

burdens reported in the world so far⁴.

These compounds are also detectable in urban and rural air¹⁰, indicating the potential for long-distance atmospheric transport. It seems that BDEs are an important — but generally unrecognized — persistent organic pollutant in the United States. Extensive use of Penta and the high burden of BDEs in land-applied biosolids may facilitate environmental dissemination of less-brominated BDEs both locally and globally.

Robert C. Hale, Mark J. La Guardia, Ellen P. Harvey, Michael O. Gaylor, T. Matteson Mainor, William H. Duff
 Department of Environmental Science, Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, Virginia 23062, USA
 e-mail: hale@vims.edu

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Neuroadaptation

Incubation of cocaine craving after withdrawal

Relapse to cocaine addiction is frequently associated with subjective reports of craving, a poorly understood state that precedes and accompanies cocaine-seeking behaviours¹. It has been suggested² that over the first few weeks of withdrawal from cocaine, human addicts become sensitized to drug-associated environmental cues that act as external stimuli for craving, although the evidence for this is inconsistent³. Here we provide behavioural evidence from laboratory animals suggesting that the onset of craving is delayed and that craving does not decay, but rather increases progressively, over a two-month withdrawal period.

We modelled cocaine-craving behaviour by using rats trained to press a lever to receive an intravenous injection of cocaine and then testing them under conditions in which lever-pressing could continue but the cocaine reward was no longer given. In this model, lever-pressing drops to almost zero ('extinguishes') but can be temporarily reinstated by giving the animal an unearned 'priming' injection of the drug⁴, by

administering some forms of stress⁵, or by presenting drug-associated cues⁶ — factors that are known to provoke drug craving in human addicts^{1,7,8}.

We trained seven groups of rats to press the lever for intravenous cocaine injection (0.5 mg per kg body weight per lever-press). Individual rats lived in a chamber that had a retractable lever. Each training session began with insertion of the lever and illumination of a red house light. At the end of each session, the house light was turned off and the lever retracted. A 5-second tone–light signal accompanied each earned injection. After 10 days of 3-hour training sessions twice daily, in which the animals came to earn 55.3 ± 2.7 infusions per day, they were withdrawn from cocaine for 1, 2, 4, 7, 15, 29 or 60 days. During the withdrawal period, the lever was retracted and the house light was kept off.

We subsequently tested each group under two extinction conditions in which cocaine reward was withheld. First, we assessed resistance to extinction in the presence of the house light and the lever — cues that during training had indicated drug availability — but in the absence of the light and tone that were previously paired with drug injection. The animals were allowed to lever-press for six to eight 1-hour sessions (separated by 5-min intervals, during which the lever was retracted and the house light turned out) until their response fell to less than 15 presses per session. We found that lever-pressing was minimal in rats that had been deprived of cocaine for a single day and maximal in animals that had been deprived for 60 days (Fig. 1a).

The second test of cocaine seeking was a cue-induced reinstatement test conducted 5 min after the last of the extinction sessions. This test began with a 5-second presentation of the tone–light signal that had previously accompanied cocaine injection; each lever-press in this test resulted in another presentation of the tone–light signal⁶. In this test, the animals were not only exposed to the cues that would normally signal cocaine availability, but they were also exposed to the conditioned reinforcing cues that previously confirmed cocaine reward. Again, response was minimal after a single day of cocaine deprivation and maximal after 60 days (Fig. 1b). We found a linear increase over 2 months of cocaine withdrawal in the rats' sensitivity to similar drug-associated environmental cues that stimulate cocaine craving in humans¹.

Our results are consistent with clinical observations in humans² and suggest that a delayed-onset craving syndrome develops or 'incubates' during the first 2 months of cocaine abstinence, and probably lasts for longer. Although the mechanisms responsible for this incubation are not known, the intensification of cocaine seeking described

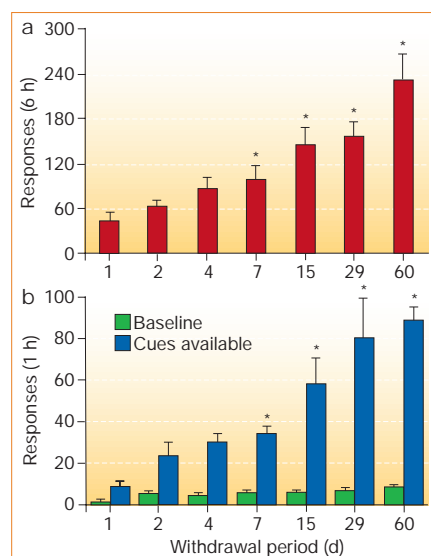


Figure 1 Persistence of a cocaine-seeking habit as a function of time since the last day of self-administration of cocaine. **a**, Mean (\pm standard error) number of non-reinforced responses on the lever previously associated with cocaine, from six extinction sessions in the presence of the house light and lever cues that were previously associated with cocaine availability. **b**, Mean (\pm standard error) number of non-reinforced responses on the lever previously associated with cocaine in the subsequent presence of the light–tone signal (conditioned reinforcer) that was previously associated with earned cocaine injections. Baseline data are from the previous extinction session. *Different from day 1 ($P < 0.01$).

here develops over a period when most of the neuroadaptations that accompany withdrawal from chronic cocaine addiction are in progressive decline^{9–11}.

The time course of this intensified drug seeking is similar to that of psychostimulant sensitization, which becomes progressively stronger with increasing abstinence for periods of up to several weeks^{12,13}. Whatever the mechanism by which craving is incubated, our evidence is inconsistent with the view that cocaine craving decays progressively after cessation of drug use. It suggests instead that the individual is most vulnerable to relapse at times well beyond the acute phase of drug withdrawal.

Jeffrey W. Grimm, Bruce T. Hope, Roy A. Wise, Yavin Shaham

Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, Maryland 21224, USA
 e-mail: yshaham@intra.nida.nih.gov

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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 chloride⁷. 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 previously¹⁰.

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

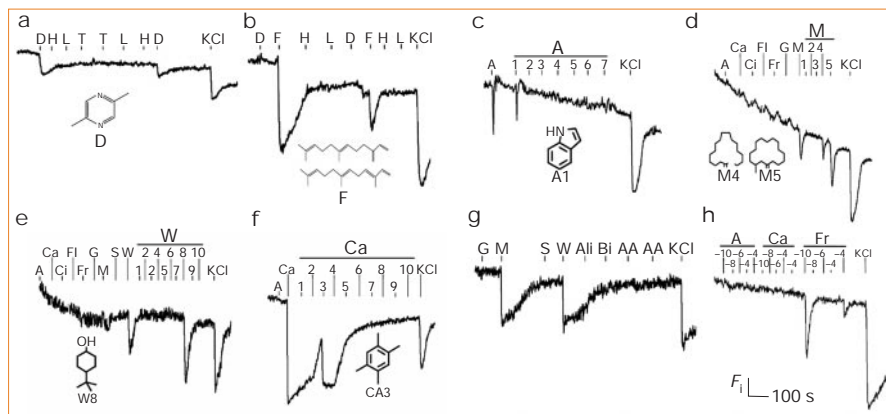


Figure 1 Responses of single vomeronasal neurons (VNs) to pheromones and odorants. Calcium imaging⁷ was used on dissociated VNs containing Fura-2 during exposure by perfusion to pheromones (a, b) or odorant mixes/odorants (c–h) (4 seconds each) and then to 100 mM KCl. Fluorescence emission (at 510 nm) 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 (Al) 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), hexadecanolid (M4), muscone (M5), and durenene (Ca3); as well as (not shown) *p*-cresol (A2), eucalyptol (Ca1), isoborneol (Ca4), borneol (Ca5), fenchone (Ca6), butyrophenone (Ca7), methylanisole (Ca8), myrtenal (Ca9), phenylfleur (F15), dimethyl-3-octanol (F18), helional (F110), pentadecalactone (M3) and aubepine (S1).

activated by 18 single odorants classified as animalic, camphoric, floral, musky, sweet or woody (Fig. 1).

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 pheromones¹⁰, 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 activity¹. 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.

Mehran Sam*, **Sadhna Vora***, **Bettina Malnic***, **Weidong Ma†**, **Milos V. Novotny†**, **Linda B. Buck***

*Howard Hughes Medical Institute, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA

e-mail: lbuck@hms.harvard.edu

†Institute for Pheromone Research, Department of Chemistry, Indiana University, Bloomington, Indiana 47405, USA

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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.