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LESIONS THAT FUNCTIONALLY DISCONNECT THE ANTERIOR AND POSTERODORSAL SUB-REGIONS OF THE MEDIAL AMYGDALA ELIMINATE OPPOSITE-SEX ODOR PREFERENCE IN MALE SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*)

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Abstract—In many rodent species, such as Syrian hamsters, reproductive behavior requires neural integration of chemosensory information and steroid hormone cues. The medial amygdala (MA) processes both of these signals through anatomically distinct sub-regions; the anterior region (MeA) receives substantial chemosensory input, but contains few steroid receptor-labeled neurons, whereas the posterodorsal region (MePD) receives less chemosensory input, but contains a dense population of steroid receptors. Importantly, these sub-regions have considerable reciprocal connections, and the goal of this experiment was therefore to determine whether interactions between MeA and MePD are required for male hamsters' preference to investigate female over male odors. To functionally disconnect MeA and MePD, males received unilateral lesions of MeA and MePD within opposite brain hemispheres. Control males received either unilateral lesions of MeA and MePD within the same hemisphere or sham surgery. Odor preferences were measured using a 3-choice apparatus, which simultaneously presented female, male and clean odor stimuli; all tests were done under conditions that either prevented or allowed contact with the odor sources. Under non-contact conditions, males with asymmetrical lesions investigated female and male odors equally, whereas males in both control groups preferred to investigate female odors. Under contact conditions, all groups investigated female odors longer than male odors, although males with asymmetrical lesions displayed decreased investigation of female odors compared to sham males. These data suggest that MeA–MePD interactions are critical for processing primarily the volatile components of social odors and highlight the importance of input from the main olfactory system (MOS) to these nuclei in the regulation of reproductive behavior. More broadly, these results support the role of the MA in integrating chemosensory and hormone information, a process that may underlie social odor processing in a variety of behavioral contexts.

Key words: reproductive behavior, social behavior, olfaction, chemosensory, sexual preference.

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Abbreviations: ANOVA, analysis of variance; AOB, accessory olfactory bulb; AOS, accessory olfactory system; ASYM, asymmetrical lesion group; BNST, bed nucleus of the stria terminalis; MA, medial amygdala; MeA, medial amygdala, anterior region; MePD, medial amygdala, posterodorsal region; MOS, main olfactory system; MPOA, medial preoptic area; SEM, standard error of the mean; SHAM, sham surgery group; UNI, unilateral lesion group.

In many rodent species, including Syrian hamsters, social behavior relies heavily on the perception of chemosignals released from conspecifics (Johnston, 1983; Hull et al., 2002; Beauchamp and Yamazaki, 2003). In the context of reproductive behavior, male hamsters are highly attracted to female odors (Murphy, 1973; Johnston, 1974; Landauer et al., 1977) and these chemosignals serve as the primary signal to initiate male copulatory behavior (Murphy, 1973; Johnston, 1975, 1986). Social odors are processed by two, anatomically distinct chemosensory systems; sensory receptors of the main olfactory system (MOS), located in the main olfactory epithelium, respond best to low molecular weight, volatile components of social odors, whereas sensory receptors of the accessory olfactory system (AOS), located in the vomeronasal organ, are thought to process high molecular weight, non-volatile components of social odors (Meredith, 1991; Restrepo et al., 2004; Keller et al., 2009). Together, these systems regulate most aspects of rodent social behavior, including the attraction to, and preference for, opposite-sex odors (Murphy and Schneider, 1970; Rowe and Edwards, 1972; Powers et al., 1979; Edwards et al., 1990; Keverne, 2004; Keller et al., 2009).

In addition to chemosensory cues, male reproductive behavior is also regulated by internal signals of reproductive state via changes in circulating levels of gonadal steroid hormones (Beyer et al., 1976; Morin and Zucker, 1978; Hull et al., 2002). In hamsters, testosterone and its primary metabolites, estradiol and dihydrotestosterone, are critical not only for the expression of male copulatory behavior (Morin and Zucker, 1978; Powers et al., 1985), but also for male's attraction to investigate female odors (Steel, 1982; Powers and Bergondy, 1983; Powers et al., 1985; Petrulis and Johnston, 1995). Consequently, the expression of reproductive behavior in male hamsters involves the neural integration of chemosensory and hormonal cues (Wood, 1998).

The medial amygdala (MA) has been suggested as one candidate site for integrating chemosensory and hormonal cues, as it is receives both types of information (Wood, 1998). Functionally, the MA plays a critical role in odor-guided reproductive behaviors in many rodent species, including hamsters (Lehman et al., 1980; Petrulis and Johnston, 1999; Maras and Petrulis, 2006), rats (Kondo, 1992; Kondo et al., 1997; Stark et al., 1998), and gerbils (Heeb and Yahr, 2000). Detailed analysis of the distinct sub-regions within MA, however, suggests that chemosensory and hormonal signals are processed separately within

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this nucleus. Indeed, the anterior medial amygdala (MeA) has extensive connections with both the MOS and AOS, via direct projections from the olfactory bulbs, as well as indirect projections through secondary chemosensory structures (Scalia and Winans, 1975; Kevetter and Winans, 1981b; Lehman and Winans, 1982; Coolen and Wood, 1998; Kang et al., 2009). Although the posterodorsal region of the MA (MePD) receives some input from the accessory olfactory bulbs (AOB), these projections are less substantial than compared to MeA, and the MePD has much more limited connections with the MOS (in particular the secondary nuclei of the MOS) than compared to MeA (Scalia and Winans, 1975; Kevetter and Winans, 1981b; Lehman and Winans, 1982; Coolen and Wood, 1998). The processing of steroid hormone information also appears to be separated within MA, as the vast majority of steroid receptor-containing neurons are localized specifically within MePD, not MeA (Doherty and Sheridan, 1981; Wood et al., 1992; Wood and Newman, 1993).

In addition to these anatomical data, several lines of evidence suggest functional differences between MeA and MePD. For example, lesions of MeA completely eliminate male hamster copulatory behavior (Lehman et al., 1980), similar to deficits observed following destruction of the olfactory bulbs (Murphy and Schneider, 1970), whereas lesions restricted to MePD only alter the temporal pattern of the male copulatory sequence (Lehman et al., 1983). More recently, we have shown that, although both MeA and MePD are critical for the preference to investigate opposite-sex odors in male hamsters, these sub-regions regulate distinct aspects of social odor investigation (Maras and Petrulis, 2006). Specifically, MeA appears to function as a chemosensory filter to identify or categorize the sexual/social relevance of odors in the environment, whereas MePD may be critical for generating attraction specifically to opposite-sex odors (Maras and Petrulis, 2006). These behavioral findings are supported by several immediate-early gene studies that find that neurons within MeA respond to a wide variety of social odors, whereas neurons within MePD respond specifically to sexually relevant odors (Day et al., 2004; Meredith and Westberry, 2004; Kiyokawa et al., 2005; delBarco-Trillo et al., 2009; Samuelsen and Meredith, 2009).

Taken together, these data suggest that MeA and MePD differentially process chemosensory and steroid hormone cues. Critically, substantial reciprocal fibers connect MeA and MePD (Gomez and Newman, 1992; Coolen and Wood, 1998), providing a substrate for the neural integration of these cues. We therefore hypothesized that interactions between MeA and MePD are required for appropriate behavioral responses to social odors in male hamsters. To test this hypothesis, we compared opposite-sex odor preferences displayed by males in which MeA and MePD connections were either disrupted or intact. As the connections between MeA and MePD do not constitute a discrete, identifiable pathway (Coolen and Wood, 1998), we used an asymmetrical lesion technique, in which males received a combination of unilateral lesions of MeA and MePD in opposite brain hemispheres, to disconnect these nuclei. This technique takes advantage of three important facts: (1) the MeA and MePD each regulate distinct aspects of odor processing (Wood, 1997; Meredith and Westberry, 2004; Maras and Petrulis, 2006), (2) the connections between MeA and MePD are almost exclusively ipsilateral (Gomez and Newman, 1992; Coolen and Wood, 1998), and (3) unilateral lesions of MeA or MePD by themselves do not affect opposite-sex odor preferences in male hamsters (Maras and Petrulis, 2006). Thus, the asymmetrical lesion technique (hereby referred to as a "functional disconnection") leaves each nucleus sufficiently intact to generate behavior, but prevents these nuclei from communicating with each other. These results provide the first evidence that the interactions between chemosensory and steroid-sensitive sub-regions of the MA are indeed critical for the attraction to sexual odors and further support the concept of MA as a critical node in the regulation of odor-guided aspects of social behavior (Newman, 1999).

EXPERIMENTAL PROCEDURES

Animals

Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratory (Wilmington, MA, USA) at 8 weeks of age. Subjects were sexually naïve males (4–6 months old, 120–150 g) that had been singly housed upon arrival. A separate group of male and female hamsters (3–8 months old) were used to provide social odor stimuli. Subjects were unrelated to, and had no previous contact with, these odor donor animals. All animals were housed in solid-bottom Plexiglas cages (Ancare, Bellmore, NY, USA, 36 cm×30 cm×16 cm) and were maintained on a reversed 14 h light/10 h dark photoperiod (lights off/on at 9 AM/7 PM). Food and water were available *ad libitum*. All animal experiments 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 were 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.

Surgical procedures

Male subjects were randomly assigned to one of three experimental groups. One group of males received asymmetrical electrolytic lesions of MeA and MePD (i.e. unilateral lesion of MeA combined with a unilateral lesion of MePD in the opposite hemisphere of the brain; ASYM, $n=35$). Seventeen of these males received MeA lesions in the left hemisphere, whereas 18 of these males received MeA lesions in the right hemisphere. To control for the effects of unilateral lesions of MeA and MePD, a second group of males received unilateral electrolytic lesions of MeA or MePD within the same hemisphere of the brain (UNI, total $n=14$; left $n=7$, right $n=7$). Thus, ASYM and UNI males differed only in the functional connection between MeA and MePD. Finally, a third group of males received sham lesion surgery (SHAM, $n=12$), in which there was no damage to MeA or MePD.

All males were anesthetized with 2% isoflurane anesthesia and placed into a stereotaxic apparatus so that the skull was flat. The temporal muscles were retracted from the skull and small holes were drilled to expose the surface of the brain. We used a combination of multiple small electrolytic lesions in order to generate maximal damage of MeA or MePD (Maras and Petrulis, 2006), while limiting collateral damage to nearby nuclei or major fiber tracts (Table 1). Electrolytic lesions were made by lowering a platinum/iridium electrode (0.25 mm diameter, 0.45 mm uninsu-