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Citation	Ben-Shaul, Yoram, Larry C. Katz, Richard Mooney, and Catherine Dulac. 2010. In-vivo vomeronasal stimulation reveals sensory encoding of conspecific and allospecific cues by the mouse accessory olfactory bulb. Proceedings of the National Academy of Science 107(11): 5172-5177.
Published Version	doi:10.1073/pnas.0915147107
Accessed	February 19, 2015 10:30:36 AM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:9887624
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(Article begins on next page)

Biological Science: Neuroscience

I*n-vivo* Vomeronasal Stimulation reveals Sensory Encoding of Conspecific and Allospecific Cues by the Mouse Accessory Olfactory Bulb

Y. Ben-Shaul[1], L.C. Katz[2*], R. Mooney[2], and C. Dulac[1]

[1]Howard Hughes Medical Institute, Department of Molecular and Cellular Biology
Harvard University, Cambridge, MA 02138

[2]Department of Neurobiology, Duke University School of Medicine, Durham, NC 27710

Corresponding author: Catherine Dulac
*: deceased

The rodent vomeronasal system plays a critical role in mediating pheromone-evoked social and sexual behaviors (1-4). Recent studies of the anatomical and molecular architecture of the vomeronasal organ (VNO) and of its synaptic target, the accessory olfactory bulb (AOB), have suggested that unique features underlie vomeronasal sensory processing. However, the neuronal representation of pheromonal information leading to specific behavioral and endocrine responses has remained largely unexplored due to the experimental difficulty of precise stimulus delivery to the vomeronasal organ (VNO). To determine the basic rules of information processing in the vomeronasal system, we developed a novel preparation that allows controlled and repeated stimulus delivery to the VNO, and combined this approach with multisite recordings of neuronal activity in the AOB. We found that urine, a well characterized pheromone source in mammals, as well as saliva, activate AOB neurons in a manner that reliably encodes the donor animal's sexual and genetic status. We also identified a significant fraction of AOB neurons that respond robustly and selectively to predator cues, suggesting an expanded role for the vomeronasal system in both conspecific and interspecific recognition. Further analysis reveals that mixed stimuli from distinct sources evoke synergistic responses in AOB neurons, thereby supporting the notion of integrative processing of chemosensory information.

In most animal species, the detection of pheromonal cues is essential to trigger and modulate sexual and social interactions between conspecifics. Genetic and surgical manipulations in the mouse have demonstrated the essential role of the vomeronasal system in this process (1-4). Moreover, tracing studies revealed dense projections from the vomeronasal system to hypothalamic nuclei involved in behavioral and endocrine control (5). The vomeronasal system thus offers a unique opportunity to explore principles of information processing underlying animal-animal communication and species-specific social and sexual interactions.

Although the rodent vomeronasal system is clearly involved in pheromone sensing, the range of stimuli it can detect is largely unknown (5). At one extreme, it may be exclusively dedicated to processing pheromonal (i.e. conspecific) information. Consistent with this view, several classes of conspecific cues were shown to directly activate isolated VNO preparations (see (6) for a recent review). Moreover, genetic as well as surgical VNO silencing significantly impairs behavioral responses to conspecific cues (1-2). Alternatively, the vomeronasal system may resemble the main olfactory system in which a wide variety of stimuli, including both pheromonal and non-pheromonal signals, are processed. This idea is supported by the finding that in-vitro VNO preparations can be activated by large sets of diverse chemicals (7-8).

The type and specificity of information extracted by the AOB represent another unresolved issue. AOB neurons can reliably detect conspecific sex from urinary cues (9) and strain information from as yet unidentified cues (10). One hint that the vomeronasal system can provide finer discrimination among individuals emerges from its role in the Bruce effect, (4) in which a gestating female terminates pregnancy when exposed to an unfamiliar male. However, how sex, strain and individual identity are encoded by AOB neurons remains poorly understood.

Exactly which stimulus features are encoded by the vomeronasal system depends ultimately on the nature and extent of information transmitted from the primary sensory neurons to the AOB. Unlike mitral cells in the main olfactory bulb (MOB), in which responses are shaped by input from one or a few receptor cells classes (11-12), AOB output neurons receive convergent inputs from multiple glomeruli (13-14). This architecture could provide a platform for integrative information processing that significantly differs from that observed in the main

olfactory system. However, direct physiological evidence for integrative processing by AOB neurons is still lacking.

A major challenge to resolving these issues is that stimulus delivery to the VNO requires active pumping which is only triggered during exploratory behaviors (15). The relatively low-throughput of chronic recording methods and, more fundamentally, the lack of control over stimulus uptake in freely behaving mice are not well suited for the systematic investigation of information processing in the AOB. Recently (9, 16), direct stimulus perfusion into the VNO of the anesthetized opossum and mouse has been used to show the importance of the AOB in distinguishing conspecific sex through urinary cues. However, delivery by perfusion requires relatively large samples, typically pooled from multiple individuals, hence limiting the ability to systematically address the processing of pheromonal stimuli with limited availability. Furthermore, stimulus delivery by perfusion effectively bypasses VNO pump dynamics, a physiological parameter which may play a significant role in the faithful transduction of stimulus information to downstream targets, much as sniffing does in the main olfactory system (17).

To overcome these limitations, we developed a novel experimental approach that enables repeatable and naturalistic delivery of small and physiologically relevant stimulus volumes to the intact VNO. By combining this approach with multisite electrophysiological recordings, we explored how AOB neurons respond to features in conspecific urine and saliva. These experiments revealed that AOB neurons respond to these complex stimuli in a manner that reliably encodes information about the strain, sex, as well as finer aspects that might represent the physiological state and the individual identity of conspecifics. This approach also led us to the surprising finding that cues present in a variety of predator urines can serve as potent and selective activators of AOB neurons. These results offer new insights into how the vomeronasal system functions to enable individual recognition important to social and sexual behavior, while also suggesting an expanded role for this system in the recognition of other species.

Results

A naturalistic preparation for recording neural activity in the vomeronasal system

To activate vomeronasal pumping in anesthetized animals, we implanted custom built cuff-electrodes over the cervical region of the sympathetic nerve trunk using the carotid artery as a scaffold (Fig. 1A). Initial experiments established that brief trains of biphasic current pulses applied to the sympathetic nerve (amplitude: ±100μA, frequency: 33Hz, duration: 1.6s) induced efficient uptake of fluorescent dye into the VNO (Fig. 1A). To flush the nasal cavity and VNO lumen and thus enable multiple cycles of stimulus presentation, we induced a flow of Ringer's solution from the nostril through the nasopalatine duct and to the oral cavity (18) while repeatedly activating the VNO pump (Fig. 1A). A planar array of 32 electrodes (19) was inserted into the external cellular layer (ECL) of the AOB which contains the cell bodies of mitral and tufted cells (20). Subsequent confirmation of electrode placement in the ECL was performed by histological inspection of the DiI painted electrode tracks (Fig. 1A). Neuronal activity recorded in the AOB when a small volume of diluted urine (2μl) was presented to the nasal cavity either alone or with sympathetic nerve stimulation is shown in Fig. 1B. These recordings reveal that both stimulus application and subsequent sympathetic stimulation are typically required to evoke a neuronal response in the AOB, and show that repeated sympathetic stimulation during flushing can be used to efficiently "clean" the VNO (Fig. 1B).

Basic characteristics of vomeronasal responses

Using this approach, we analyzed responses of 711 single- and 2332 AOB multi-units to a variety of stimuli. Unless otherwise indicated, all analyses refer to single unit data. Evoked responses of two single units recorded simultaneously following 6 trials of interleaved presentations of male and female urine is shown in Fig. 2A. In addition to demonstrating the repeatability and reliability of our experimental procedure, these data highlight the known (9) specificity of AOB units to male and female stimuli. The response specificity to sex- specific stimuli is further documented in SI.

Baseline firing patterns of single units were low (0.94±0.05Hz, mean ± sem), similar though slightly lower than values previously recorded in the anesthetized and behaving mouse (9-10). These lower baseline values could reflect a more effective removal of residual stimuli with our cleaning procedure, our non biased approach to finding single units, or the anesthetic state. Rate increases started 4.34±0.03 seconds following nerve stimulation, peaked at 10.9±0.10 seconds, with a half time of 12.8±0.06 seconds (Fig. 2B). Similar quantification of the temporal

profiles of rate decreases was impractical due to the low baseline rates. The average response delay (4.34s) was also similar to that measured in freely behaving mice (3.6±0.7s) (10). Because the vomeronasal signaling cascade has been estimated to require less than 0.5s (21), the response latency observed here could reflect the combined delays of sympathetic stimulation, pump activation and stimulus suction. Comparison of the temporal response profiles observed here with direct recordings of VNO pump activity in anesthetized (22) and awake animals (15), suggests that each sympathetic stimulation in our preparation triggers a single suction event. Thus, the basic vomeronasal response is significantly slower than the unitary sensory events in the main olfactory system which occurs at a typical frequency of 4-12Hz (23).

Stimulus induced responses require functional TRPC2 signaling

In contrast to wild type mice, recordings from TRPC2 -/- mice failed to reveal any responses when stimulus application to the nasal cavity was paired with sympathetic nerve stimulation. Specifically, although baseline activity from AOB neurons of adult TRPC2 -/- mice could be recorded (N=221 units including MUA, 8 recording sites from 4 males and 2 females), the distribution of stimulus-evoked response strengths was clearly different from that of WT mice (Fig 3A) and, more importantly, the rate of significant responses (p<0.01) was at chance levels (Fig. 3B). This finding indicates that the AOB responses evoked by pairing stimulus presentation with sympathetic nerve stimulation require an intact TRPC2 transduction pathway in the VNO. Thus, in contrast to recent reports that postulate residual TRPC2-independent vomeronasal activity (24), the present results employing direct measurement of electrical activity in the AOB reveal that the elimination of vomeronasal signaling is effectively complete following genetic ablation of TRPC2.

AOB readout of sex and strain

Prior studies involving chronic recordings from behaving mice revealed that AOB neurons can distinguish strain specific cues (10), but the pheromonal source of this information was not identified. Interestingly, these recordings revealed strong and selective bursts of activity correlated with the animal's investigation of both the anogenital and the facial region of conspecifics (10) suggesting that at least two bodily sources contain strain specific information.

Moreover, exocrine gland secreted peptides (ESPs) found in tears, mucosa, or saliva in a sex- and strain- specific manner have been shown to be effective vomeronasal stimuli (25-26). We explored this issue by testing the responses of AOB neurons to urine and saliva samples from mice of different sex and strain combinations. Of 107 single units tested with urinary stimuli, 40 (37%) showed a significant response to one or more stimuli (p<0.01, non-parametric ANOVA) with a prevalence of responses to only one stimulus (60%, 24 of 40, SI). Of 23 single units tested with salivary stimuli, 13 (57%) showed a significant response (p<0.01) to one or more stimuli (Fig. 4B,E). As seen with urinary stimuli (Fig. 4A), AOB neurons display responses triggered exclusively by saliva from one or few strains, by all animals of a given sex, or in more complex patterns (Fig. 4B and SI). For both urine and saliva, units responding exclusively to urine of both sexes from the same strain were not observed. Thus, both urinary and salivary stimuli can elicit highly specific responses in single units in a manner that could be used by the mouse to unambiguously detect the strain and sex of the stimulus animal.

Are the sex specific responses observed here and elsewhere merely a byproduct of the finer discrimination of strain and sex selectivity? To test this issue we analyzed the population responses to urinary and salivary stimuli using hierarchical clustering. This analysis revealed a perfect segregation of responses to male vs. female urine and an almost perfect segregation for salivary cues (Fig. 4C,D). Thus, although individual AOB neurons are often tuned to specific strain/sex combinations, sex nevertheless emerges as a primary parameter distinguishing different classes of responses in the AOB.

To explore whether AOB neuron responses to individual samples reflects the genetic background of the donor animals, we presented three distinct urine samples from males of each of three distinct strains (CBA, BalbC or C57Bl6). For each strain, two samples were collected from one individual and one was collected from a different mouse. Of 51 single-units tested, 16 showed a significant response to at least one stimulus. Population analysis of these responses showed that urinary cues from the same strain, and, for 2 of 3 strains, even from the same individual within a strain, clustered together (Fig. 4F). However, in addition to neurons that responded to all samples of a given strain, we observed specific responses to samples from an individual mouse and even to individual samples from a given mouse (Fig. 4C). The subset of neurons that differentiated samples from different mice and even different samples of the same mouse could provide the basis for detection of more subtle features, which might reflect the

individual's physiological state and identity. One should stress that these responses are not likely to reflect random fluctuations in the neuronal responses, as they are consistent across multiple presentations of the same stimuli.

Responses to allospecific stimuli

To investigate the potential role of the mouse vomeronasal system in detecting allospecific stimuli, we measured the responses of AOB single units to a mix of urinary cues from three mouse predators: bobcat, fox and rat. We found that the mix of predator urines could evoke remarkably robust responses from AOB neurons. Of 186 single units tested with both mouse and predator stimuli, an equal fraction (19%) showed a significant ($p<0.01$) response to mouse (male or female) or to predator urine. Importantly, most neurons (60%, 31 of the 51 single units that showed any response) responded specifically only to predator or mouse urine (Fig. 5 A, C). This specificity and the similar distribution of response magnitudes to mouse and to predator stimuli (Fig. 5B) suggest that mouse AOB neurons respond to predator urine because it contains specific predator-related cues, and not simply because it contains cues also common to mouse urine.

To further explore the specificity of these responses, we tested the responsiveness of individual AOB units to the urine of individual predators. Of 35 units tested, 12 showed a response to at least one of these stimuli ($p<0.01$). Of these twelve, 5 responded to only one predator stimulus in addition to the mixed predator cues (Fig. 5D), two responded to all stimuli, and the rest responded to only one of the stimuli (Fig. 5B, SI). The robustness of predator responses and the specificity of discrimination between mouse and predator cues shows that the vomeronasal system could participate in the detection and discrimination of allospecifics.

Responses to conflicting stimuli

The detection of conspecific female, male or predator cues triggers dramatically different behavioral responses. It is therefore intriguing that although about 60% of the single units (31 of the 51 responding units) are specifically activated by only one stimulus class ($p<0.01$) and thus can convey unambiguous information to downstream targets, a substantial fraction (the remaining 40%) responds to multiple stimulus classes with conflicting significance (Fig. 5A,E).

To explore how conflicting cues are processed by the AOB, we measured responses to male and female mouse urinary cues, to predator urine and to their mixtures (N=61 single units, of which 36 (59%) showed significant response (p<0.05) to at least one stimulus). The mixtures were prepared as averages of the elemental stimuli (see sup methods). Specifically, we asked whether a mix of conflicting cues generates an intermediate or a novel response, and whether the response to one cue can override others. At the population level, similarity relationships (given as correlation distances, SI) reveal that female and male mice stimuli are considerably closer to each other than each is to predator urine (0.46 vs. 0.78 and 0.82, normalized distances). This indicates that AOB activity readily allows differentiation of conspecific from non-conspecific urine stimuli. Moreover, mixes of predator with female or with male urine yielded intermediate representations between mouse and predator stimuli. Thus, the predator stimulus does not override or inhibit the response to conspecific cues. These relationships are depicted in Fig. 6A using multidimensional scaling (MDS) to approximate these distances in two-dimensional space (see methods). For comparison, also shown are distance relationships for simulated cases in which the predator responses entirely inhibits or alternatively, combines linearly with the mouse urine response. Comparison of the actual data with the two simulated cases supports the idea that responses to mixed stimuli resemble intermediate responses to the elemental stimuli.

At the single unit level, a substantial proportion of responses to the combined predator and mouse stimuli (38%, 20 of 53 cases) display intermediate magnitudes to that of the individual stimuli, consistent with the population data shown in Fig. 6A. Surprisingly, the data also reveals a prevalence of synergistic interactions between predator and mouse stimuli (42%) (Fig. 6B). In comparison, mixes of urine from distinct samples of mouse urine elicited fewer and weaker synergistic interaction (SI). Since mouse and predator stimuli are likely to contain a large number of non-overlapping components, as compared to two conspecific stimuli, this result suggests that a specific combination of components is sometimes required to elicit a robust response, thus providing direct support to the notion that AOB units integrate information from distinct components.

Discussion

The importance of the mouse vomeronasal system in processing chemical cues required for sex- and species-specific social and reproductive behavior has been documented in various studies (1-2, 4, 27). However, many fundamental aspects of vomeronasal function have not been addressed yet: Is the vomeronasal system dedicated to pheromone detection? How is the representation of social parameters achieved and at what level of resolution? How are the various parameters carrying social information computed in the first vomeronasal brain relay? The challenges posed by the unique mode of stimulus delivery to the vomeronasal organ, primarily the sympathetic activation of a vascular pump, have so far hampered in-vivo physiological investigation of vomeronasal function, leaving the range of chemical stimuli detected by the vomeronasal system and the basic rules of vomeronasal information processing largely unknown.

Here we describe an experimental preparation with several advantages for studying VNO function, including the non-invasiveness to the sensory organ structure, the engagement of natural pumping mechanism and the small volumes of test stimuli. Another essential aspect of our preparation is that it allows many cycles of presentation, and hence the responses of a given neuron to multiple stimuli can be tested repeatedly over the course of a single experiment (see Figure 1). By combining this method with multielectrode recording in the AOB of the anesthetized mouse, we confirmed the high selectivity of vomeronasal activation by male and female mouse urinary and salivary cues, and showed that when challenged with increasingly more subtle discriminations, AOB neurons can achieve multilevel distinctions, namely, among animals from different strains, among individuals from the same strain, and even among distinct samples from an individual mouse.

The wiring diagram of the AOB identified by anatomical and genetic tools has suggested that, in contrast to the MOB, integration of information by output neurons may occur across multiple chemosensory receptors (20, 28). Whereas the VNO appears to reliably convey information about sex (21, 29), an earlier study suggested that a population code is required to derive information about strain (30). In our AOB recordings we identified mostly units with selectively to sex/strain combinations, a few single-units with generalized sex responses and no units with specific responses to strain regardless of sex (Fig. 4). In contrast to VNO recordings, we also observed single-units that generalized across all male samples of the same strain or

across distinct samples from the same individual (Fig. 4). Thus, at least with respect to encoding strain/gender combinations, the AOB appears to derive a sparser representation than the VNO (compare Fig. 4A with Fig. 2 in (30)).

We found here that many AOB units could respond to cues present in predator urine. These findings suggest that in addition to serving an important role in individual recognition of conspecifics, especially in a reproductive and social context, the vomeronasal system of the mouse is also likely to serve an important role in recognizing animals of other species, perhaps especially predators. Responses of AOB neurons to predator urines were robust in magnitude and frequency, and appear highly specific, even among distinct predator species. Intriguingly, reproductive processes can be affected by the presence of predator stimuli (31). Because the behavioral (1) and physiological (4, 32) involvement of the vomeronasal system in reproductive processes is well established, one possibility is that predator odors act via the VNO to affect such reproductive behaviors and physiology. However, predator-related responses have been recently detected in the main olfactory system (33) raising the question about the respective role of each chemosensory system in this process. The slow timing of AOB activation after stimulus detection, in the order of several seconds compared to fraction of seconds in the main olfactory system, together with the need for contact for VNO stimulus detection suggest that the vomeronasal system may provide a means to survey the presence of predators within the ecological niche. This, in turn, could provide long term modulation of the mouse's reproductive physiology, whereas predator detection by main olfactory neurons may provide real time identification, and hence immediate avoidance of the predators. Previous work showed the importance of the vomeronasal system for predator and prey detection in reptiles (34-35), and more recent studies implicated the rodent vomeronasal system in detecting predator cues as well (35-37). However, this is the first demonstration that such odorants elicit robust, frequent and specific electrical activity in the mouse AOB.

Cues from conspecifics and predators provide ecologically conflicting information that could lead to strikingly different behavioral/physiological outputs, the former by enticing social and reproductive interactions, the latter arousing fear and delaying reproduction. How would animals resolve the simultaneous detection of conflicting stimuli? In one scenario, information about conflicting classes of stimuli are already processed at early stages by largely independent units that in turn convey the prospect for friendly or harmful encounters to distinct effector

nuclei in the amygdala and hypothalamus. In addition, some cross-inhibition in the response to both stimuli presented simultaneously may exist, such that the anticipation of a mating partner for example can be dampened by the detection of a possible threat. We indeed identified AOB neurons that displayed specific responses to either female or male mice, or to predators. However, we also detected surprisingly large cohorts of neurons that responded to both mouse and predator urine, which is hard to reconcile with a highly segregated opponent mechanism. What is the functional significance of this dual specificity? One possibility is that features used to distinguish between two classes of conspecific stimuli may also be present in predator urine. Alternatively, the feature space defined by AOB neurons may not be explicitly related to the ecological significance of the stimuli. Instead, AOB processing might be optimized to extract statistical features of stimuli and maximize the efficiency of information transfer to downstream targets regardless of their ethological value. Under this scenario, the significance of the various response patterns is relegated to subsequent processing stages in the amygdala and hypothalamus. Finally, the synergistic responses to mouse/predator odors uncovered in our study may provide a sensory alert for the presence of conflicting cues. According to this hypothesis, AOB processing would not only encode chemical features, but also attribute salience to specific combination of cues. Regardless of their significance, the observation of these synergistic responses provides direct physiological support to the notion that some AOB mitral cells effectively integrate information from multiple glomeruli.

Although we did not test all single units with all stimuli, summing the frequencies of responses to each of the stimuli tested indicates than on average, single units respond to more than one type of stimulus. Indeed, some units display responses across distinct stimulus classes (i.e. conspecific and predator urine, Fig. 5). Considering the large space of possible conspecific urine stimuli in natural settings, it is striking that responses were found to the few individual samples tested. These observations suggest that single units cannot unambiguously encode the identity of the stimulus. Instead, it seems that activity across a population is required to extract information about stimuli and the relationships between them. This is supported by our population level analyses, which show that small ensembles can reliably encode the relationships between stimuli (Fig. 4 D-F) even when single units often provide ambiguous information about these stimuli.

Lastly, we have described a powerful experimental platform that, in addition to providing insights about AOB function, should be further instrumental in exploring the processing of vomeronasal as well as olfactory chemosensory information in amygdala and hypothalamic behavioral control centers in order to generate physiologically relevant responses.

Methods

Surgical procedures

Experiments were performed under NIH, Duke University, and Harvard University guidelines. Adult sexually naïve BalbC mice were anesthetized with 100-150mg/kg ketamine and 6-10mg/kg xylazine and maintained with 0.5-2% isoflurane in pure O_2. The trachea was exposed, cut, and a polyethylene tube (I.D. 0.76mm, O.D. 1.22mm) was inserted and glued with Vetbond (3M, St. Paul, MN). A cuff electrode was placed around the sympathetic trunk and carotid artery (for mechanical support) and held in place by Vetbond. The cervical cavity was resealed with Vetbond. See SI for details for details on all experimental procedures.

Suction of fluids from the nasal cavity

The metal part of the stereotax mouth bar was covered by a polyethylene tube with a small triangular aperture apposed to the oral part of the naso-palatine duct (SI). Suction was induced by a solenoid-controlled vacuum line.

Stimulation of the sympathetic nerve trunk

Cuff electrodes were made from platinum foil (0.001", ESPI, Ashland, OR) wrapped around a 27G needle. A Teflon coated platinum wire was soldered to the cuff and to a connector pin. Silicone (NuSil Silicone Technology, Lake Mary, FL) was used to insulate the external part of the cuff. A TDT electrophysiology station (TDT, Alachua, FL) was used to deliver stimuli (duration: 1.6s, amplitude: ±100µA, frequency: 30Hz, waveform: 2ms negative - 0.2ms delay - 2ms positive). No obvious effects on heart rate in responses to stimulation were observed. Moreover, there was no consistent change in neuronal response properties as a possible outcome of sympathetic stimulation.

Electrophysiology and data recording

A craniotomy was made over the olfactory bulbs and the dura was removed. Multisite probes (NeuroNexus Technologies, Ann Arbor, MI) were dipped in DiI and advanced at an angle of 15°-30° (from the vertical). Penetration sites were subsequently examined to confirm positioning within the AOB. Data was sampled at 25 KHz, filtered (300-5000Hz) and saved using a TDT electrophysiology system (TDT, Alachua, FL). Spike sorting was performed with custom MATLAB (Mathworks, Natick, MA), KlustaKwik (38) and Klusters (39) programs. Clusters with distinct waveforms, no overlap with other classes and a refractory period were designated as single units. Other spikes were designated as multi-units (MUA).

Data Analysis

Responses were quantified as the rate change following stimulation (30s) relative to the preceding 10 seconds. Response significance for a given stimulus was determined with a one-way non-parametric ANOVA with the set of post stimulation rates (over a 30s period) compared to the set of all pre-stimulation rates. All data analyses were performed with custom written or built in MATLAB code.

Stimuli

Urine was collected from adult female and male mice (Charles River Laboratories, Wilmington, MA, and Jackson Laboratories, Bar Harbor, Maine). Fresh urine samples were immediately frozen and then stored in -80ºC. For stimulation, urine was diluted in water or in Ringer's solution (1/100). Saliva was collected by from the oral cavity using a micropipette. Salivation rate was increased by pilocarpine-HCL injections (10mg/kg, IP) several minutes prior to collection. Freshly frozen bobcat and fox urine was kindly given by PredatorPee (Lincoln, Maine). Rat urine was collected similarly to mouse urine. Artificial urine and Ringer's solution were prepared as described in (21).

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Figure Legends

Fig. 1: Experimental setup.

A. Schematic of the mouse nasal cavity and relevant olfactory structures. Inset (left): coronal section through a mouse VNO showing selective uptake of DiI ipsilateral to nerve stimulation. L: VNO lumen. BV: Blood vessel. Inset (right): Sagittal AOB section showing a DiI painted probe within the external cell layer (ECL). GL: glomerular layer, Gr: granule cell layer. **B.** Abbreviated sequence of events in a single trial showing the serial application of mouse urine followed by sympathetic stimulation and flushing. Each panel includes a cartoon of the presumed state of VNO, a raw electrode signal (stimulation artifacts clipped) and the firing rates.

Fig. 2: Sex specificity of responses and temporal response profiles

A. Sympathetic stimulation induced spike times in six interleaved presentations of dilute male or female mouse urine from two simultaneously recorded neurons (orange and green). Bottom: averaged responses (mean±SEM). Time 0: sympathetic stimulation. **B.** Histograms of start time (latency), peak time and half time of individual responses relative to the onset of sympathetic stimulation (time 0)

Fig. 3: Dependence of AOB responses on functional TRPC2 signaling

A. The histograms show the percentages of stimulus induced rate changes of individual units to the most effective stimulus (stimuli were male, female and predator urine). **B.** Percent of significant responses ($p<0.01$) to mouse urine in wild type (WT) and TRPC2$^{-/-}$ mutant mice. Data in this analysis includes MUA. (N =1195: WT, 221: TRPC-/-).

Fig. 4: Responses to urine and saliva from different strains and sex combinations.

A. Responses of three different single units to urine from mice of distinct sex and strain. Here and in other figures, significant responses ($p<0.01$, either positive or negative) are shown on a gray background. Strains are indicated at the top (BC: BalbC, C57: C57 Black6) **B.** Same as A for saliva stimuli. **C.** Responses to distinct urine samples. For each strain, the samples ending with -1 and -1B denote two different samples from the same individual while the sample ending with -2 is from a different individual. **D-F:** Hierarchical clustering of population responses to the stimuli in A (N=23), B (N=13) and C (N=16). Panels A-C show responses of distinct units.
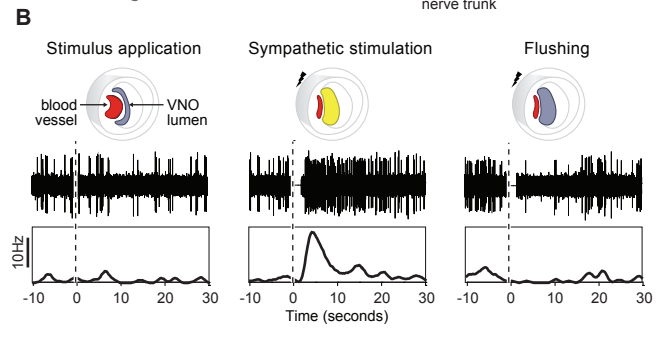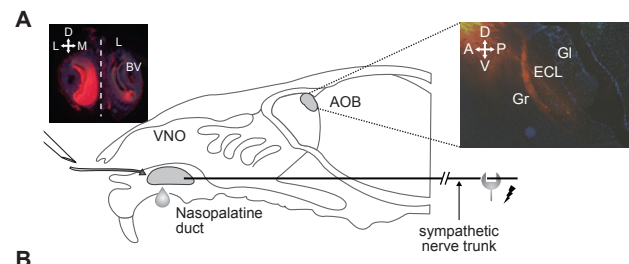
Fig. 5: Responses to predator urine

A. Percentages of units with significant responses ($p<0.01$) to each of the stimulus combinations (N=51 single units responding to at least one stimulus). Stimulus categories (male mouse urine, female mouse urine and predator urine) are indicated on the outer border of each slice. Mouse urine stimuli were pooled from three different strains (BalbC, C57 Black6, CBA). **B.** Response magnitudes of these single units to mouse and predator urine. **C.** Three single units with exclusive responses to female mouse, male mouse, or combined predator urine. **D.** Single units responding differentially to urine from different predators. The icons designate, from left to right, bobcat, fox, rat and combined urine from these sources. **E.** Single units responding across stimulus categories.
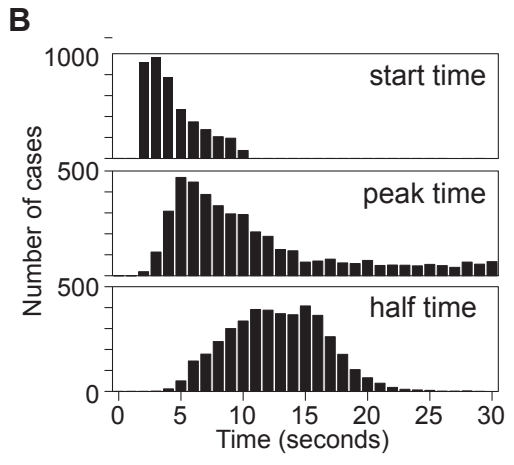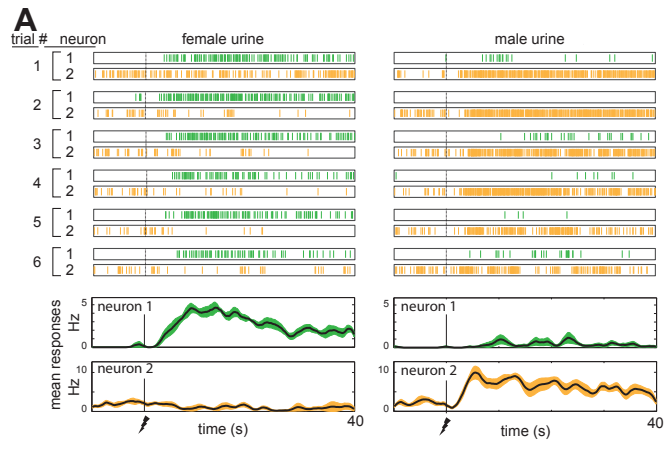
Fig. 6: Responses to conflicting stimuli

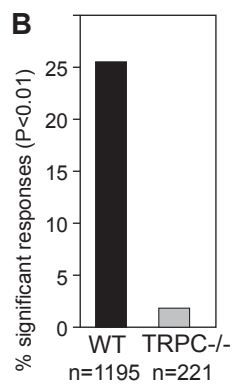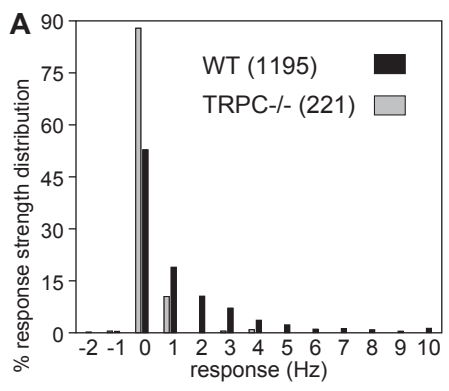**A.** Relationship of population responses to the elemental stimuli and to their combinations. Multidimensional scaling (MDS) was used to represent similarity relations on two dimensions. Each circle represents the population response to one stimulus. The color of the circle indicates the type of stimulus (see legend) and the numeral indicates whether the circle represents actual

(1) or simulated responses to combined stimuli (2 and 3). The "predator inhibition" simulation models the response were predator urine entirely inhibits the response to mouse urine.

B. Responses of individual units to mixed stimuli. Each line represents the response of one single unit to one mix. The edges of the line are defined by the responses of a given unit to the two elemental stimuli (male mouse urine and predator urine, or female mouse urine and predator urine) whereas the "+" indicates the response of the same unit to their mixture. Responses are categorized into those showing suppression, intermediate or synergistic interactions following mixing. N = 53 cases. Male and female mouse urine was pooled from three different strains as described in Fig 5.

A

B

Stimulus application

Sympathetic stimulation

Flushing

blood vessel — VNO lumen

10Hz

-10   0   10   20   30
-10   0   10   20   30
-10   0   10   20   30
Time (seconds)

A

trial # | neuron — female urine — male urine

mean responses Hz

neuron 1 — female urine — neuron 1 male urine
neuron 2 — female urine — neuron 2 male urine

time (s) — 40

B

Number of cases

start time
peak time
half time

Time (seconds)

A Female urine   Male urine

C57   BC   FVB   C57   BC   FVB

Hz

time (s)

B Female saliva   Male saliva

C57   BC   CBA   C57   BC   CBA

Hz

time (s)

C Individual Male Samples

CBA-1 CBA-1B CBA-2   BC-1 BC-1B BC-2   C57-1 C57-1B C57-2

Hz

time (s)

D M/F Urine

distance

BC (M) FVB (M) C57 (F) C57 (F) BC (F) FVB (F)

E M/F Saliva

C57(M) CBA (M) BC (M) CBA (F) C57 (F) BC (F)

F Male Urine

CBA-1 CBA-1B CBA-2 BC-1B BC-1 BC-2 C57-1 C57-1B C57-2

A Response Frequencies

B Response Strength

C

D bobcat fox rat all

E

A

B

Responses to combined stimulus sets
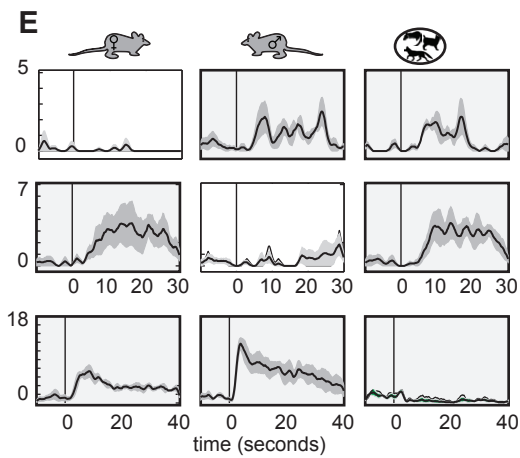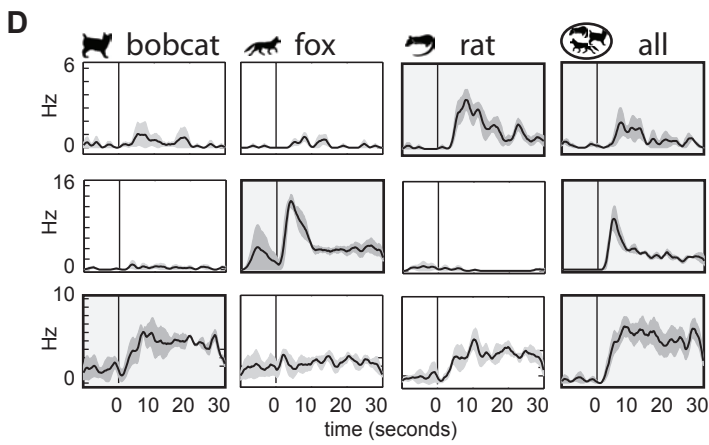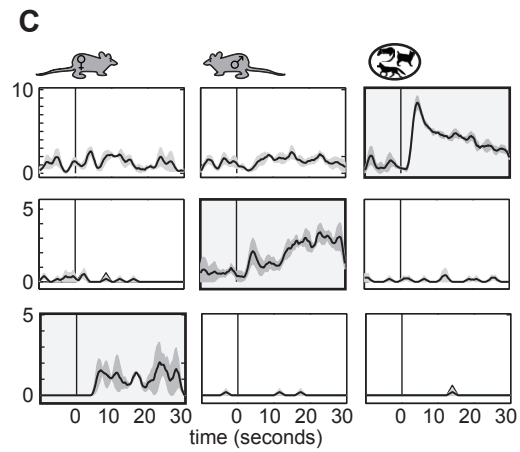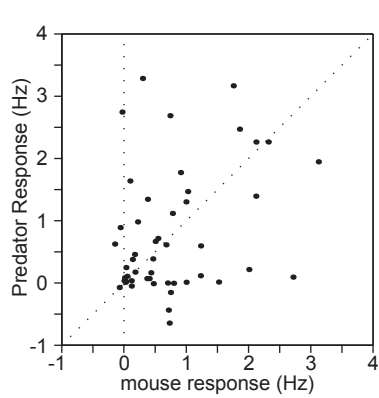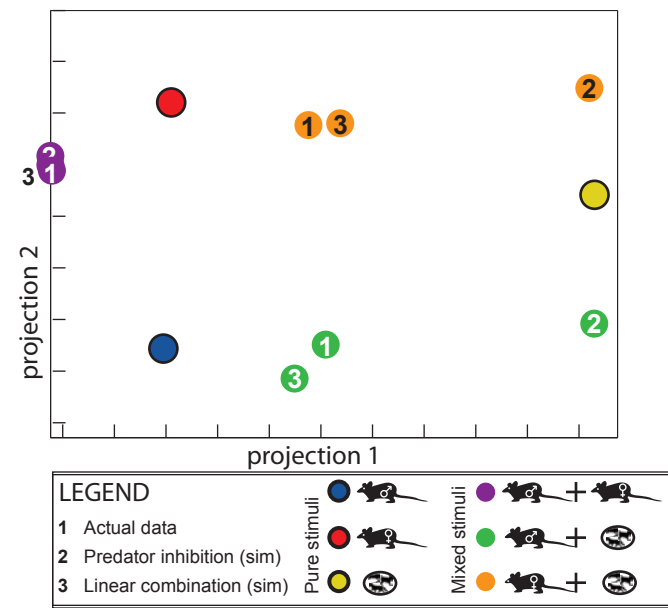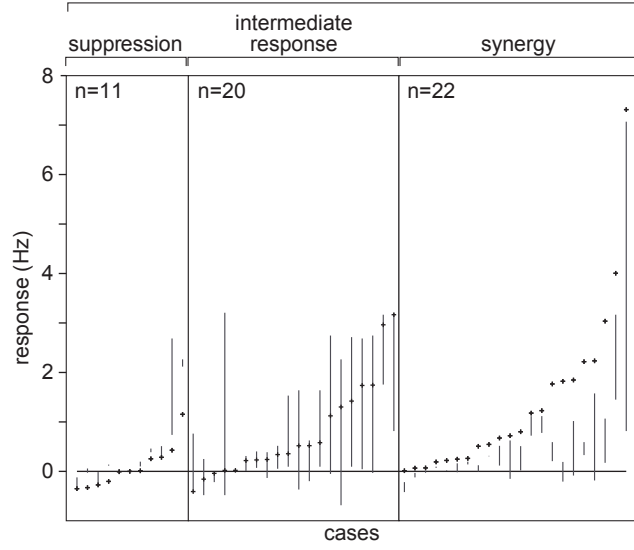
Supplementary data: Figure legends and methods

Supplementary Fig. 1: *The experimental system*

A. Timeline showing events in a single trial. Top trace indicates stimulus application (yellow drop = single application of test stimulus (1-2µl), blue drops = continuous application of ~3ml Ringer's solution). Also shown are epochs of sympathetic trunk stimulation trains (ST stims) and suction valve status. The electrode trace shows a continuous trace from such a trial, in which stimulation artifacts have been clipped. The event names are indicated below the electrode trace. Initially, the trial sequence comprised the following intervals: application to first stimulation (30s), first stimulation to second stimulation (30s), second stimulation to clean start (30s), clean start to third stimulation (30s), third stimulation to fourth stimulation (30s). In subsequent experiments, the second post application stimulation was not used, and the following intervals were used: application to first stimulation (20s), first stimulation to clean start (40s), clean start to second stimulation (20s), second stimulation to third stimulation (20s). The second post application stimulus was omitted after observing that the first stimulation was considerably more effective than the second. The other intervals were modified to minimize the time required for a single trial. **B**. Bottom view of the mouse oral cavity showing how the aperture in the suction tube is placed directly beneath the oral opening of the nasopalatine duct. **C.** Histograms of baseline firing rates and peak responses (firing rates) for single units. The number of units used for calculation of baselines is smaller than that for responses because only units that showed stability during the entire recording session were used for calculating baseline rates. Baselines rates were derived from the average rates across all 10-second periods at the beginning of a trial prior to stimulus application. **D.** Selecting the working dilution for urine. Left: mean response profiles of one unit to serial dilutions of male mouse urine. Center: quantification of same responses (mean ± SEM). Right: Tuning profiles for several units. Shown are means ± SEM. A 1/100 dilution was chosen for further experiments as it provides robust signals, is not saturating, does not induce non specific depolarization due to potassium ionic gradients, and is also consistent with working ranges of urine in VNO slices. E. Frequency of responses during application, stimulation, and stimulation during cleaning a set of 22551 units * stimulus

combinations. The figure shows that consistent responses (defined when a majority of trials for a given stimulus shows a change in the same direction) are more often rate increases than decreases, and also that most occur following nerve stimulation.

Supplementary Figure 2: *AOB readout of sex-specific cues*

A. Examples from three single units showing activation by female, male and female, or male urine, respectively. Averaged responses (±SEM) are shown. **B**. Sex specificity index (SSI) histograms for single-units recorded in male (white) and female (black) AOB. The SSI is defined as (Rm-Rf)/(Rm+Rf), where Rm and Rf represent the responses to male and female urine, with values of -1 and 1 corresponding to exclusive selectivity to female or male urine, respectively. The rare cases of negative modulations were clipped to zero to keep the SSI within the range -1 to 1. The SSI distribution is shown for 36 single units responsive to either male, female or both urine stimuli. 63% of the single units (23 out of 36) displayed an SSI with an absolute value larger than 0.5, thus displaying a sex-specific bias in their response. Our data also does not reveal any significant sexual dimorphism ($p > 0.05$, ranksum test) in the SSI distribution of units from male and female AOBs. Thus, in both female and male mice, information about the sex of a conspecific can be reliably encoded in the activity of single units in the AOB.

Supplementary Figure 3: *Response specificity of single units*

A. Responses from all 40 single units showing a significant response to urine from at least one strain/sex combination. Each row corresponds to one unit and each box indicates whether a significant ($p < 0.01$) increase (black), decrease (gray) or no significant change (white) occurred. **B.** Histograms of the number of significant stimuli for each single unit for the stimulus set shown in panel A (N-107 single units). **C.** Same as in A, only responses are to salivary stimuli (N=13). **D.** Same as panel B, but for salivary stimuli (N=23). **E.** Response patterns of all units responsive to at least one of the individual male urine stimuli. **F.** Response patterns of units responsive to predator stimuli. The icons designate (from left to right, bobcat, fox, rat and combined urine mix).

Supplementary figure 4: *Responses to male urine from distinct strains and mixes of strains*

In the experiment shown in this figure, we tested responses to male urine stimuli and to their mixes. Mixes were prepared so that individual compounds were always present at levels intermediate between those of the composing stimuli, a key experimental feature to avoid saturating levels of common urinary components. **A.** Correlation distance (defined as 1-correlation coefficient) between population responses to pure male urine stimuli and to their mixes. Distances are indicated along the edges of the triangles. In all cases, population responses to the mixes are more similar to the pure stimuli as compared to the similarity of the two pure stimuli to each other. **B.** Responses of individual units to elemental stimuli and to mixes. Each line represents the response of one single unit to one mix. The edges of the line are defined by the responses of a given unit to the two elemental stimuli whereas the "+" indicates the response of the same unit to their mixture. Responses are categorized into those showing suppression, intermediate or synergistic interactions following mixing. Thus, in each of these categories, the "+" is under, within, or above the corresponding line. N = 166 responses. **C.** Examples of responses to each of these categories. Each of the three panels shows the response of one single unit to three pure male urine stimuli and to their three pair wise mixes. The unit on the left shows suppression of C57Bl6 with CBA urine, the unit at the center shows an intermediate response to mixes of C57Bl6 urine with other stimuli, and the unit on the right shows a synergistic response to C57Bl6 with CBA urine.

The responses can be grouped into three categories in which responses to mixes of stimuli were weaker, intermediate, or stronger than the two elemental stimuli, respectively. Surprisingly, less than half of the single unit responses to mixtures were intermediate between elemental responses (44%, 73 of 166 cases). In fact, most cases show suppression (31%, 51 cases) or augmentation (25%, 42 cases), neither of which are consistent with monotonic responses to one component. Importantly, mixtures did not evoke entirely novel responses from single units. Indeed, we never detected responses to mixtures of stimuli that were otherwise ineffective individually, nor did we observe a lack of responses to blends of effective single compounds. Thus, our data support the notion that the activity of most AOB neurons reflects features that are more complex than levels of individual compounds yet do not provide entirely novel higher-level feature representations of strain or individual identity.

	F	M	P	F+M	M+P	F+P
F	0					
M	0.46	0				
P	0.78	0.82	0			
F+M	0.25	0.29	1	0		
M+P	0.55	0.17	0.5	0.5	0	
F+P	0.39	0.49	0.5	0.37	0.41	0

Supplementary Table 1: Normalized correlation distances (defined as 1-correlation coefficient) between population level responses to pure stimuli and their mixtures. F: female urine, M: male urine, P: predator urine. These distances were used to calculate the approximate distances shown in Figure 6a using multidimensional scaling.

Experimental procedures

Surgical procedures

All experiments were performed in strict compliance with the National Institute's of Health and Duke University Institutional Animal Care and Usage Committee guidelines or Harvard University. Adult sexually naïve BalbC male and female mice (Charles River Laboratories, Wilmington, MA or Jackson Laboratories, Bar Harbor, Maine) were initially anesthetized with 100-150mg/kg ketamine and 6-10mg/kg xylazine and subsequently maintained with 0.5-2% isoflurane in pure O_2. The trachea was exposed by moving overlying gland tissue and separating the right and left sternohyoid muscles. An incision was made with fine scissors between the second and third tracheal rings and a 15mm length polyethylene tube (I.D. 0.76mm, O.D. 1.22mm, Franklin Lakes, NJ) was inserted in the caudal end of the cut trachea and held in place with a small amount of Vetbond glue (3M, St. Paul, MN). To prevent efflux of fluid from the cervical cavity to the nasal cavity and the VNO, the upper end of the trachea was blocked with a ~5mm polyethylene tube (I.D. 0.76mm, O.D. 1.22mm) closed at one end with wax and secured withVetbond. The sympathetic nerve trunk was gently separated from connective tissue in the

region posterior to the superior cervical ganglion using fine forceps and a micro-hook tool (FST, Foster City, CA). A cuff electrode (see below) was placed around the sympathetic trunk and the carotid artery, the latter serving as mechanical support. The electrode was held in place by a small amount of Vetbond placed around the connector wire and the cervical cavity was resealed with Vetbond. The mouse was then placed in a custom built stereotaxic apparatus.

Suction of fluids from the nasal cavity

The metal part of the stereotax mouth bar was covered by a polyethylene tube with a small triangular aperture that was apposed to the oral part of the naso-palatine duct (see Supplementary Figure 1). The plastic tubing was connected to a solenoid-controlled vacuum line which was only opened for flushing. Surgical silk sutures (6/0, CP Medical, Portland, OR) were inserted into the cheek skin to gently pull them laterally to prevent occlusion of the aperture during suction. A tracheotomy (see above)was performed prior to these procedures to prevent fluid entry into the lungs.

Stimulation of the sympathetic nerve trunk

Cuff electrodes were fabricated by cutting a small piece (0.5 x 2.5-3mm) of platinum foil (thickness 0.001", ESPI Metals, Ashland, OR) that was shaped into a cuff by wrapping around an injection needle (27G). A Teflon coated platinum wire (0.002" bare, 0.004" coated, A-M Systems, WA) was soldered to the cuff electrode on one side and to a connector pin on the other. Silicone coating (MED10-6640, NuSil Silicone Technology, Lake Mary, FL) was applied to the cuff electrode and cured overnight at 100°C. Current was applied using a TDT (TDT, Alachua, FL) Rx-7 stimulator and MS16 stimulus isolator controlled by an RZ2 processor. Stimulation parameters: duration: 1.6s, amplitude: biphasic ±100μA, frequency: 30Hz, waveform: 2ms negative - 0.2ms delay - 2ms positive.

Sequence of events during a single trial

Two similar protocols were used for a single trial sequence. Changes in the second protocol were introduced to minimize the time required for a single trial to allow for more repetitions in a session. The parameters used for most experiments (the optimized protocol) are listed first, and those used for the initial experiments appear in parenthesis. Stimuli were applied to the nasal

cavity by placing 2µl (1µl) of sample directly on the nostril. After a delay of 40s (30s), a stimulation train was applied to the sympathetic nerve trunk as described above. In a minority of experiments, a second stimulus train, separated by 30s from the first was also applied. Since in almost all cases the stronger response occurred in response to the first stimulation, the second was subsequently omitted. The flushing procedure began 20s (30s) after the second stimulation by opening the vacuum line leading to the nasopalatine duct and applying ~3ml of Ringer's solution to the nostril using a micropipette. The wash solution flowed from the nostril through the nasal cavity and into the collection receptacle through the nasopalatine duct. During the wash procedure two sympathetic trunk stimulation trains, separated by 20s (30s) were applied. Flushing was complete when the vacuum line was closed 20s (30s) seconds after the last stimulus train. See Supplementary Figure 1A for a graphic description of this sequence.

Structure of a session

After the electrodes were positioned in the recoding sites, baseline activity was recorded for several minutes. This period was used to extract parameters for subsequent spike detection and sorting (see below). An experiment comprised pseudo-random presentation of the samples from the stimulus set, each presentation followed by flushing of the nasal cavity and VNO as described above. The number of repetitions of each stimulus presentation depended on the stability of the preparation (often 2-3 hours) and the number of stimuli in the set (usually 6-10 stimuli) but typically was in the range of 4-6.

Electrophysiology

Recordings were made with 32-channel probes (NeuroNexus Technologies, Ann Arbor, MI). The probes used comprised 4 shanks, each with 8 recording sites. Inter-shank (horizontal) spacing was 200µm, and within shank (vertical) spacing was either 50µm or 100µm. Prior to each electrode penetration, probes were dipped in DiI solution (1mM in ethanol, Invitrogen, Carlsbad, CA) to allow identification of penetration tracts after the experiment. For recording activity from the AOB, a small cut was made over the skull and a craniotomy was performed over the olfactory bulbs, rostral to, but close as possible to the rhinal sinus. The dura was removed around the penetration site and probes were advanced at an angle of 30° or 15° (from the vertical). Clear single-unit neuronal activity was first typically recorded from the

Mitral cell layer of the main olfactory bulb at a depth of ~400μm. After that, a region devoid of clear spikes was traversed and finally distinct spikes of low spontaneous rate were recorded from the external cell layer of the AOB. The spiking rate and temporal firing properties of these neurons (i.e. non-periodic and non-bursting Poisson-like firing patterns) were very similar to those observed in awake behaving recordings from mice (1). The probes were positioned in attempt to maximize the number of sites with clear spikes, but site selection was not made based on response properties. Following the experiment, penetration sites were examined to confirm electrode penetration tracts within the AOB external cellular layer. Usually, sections were counterstained with DAPI and in minority of cases also with BS-Lectin to highlight the location of the glomerular layer (2). In some cases, the most lateral recording sites (i.e. the first and/or fourth shank) recorded from units that were not responsive to sympathetic trunk stimulation, but did show rate changes during the wash procedure, most likely due to the air flow induced by suction. These sites probably targeted the granule cell layer of the MOB and were excluded from analysis.

Data recording and spike sorting

32 channels were continuously sampled at 25 KHz and band-pass filtered (300-5000Hz) using an RZ2 processor, PZ2 preamplifier, and two RA16CH head-stage amplifiers (TDT, Alachua, FL). Binary data files containing electrode signals and discrete experimental events were processed using TDT ActiveX software and custom MATLAB software (Mathworks, Natick, MA). Custom MATLAB programs were used to extract spike waveforms from the continuous data and to calculate their projections on principle components. These coefficients were then input to KlustaKwik (3) for automatic classification and then manually adjusted using Klusters (4). Spike clusters were evaluated by spike shapes, projections on principle component space and autocorrelation functions. Spikes in clusters with distinct spike shapes, no overlap with other classes and a clear trough in the autocorrelation function were designated as single units. Spikes in clusters that seemed to contain a substantial portion of spikes from at least another cluster were designated as multi-units. Generally, since the number of contributing neurons cannot be ascertained, there is a wide heterogeneity in the quality of multi-unit data but at least in some cases, multi-unit data may comprise spikes from only two neurons.

Data Analysis

Generally, responses were quantified during the time period following the stimulation period subsequent to stimulus application. Unless stated otherwise, response magnitudes were defined as the rate change following stimulation (30s) relative to the 10 seconds prior to stimulation. For display purposes, spike rates were calculated by binning spike times in 0.2 second bins and then smoothed by convolving with a triangular window (3s width). Response significance for a given stimulus was determined with a one-way non-parametric ANOVA (Kruskall-Wallis test) in which the set of post stimulation rates for that stimulus (over a 30s period) was compared to the set of all baseline rates (the set of all pre-stimulation rates).

In a minority of cases, we also observed responses immediately during application, often also followed by stimulation induced responses. When such responses occurred, they were typically associated with a given experimental preparation; they did not appear sporadically in individual trials. These responses could be due to direct entry into the VNO (presumably possible in some experimental preparations) or to contact-induced activation of the VNO pump (presumably when anesthesia levels were relatively low). Overall, these responses occurred in less than 5% of the experiments. In the small number of cases where application induced responses were larger than stimulation induced response, we regarded the response as the post application change in firing rate.

There was no obvious trend for responses diminishing or increasing in magnitude during the course of an experiment. To quantify this statistically, we compared rate modulations for all the cases of single units responding to a given strain/sex combinations (the same population of neurons used for the analysis in Figure 4A).The dataset includes 137 cases of units x stimulus combinations with a significant response at the 0.05% level. We then conducted a paired rank-sum test to detect a consistent difference between the first and last trial (typically 6 trials per unit x stimulus combination, mean = 5.9 trials). The null hypothesis that rates in the first and last trials originate from the same distribution was not rejected (p=0.1206).

In some cases, units showed strong responses during a subset of trials and no responses during other trials, typically either gaining responsiveness or losing it as the session progressed. Multiple reasons could account for these sources of non-stationarity. These include the states of the sympathetic nerve trunk and stimulating electrode, the nasal cavity and the VNO, the AOB and recording electrodes, or the general state of the mouse. These sources of variability are

generally included in our analysis, but in some instances, when there was an obvious transition from a responsive to a non-responsive state, the outlying trials were input into a database and automatically excluded from further analysis. Trial exclusion was conducted independently of data analysis and does not affect the main conclusions.

All data analyses were performed with custom MATLAB code except for statistical testing, hierarchical clustering, and multi dimensional scaling for which built-in MATLAB functions were used. For hierarchical clustering (Figures 3,4), we used a *Euclidean* distance among the vectors representing the population response profiles, and *average* cluster distances for the linkage function (with the MATLAB *dendrogram* function). For this analysis, only significant responses ($p<0.01$) were used.

Multidimensional scaling (Figure 6): For multi-dimensional scaling, we used classical multidimensional scaling (the MATLAB *cmdscale* function) with a *correlation* distance function. Only significant responses ($p<0.05$) were considered. Note that for each of the three conditions (pure stimuli and the two simulations), the multidimensional scaling axes and projections had to be calculated separately. The algorithm produces the "best" two-dimensional space to represent the distance relationships, but these are not perfectly faithful to all the pairwise distances. In each instance of the scaling then, the deviations from the actual distances vary slightly. This is the reason why the pure stimuli do not assume exactly the same positions under the three conditions, even though the actual distances between them are identical. This also explains why the mixed stimuli in the linear combination model do not lie exactly halfway between the two pure stimuli. The predator inhibition model was defined as follows: for all single units that showed a response to a pure mouse stimulus but not to a pure predator stimulus, the firing rates were set to zero for the mixed predator and mouse urine.

Response detection and temporal parameters

For the analysis in of temporal features of individual responses (Fig. 2B) we employed the following criterion for a response. A response was defined as excitatory if during the post

stimulation period the spiking rate increased above the maximum rate calculated during the preceding 10 seconds. To minimize false positives, rate elevations were required to last at least 5 seconds and begin no later than 10 seconds after the stimulation event. Inhibitory responses were detected similarly with the difference that segments were required to show a rate *lower* than the *minimal* rate calculated during the preceding 10 seconds. A unit was considered to respond to a stimulus by an increase if an increase was detected in a majority of trials (trial number ranging from 2-5). Similarly, a unit was considered to respond with a decrease if in a majority of trials it showed a decrease to the stimulus. Latency was defined as the start time of the first segment showing an increase, peak response was the time in which the response showed a maximal rate increase, and half time was defined as the time by which 50% of the spikes during the 30s post-stimulation period occurred.

Stimuli

Urine was collected from adult female and male mice of the BalbC, FVB/N, CBA or C57Bl6 strains. All mice were ordered from Charles River Laboratories (Wilmington, MA) or Jackson Laboratories (Jackson, Bar Harbor, Maine). For pooled stimuli (used in Figures 2,3) mice were left overnight in a closed round chamber overlying a wire mesh and funnel which was connected to a collection tube. The collection tube was immersed in a receptacle with dry ice so that urine was immediately frozen. Urine was diluted in water or in Ringer's solution. Male and female urine comprised pooled urine from all three strains. Other urine samples were collected from individual mice by holding the mouse over a clean plastic bag and collecting the urine (if the mouse urinated while being held) into a microcentrifuge tube that was immediately placed in liquid nitrogen and later kept in -80ºC. A standard dilution of urine of 1/100 was used throughout our experiments as it typically elicits robust signals with minimal risk of non-specific responses due to high potassium. Saliva was collected by holding mice and sucking saliva from the oral cavity using a micropipette. Salivation rate was increased by injecting pilocarpine-HCL (10mg/kg, IP) several minutes prior to saliva collection. Freshly frozen bobcat and fox urine was kindly given by PredatorPee (Lincoln, Maine). Rat urine was collected by restraining rats and collecting urine drops into plastic vials and immediately freezing in -80ºC. All urine samples

were diluted, aliquoted, and frozen until the day of use. Artificial urine and Ringer's solution were prepared as described in (5), except that glucose was not included in the Ringer's solution. Mixed urine stimuli (either from distinct male mice as shown in supp figure 4, or from mouse and predator urine as shown in Figure 6) were prepared so as to keep the total urine concentration constant at 1/100. For example, a mouse and predator mix contained 0.5% mousr urine, 0.5% predator urine and 99% solute (ringers). This design ensures that the concentration of each individual component in the mix is bounded by its levels in the elemental mixes.

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A

Application

ST stim

Valve open

Time (sec)

application   stimulation   stimulation   Clean Start   stimulation   stimulation

Time

B

pipette tip applied
to nostril

oral opening
of nasopalatine duct

waste collection

C

baselines and peak responses of single units

Baselines   (stable singles)   n=495   : 0.94 +/-0.05
Responses   (all singles)   n=711   : 6.98 +/-0.17

% neurons

Hz

D

Hz

Time (seconds)

Hz

Dilution

Hz

Dilution

E

N = 22551 unit * stim combinations

% consistent reponse

increases
decreases

App   Stim   cStim

epochs

male and female mouse saliva and urine

A

Female urine | Male urine

C57 | BC | FVB | C57 | BC | FVB

B

C

Female saliva | Male saliva

C57 | BC | CBA | C57 | BC | CBA

D

E strain repeats

CBA-1 CBA-1B CBA-2 BC-1 BC-1B BC-2 C57-1 C57-1B C57-2

F

specific predators

A

Distance

mix
0.24   0.18
n=53
BC C57
0.36

mix
0.26   0.18
n=51
CBA C57
0.44

mix
0.20   0.16
n=53
BC .... CBA
0.36

B   Responses to mixtures and to elemental stimuli

suppression   intermediate response   synergy

n=51   n=73   n=42

response (Hz)

6

4

2

0

-2

C

C57

C57 + BC   BC

C57 + CBA   BC + CBA   CBA

Time (seconds)

C57

C57 + BC   BC

C57 + CBA   BC + CBA   CBA

Time (seconds)

C57

C57 + BC   BC

C57 + CBA   BC + CBA   CBA

Time (seconds)