iScience, Volume *25*

Supplemental information

Self-directed orofacial grooming promotes social attraction in mice via chemosensory communication

Yun-Feng Zhang, Emma Janke, Janardhan P. Bhattarai, Daniel W. Wesson, and Minghong Ma

Supplemental Figures

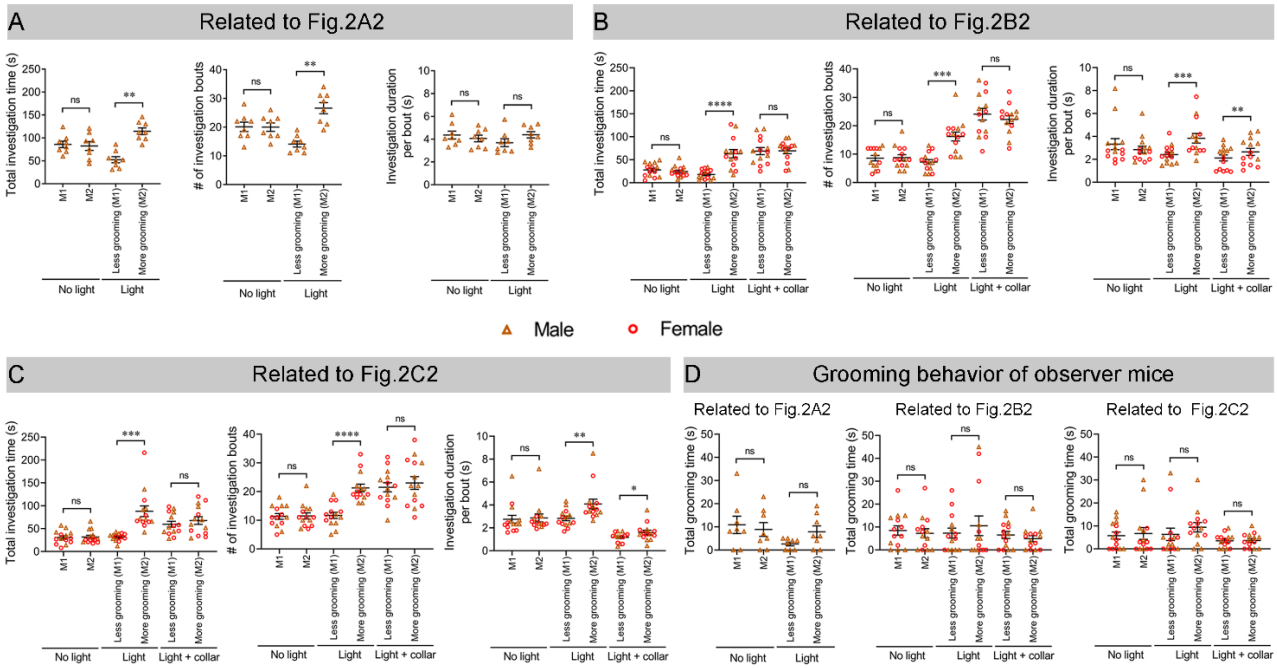

Figure S1. Observer mice spend more time investigating mice that groom more, Related to Figure 2.

(A) Mice stimulated by blue (more grooming) or green light (less grooming) in uncovered cups (with visual cues). Investigation behavior of observer mice (n = 8 male) with female light-stimulated mice. (B-D) Mice stimulated by blue (more grooming) or green light (less grooming) in covered cups (without visual cues). (B) Investigation behavior of observer mice (n = 13 males and 14 females) with female light-stimulated mice. (C) Investigation behavior of observer mice (n = 7 males and 7 females) to male light-stimulated mice. (D) Observer mice show similar durations of self-grooming near blue or green light-stimulated mice. Left, light-stimulated mice in uncovered cups (with visual cues). Self-grooming behavior of observer mice (n=8 male) near female blue or green light-stimulated mice. Middle and right, light-stimulated mice in covered cups (without visual cues). Middle, self-grooming behavior of observer mice (n = 13 males and 14 females) near female blue or green light-stimulated mice. Right, self-grooming behavior of observer mice (n = 7 males and 7 females) near male blue or green light-stimulated mice. Data are shown as the mean ± s.e.m. Two-tailed paired student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns, not significant. Results of statistical analyses are included in **Table S2**.

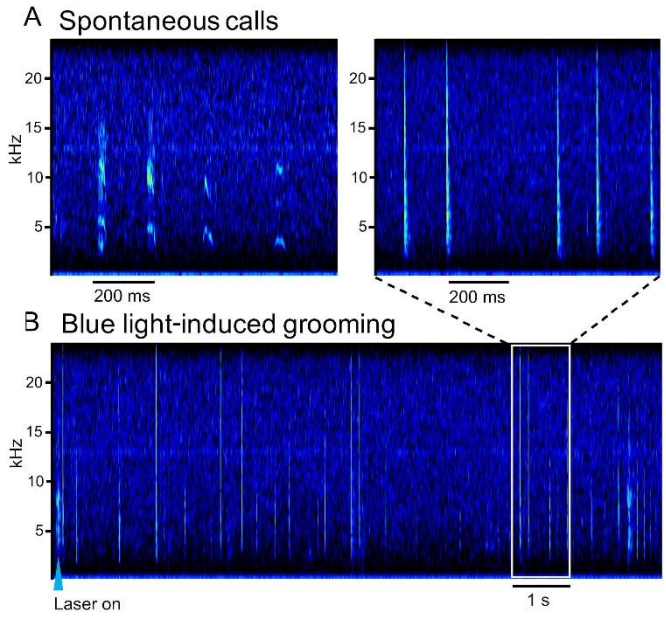

Figure S2. Mice do not emit discernable vocalizations during self-grooming, Related to Figure 2.
Sonograms of spontaneous audible calls (A) and sounds associated with grooming strokes (B). No obvious ultrasonic vocalizations between 20 to 96 kHz in these recordings (not shown). Inset, an enlarged view of the rectangle area in (B). Whereas spontaneous calls, as expected, were restricted within frequency bands, self-grooming was associated with broad-band acoustic events which corresponded in time to movement of the fiber optic tether. Similar results were obtained from 6 mice recorded.

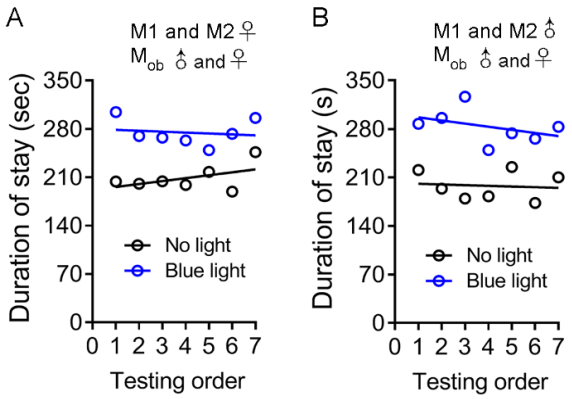

Figure S3. The testing order of observer mice does not impact the time spent in the side of blue light-stimulated mouse, Related to Figure 2.
The duration of stay in the side of blue light-stimulated mouse (more grooming) is plotted against the testing order of observer mice. (A) Female D3-ChR2 mice with no light or blue light stimulation in the OT. (B) Male D3-ChR2 mice with no light or blue light stimulation in the OT. Each data point is an average of four observer mice (two male and two female). Pearson's linear regression analysis. In A, No light: $r = 0.492$, $p = 0.262$; Blue light: $r = -0.151$, $p = 0.747$. In B, No light: $r = -0.103$, $p = 0.826$; Blue light: $r = -0.400$, $p = 0.374$.

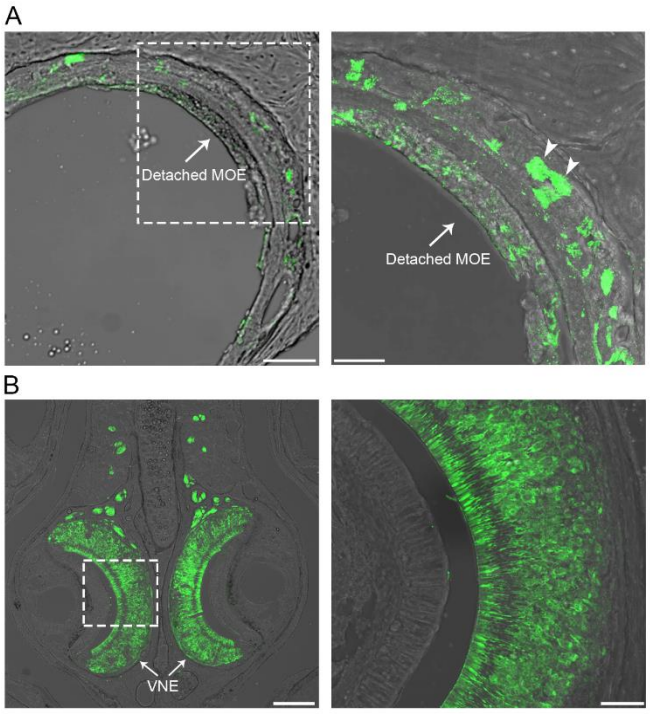

Figure S4. Methimazole treatment ablates the main olfactory epithelium but leaves the vomeronasal epithelium intact, Related to Figure 3.

Four days post methimazole treatment, coronal sections of the nose were processed for immunoreactivity to the olfactory marker protein (OMP), which labels mature olfactory sensory neurons. (A) Left, a representative image showing the detached main olfactory epithelium (MOE) without obvious OMP+ olfactory sensory neurons (OSNs). Right, an enlarged view of the dotted rectangle area in the left panel. Arrowheads denote OSN axon bundles. Scale bars = 100 (left) and 50 μm (right). (B) Left, a representative image (coronal section) showing the intact vomeronasal epithelium with abundant OMP+ sensory neurons. Right, an enlarged view of the dotted rectangle area in the left panel. Scale bars = 200 (left) and 50 μm (right). MOE, main olfactory epithelium; VNE, vomeronasal epithelium. Similar results were obtained from 3 mice.