Encoding Pheromonal Signals in the Accessory Olfactory Bulb of Behaving Mice

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Many mammalian species rely on pheromones—semiochemicals produced by other members of the same species—to communicate social status and reproductive readiness. To assess how the central nervous system integrates the complex repertoire of pheromones, we recorded from single neurons in the accessory olfactory bulb, a nucleus that processes pheromonal signals, of male mice engaged in natural behaviors. Neuronal firing was robustly modulated by physical contact with male and female conspecifics, with individual neurons activated selectively by specific combinations of the sex and strain of conspecifics. We infer that mammals encode social and reproductive information by integrating vomeronasal sensory activity specific to sex and genetic makeup.

For mammals ranging from mice to elephants, pheromones trigger neuroendocrine responses and reproductive and social behaviors (*1–3*). In rodents, semiochemicals are detected by two separate neural pathways, the main and the accessory olfactory systems. In the main olfactory system, olfactory receptor neurons in the nasal epithelium are activated by small, volatile odorants either passively by respiration or actively by sniffing. In the accessory olfactory system, sensory neurons in the vomeronasal organs (VNO), located at the base of the nasal septum, access nonvolatile stimuli by active dilation and constriction (pumping) of the VNO during direct nasal contact with sources of pheromones (*4*, *5*). Olfactory receptor neurons project to the main olfactory bulb (MOB), whose mitral/tufted cells project in turn to the olfactory cortex (*6*). In contrast, vomeronasal receptor neurons innervate the accessory olfactory bulb (AOB), whose mitral cells project to hypothalamic areas by way of the medial amygdala (*7*). Whereas large lesions of the main olfactory pathway leave reproductive behaviors intact (*8*), lesions or genetic inactivation of the VNO to AOB pathway severely compromises sex discrimination and mating behaviors (*9–12*), suggesting that the accessory olfactory pathway is critical for encoding pheromonal cues.

The VNO to AOB pathway possesses unique features for detecting pheromones. Vomeronasal sensory neurons use receptors and heterotrimeric GTP-binding proteins distinct from those of olfactory epithelial neurons (*13*, *14*); they are also more sensitive and selective to sensory stimuli than olfactory neurons (*15–17*). The axons of vomeronasal neurons bearing the same receptor form multiple glomeruli in the AOB, whereas those of the main olfactory epithelium converge to a single glomerulus in the MOB (*18–21*). The mitral cells in the mammalian AOB and MOB also have markedly different dendritic architectures (*22*, *23*).

Although VNO sensory neurons in vitro can be activated by dilute urine (a rich source of pheromones) (*24*, *25*) or by solutions containing putative mouse pheromones (*15*, *26*, *27*), how the AOB represents the diversity of social signals in natural behavioral contexts remains mysterious. Whether AOB neurons encode pheromonal signals by integrating peripheral sensory information and, if so, how such integration is accomplished are also unknown. Because the accessory olfactory pathway receives chemical stimuli by the active pumping of the VNO (*28*), the response properties of AOB neurons can only be determined in behaving, conscious animals. To accomplish this task, we used a miniature motorized drive to record the activity of single neurons in the AOB of male mice while they investigated conspecifics differing by sex, genetic makeup, and hormonal state. We found that the excitatory and inhibitory circuitry in the AOB generates unique response signatures in AOB mitral cells that endow them with substantial selectivity, providing a neural substrate for distinguishing the genetic makeup and gender of conspecifics.

Response kinetics of AOB cells. Sexually mature male mice of one of two genetic strains (BALBc or CBA) were implanted with miniature microdrives (*29*) that allowed three microelectrodes to be independently advanced or retracted through the AOB (*30*). On days of recording, a flexible cable was attached to the microdrive and connected to a torqueless, feedback-controlled commutator (fig. S1). The test animals were then free to move around the test arena and interact with stimulus animals. To minimize copulatory and aggressive behaviors from test animals, we examined the responses to pheromonal cues by introducing lightly anesthetized stimulus animals, which were avidly investigated by test animals. After initially circling the stimulus animals, test animals approached and then repetitively poked the bodies of the stimulus animals with their snouts (Fig. 1, A to C). Bouts of physical contact lasted 3 to 67 s (median = 5 s; nine test animals; 47 recording sessions; 1879 bouts). We achieved stable recordings of AOB neurons from behaving mice with excellent signal/noise ratios. Cells recorded in the AOB were likely from the layer of mitral cells, on the basis of depth measurements, electrophysiological signatures, and electrolytic lesions (see below). In the limited confines of the arena, test animals received strong, volatile signals from the stimulus animals; even human noses could smell them. However, these putative AOB mitral cells were activated only after test animals directly contacted and actively investigated the bodies, primarily the anogenital or head/face regions, of stimulus animals (Fig. 1). Responses consisted of a gradual elevation, over many seconds, of neuronal firing rates from baseline (2 to 5 Hz) to >20 Hz (Fig. 1, D and E). To verify that the responses were not due to the investigative activity alone, we introduced clean fake mice into the arena. The test animals were very interested in these fake mice, pushing them with their snouts and burying their noses into the "facial" and "anogenital" areas of the models. However, these activities did not evoke any neuronal response ($n = 8$ cells from 7 animals). These observations indicate that investigative activity provided the VNO access to nonvolatile stimuli, and that direct contact with conspecific individuals was required for activation of AOB cells.

Whereas the main olfactory epithelium has rapid and direct contact with volatile odorants, sensory neurons in the VNO access soluble stimuli through a narrow entrance duct in the nasal cavity. Some volatile odor–guided behaviors, such as recognizing predator odors, may place a premium on speed, whereas those mediated by pheromones, such as distinguishing male and female conspecifics, may place a premium on accuracy over speed. To examine how rapidly neurons in the AOB responded to social cues, we analyzed the activity of 14 cells from nine test animals in response to the introduction of a stimulus animal. Using the time-synchronized videotapes of behavior, we aligned neural responses with the start of the initial bout of investigation and averaged the responses (Fig. 2, A and B).

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Unlike neurons in the MOB, which invariably responded to volatile odorants with rapid and abrupt changes in firing frequency, the activity of AOB cells gradually increased following the initiation of active investigation. The latency from initial contact to the point when neuronal activity rose above the resting level was 3.6 ± 0.7 s (mean ± SEM, $n = 20$). Peak responses (23.6 ± 2.0 Hz) were delayed 18.6 ± 2.6 s from the initial contact. In many cases, firing rates remained strongly elevated for 10 to 30 s (median = 11.9 s) after a bout of direct interaction (Fig. 2, A and B). Compared with processing times in other sensory systems, including the MOB, detection of pheromonal cues by the AOB is far slower. This suggests that the accessory olfactory system is not designed to sense fleeting changes in the chemical environment, although responses are sufficiently rapid for detection of the social and reproductive status of conspecifics.

Inhibitory responses, like the excitatory responses, also began a few seconds after the initiation of direct contact (delay to significant reduction from the resting level = 5.0 ± 0.9 s; $n = 20$ trials, 16 cells) (Fig. 2, C to E). Cells gradually reached an inhibitory maximum, which was manifested as a nearly complete silencing of neuronal activity that persisted for up to 60 s (delay to inhibitory trough = 16.1 ± 2.1 s). Inhibition was also frequently observed immediately following strong excitatory responses (Fig. 2, F to H), possibly resulting from self-inhibition between mitral and granule cells or from intrinsic properties of the mitral cells themselves.

The slow activation and inactivation of AOB neurons reflect the aggregate delays of stimulus access, ligand binding, and the activation of vomeronasal receptor neurons. In VNO slices, VNO receptor neuron activation by dilute urine is about 1 s slower than potassium chloride–induced excitation (*25*), suggesting that ligand-mediated activation of VNO receptor neurons requires about 1 s. The VNO starts actively pumping immediately after introduction of novel stimuli (*28*); delivering water-soluble stimuli to the VNO probably requires 2 to 3 s following direct contact with a stimulus source. The prolonged excitation or inhibition of AOB neurons following the cessation of contact may reflect a slow process of clearing stimuli from the VNO, as has been proposed for the elephant VNO (*31*).

Selective responses of AOB cells. To explore the selectivity of AOB cells, we recorded 43 single units in nine male mice (five BALBc, four CBA). Stimulus animals consisted of male and female BALBc, C57BL/6, and CBA mice, as well as castrated male CBA mice. We quantified responses by calculating a response index (RI) that reflects the fold change in basal firing rates evoked by direct contact (decrease or increase, range −3.4 to 10.4) (*30*).

Sensory neurons expressing the same receptors project to multiple glomeruli in the AOB, and AOB mitral cells innervate multiple glomeruli (*19–21*). This apparent anatomical substrate for integration, coupled with the rich repertoire of pheromones in the urine and other bodily secretions, led us to anticipate that these neurons would respond to a broad range of stimulus animals. Nevertheless, in behaving animals, natural stimuli evoked exquisitely specific neural responses. Of the 222 trials with stimulus animals, significant excitatory responses were evoked from 38 trials (17%) (supporting online text). Of these, 25 trials (11%) were classified as strong, with a response index > 3. These ratios of responding cells are potentially biased by cell selection during recording (cells with low or absent spontaneous activity may have been missed) and by our choice of stimulus animals that included strains believed to have especially robust pheromone release. The vast majority of responsive units were excited selectively by a combination of the strain and sex of stimulus animals. For example, the cell in Fig. 3, A to G, was recorded from a CBA male and was strongly activated exclusively by the BALBc male stimulus animal (RI > 6; Movie S1). It was strongly inhibited by the CBA female stimulus animal (RI < -2) and more weakly inhibited by the C57BL/6 female stimulus animal (RI $= \sim -1$). The three other stimulus animals had no effect on the cell's firing rate. Of 34 units for which at least four stimulus animals of different strain and sex were tested, 10 responded strongly to only one type of animal (Fig. 3H; fig S2A). In two cases, cells responded to both sexes of the same genetic strain. The neuron illustrated in Fig. 3, I to O, recorded from a BALBc male, responded strongly to both males and females of the BALBc strain, although its response to the male was consistently stronger than to the female. Similarly, another neuron (from a CBA male) responded to both males and females of the CBA strain (Fig. 3P). Broad selectivity—responses to four of six stimulus animals—was only observed for one cell (Fig. 3Q). We did not encounter neurons whose responses generalized to either all stimulus males or all females. Six units (18%) had no significant response (neither excitatory nor inhibitory) to any of the stimulus animals (fig. S2B).

Male mice in their home territory attack other sexually active males, but not castrated animals. This distinction is mediated by the VNO to AOB pathway (*10*). We therefore tested whether AOB neurons distinguished castrated males from intact males. Out of 15 units tested with CBA male castrates, excitatory responses were evoked from two units, one of which responded strongly (Fig. 3R). This neuron did not respond to a CBA female stimulus animal, suggesting that the castrated animal exhibited a pheromonal repertoire that was neither male nor female.

Inhibitory responses were also markedly selective. Of the 222 trials of initial presentation of stimulus animals to 43 cells, significant inhibitory responses were evoked from 33 trials (15%), 23 of which (10%) had RIs of less than −2, indicating very strong inhibition. Each neuron was strongly inhibited by one or two types of stimulus animal, indicating a narrow selectivity for inhibitory responses (Fig. 4). In the MOB, inhibitory dendrodendritic synapses from granule cells are located primarily on mitral cell's extensive basal dendrites (*32*, *33*). This organization has been proposed to underlie lateral inhibition in the MOB, providing a mechanism for sharpening the selectivity of olfactory responses (*34*, *35*).

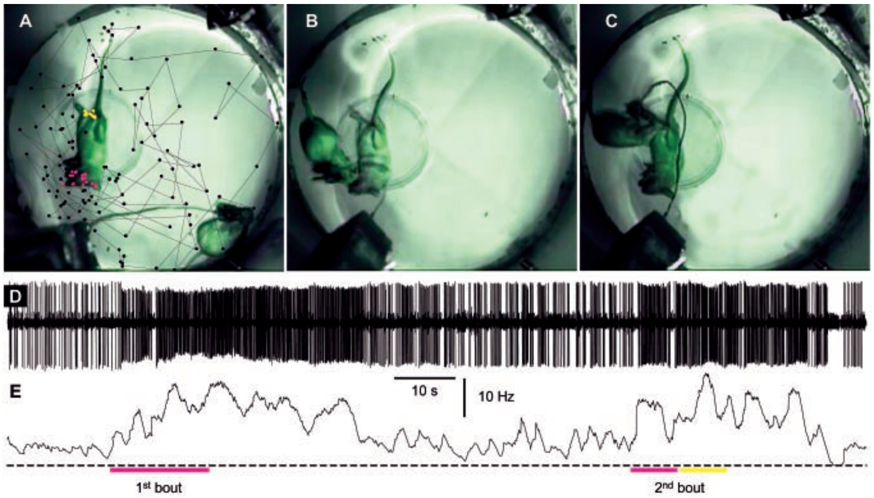

Fig. 1. Representative response patterns of neurons in the AOB of behaving mice. (**A**) Traces of the movements (dashed lines) by a test animal (BALBc male) following introduction of an anesthetized stimulus animal (BALBc female). Dots indicate the position (sampled at 1 Hz) of the test animal's snout. Colored dots indicate direct contact between the snout of the test animal and the body of the stimulus animal. Pink dots, investigation of the face and head; yellow dots, investigation of the anogenital region. (**B**) A video frame showing the investigation by the test animal of the head area of the stimulus animal. (**C**) A frame showing active investigation of the anogenital area. (**D**) Recording of the activity of a single-unit (unit 9.2x) at rest and following the animal's investigation of the stimulus animal. (**E**) Mean firing rate of the unit shown in (D). Pink bars correspond to the pink dots in (A), yellow bar corresponds to the yellow dots in (A). Dashed line indicates mean firing rate of 0 Hz. (D) and (E) share the same time scale.

Unlike MOB mitral cells, AOB mitral cells have short basal dendrites, and much of their inhibitory input forms on multipolar apical dendrites (*23*, *36*, *37*). This difference has led to proposals that dendrodendritic connections between mitral and granule cells in the AOB mainly perform recursive self-inhibition rather than lateral inhibition (*38*). However, our finding that different stimulus animals mediate inhibition and excitation in the same neuron implies substantial, but selective, lateral inhibition in the AOB. The narrow tuning of inhibitory responses in the AOB (Fig. 4) suggests that the absence of broad excitatory responses in AOB mitral cells (Fig. 3) derives from the selectivity of their excitatory drive and not from sharpening by broad lateral inhibition. It also suggests that a given mitral cell might be connected, by way of inhibitory granule cells, to a population of mitral cells whose response profiles are similar, rather than to mitral cells activated by a range of different receptors.

To test the response reproducibility, we repeated the presentation of the same stimulus animals and measured the RI ($n = 110$ trials, from 29 of the 34 cells described above). Although the duration and sequence of exploring body parts of stimulus animals by test animals differed between the two sets of trials, the correlation of the RI for same cells between sessions was highly significant ($r = 0.72$, $P < 0.0001$, df = 108). The greatest variation was observed for the weakest responses, whereas strong excitatory or inhibitory responses were very reliable (Fig. 3G). To verify that the specificity of responses was not due to peculiarities of the particular stimulus animals used, for a subset of 14 cells (44 trials) we presented test animals with two entirely different sets of stimulus animals consisting of the same strains and sexes. The same degree of response selectivity was evident, and the correlation of RI between the two stimulus sets was very high ($r = 0.69$, $P < 0.0001$) (Fig. 3O).

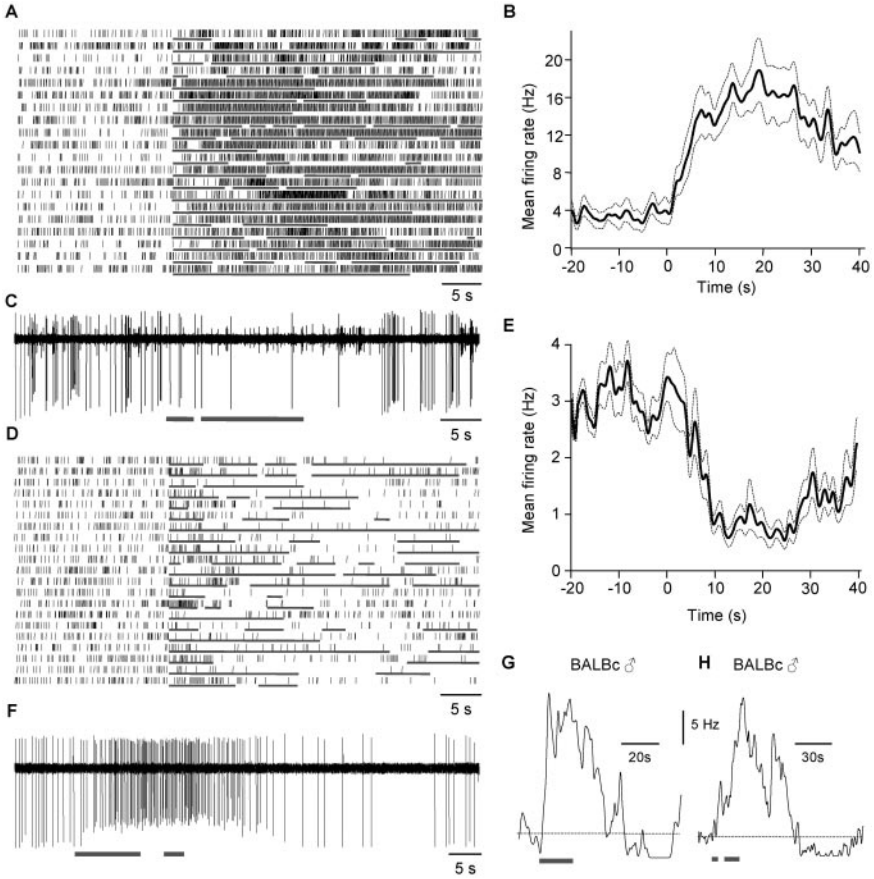

Fig. 2. Response kinetics of AOB neurons. (**A**) Raster plots of the activation patterns of 14 cells (from 9 test animals) during the initial investigation of a variety of stimulus animals. Plots are aligned with the initiation of direct contact. Gray bars beneath rasters represent periods of direct contact between the snout of test animals and the body of stimulus animals. (**B**) Averaged neuronal responses for the trials in (A). Mean firing rates were calculated from the raster plots and averaged (thick line). The thin dashed lines represent ±1 SEM. (**C**) A sample trace showing a representative inhibitory response following direct contact between a BALBc male test animal and a C57Bl/6 female stimulus animal. (**D**) Raster plots of the inhibitory responses from a sample of 16 cells (from 8 test animals) to the initial investigation of stimulus animals. (**E**) Averaged inhibitory responses for the trials in (D). (**F**) A recording of a single unit from a BALBc male test animal showing a period of inhibition following strong excitation elicited by a BALBc male stimulus animal. (**G** and **H**) Mean firing rates showing two additional examples of inhibition following strong excitation. Dashed lines, mean firing rates.

Responses to facial and anogenital contact. Most semiochemical research in rodents has focused on compounds present in urine, the preputial glands, and vaginal secretions in the anogenital region. In our recording sessions, however, the first interactions between test animals and either anesthetized or behaving stimulus animals typically involved investigating the face and mouth areas. Test animals investigated the face and head of anesthetized stimulus animals more frequently (40% of bouts, 1879 bouts, nine test animals) and for longer times (51% of total investigation time of 16,862 s, mean 11.7 s/bout) than the anogenital areas (30% of bouts, 22% of total investigation time, mean 6.8 s/ bout). Indeed, the facial areas of the stimulus animals evoked some of the most robust responses (Fig. 1 and Movie S1). In most cases, when test animals were allowed to freely explore the stimulus animals, the neuronal responses to different body parts of the stimulus animals were similar in specificity and degree. However, four neurons were differentially activated by investigation of facial and anogenital regions. In three cases, the facial area of a subset of stimulus animals evoked robust excitatory responses, whereas investigation of the anogenital area of all stimulus animals had no effect on the cells' firing rates (Fig. 5; fig S2C). In one case, the facial area of one type of stimulus animal evoked a strong inhibitory response, whereas the anogenital area of all stimulus animals elicited no clear response. Although pheromones initially produced by anogenital areas might have been transferred to the head by grooming, it is more likely that the differential responses of some neurons to the facial area were due to semiochemicals from several scent glands found in the facial area, such as the Harderian glands and the submaxillary salivary glands (*39*, *40*). Our recordings suggest that semiochemicals from these glands are accessible to the VNO and can communicate gender and genetic identity with fidelity similar to that of better-known sources of pheromones.

Distinctions between AOB and MOB neurons. Several lines of evidence suggest that we recorded from AOB mitral cells. When advancing electrodes through the frontal cortex to the olfactory bulb, we first encountered cortical regions dominated by "chattering" spikes and a zone of inactivity over the next 200 to 300 μm. This was followed by a layer about 100 μm thick, in which multiple units were typically recorded from single electrodes. Single units isolated in this layer had low spontaneous activity (2.8 ± 0.2 Hz; range 1.2 to 5.9 Hz, $n = 65$) (Fig. 6, A and B). Although short "runs" of accelerated firing (~10 Hz, 1 to 2 s) were often observed, these cells did not burst at rest (percentile of interspike interval < 10 ms = 0.4 ± 0.2%;

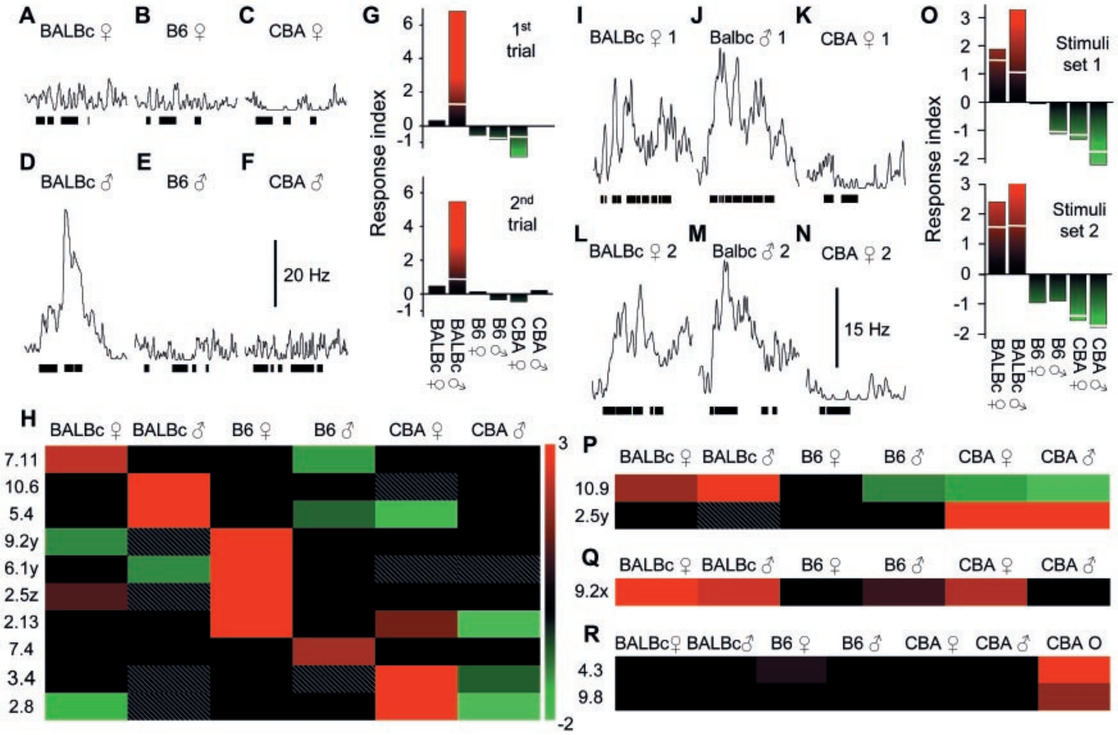

Fig. 3. Selectivity and reproducibility of AOB neuron responses to conspecifics. (**A** to **F**) Meaning firing rates of an AOB mitral cell from a CBA male (unit 5.4) in response to six anesthetized stimulus animals of the indicated sex and genetic strain. In this and subsequent figures, B6 denotes C57BL/6. (**G**) Response index of unit 5.4 following two separate presentations of the same stimulus animals. Upper panel uses the data in (A) to (F). Bottom panel shows results when the same stimulus animals as in (A) to (F) were presented a second time. Red, excitation; green, inhibition; horizontal white lines, 5% significance level. (**H**) Summary of response indices of neurons that responded selectively to a combination of strain and sex. Significant responses are indicated in color and nonsignificant responses are shown in black. Color scale at the right represents response indices ranging from −2 to 3. Hatched boxes indicate stimulus animals not tested; numbers to the left are identifiers of single units. (**I** to **N**) Reproducibility of responses to different stimulus animals of the same sex and genetic background. Mean firing rates of unit 10.9 during the initial investigation of a group of three stimulus animals (I to K) and during initial investigation of a new group of stimulus animals of the same sexes and strains (L to N). (**O**) Response indices of unit 10.9 to the first and second set of stimulus animals. (**P**) Response index plots of two cells that were selectively excited by the strain of stimulus animal, independent of sex. (**Q**) Response index plots of a unit that was excited by the majority of stimulus animals. (**R**) Response index plots of two units activated selectively only by castrated CBA males (CBA O). Conventions and color scale used in (P) to (R) are the same as in (H).

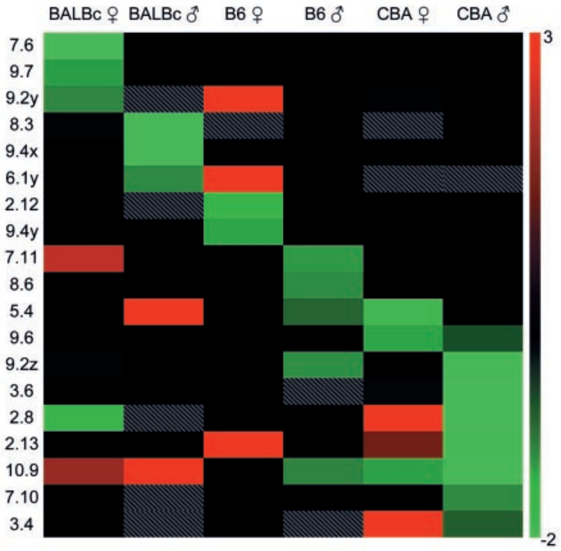

Fig. 4. Summary of response indices indicating the selectivity of inhibitory responses. Response indices of 19 units that were significantly inhibited by at least one stimulus animal are indicated by green bars. Conventions used are the same as in Fig. 3H.

$n = 18$ cells, 9 animals). Postmortem examination of electrode tracks revealed that electrodes entered the AOB ($n = 9$ animals). In four cases where electrolytic lesions were performed following recordings in this layer, the centers of the lesions were located in the AOB mitral cell layer, suggesting that these units with low spontaneous activity were AOB mitral cells (Fig. 6G). A very different population of neurons, corresponding to MOB mitral cells, was encountered from a thin layer less than 100 μm thick and 700 to 1300 μm deep to the putative AOB mitral cell layer (the exact depth of which depended on the angle of electrode penetration). At resting states, these cells were significantly more active than the putative AOB cells (21.2 ± 1.9 Hz, $n = 14$; unpaired *t* test versus AOB cells, $t = 26.5$, $P < 0.001$, df = 79) (Fig. 6, C and D). Whereas the putative AOB mitral cells showed no clear respiratory rhythm in their activity (power spectrum analysis, $n = 18$), a substantial number of the putative MOB cells (9 out of 16) fired rhythmically at the respiratory frequency (3 to 5 Hz), suggesting that they were indeed MOB mitral cells.

The putative AOB cells in behaving mice did not respond to volatile odorants. Although "common" volatile odorants such as amyl acetate activate VNO neurons in vitro (*27*) and weakly stimulate AOB neurons in anesthetized animals (*5*), when presented on swabs near the animal's nostril they did not produce an obvious change of activity in any AOB cell tested ($n = 16$) (Fig. 6E). Similarly, no activity change was observed following presentation of four purified putative pheromones (2-heptanone, 2,5-dimethylpyrazine, E,E-α-farnesene, and E-β- farnesene; 1% on swabs) that strongly and selectively activate VNO cells in vitro ($n = 5$) (*15*). In contrast, high concentrations of two volatile odorants (amyl acetate or butanal) reliably elicited strong excitation or inhibition in almost all MOB neurons (15 out of 16) (Fig. 6F). Purified pheromones also activated many MOB neurons (5 out of 7) when presented near the animal's nostrils with swabs. The absence of response to volatile odorants by AOB neurons probably reflects the inability of the stimulus to reach the VNO in behaving animals, rather than an absolute insensitivity of the VNO receptor neurons to these stimuli.

Discussion. By recording from the AOB of behaving mice during natural investigative behaviors, we have uncovered some of the principles used to encode pheromonal social and reproductive cues in the poorly understood accessory olfactory pathway of the central nervous system. We find that activation of AOB neurons requires direct contact between the snout of test mice and the face or anogenital regions of stimulus animals. Both the prolonged excitation and inhibition of AOB cells evoked by stimulus animals are surprisingly selective to a combination of the genetic makeup and sex of conspecifics. Finally, AOB cells have physiological characteristics distinct from those of MOB cells in terms of resting activity and sensory responses.

These first insights into the encoding of pheromonal cues by AOB neurons required recording the activities of single units from behaving animals. Prior observations of weak or absent sensory responses in the AOB probably reflect an inability to activate properly the pumping mechanism of the VNO in anesthetized animals. The absence of responses to volatile odorants (Fig. 6E) and the requirement of direct contact between test and stimulus animals for activation of AOB cells (Fig. 2) are consistent with the idea that the VNO primarily accesses water-soluble odorants bound to large, nonvolatile proteins through active VNO pumping during chemoinvestigation (*4*, *28*, *41*).

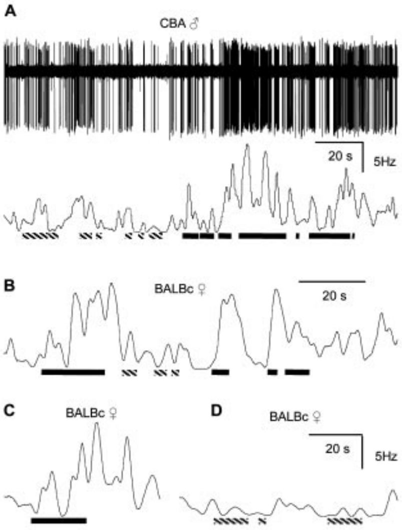

Fig. 5. Response of AOB neurons to the face/head and anogenital areas of stimulus animals. (**A**) Raw recording (upper panel) and the mean firing rate (lower panel) of a single neuron (unit 5.2y) from a CBA male during investigation of the anogenital region (hatched bars) or head/face area (filled black bars) of an anesthetized CBA male. (**B** to **D**) Mean firing rate of another neuron (unit 10.4) in a BALBc male during investigation of the head/face region or anogenital area of an anesthetized BALBc female (B) and in response to investigation of the isolated head /face (C) and isolated anogenital area (D) of the same BALBc female. In (C), the stimulus animal was placed in a plexiglass tube, leaving only the head/ face region accessible. Conventions used are the same as in (A).

Individual AOB neurons respond selectively to stimulus animals of specific combinations of sex and strain. Urine from a single mouse strain activates a substantial proportion of VNO neurons in vitro (*25*). In a natural environment, conspecifics almost certainly exude a richer and more complex repertoire of pheromonal cues than those present in urine alone (*42*). The apparently divergent connections between vomeronasal cells and AOB mitral cells suggest that AOB mitral cells might receive inputs from several different populations of vomeronasal receptor neurons (*19*, *20*, *23*). These features initially led us to predict that AOB cells would respond broadly to conspecifics. Surprisingly, we found that the responses of AOB cells were exquisitely tuned to the combination of sex and genetic makeup.

Do these selective response fields reflect the selectivity of the receptor neurons in the VNO or do they arise by integrative processes within the AOB? Vomeronasal receptor neurons respond with high sensitivity and selectivity to purified pheromones; each VNO receptor neuron may be exclusively activated by a specific pheromone molecule (*15*). For example, the putative pheromone 2-heptanone is a preferred ligand of the V1rb2 VNO receptor (*26*). Moreover, the multiple apical dendrites of AOB mitral cells innervate glomeruli formed by a single receptor population of VNO neurons, suggesting that each AOB mitral cell receives excitatory input from one type of receptor neuron (*21*). In principle, then, the highly selective responses of the putative AOB mitral cells could reflect the specific excitatory drive from the selective activation of a single type of VNO receptor. Selective receptor activation might itself have resulted from unique pheromonal components and/or their differential binding to the major urinary proteins released by conspecifics of different genetic backgrounds (*43*). This model of selectivity is supported by results showing selective responses of rat VNO neurons to urine from rats of different strain and sex (*24*). The inhibitory response fields of putative AOB mitral cells exhibit a similar specificity in their tuning, suggesting that the inhibitory drive onto a given mitral cell arises from activation of a very restricted population of VNO receptor types. The apparently greater selectivity of AOB neurons compared with that of VNO sensory neurons (*25*) may indicate that the identity and concentrations of pheromones actually reaching the VNO of behaving animals are different from those applied directly to receptor neurons in vitro.

The high selectivity to the conspecifics of the AOB mitral cells, which are just one synapse away from receptor neurons, is reminiscent of the exquisite feature selectivity of "face cells" sparsely distributed in the inferotemporal lobe in primates (*44*). Mice may create a "pheromonal image" of conspecifics by using a relatively small population of neurons with highly specific properties, rather than by extracting such an image from a large population of broadly tuned neurons. These findings are consistent with a model in which conspecific individuals activate "labeled lines" that can convey information about their gender and genetic makeup. Nonetheless, integration is likely required for encoding the complete pheromonal images of conspecifics. The number of mouse strains and substrains is large, and the number we actually tested is small. Given that a substantial number of cells responded in

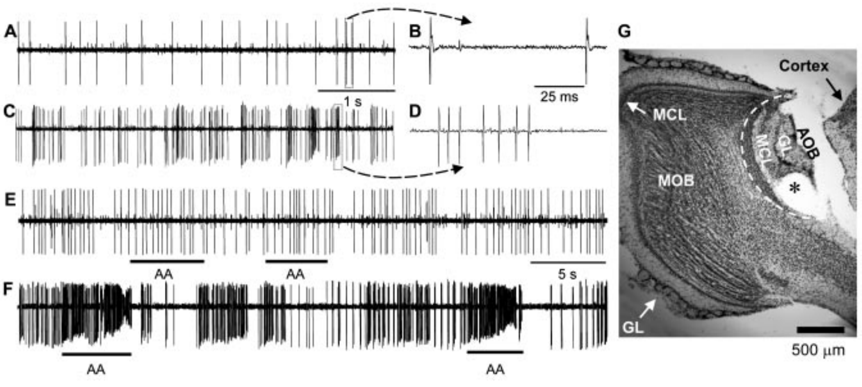

Fig. 6. Spontaneous firing patterns and responses to volatile odorants of mitral cells in the AOB and MOB. (**A**) Spontaneous activity of a putative AOB mitral cell recorded from a behaving BALBc male mouse. These cells typically fire at 2 to 4 Hz at rest. (**B**) Enlarged view of the spike waveforms in (A). (**C** and **D**) Spontaneous activity of a putative MOB mitral cell from a behaving BALBc male mouse. These cells typically fire at ~20 Hz at rest. (**E**) Response of an AOB mitral cell from a behaving BALBc male to a volatile odorant (amyl acetate, AA) presented by a swab near the test animal's nostril (bars). (**F**) Response of the MOB cell shown in (C) and (D) to amyl acetate (bars). (**G**) Cresyl violet–stained section showing a large electrolytic lesion at a recording site within the AOB. Asterisk indicates the center of the lesion in the mitral cell layer; dashed white line demarcates the border between the AOB and the MOB; MC, mitral cell layer; GL, glomerular layer. (A) and (C), (B) and (D), and (E) and (F) share their respective scale bars. (A), (B), and (E) are from the same unit as in Fig. 1, D and E.

some manner to one of the strains used, animals with different genetic backgrounds must have overlapping pheromonal composition and thus activate overlapping populations of AOB cells, implying that some mechanisms providing additional discriminatory power will be required. We uncovered considerable evidence for integration of pheromonal signals from multiple populations of receptors in the form of inhibitory signals, which are almost certainly mediated by local circuits within the AOB, because no natural stimuli have been reported to inhibit the activity of VNO sensory neurons (*24*, *25*).

In this study, we found that neurons were activated selectively by the strain and sex of a restricted subset of stimulus animals. These are two important cues for guiding many aspects of animal behavior (*10*, *11*, *45*). However, the accessory olfactory system mediates a far larger repertoire of behavioral and neuroendocrine events, many of which require more precise information about the social and reproductive status of other conspecifics, including dominance, mating receptivity, and individuality (*3*). Additionally, the AOB is divided into rostrolateral and caudomedial regions, each of which receives inputs from separate layers in the VNO expressing distinct families of receptors (*46–48*). These two subdivisions of AOB likely mediate different aspects of behavior (*49*) and may have distinct physiological responses to conspecifics.

References and Notes

1. M. R. Murphy, G. E. Schneider, *Science* **167**, 302 (1970).
2. L. E. Rasmussen, B. A. Schulte, *Anim. Reprod. Sci.* **53**, 19 (1998).
3. M. Halpern, *Annu. Rev. Neurosci.* **10**, 325 (1987).
4. C. J. Wysocki, J. L. Wellington, G. K. Beauchamp, *Science* **207**, 781 (1980).
5. M. Meredith, R. J. O'Connell, *J. Physiol. (London)* **286**, 301 (1979).
6. J. W. Scott, R. L. McBride, S. P. Schneider, *J. Comp. Neurol.* **194**, 519 (1980).
7. M. N. Lehman, S. S. Winans, J. B. Powers, *Science* **210**, 557 (1980).
8. J. B. Powers, S. S. Winans, *Physiol. Behav.* **10**, 361 (1973).
9. ———, *Science* **187**, 961 (1975).
10. L. Stowers, T. E. Holy, M. Meister, C. Dulac, G. Koentges, *Science* **295**, 1493 (2002).
11. B. G. Leypold *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **99**, 6376 (2002).
12. K. Del Punta *et al.*, *Nature* **419**, 70 (2002).
13. A. Berghard, L. B. Buck, *J. Neurosci.* **16**, 909 (1996).
14. C. Dulac, R. Axel, *Cell* **83**, 195 (1995).
15. T. Leinders-Zufall *et al.*, *Nature* **405**, 792 (2000).
16. P. Duchamp-Viret, M. A. Chaput, A. Duchamp, *Science* **284**, 2171 (1999).
17. B. Malnic, J. Hirono, T. Sato, L. B. Buck, *Cell* **96**, 713 (1999).
18. P. Mombaerts *et al.*, *Cell* **87**, 675 (1996).
19. I. Rodriguez, P. Feinstein, P. Mombaerts, *Cell* **97**, 199 (1999).
20. L. Belluscio, G. Koentges, R. Axel, C. Dulac, *Cell* **97**, 209 (1999).
21. K. Del Punta, A. Puche, N. Adams, I. Rodriguez, P. Mombaerts, *Neuron* **35**, 1057 (2002).
22. K. Mori, K. Kishi, H. Ojima, *J. Comp. Neurol.* **219**, 339 (1983).
23. K. Mori, *Prog. Neurobiol.* **29**, 275 (1987).
24. K. Inamura, Y. Matsumoto, M. Kashiwayanagi, K. Kurihara, *J. Physiol.* **517**, 731 (1999).
25. T. E. Holy, C. Dulac, M. Meister, *Science* **289**, 1569 (2000).
26. C. Boschat *et al.*, *Nature. Neurosci.* **5**, 1261 (2002).
27. M. Sam *et al.*, *Nature* **412**, 142 (2001).
28. M. Meredith, *Physiol. Behav.* **56**, 345 (1994).
29. M. S. Fee, A. Leonardo, *J. Neurosci. Methods* **112**, 83 (2001).
30. Supplementary material is available on *Science* Online.
31. J. Lazar, D. R. Greenwood, L. E. Rasmussen, G. D. Prestwich, *Biochemistry* **41**, 11786 (2002).
32. C. E. Jahr, R. A. Nicoll, *Science* **207**, 1473 (1980).
33. W. R. Chen, W. Xiong, G. M. Shepherd, *Neuron* **25**, 625 (2000).
34. M. Yokoi, K. Mori, S. Nakanishi, *Proc. Natl. Acad. Sci. U. S. A.* **92**, 3371 (1995).
35. M. Luo, L. C. Katz, *Neuron* **32**, 1165 (2001).
36. C. Jia, W. R. Chen, G. M. Shepherd, *J. Neurophysiol.* **81**, 345 (1999).
37. W. Reinhardt, N. K. MacLeod, J. Ladewig, F. Ellendorff, *Neuroscience* **10**, 131 (1983).
38. P. A. Brennan, E. B. Keverne, *Prog. Neurobiol.* **51**, 457 (1997).
39. D. Thiessen, M. Rice, *Psychol. Bull.* **83**, 505 (1976).
40. W. D. Booth, *J. Endocrinol.* **55**, 119 (1972).
41. Z. Bocskei *et al.*, *Nature* **360**, 186 (1992).
42. E. S. Albone, *Mammalian Semiochemistry: The Investigation of Chemical Signals Between Mammals* (Wiley, New York, 1984).
43. J. L. Hurst *et al.*, *Nature* **414**, 631 (2001).
44. M. P. Young, S. Yamane, *Science* **256**, 1327 (1992).
45. M. Bruce, *Nature* **184**, 105 (1959).
46. K. Imamura, K. Mori, S. C. Fujita, K. Obata, *Brain Res.* **328**, 362 (1985).
47. C. Jia, M. Halpern, *Brain Res.* **719**, 117 (1996).
48. J. Ryba, R. Tirindelli, *Neuron* **19**, 371 (1997).
49. A. Kumar, C. A. Dudley, R. L. Moss, *J. Neurosci.* **19**, RC32 (1999).
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Materials and Methods

Figs. S1 to S2

References

Movie S1

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REPORTS

Gigahertz Electron Spin Manipulation Using Voltage-Controlled g-Tensor Modulation

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We present a scheme that enables gigahertz-bandwidth three-dimensional control of electron spins in a semiconductor heterostructure with the use of a single voltage signal. Microwave modulation of the Landé g tensor produces frequency-modulated electron spin precession. Driving at the Larmor frequency results in g-tensor modulation resonance, which is functionally equivalent to electron spin resonance but without the use of time-dependent magnetic fields. These results provide proof of the concept that quantum spin information can be locally manipulated with the use of high-speed electrical circuits.

Control of electron spin forms the basis for spintronics (*1*) and related solid-state quantum information technologies (*2*). In the latter case, coherent control over individual spins, together with controlled exchange interactions, form a gate set that is known to be universal for quantum computation (*3*). For example, in quantum computing experiments using nuclear magnetic resonance (*4*), nuclear spins are manipulated with the use of a combination of static and radio-frequency magnetic fields. For qubits based on electron spins in semiconductors, local control over magnetic interactions is required. Early proposals suggested the use of electrical gates to bring electrons into contact with magnetic or high g-factor environments (*3, 5*). Subsequent ideas included methods to shift individual electrons into resonance with a global microwave

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