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Non-neural androgen receptor promotes androphilic odor preference in mice

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ABSTRACT

In mice, male-typical preference for female olfactory cues results largely from sexually differentiated testosterone production. It is currently unclear on which cells and tissues testosterone acts to produce male-typical preference for female olfactory cues. To further address the site of androgen action on olfactory preference, we have developed a loxP-based transgenic mouse that overexpresses androgen receptors (AR) only when activated by Cre. We used this transgene to overexpress AR globally in all tissues using a CMV-Cre driver and a Nestin-Cre driver to overexpress AR selectively in neural tissue. We then examined olfactory preference in transgenic and wildtype (Wt) littermates by simultaneously exposing animals to female-soiled, male-soiled and clean bedding. Ubiquitous overexpression of AR in CMV-AR mice increased preference for male bedding, whereas neural-specific AR overexpression in Nestin-AR transgenic mice did not differ from wildtype siblings in olfactory preference. Neural activation of olfactory brain areas in response to female-soiled bedding was also evaluated in these mice by measuring FOS immunoreactivity. This revealed a decrease in neural activity along the accessory olfactory pathway that accompanied the decrease in preference for female odors in CMV-AR males, compared to both Nestin-AR and Wt male siblings. Together, results indicate that androgens act via non-neural AR to mediate olfactory preference and neural responses to olfactory stimuli, and further suggest that AR in non-neural tissues can promote androphilic odor preferences in male mice.

Introduction

For many species, social and sexual behaviors are dependent on olfactory chemosensory cues provided by conspecifics (for review, see McClintock, 1998). In response to chemosensory cues, neural activity is observed throughout the olfactory pathways that correspond to sexual preference. For example, in humans, pheromone-like compounds activate olfactory networks differently depending on sexual preference, independent of biological sex (Savic et al., 2005; Berglund et al., 2006). Similarly in rodents, neural activity patterns in response to male and female odors correspond to their preference in odors (for review, see Brown, 1979; e.g. Bakker et al., 1996; Halem et al., 1999; Bodo and Rissman, 2007).

Studies of AR loss of function mutations suggest that the organization of a male-typical olfactory preference in mice is dependent on AR, but not on AR in neural tissues. Bodo and Rissman (2008) showed that treatment with a non-aromatizable metabolite of testosterone, dihydrotestosterone (DHT), given on the first day of birth, is sufficient to produce a male-typical olfactory preference in adulthood. Models of AR insensitivity have also indicated that AR is necessary for a male-typical olfactory preference. For example, male mice with global androgen insensitivity (testicular feminization mutation; Tfm) are more female-typical in many aspects of their brain and behavior (for review, see Zuloaga et al., 2008; Charest et al., 1991; Gaspar et al., 1991; He et al., 1991), including a female-typical olfactory preference (e.g. an equal preference for male and female soiled bedding or a preference for male bedding; Moncho-Bogani et al., 2002). Furthermore, neural activity along the accessory olfactory pathway in response to male-soiled bedding is female-typical in Tfm males (Bodo and Rissman, 2007). Unlike many behaviors affected by AR insensitivity that can be restored with estrogen (E) treatment in adulthood, olfactory preference is consistently sex-reversed, even with adult E treatment (Bodo and Rissman, 2007). Interestingly, in male mice lacking AR only in the nervous system (Nestin-ARKO), olfactory preference remains completely male-typical, and neural activation in response to female-soiled bedding remains unaltered (Raskin et al., 2009).

To further address this question of site of action, we compared two models of AR overexpression (Swift-Gallant et al., 2015). The first model overexpresses AR only in neural tissue (Nestin-AR) and the second overexpresses AR globally in both neural and non-neural tissue (CMV-AR). We previously reported that mice with global overexpression of AR did not show a preference for anogenital investigation of male and female stimulus animals, whereas both Wt males and males

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with neural AR overexpression preferentially investigated female stimulus mice (Swift-Gallant et al., 2015). Here, we compared olfactory preference and neural activity in response to female-soiled bedding of these two models. We hypothesized that androgens act on AR in non-neural cells to sexually differentiate behaviors and neural activity patterns associated with olfactory preference. Therefore, we predicted that males with global AR overexpression would exhibit changes in olfactory preference compared to both males with neural AR overexpression and wildtype animals. Results support our hypothesis that androgens act via non-neural AR to mediate male-typical olfactory preference, and indicate that global hypersensitivity to androgens can lead to an increased preference for same-sex odors in male mice.

Methods

Experimental animals

All mice (C57Bl/6 background) were between 3 and 5 months of age at the time of behavioral testing, and were 4–6 months of age upon collection of tissue. Animals were kept on a 12:12 dark/light cycle at 21–23 °C and had ad libitum access to Harlan chow (Harlan Laboratories, Inc.). All procedures adhered to Canadian Council on Animal Care Regulations and were approved by the Local Animal Care Committee at the University of Toronto, Mississauga (UTM).

Nestin-Cre and CMV-Cre mice were purchased from Jackson Laboratories (Stock #003771; 006054), and were subsequently bred with animals in colony with the CMV-STOP-AR transgene (Swift-Gallant et al., 2015). Males and females of all four genotypes were included in this experiment: wildtype (Wt) littermates (male $n = 16$, female $n = 6$), littermates with the only one of the two necessary mutations mutation (Wt/CMV-STOP-AR, CMV-Cre/Wt, and Nestin-Cre/Wt single mutant mice; male $n = 16$, female $n = 24$), mice with both CMV-STOP-AR and CMV-Cre (CMV-AR; male $n = 14$, female $n = 16$), and mice with both CMV-STOP-AR and Nestin-Cre (Nestin-AR; male $n = 12$, female $n = 10$). Genotyping was conducted as previously described (Swift-Gallant et al., 2015).

Stimulus animals

Wildtype stimulus males and females were purchased from Charles River Laboratories. At 7–10 weeks of age, stimulus females implanted with a Silastic capsules (1.98 mm id/3.17 mm od) containing dissolved 17β-estradiol in sesame oil (50 μg in 0.025 ml) and sealed with Silastic Medical Adhesive Silicone (Dow corning, Midland, MI, USA). On behavioral testing days, stimulus females were treated with progesterone subcutaneously (500 μg/0.1 ml corn oil) 2–5 h prior to behavioral tests to induce estrus. Both male and female stimulus animals were given sexual experience (2–3 copulation tests) a minimum of one week prior to testing with experimental animals.

Olfactory preference behavioral paradigm

Experimental animals underwent an olfactory preference test and were exposed to female-soiled bedding prior to tissue collection. Behavioral testing chambers were cleaned with 10% EtOH between each test. Experimental animals were placed in a 10-l aquarium with clean corncob bedding for a 10-min habituation period, followed by a 10-min test. In both the test and habituation phases, three circular ceramic ramekins (4 cm high × 6 cm in diameter) were placed in the center of the aquarium in a straight-line equidistance apart and filled with stimulus bedding (described below). In the habituation phase, all three ramekins were filled with clean bedding. In the test phase, mice were place in an aquarium with one ramekin filled with female soiled bedding, one with male soiled bedding and the third ramekin filled with neutral clean bedding. The order of the ramekins was randomized between trials and unknown to the observer during coding. Video recordings of the test phase were coded using Noldus Observer XT 10.5.

Stimulus bedding

Clean corn cob bedding from vivarium stock was provided to both male and female stimulus animals 48 h prior to collection of bedding for olfactory preference tests (Bed-o'Cobs 1/4 from The Andersons lab

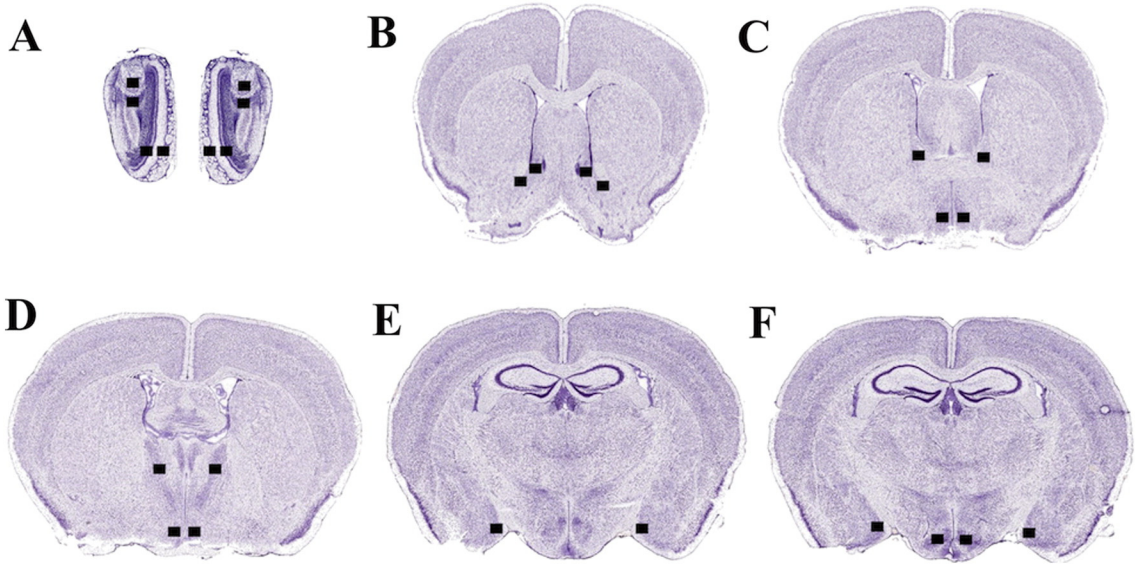

Fig. 1. Images captured for FOS-ir analysis. All images of the bulbs were captured at 1000× magnification, and all other regions of the brain were captured at 400× magnificent. A) Bilateral images of the granule cell layer and mitral layer of the AOB, and of the glomeruli and mitral cell layer of the MOB, B) Bilateral images of the core and shell of the NAcc, C and D) Bilateral images of the MPOA and BNST were captured for two sections. E and F) Bilateral images of the MePD were captured for two sections. F) Bilateral images of the VMH. Image credit: Thionin stained images obtained from the Allen Institute for Brain Science website at www.alleninstitute.org.