Figures and figure supplements

Sex-specific processing of social cues in the medial amygdala

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Figure 1. Experimental system for recording MeA sensory responses. (A) Vomeronasal and olfactory structures are shown in yellow and blue, respectively. Multichannel electrophysiological probes are stereotaxically positioned in the MeA to continuously record neural responses to sensory stimulation. VNO stimulus presentation (orange arrow) is achieved by placing nonvolatile stimuli in the nostril followed by electrical stimulation of the sympathetic nerve trunk (SNT) to activate the VNO pump and permit access of stimuli into the VNO. VNO stimuli are washed out through the NPD. MOE stimulation is achieved by controlling airflow of volatile stimuli into the nostril (blue arrow), which access the MOE, and are eliminated through a tracheotomy. (B) Diagram illustrating a coronal section through the posterior MeA with red dots indicating the expected dorsal–ventral distribution of recording sites. A single fluorescent electrode tract accurately targeted to MeA is shown in the inset. (C) Timecourse of VNO and MOE stimulation trials (top). Electrophysiological signals recorded from a single MeA electrode during four successive trials reveal a well-isolated unit responding only to female stimuli following electrical stimulation of SNT (stimulation artifacts are evident at 20 s). (D) Percentage of MeA units responding to VNO vs MOE stimulation with chance rates indicated by a horizontal dashed line. (E) Sagittal section of whole brain showing DAPI staining and the site of MeA FluoroGold iontophoresis. (F) Dense retrograde labeling of AOB projection neurons. (G) Fraction of AOB (99.8%) and MOB (0.2%) neurons that are retrogradely labeled by FluoroGold iontophoresis in the MeA. DOI: 10.7554/eLife.02743.003
Figure 1—figure supplement 1. Clustering and analysis of multichannel electrophysiological recordings. DOI: 10.7554/eLife.02743.004
Figure 1—figure supplement 2 Baseline electrophysiological characteristics of MeA responses.
DOI: 10.7554/eLife.02743.005
Figure 1—figure supplement 3  MOE-driven responses in the MOB and PLCO.
DOI: 10.7554/eLife.02743.006
Figure 2. MeA sensory responses to VNO stimuli. (Left) Each row shows the responses elicited in a single MeA unit by four different VNO stimuli, with each stimulus presented 5 times. The order of stimulus presentation was randomized during the experiment, but is shown grouped by stimulus for clarity. (Right) Histograms showing the mean response and standard error (shaded region) for each unit. Responses were aligned to the onset of stimulus presentation. All significant responses (p<0.01; Nonparametric ANOVA) are indicated by an asterisk in the top right corner of the average histogram plots. Response magnitudes for each unit were normalized to the maximum response for all stimuli. Colored bars (top and bottom) indicate the 40 s epoch following stimulus presentation that was considered for all analyses.

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Figure 2—figure supplement 1. MeA units responsive to different stimulus categories are spatially segregated.
DOI: 10.7554/eLife.02743.008
Figure 2—figure supplement 2. AOB sensory responses to VNO stimuli.
DOI: 10.7554/eLife.02743.009
Figure 3. Decreased frequency and increased specificity of sensory responses in the MeA compared to the AOB. (A) The percentage of single AOB units (dashed curves) and MeA units (solid curves) exhibiting statistically significant responses to male (blue), female (red), and predator (green) vomeronasal stimuli as the threshold for inclusion was varied from $p<0$ to $p<0.2$ (abscissa). The diagonal gray line indicates the predicted false positive rate. (B) Distribution of response selectivity ('Materials and methods') showing a shift towards higher specificity in MeA (solid line) as compared to the AOB (dashed line). (C) Selectivity of sensory responses for units recorded in the adult AOB (197 units from male and female animals). (D) Selectivity of sensory responses for units recorded in the adult MeA (274 units from male and female animals). Each point represents the response profile of an individual unit, with at least one significant response, to male, predator, and/or female stimuli. Points located near a vertex (more frequent in the MeA) represent units that respond most strongly for the stimulus indicated at that vertex whereas points at the center (more frequent in the AOB) represent units that respond similarly to all stimuli. Insets (C and D) show correlation between responses for each pair of stimuli. DOI: 10.7554/eLife.02743.010
**Figure 3—figure supplement 1** Leverage analysis for stimulus response correlations.  
DOI: 10.7554/eLife.02743.011
Figure 3—figure supplement 2. Principal components analysis of MeA categorization data. DOI: 10.7554/eLife.02743.012
Figure 4. Sexual dimorphism of adult MeA responses. (A) Responses of AOB neurons to vomeronasal stimuli in adult male (210 units) and female (64 units) mice. (B) Responses of MeA neurons to vomeronasal stimuli in adult male (106 units) and female (91 units) mice. (C) Responses of MeA neurons to vomeronasal stimuli in juvenile male (37 units) and female (50 units) mice. Units shown in panels A–C are the same data shown in Figure 3C,D but classified according to the sex of the animal recorded. Blue circles indicate units recorded from male mice and red squares indicate data recorded from female mice. (D–F) Sex-specificity ('Materials and methods') histograms are shown for all units recorded from male (blue) and female (red) animals in the adult AOB (D), adult MeA (E) and juvenile MeA (F). Horizontal lines (above) indicate the mean and 95% confidence interval (bootstrap CI) of the mean for each distribution. Data collected from males vs females were only different in the adult MeA (AOB: p=0.26 adult MeA: p<0.00001; juvenile MeA: p=0.18; permutation tests).

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Figure 4—figure supplement 1. Sexual dimorphism in the dominance of predator versus conspecific responses.

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Figure 5. Importance of aromatase signaling for the development of sexually dimorphic MeA responses. (A) Population summary of MeA responses to male, female, and predator stimuli recorded from adult male ArKO mice (light blue circles) or heterozygous male littermates (dark blue diamonds). All plotted units responded significantly to at least one stimulus. (B) Sex-specificity histograms for units recorded from ArKO males (blue fill), heterozygous male littermates (dark blue; no fill), and wild-type juvenile males (light blue; no fill). Horizontal lines indicate the mean and 95% confidence interval of the mean of each distribution. (C) Matrices of correlation for the population responses between pairs of sensory stimuli for heterozygous males (top) and ArKO males (bottom). DOI: 10.7554/eLife.02743.015
Figure 6. Estrogen influences the development of sexually dimorphic MeA responses. (A) Population summary of MeA responses to male, female, and predator stimuli recorded from adult untreated female mice (red squares) or estrogen treated adult females (dark red triangles). (B) Sex-specificity histograms for units recorded from estrogen-treated adult females (dark red fill), adult untreated females (red; no fill), and untreated adult males (blue; no fill) for comparison. Estrogen treatment reduced responses in adult female mice to male stimuli (leftward) and increased the frequency of responses to same-sex stimuli (rightward). Horizontal lines indicate the bootstrapped 95% confidence interval for the mean of each distribution. (C) Comparison of the dorsal-ventral locations of male vs predator responsive units (blue) and female vs predator responsive units (red; see Figure 2—figure supplement 1) in the MeA of estrogen treated females. Both male responsive and female responsive units are dorsal to predator responsive units.

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