10. White, F. J. & Kalivas, P. W. *Drug Alcohol Depend.* **51,** 141–153 (1998).
11. Sarnyai, Z., Shaham, Y. & Heinrichs, S. C. *Pharmacol. Rev.* (in the press).
12. Vanderschuren, L. J. & Kalivas, P. W. *Psychopharmacology* **151,** 99–120 (2000).
13. Robinson, T. E. & Berridge, K. C. *Brain Res. Rev.* **18,** 247–291 (1993).

Neuropharmacology

Odorants may arouse instinctive behaviours

The prevailing view of the mammalian olfactory system is that odorants are detected only in the nasal olfactory epithelium, whereas pheromones are generally detected in the vomeronasal organ[1–3]. Here we show that vomeronasal neurons can actually detect both odorants and pheromones. This suggests that in mammals, as in insects[4–6], odorous compounds released from plants or other animal species may act as 'semiochemicals' — signalling molecules that elicit stereotyped behaviours that are advantageous to the emitter or to the receiver.

To investigate the function of the vomeronasal organ, we used calcium imaging of single murine vomeronasal neurons containing Fura-2 dye[7,8]. As with olfactory neurons in the nose, resting concentrations of intracellular calcium were about 20–40 nM in vomeronasal neurons and were increased to about 120–150 nM by 100 mM potassium chloride[7]. We found that vomeronasal neurons from both males and females respond to six mouse pheromones that stimulate aggression, subordination or alteration in puberty onset or oestrus (Fig. 1a, b)[9]. Individual pheromones (100 μM) stimulated 0.3–0.7% of the neurons, most of which responded to only one pheromone, as shown previously[10].

Surprisingly, mouse vomeronasal neurons also detect odorants (Fig. 1; Table 1). We assembled 82 odorants (50 μM each) in 9 mixes and found that neurons responded to several of these mixes. Vomeronasal neurons that responded to an individual mix were tested against each odorant in the mix. Altogether, 0.1–1.5% of neurons responded to a single mix (Table 1). Neurons were also activated by 18 single odorants classified as animalic, camphoric, floral, musky, sweet or woody (Fig. 1).

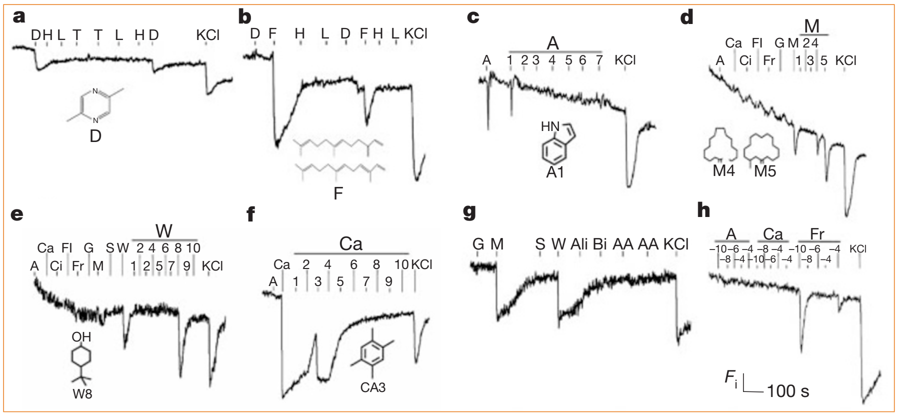

Figure 1 Responses of single vomeronasal neurons (VNs) to pheromones and odorants. Calcium imaging[7] was used on dissociated VNs containing Fura-2 during exposure by perfusion to pheromones (**a, b**) or odorant mixes/odorants (**c–h**) (4 seconds each) and then to 100 mM KCl. Fluorescence emission (at 510 nm) from cells illuminated at 380 nm was monitored (F_i, fluorescence intensity in arbitrary units)[7]. Scale is the same for all traces. The lack of response to all concentrations (10^{-10}–10^{-4} M) of an odorant in **h** may be due to transient desensitization. Odorant (and other) mixes: animalic (A), camphoric (Ca), citrus (Ci), floral (Fl), fruity (Fr), green minty (G), musky (M), sweet (S), woody (W), aliphatic (Ali) and amino acids (AA). Pheromones: dehydro-*exo*-brevicomin (B), 2-heptanone (H), 2,5-dimethyl pyrazine (D), 2-*sec*-butyl-4,5-dihydrothiazole (T), E,E-α-farnesene plus E-β-farnesene (F) and lactol (L). Single odorants that elicited responses: indole (A1), hexadecanolide (M4), muscone (M5), patchone (W8) and durene (Ca3); as well as (not shown) *p*-cresol (A2), eucalyptol (Ca1), isoborneol (Ca4), borneol (Ca5), fenchone (Ca6), butyrophenone (Ca7), methylanisole (Ca8), myrtenal (Ca9), phenafleur (Fl5), dimethyl-3-octanol (Fl8), helional (Fl10), pentadecalactone (M3) and aubepine (S1).

We found that, like olfactory neurons[7,8], vomeronasal neurons were activated by more than one odorant or mix, but they can also distinguish between highly related odorants such as indole and skatole, which differ by a single methyl group (Fig.1c). Like pheromones[10], the three odorant mixes tested activated vomeronasal neurons ($n=6$) at 10^{-10} M (Fig. 1h), which is much less than is required for an olfactory neuron to respond, indicating that the vomeronasal organ is highly sensitive to low concentrations of both pheromones and odorants.

Vomeronasal neurons detected many of the odorants we tested (18 out of 82). It is unlikely that so many odorants would be released from mice as pheromones. But why does the vomeronasal organ detect odorants? In contrast to the olfactory epithelium, there is no direct pathway from this organ to the higher cortical areas involved in odour perception and discrimination[1–3]. Instead, inputs are targeted to the amygdala and hypothalamus, areas that control hormone levels, emotions, basic drives and instinctive behaviours. Like pheromones, some odorants may stimulate innate behavioural or physiological responses.

As in insects, certain odorants may act in mammals as semiochemicals that influence behaviour. Volatile chemicals emitted by plants can elicit oviposition or pollination in insects, and those released from prey can stimulate prey-finding behaviours[4–6]. A prey protein detected by the vomeronasal organ of the garter snake also induces tracking activity[1]. Certain odorants in the natural habitat of mice may similarly provide cues that signal the presence of a predator or indicate the suitability of a particular site for feeding or nesting.

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1. Halpern, M. *Annu. Rev. Neurosci.* **10,** 325–362 (1987).
2. Wysocki, C. J. & Meredith, M. in *The Neurobiology of Taste and Smell* (eds Finger, T. E. & Silver, W. L.) 125–150 (Wiley, New York, 1987).
3. Buck, L. B. *Cell* **100,** 611–618 (2000).
4. Binder, B., Robbins, J. & Wilson, R. *J. Chem. Ecol.* **21,** 1315–1327 (1995).
5. Dobson, H., Groth, I. & Bergstroem, G. *Am. J. Bot.* **83,** 877–885 (1996).
6. Kielty, J., Allen-Williams, L., Underwood, N. & Eastwood, E. *J. Insect Behav.* **9,** 237–250 (1996).
7. Sato, T., Hirono, J., Tonoike, M. & Takebayashi, M. *J. Neurophysiol.* **72,** 2980–2989 (1994).
8. Malnic, B., Hirono, J., Sato, T. & Buck, L. B. *Cell* **96,** 713–723 (1999).
9. Novotny, M., Jemiolo, B. & Harvey, S. in *Chemical Signals in Vertebrates* (eds Macdonald, D. W., Muller-Schwarze, D. & Natynczuk, S. E.) 1–21 (Oxford Univ. Press, Oxford, 1990).
10. Leinders-Zufall, T. *et al. Nature* **405,** 792–796 (2000).

Table 1 Responsiveness of vomeronasal neurons to odorants

Odorant mix	*Neurons tested**	*Responsive neurons (%)*
Animalic	1,045	13 (1.2)
Camphoric	973	15 (1.5)
Citrus	731	2 (0.3)
Floral	719	4 (0.6)
Fruity	848	5 (0.6)
Green minty	696	1 (0.1)
Musky	696	4 (0.6)
Sweet	626	3 (0.5)
Woody	596	4 (0.7)

*Number of KCl-responsive vomeronasal neurons tested with each odorant mix. Some neurons were tested with all mixes and others with a subset of mixes.