA, BNST, and MPOA. Indeed, lesions or knife cuts of the stria terminalis produce copulatory deficits in sexually-experienced male hamsters (Lehman et al. 1983) and rats (Kondo and Yamanouchi 1995). Similarly, asymmetrical lesions that functionally disconnect MA from MPOA severely disrupt copulatory behavior in sexually-experienced male rats (Kondo and Arai 1995), suggesting that the connections between MA and MPOA are critical for copulation. Together, this evidence suggests that previous reports of BNST lesion-induced copulatory deficits in sexually-experienced males were due to deafferentation of MPOA rather than damage to BNST itself.

Alternatively, previous reports of BNST lesion-induced deficits in copulatory behavior in sexually-experienced males may be due to lesion damage extending to anterior BNST and the most ventral portion of pBNST. The ventral pBNST in particular may be critical for male copulatory behavior, as excitotoxic lesions of this area alone eliminate mating in sexually-experienced male rats (Finn and Yahr 2005). Similarly, asymmetrical lesions that functionally disconnect ventral pBNST from the sexually dimorphic area of the hypothalamus eliminate mating in sexually-experienced male gerbils (Sayag et al. 1994). In the current study, however, we were unable to damage the most rostral areas of pBNST and ventral pBNST simultaneously without incurring substantial damage to adjacent posterior nuclei, including MPOA. It is therefore possible that additional damage to ventral pBNST may account for the deficits in copulatory behavior observed in sexually-experienced males in previous studies and we would predict that lesions of this area alone may disrupt copulation in male hamsters.

Role of Sub-Nuclei within pBNST

In this study, lesion damage was primarily restricted to BNSTpm, BNSTpi, and BNSTpl. Given the known differences in odor-processing and connectivity within these subnuclei, it is likely that they contributed differentially to the observed deficits in reproductive behavior. For example, BNSTpm contains dense populations of steroid receptor-containing neurons that are activated during copulatory behavior in male hamsters (Kollack and Newman 1992). In contrast, BNSTpi contains fewer steroid receptors and shows increased Fos expression in response to both reproductive odors and agonistic encounters in male hamsters (Kollack-Walker and Newman 1995), as well as exposure to predator odors in male rats (Dielenberg et al. 2001). Furthermore, the connections of BNSTpm are restricted principally to other steroid-responsive regions within the limbic system, whereas BNSTpi has more extensive connections throughout the limbic system and related areas (Wood and Swann 2005). These functional and anatomical dissociations suggest that damage to BNSTpm may cause deficits in males' motivation to approach and interact with sexual odors, whereas damage to BNSTpi may cause deficits in categorizing all social odors. BNSTpl also sustained significant damage in most pBNST-X males, raising the possibility that the post-lesion deficits observed in the current study were due to BNSTpl damage. Unlike BNSTpi and BNSTpm, BNSTpl is connected primarily with neural areas that are involved in motivated behaviors other than reproduction (i.e. basolateral and central amygdala, lateral hypothalamus) (Wood and Swann 2005), making it unlikely that BNSTpl contributed to the observed deficits in reproductive behavior. Future studies would need to delineate which of

these anatomically separate and functionally dissociated subnuclei is most critical for the different aspects of male reproductive behavior.

Conclusion

Together, these results suggest that pBNST plays a unique and critical role in the mediation of male reproductive behavior. Our findings demonstrate that pBNST mediates the approach and investigation of volatile opposite-sex odors in sexually-naïve males, but plays a more modulatory role when these odors are directly contacted or after prior sexual experience. Despite confirming one aspect of previous findings, we found no support for the idea that pBNST alone is critical for copulatory behavior in sexually-experienced males. Indeed, pBNST appears to be important for the timing of the copulatory sequence in sexually-naïve males only, suggesting previous reports of lesion-induced deficits in sexually-experienced males may be due to disruption of social odor information transfer between MA and MPOA or to more ventral pBNST damage. Ongoing studies will determine whether the functional connections between MA, pBNST, and MPOA are critical for the appropriate behavioral response to social odors.

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ABBREVIATIONS

AGI	anogenital investigation
AOS	accessory olfactory system
BNSTpi	posterointermediate bed nucleus of the stria terminalis
BNSTpl	posterolateral bed nucleus of the stria terminalis
BNSTpm	posteromedial bed nucleus of the stria terminalis
E	ejaculations
EXP	sexually-experienced
HBI	head-body investigation
I	mounts with intromissions
I-E	intromissions to ejaculation
LI	long intromissions
M	mounts without intromissions
MA	medial amygdale
ME	mount efficiency
MOS	main olfactory system
MPOA	medial preoptic area
NeuN	Neuronal Nuclei protein
NVE	sexually-naïve
pBNST	posterior bed nucleus of the stria terminalis

pBNST-X	posterior bed nucleus of the stria terminalis lesion
PEI	post-ejaculatory interval
SG	self-grooming
SHAM	posterior bed nucleus of the stria terminalis sham surgery
VNO	vomeronasal organ

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Figure 1. Lesion Reconstruction

a) Coronal sections through rostral to caudal extent of pBNST showing largest (light gray) and smallest (dark gray) lesion included in the pBNST-X group. Immunohistochemical localization of NeuN protein was used to visualize cell loss in males with excitotoxic lesions (b) compared to males with SHAM lesions (c).

Figure 2. Investigation Times for Non-Contact Preference Test

a) In NVE males, lesions of pBNST eliminated preference for opposite-sex odors, whereas
 b) preference for opposite-sex odors remained intact in EXP males. Dissimilar letters indicate significant differences in investigation duration within lesion group, $P < 0.05$. * and # indicate significant differences in investigation duration between lesion groups, $P < 0.05$. Data expressed as mean $\pm$ standard error of means.

Figure 3. Investigation Times for Contact Preference

Lesions of pBNST significantly decreased males' investigation of female odors in a) NVE and b) EXP males, but preference for opposite-sex odors remained intact for both groups. Dissimilar letters indicate significant differences in investigation duration within group, $P < 0.05$. * and # indicate significant differences in investigation duration between groups, $P < 0.05$. Data expressed as mean $\pm$ standard error of means.

Figure 4. Investigation Times for Odor Discrimination Test

a) Both SHAM and pBNST-X NVE males a) habituated to repeated presentations of male odors and dishabituated to a novel female odor and b) habituated to strawberry or baby powder odors and dishabituated to a novel non-social odor. * indicates a significant decrease between the first and fourth presentations, $P < 0.05$, # indicates a significant increase between the fourth and fifth presentations, $P < 0.05$. Data expressed as mean $\pm$ standard error of means.

Figure 5. Latencies to Mating Events

In NVE subjects, pBNST-X males had significantly longer latencies to display mounts, intromissions, ejaculations, and long intromissions than SHAM males. In EXP subjects, pBNST-X and SHAM males did not differ in their latency to any mating event. * indicates significant differences between lesion groups, $P < 0.05$. Data expressed as mean $\pm$ standard error of means.

Table 1
Summary of Odor Preference measures from males with incomplete lesion damage to pBNST

Unilateral or bilateral, partial lesions of pBNST do not disrupt NVE or EXP males' preference to investigate female odors more than male or clean odors in a) Non-Contact or b) Contact tests. Data expressed as mean $\pm$ standard error of means.

	a) Non-Contact			b) Contact		
	Female	Male	Clean	Female	Male	Clean
NVE						
Unilateral (n = 6)	42.31 $\pm$ 9.07	20.84 $\pm$ 3.91	12.33 $\pm$ 5.10	59.42 $\pm$ 11.55	20.13 $\pm$ 2.68	13.13 $\pm$ 2.93
Partial (n = 3)	47.76 $\pm$ 5.53	17.4 $\pm$ 6.68	6.77 $\pm$ 1.52	57.61 $\pm$ 4.14	21.39 $\pm$ 10.47	6.04 $\pm$ 3.46
EXP						
Unilateral (n = 1)	42.86	7.74	5.06	88.83	32.41	24.13
Partial (n = 3)	57.83 $\pm$ 15.95	20.87 $\pm$ 6.43	7.12 $\pm$ 1.15	65.26 $\pm$ 9.27	16.43 $\pm$ 2.90	13.61 $\pm$ 3.63

Table 2
Total number, duration, and derived measures of mating events

In NVE and EXP subjects, pBNST-X males did not differ from SHAM males in the a) total number or b) duration of any mating event, nor did they differ in any c) derived measure of copulation. Data expressed as mean $\pm$ standard error of means.

	<u>NVE</u>		<u>EXP</u>	
	SHAM	pBNST-X	SHAM	pBNST-X
a) Total Number				
M	46.87 $\pm$ 2.93	42.75 $\pm$ 4.59	63.57 $\pm$ 3.33	66.60 $\pm$ 2.28
I	21.63 $\pm$ 1.53	20.00 $\pm$ 3.20	34.02 $\pm$ 2.05	33.58 $\pm$ 1.69
E	5.50 $\pm$ 0.50	5.83 $\pm$ 0.92	8.18 $\pm$ 1.34	9.67 $\pm$ 0.62
LI	11.00 $\pm$ 0.50	5.92 $\pm$ 1.40	7.56 $\pm$ 2.28	7.78 $\pm$ 1.46
b) Total Duration				
AGI	102.13 $\pm$ 19.92	115.05 $\pm$ 13.7	105.50 $\pm$ 18.29	60.10 $\pm$ 14.68
HBI	228.88 $\pm$ 30.05	237.08 $\pm$ 46.4	135.80 $\pm$ 33.98	79.20 $\pm$ 13.23
SG	188.59 $\pm$ 41.80	237.08 $\pm$ 46.4	350.70 $\pm$ 50.50	379.30 $\pm$ 22.85
c) Derived Measures				
PEI	39.61 $\pm$ 4.71	37.81 $\pm$ 6.23	41.32 $\pm$ 5.63	38.81 $\pm$ 4.74
I-E	4.21 $\pm$ 1.11	4.89 $\pm$ 0.62	3.29 $\pm$ 1.03	4.16 $\pm$ 0.98
ME	0.32 $\pm$ 0.04	0.32 $\pm$ 0.06	0.34 $\pm$ 0.07	0.33 $\pm$ 0.09

Table 3
Summary of Copulatory Behavior measures from males with incomplete lesion damage to pBNST

Unilateral or bilateral, partial lesions of pBNST do not disrupt the a) total number or b) latency to express any mating event in NVE and EXP males. Data expressed as mean $\pm$ standard error of means.

	SHAM (n = 8)	NVE		Partial (n = 3)	SHAM (n = 8)	EXP	
		Unilateral (n = 6)	Unilateral (n = 1)			Unilateral (n = 1)	Partial (n = 3)
a) Total Number							
M	46.87 ± 2.93	52.50 ± 4.47	61.66 ± 3.47	65.22 ± 3.33	60.0	71.66 ± 7.31	
I	21.62 ± 2.77	25.00 ± 3.13	29.33 ± 2.78	34.02 ± 2.05	25.0	42.66 ± 6.01	
E	5.5 ± 0.50	7.16 ± 0.69	10.00 ± 1.08	8.18 ± 1.34	6.0	8.66 ± 1.20	
LI	11.25 ± 2.77	9.50 ± 2.93	7.00 ± 2.15	7.56 ± 2.28	14.0	8.00 ± 0.81	
b) Latencies (s)							
M	77.80 ± 8.29	123.92 ± 35.45	84.30 ± 23.76	101.80 ± 39.99	86.33	99.18 ± 29.6	
I	98.74 ± 8.26	147.97 ± 32.78	100.33 ± 26.46	130.60 ± 34.92	104.42	117.11 ± 28.29	
E	158.63 ± 6.49	173.31 ± 58	180.07 ± 20.35	223.00 ± 30.88	220.25	246.14 ± 75.68	
LI	512.88 ± 87.99	649.70 ± 82.19	714.97 ± 68.28	990.60 ± 70.45	784.02	936.62 ± 55.34	