Chemosensory Cues are Essential for Mating-Induced Dopamine Release in MPOA of Male Syrian Hamsters

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The medial preoptic area (MPOA) is crucial for male sex behavior. Dopamine (DA) is released in MPOA during copulation, and contributes to the reinforcing effects of mating. The aim of the present study was to identify sensory stimuli responsible for mating-induced DA release. Specifically, we determined if chemosensory cues are essential for mating-induced MPOA DA release using *in vivo* microdialysis in male Syrian hamsters. Hamsters were used because chemosensory cues from the olfactory mucosa and vomeronasal organ are essential for sexual behavior in this species. Sexually experienced adult male hamsters were implanted with a microdialysis guide cannula over MPOA. At the same time, males received sham olfactory bulbectomy (Sham Bx, $n = 11$), bilateral bulbectomy (Bibx, $n = 6$), or unilateral bulbectomy (Ubx) ipsilateral (Ipsi Ubx, $n = 9$) or contralateral (Contra Ubx, $n = 8$) to the microdialysis probe. This model takes advantage of the predominantly ipsilateral projections of the olfactory bulbs. Microdialysis samples were collected from the MPOA during baseline, exposure to a receptive female, and after removal of female. Extracellular DA was measured using high-performance liquid chromatography with electrochemical detection. During mating, DA increased in MPOA of Sham Bx males (to $146.7 \pm 17.5\%$ of baseline). Bibx males did not mate, and MPOA DA did not increase ($96.1 \pm 15.8\%$ of baseline). Although both groups of Ubx males mated to ejaculation, MPOA DA increased significantly only in Contra Ubx males (to $161.8 \pm 35.3\%$ of baseline), and not in males with Ipsi Ubx ($107.6 \pm 11.5\%$ of baseline). The results demonstrate that chemosensory cues are essential for MPOA DA release during mating in male Syrian hamsters.

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INTRODUCTION

The neural control of male sexual behavior requires dopamine (DA) release in the medial preoptic area (MPOA). MPOA DA contributes to sexual motivation, genital reflexes, and copulation (Hull, 1995). In males of most species, lesions of MPOA severely impair male sex behavior (Hull *et al*, 2002). Neurotoxic lesions of MPOA DA neurons with 6-hydroxydopamine impaired copulation as well (Bazzett *et al*, 1992; Dhawan *et al*, 1998). Moreover, copulation in male rats is stimulated by DA agonists in MPOA (Hull *et al*, 1986), and inhibited with DA antagonists (Warner *et al*, 1991). Sexual motivation, measured as choice of a female in an X-maze, is also inhibited by DA antagonists (Warner

et al, 1991; Moses *et al*, 1995). As measured by *in vivo* voltammetry (Louilot *et al*, 1991) or microdialysis (Hull *et al*, 1995) in male rats, noncontact exposure to a female elicits DA release in MPOA; copulation further stimulates MPOA DA release. DA is also released with mating and sexually relevant stimuli in other forebrain sites, including the nucleus accumbens (Mitchell and Gratton, 1991) and the paraventricular hypothalamic nucleus (Melis *et al*, 2003).

The aim of the present study was to determine if chemosensory cues are necessary for MPOA DA release during mating in male Syrian hamsters. Neural pathways from the olfactory bulbs to MPOA are well established. Chemosensory cues from the olfactory mucosa and vomeronasal organs are essential for male hamster sexual behavior (Murphy and Schneider, 1970), and previous studies have established that chemosensory stimuli elicit DA release in various brain regions in male rodents (Pfaus *et al*, 1990; Damsma *et al*, 1992; Hull *et al*, 1995; Schulz *et al*, 2003). However, MPOA also receives other sensory stimuli during mating, including ascending somatosensory input from the penis (Greco *et al*, 1998; Truitt and Coolen, 2002; Coolen *et al*, 2003; Truitt *et al*, 2003). While previous studies have shown that chemosensory cues alone are sufficient for DA release in MPOA, whether chemosensory

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stimuli are essential for MPOA DA release during mating has not yet been determined.

Our approach was to measure DA in MPOA by *in vivo* microdialysis with high-performance liquid chromatography with electrochemical detection (HPLC-EC) during mating in sexually experienced male hamsters. Males were unilaterally bulbectomized to eliminate chemosensory input in ipsilateral or contralateral MPOA. Unilateral olfactory bulbectomy takes advantage of the predominantly ipsilateral projections of the olfactory bulbs (Davis *et al*, 1978), and does not prevent normal sexual behavior (Winans and Powers, 1974). By contrast, bilateral removal of the olfactory bulbs immediately and permanently abolishes mating (Murphy and Schneider, 1970). If chemosensory cues are

essential for MPOA DA release during copulation, removal of the ipsilateral olfactory bulb will block the mating-induced increase in MPOA DA (see Figure 1 for experimental model). The present study compared mating-induced MPOA DA release in male hamsters with ipsilateral (Ipsi UBx), contralateral (Contra UBx), bilateral (BiBx), or sham (Sham Bx) olfactory bulbectomy.

MATERIALS AND METHODS

Animals

Adult male Syrian hamsters (120–160 g BW) were obtained from Charles River Laboratories (Wilmington, MA). Hamsters were housed individually under a long-day photoperiod (14L:10D) with food and water available *ad libitum*, unless otherwise stated. Additional sexually mature females were used as stimulus animals.

Initially, all males were screened in 1 or 2 10-min sessions in their home cage for sexual behavior with a receptive female. Only males that achieved at least one ejaculation were used in the study. Stimulus females had been previously ovariectomized via dorsal incision under sodium pentobarbital anesthesia (100 mg/kg, Abbott Laboratories, North Chicago, IL), and were treated chronically with estradiol-17B (Sigma Chemical Co, St Louis, MO) via a 4-mm Silastic capsule implanted subcutaneously (s.c.) immediately after ovariectomy (i.d.: 1.98 mm; o.d.: 3.18 mm; Dow Corning, MI). These estradiol implants are widely used to maintain constant physiologic concentrations of estradiol in circulation (Takahashi and Lisk, 1985). To induce lordosis, each female received 350 µg progesterone (2.5 mg/ml in sesame oil; Sigma) s.c. 4 h before testing.

Surgery

Surgical procedures were carried out under aseptic conditions according to 'Principles of laboratory animal care' (NIH Publication No. 86–23, revised 1985). Hamsters were anesthetized with sodium pentobarbital and secured in a Kopf stereotaxic apparatus with lambda and bregma in the same horizontal plane. The olfactory bulb was removed by aspiration through a hole in the overlying bone (Winans and Powers, 1974), and the resulting cavity was packed with sterile gel-foam. A microdialysis guide cannula (CMA/12, CMA, N. Chelmsford, MA) was lowered to 1 mm above MPOA (AP: +1.6 mm; ML: ±0.5–0.8 mm; and DV: –6.5 mm from bregma), and secured to the skull with stainless-steel screws and dental acrylic. Stereotaxic coordinates for the MPOA were according to the hamster brain atlas of Morin and Wood (2001). A dummy cannula was inserted to prevent the entry of foreign material. After surgery, males were allowed to recover for at least 5 days before mating and microdialysis sampling.

Microdialysis

Mating was tested in a 30 cm³ glass aquarium. To obtain a stable baseline of DA release, the microdialysis probe (CMA/12, 1 mm dialysis membrane) was inserted at least 4 h before testing, and perfused with artificial cerebrospinal fluid (aCSF, pH 7.4, Harvard Apparatus, Holliston, MA) via

Figure 1 Experimental model depicting the relationship between the olfactory system and the microdialysis probe (filled circles) in male hamsters with ipsilateral (top) and contralateral (bottom) bulbectomy. Chemosensory cues relayed through the olfactory bulb (OB) continue through the medial amygdala (Me) and bed nucleus of stria terminalis (BST) to the medial preoptic area (MPOA). Somatosensory cues are transmitted to the MPOA via the midbrain SPF (not shown).

FEP tubing (1.2 $\mu\text{l}/100\text{ mm}$, CMA) connected through a fluid swivel (375/D/22QM, Instech, Plymouth Meeting, PA) mounted on a balance arm (SMCLA, Instech). The flow of aCSF (1.5 $\mu\text{l}/\text{min}$) began immediately after probe insertion, and was controlled by a Harvard Model 11 syringe infusion pump. Dialysate from the probe was collected through the same FEP tubing into a 250 μl centrifuge tube.

Dialysate samples (15 μl) were collected every 10 min. Basal DA release was determined from the average of the last three baseline samples. Subsequent samples were expressed as a percentage of the mean baseline value. For mating behavior testing, a receptive female was introduced for 20 min (two dialysis samples), and copulation was observed. The female was then removed, and dialysis sampling continued for an additional 30 min (three post-mating dialysis samples).

Dialysate samples (10 μl) were loaded via a Rheodyne injector valve (Model #9125, Rheodyne Inc., Cotati, CA), isolated on a reverse-phase column (ESA MD-150), and quantified using an ESA Coulochem II detector (ESA model 5200) comprising a guard cell (+350 mV, ESA model 5020) and an analytical cell (ESA model 5014B) with two electrodes in series. The potential of the first electrode was set at -150 mV (100 μA gain), and the second electrode was set at +125 mV (2 nA gain) to detect DA. Flow rate of the mobile phase (ESA MD-TM) was 0.6 ml/min using an ESA pump (Model 582). Data were collected using PowerChrom software (AD Instruments, Mountain View, CA) linked to a Macintosh computer. The detection threshold for DA was $\geq 100\text{ fg}$ at 3:1 SNR.

Histology

At the end of the experiment, each male was deeply anesthetized, and perfused through the aorta with 150 ml of 0.1 M sodium phosphate-buffered saline containing 0.1% sodium nitrite for vasodilation, followed by 250 ml of 0.1 M sodium phosphate buffer containing 4% paraformaldehyde. Brains were removed and postfixed in the perfusion fixative for 1 h at room temperature and then cryoprotected overnight in buffer with 20% sucrose at 4°C. Bulbectomies were evaluated through gross inspection. Probe placement was verified histologically in 60 μm coronal brain sections cut on a freezing microtome and stained with cresyl violet.

Statistical Analysis

Baseline DA was calculated as the mean of the last three baseline samples. DA content in dialysates from Ipsi Bx, Contra Bx, BiBx, and Sham Bx males collected during and after mating (expressed as the percent of baseline DA) were compared by two-factor repeated measures analysis of variance (ANOVA, group $\times$ sample period; Statview 5.0.1, SAS Institute, Cary, NC). Significant effects were further analyzed by factorial ANOVA with Fisher's PLSD *post hoc* test. In all analyses, $p < 0.05$ was considered significant.

RESULTS

When paired with a receptive female for 20 min, all Sham Bx ($n = 11$) males mated to ejaculation. Likewise, Ipsi Ubx ($n = 9$) and Contra Ubx ($n = 8$) males expressed at least one

ejaculation during pairing. One Contra Ubx male failed to ejaculate, and his data were omitted. None of the Bibx males ($n = 6$) showed significant sexual activity (mounts, intromissions, or ejaculations).

Figure 2 demonstrates probe placement in rostral MPOA from a representative animal, M468. As determined by gross inspection of the brain post-mortem, all olfactory bulbectomies removed at least 75% of the bulb tissue rostrally. As bulbectomy interrupts afferent chemosensory input through the cribriform plate, any remaining remnants of the caudal olfactory bulbs were deafferented. In histologic sections, microdialysis probes were distributed throughout the rostrocaudal length of MPOA, from the rostral medial preoptic nucleus (MPN) at the level of anteroventral periventricular nucleus to mid-caudal MPN at the level of the magnocellular MPN.

Figure 3 illustrates DA measured from a representative Sham Bx male at baseline (middle) and during mating (bottom), in comparison with a 0.39 pg/ μl DA standard (top) analyzed on the same day. In the standard sample, DA was recorded at 4.59 min with a peak height of 0.41 nA. At baseline, DA release was 0.12 nA. During mating, DA release in MPN reached a peak height of 0.20 nA.

Figure 4 presents mean DA levels at baseline, during mating, and after mating in Sham Bx, Bibx, Ipsi Ubx, and Contra Ubx males. As determined by two-factor repeated measures ANOVA, there were significant differences in

Figure 2 Photomicrograph of microdialysis probe placement in rostral MPOA from a representative animal, M468. III: third ventricle; ac: anterior commissure; BSTpi: posterointermediate bed nucleus of the stria terminalis; BSTpm: posteromedial bed nucleus of the stria terminalis; fx: fornix; LS: lateral septum; LV: lateral ventricle; MPN: medial preoptic nucleus.

Figure 3 Microdialysis chromatograms obtained from dialysates of MPOA in a representative male hamster at baseline (middle) and during mating (bottom), compared with a 0.39 pg/μl DA standard (top).

DA content by group $F(3,150)=3.99$, across the sample period $F(5,150)=4.37$, and a group $\times$ sample interaction $F(15,150)=1.85$. As determined by factorial ANOVA with *post hoc* analysis, MPOA DA in Sham Bx males increased significantly to $146.7 \pm 17.5\%$ of baseline within the first 10 min after introduction of the female ($p < 0.05$). DA release remained elevated ($148.3 \pm 23.8\%$) during the 20 min mating test, and then decreased significantly to basal levels ($109.5 \pm 10.8\%$) 10 min after the female was removed ($p > 0.05$ vs baseline). By contrast, MPOA DA was not

Figure 4 DA measured from dialysates of MPOA during and after mating in male hamsters, expressed as a percentage of baseline release. Top: Mean $\pm$ SEM DA release in Sham Bx (black, $n=11$) and BiBx (white, $n=6$) males. Bottom: Mean $\pm$ SEM DA release in Contra Ubx (black, $n=8$) and Ipsi Ubx (white, $n=9$) males. Shading represents samples collected during mating. Asterisk indicates significant difference compared to baseline.

elevated above baseline in nonmating Bibx males exposed to a female. In the presence of the female, DA levels in Bibx males remained at baseline (96.1 ± 15.8 , $95.7 \pm 14.6\%$, $p > 0.05$). When the female was removed, DA release decreased significantly below baseline (to $58.6 \pm 11.1\%$ in the third postmating test, $p < 0.05$).

Although Ipsi Ubx and Contra Ubx males both mated to ejaculation, MPOA DA release was elevated only in males with Contra Ubx. However, in Contra Ubx males, the mating-induced increase in DA release was delayed and attenuated relative to Sham Bx males. DA levels remained at baseline during the first 10 min with the stimulus female

($103.1 \pm 5.7\%$). DA then rose significantly above baseline during the second 10 min of the mating test ($161.8 \pm 35.3\%$, $p < 0.05$), but decreased to $149.8 \pm 20.5\%$ of baseline ($p > 0.05$) 10 min after the female was removed. In Ipsi Ubx males, DA remained at or near baseline levels throughout the 20 min test with the stimulus female (107.6 ± 11.5 , $114.2 \pm 15.8\%$) and during postmating sampling ($112.2 \pm 11.3\%$ in postmating 1, $p > 0.05$ vs baseline DA).

DISCUSSION

The present study demonstrates that chemosensory cues are necessary for mating-induced MPOA DA release in male Syrian hamsters. DA is associated with reinforcement and reward, and sexual activity is rewarding for males (see Pfau, 1997). As shown previously in a number of species, extracellular DA increases in MPOA when males mate with a receptive female (see Melis and Argiolas, 1995). Male hamsters that are bilaterally bulbectomized do not mate, and hence DA does not increase. We used Ubx to take advantage of the predominantly ipsilateral projections of the olfactory bulbs (Davis *et al*, 1978). Ubx males can mate, but they do not receive chemosensory stimuli ipsilateral to the lesioned bulb. The males with Ubx ipsilateral to the microdialysis probe did not show an increase in DA. In contrast, DA did increase in males with Ubx contralateral to the microdialysis probe, although the DA peak was delayed and attenuated relative to bulb-intact controls. These data demonstrate that somatosensory cues alone are not sufficient for mating-induced DA release in MPOA.

It is well established that MPOA is a higher organizing center, which coordinates sensory and humoral signals to control the expression of male copulatory behavior in a wide variety of species (see Hull *et al*, 2002). MPOA lesions severely impair or eliminate male sexual activity, and electrical stimulation of MPOA activates copulation. Furthermore, mating activates Fos expression in MPOA and its afferents (Baum and Everitt, 1992; Kollack and Newman, 1992; Coolen *et al*, 1998). Although a variety of neurotransmitters in MPOA have been implicated in the control of male sexual behavior, there is considerable evidence that DA plays a key role (Melis and Argiolas, 1995). Treatment with DA receptor antagonists into MPOA (Warner *et al*, 1991) or lesions of MPOA DA neurons and terminals with 6-hydroxydopamine (Bazzett *et al*, 1992; Dhawan *et al*, 1998) impair copulation in male rats. Conversely, stimulation of DA receptors in MPOA with the DA agonist, apomorphine, enhances the rate of copulation in male rats (Hull *et al*, 1986). Likewise, apomorphine can overcome mating deficits in castrated rats (Scaletta and Hull, 1990). Furthermore, increases in extracellular DA in MPOA during copulation have been measured by microdialysis and *in vivo* voltammetry (Hull *et al*, 1995; Louilot *et al*, 1991).

The present study furthers our understanding of the specific sensory cues that elicit DA release in MPOA during mating. Chemosensory and somatosensory stimuli are two major afferent inputs to MPOA that are relevant for copulation in male rodents (Hull *et al*, 2002), and it is likely that both contribute to DA release during sexual

activity. In male rats, extracellular DA increases in MPOA when a female is presented behind a screen, but mating further stimulates DA release (Damsma *et al*, 1992; Hull *et al*, 1995). Schulz *et al* (2003) showed increased dopaminergic activity in MPOA of adult male hamsters exposed to female pheromones. Although the foregoing observations suggest that chemosensory cues can elicit DA release, they do not address whether chemosensory cues are essential for DA release during mating.

The Syrian hamster is an excellent model to test this question because male hamsters require chemosensory cues for copulation (reviewed in Wood and Swann, 2000). Bilateral removal of the olfactory bulbs immediately and permanently abolishes sexual behavior (Murphy and Schneider, 1970). By contrast, olfactory bulbectomy does not eliminate copulation in sexually experienced male rats (Bermant and Taylor, 1969). However, olfactory bulbectomy has been used as a model of depression in rats (Kelly *et al*, 1997), and depression is known to alter DA release (Yadid *et al*, 2001). It may be that similar mechanisms are responsible for the postcopulatory decrease in DA in Bx males from the present study.

Chemosensory cues are transduced in the olfactory mucosa and vomeronasal organ, which project to the olfactory bulbs (see Wood and Newman, 1995). The olfactory bulbs relay this information to MPOA via the medial amygdaloid nucleus (Me). A key advantage of the olfactory system is that the efferent projections of the olfactory bulbs are massively ipsilateral. In tract-tracing studies of hamster olfactory bulb, no contralateral labeling in the ventral forebrain or amygdala was observed with injections of anterograde tracers into either the main or accessory olfactory bulbs (Davis *et al*, 1978). Connections between paired subnuclei of Me and MPOA through the anterior commissure link the two hemispheres (Maragos *et al*, 1989; Gomez and Newman, 1992). However, studies combining intracerebral testosterone implants with unilateral olfactory bulbectomy demonstrated that indirect contralateral projections are insufficient to stimulate sexual behavior (Wood and Newman, 1995).

The results of the present study complement earlier work on the role of Me in mating-induced MPOA DA release in male rats (Dominguez *et al*, 2001; Dominguez and Hull, 2001). Dominguez *et al* (2001) showed that lesions of Me impair DA release in MPOA during mating. Subsequently, they demonstrated that microinjections of the DA agonist, apomorphine, in MPOA reversed the copulatory impairment caused by Me lesions (Dominguez *et al*, 2001). As Me is an essential relay for chemosensory cues to midline hypothalamic nuclei, bilateral Me lesions in male hamsters eliminate copulation, similar to the effects of olfactory bulbectomy (Lehman *et al*, 1980; Lehman and Winans, 1982). Taken together, these data suggest that chemosensory cues relayed through the olfactory bulbs to MPOA via Me are essential for DA release in MPOA during male copulatory behavior.

At the present time, it is not known whether the chemosensory stimuli essential for DA release are transduced in the olfactory mucosa or vomeronasal organ because olfactory bulbectomy damages the main and accessory olfactory bulbs. In the control of male hamster mating behavior, nonvolatile cues detected in the

vomeroneasal organ are more important than volatile odors transduced in the olfactory mucosa. Selective destruction of the olfactory mucosa by nasal irrigation with zinc sulfate does not interrupt mating, while severing the vomeronasal nerves blocks sexual activity in 0–44% of males (Winans and Powers, 1977). However, combining zinc sulfate with vomeronasal nerve cuts eliminates copulation. In male rats, activation of the accessory olfactory bulb by female-soiled bedding (Mitchell and Gratton, 1991) or electrical stimulation (Mitchell and Gratton, 1992) stimulates DA release in the nucleus accumbens. Thus, it seems likely that mating-induced MPOA DA release is largely regulated through vomeronasal input. In this regard, vomeronasal lesions are less effective in male hamsters with prior sexual experience (Meredith, 1986). Accordingly, the effects of vomeronasal lesions on MPOA DA release should be examined in sexually naïve males.

Somatosensory stimuli are also important for copulation. Tactile sensations from the penis are relayed to MPOA via the midbrain subparafascicular nucleus (SPF), which also sends projections to Me (Greco et al, 1998; Truitt and Coolen, 2002; Coolen et al, 2003; Truitt et al, 2003). Although chemosensory cues activate Fos in MPOA and Me, a subset of neurons within an interconnected circuit of the MPN, posterior Me, and SPF is activated only following ejaculation (Coolen et al, 1998). This suggests the possibility of an 'ejaculation-specific' neural circuit. Nonetheless, the lack of DA response in Ubx males in the present study demonstrates that somatosensory stimuli alone are not adequate to elicit DA during mating in male hamsters. The present study extends the earlier work of Baum and Everitt (1992) demonstrating that unilateral lesions of SPFP failed to reduce Fos in ipsilateral MPOA. Owing to the ipsilateral projections of the olfactory bulbs, Ubx males receive bilateral somatosensory stimuli, but only unilateral chemosensory cues. Thus, while somatosensory stimuli may enhance DA release during male hamster sexual behavior, they are not sufficient in the absence of chemosensory input.

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