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The bed nucleus of the stria terminalis is critical for sexual solicitation, but not for opposite-sex odor preference, in female Syrian hamsters

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ABSTRACT

Successful reproduction in vertebrates depends critically upon a suite of precopulatory behaviors that occur prior to mating. In Syrian hamsters (*Mesocricetus auratus*), these behaviors include vaginal scent marking and preferential investigation of male odors. The neural regulation of vaginal marking and opposite-sex odor preference likely involves an interconnected set of steroid-sensitive nuclei that includes the medial amygdala (MA), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA). For example, lesions of MA eliminate opposite-sex odor preference and reduce overall levels of vaginal marking, whereas lesions of MPOA decrease vaginal marking in response to male odors. Although BNST is densely interconnected with both MA and MPOA, little is known about the role of BNST in female precopulatory behaviors. To address this question, females received either bilateral, excitotoxic lesions of BNST (BNST-X) or sham lesions (SHAM), and were tested for scent marking and for investigatory responses to male and female odors. Whereas SHAM females vaginal marked more to male odors than female odors on two days of the estrous cycle, BNST-X females marked at equivalent levels to both odors. This deficit is not due to alterations in social odor investigation, as both BNST-X and SHAM females investigated male odors more than female odors. Finally, BNST lesions did not generally disrupt the cyclic changes in reproductive behaviors that occur across the estrous cycle. Taken together, these results demonstrate that BNST is critical for the normal expression of solicitational behaviors by females in response to male odor stimuli.

Introduction

Successful reproduction in most vertebrates depends critically on precopulatory or solicitation behaviors that occur prior to mating (Beach, 1976). Although these behaviors can take many species-specific forms, they all serve to facilitate or coordinate mating behavior. Precopulatory behaviors are therefore particularly important for mating coordination in species where the sexes live in isolation from each other, such as Syrian hamsters (Gattermann et al., 2001; Lisk et al., 1983; Pfaff et al., 2008). In this species, females engage in several forms of precopulatory behaviors that include vaginal scent marking and the preference to investigate opposite-sex odors. Vaginal marking is a highly stereotyped behavior typified by the female lowering her perineum to the substrate and depositing a small amount of vaginal secretion as she moves forward (Johnston, 1977). Vaginal marks likely serve to advertise a female's location to males, as females vaginal mark to form a path linking the male and female's nesting areas when tested in a large, semi-natural environment (Lisk et al., 1983), and males vigorously investigate and follow these marks (Johnston, 1974; Johnston and Schmidt, 1979; Kwan and Johnston, 1980; Lisk et al., 1983).

The expression of vaginal marking relies on the perception of odor cues from conspecifics, as females vaginal mark at much higher levels in response to males or their odors compared to odors from other females (Johnston, 1977; Maras and Petrulis, 2008; Martinez et al., 2010; Petrulis and Johnston, 1999; Petrulis et al., 1999). In hamsters as well as other vertebrates, social odors are processed by an interconnected set of forebrain neural structures that includes the olfactory bulbs, the medial amygdala (MA), and the bed nucleus of the stria terminalis (BNST) (Wood, 1998). Some of these structures have been implicated in the regulation of female precopulatory behavior. For example, disruption of the main olfactory system decreases overall levels of vaginal marking and ultrasonic vocalizations (Johnston, 1992), whereas disruption of the accessory olfactory system eliminates preferential vaginal marking to male odors (Petrulis et al., 1999). Input from the main and accessory olfactory systems converge on MA (Coolen and Wood, 1998), and bilateral electrolytic lesions centered on MA eliminate opposite-sex odor preference and decrease vaginal marking (Petrulis and Johnston, 1999; Takahashi and Gladstone, 1988). Finally, electrolytic lesions that target the entire corticomedial amygdala decrease ultrasonic vocalizations by females in response to males (Kirn and Floody, 1985).

In contrast to MA, less is known about the role of BNST in the regulation of precopulatory behaviors. BNST receives input from the olfactory system both directly (Davis et al., 1978), as well as indirectly via MA and

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other amygdaloid nuclei (Coolen and Wood, 1998; Wood and Swann, 2005) and in male hamsters, lesions of BNST eliminate opposite-sex odor preference (Been and Petrulis, 2010). In female hamsters, large bilateral electrolytic lesions of the medial preoptic area (MPOA) that also damage BNST decrease vaginal marking in response to males (Malsbury et al., 1977). Furthermore, injection of a selective oxytocin receptor antagonist into BNST of female hamsters decreases vaginal marking to male odors, without affecting marking to female odors or the preference to investigate opposite-sex odors (Martinez et al., 2010). This effect, however, cannot be specifically attributed to BNST, as injections into the underlying MPOA were also effective in decreasing marking to male odors. In order to gain a more complete understanding of the role of BNST in precopulatory behaviors, we examined the effects of site-specific, excitotoxic lesions of BNST on vaginal marking, as well as investigatory responses to male and female odors. Based on previous data, we predicted that BNST lesions would disrupt both preferential vaginal marking and odor investigation. As BNST is a steroid-responsive brain area (Krieger et al., 1976; Li et al., 1993) and vaginal marking varies with changes in circulating gonadal steroids (Takahashi and Lisk, 1983) we tested females for scent marking on multiple days of the estrous cycle (diestrous day 2 and proestrus), to determine if BNST mediates the effects of circulating gonadal steroids on vaginal marking.

Materials and methods

Experimental design

Subjects were first assessed for levels of vaginal marking to male odors in order to screen out animals that failed to mark at sufficiently high levels (see below) (Fig. 1A). Subjects then received either bilateral, excitotoxic lesions of BNST or sham surgeries. Following recovery, subjects underwent a series of behavioral tests. First, subjects were tested for their investigatory responses to male and female odors (Odor investigation tests). After an initial habituation test (Clean test), subjects were tested both when direct contact with the odor stimuli was prevented (Non-contact test) and when it was allowed (Contact test). Second, subjects were tested for scent marking responses to male or female stimuli on two days of the estrous cycle, diestrous day 2 and proestrus. Finally, to verify that BNST lesions had not disrupted the ability of females to display copulatory behavior, subjects were tested during behavioral estrus for receptive sexual responses to a sexually experienced male.

Subjects

Adult female Syrian hamsters (*Mesocricetus auratus*) were purchased from Harlan Laboratories (Indianapolis, IL, USA) at approximately 7–8 weeks of age. In addition to experimental subjects, a separate group of unrelated adult male and female Syrian hamsters was purchased from Harlan Laboratories to serve as stimulus animals. Animals were either individually housed (experimental subjects) or group housed (3–4 same-sex animals per cage; stimulus animals) in solid-bottom polycarbonate cages containing corncob bedding and cotton nesting material (Nestlets; Ancare, Bellmore, NY). Subjects and stimulus animals were maintained on a reversed light cycle (16:8 light:dark; lights out at 10 am), with all behavior testing occurring during the first four hours of the dark portion of the light cycle. Food and water were available ad libitum. Animal procedures were carried out in accordance with the 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.

Estrous cycle monitoring

Prior to behavioral testing, adult subjects were examined daily for eight consecutive days to determine stage of the estrous cycle. Subjects were gently restrained while vaginal secretion was manually extruded using a disposable probe, and the consistency of the secretion was examined for stringy consistency indicative of behavioral estrus (Orsini, 1961). Once behavioral estrus was identified, the two cycle days prior to estrus were defined as diestrous day 2 and proestrus, respectively (Johnston, 1977). Estrous cycles were also monitored for eight days following surgery to ensure that this procedure had not disrupted cyclicity. In all cases, 'day' refers to the dark phase of the light cycle.

Surgery

At two to three months of age, subjects were assigned to either a BNST lesion group (BNST-X; $n = 28$) or a sham lesion group (SHAM; $n = 14$). A matched random assignment procedure was used, such that initial levels of vaginal marking in response to male odors on proestrus were equivalent across both experimental conditions (see below). Subjects were first deeply anesthetized with 2–3% isoflurane gas (Baxter, Deerfield, IL) in an oxygen (70%) and nitrous oxide (30%)

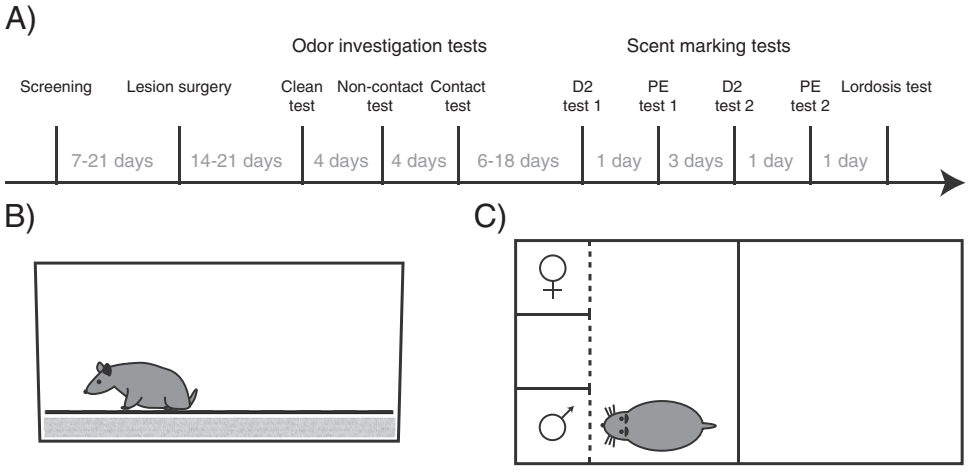

Fig. 1. Timeline and testing apparatus. A. Timeline of behavioral testing and surgeries in experiment. D2 = diestrous day 2; PE = proestrus. B. Side view of testing apparatus for scent marking tests. C. Top-down view of apparatus used for odor investigation tests.