Single compounds elicit complex behavioural responses in wild, free-ranging rats

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There is mounting evidence that single compounds can act as signals and cues for mammals and that when presented at their optimal concentration they can elicit behavioural responses that replicate those recorded for complex mixtures like gland secretions and foods. We designed a rapid bioassay to present nine compounds that we had previously identified in foods, each at seven different concentrations (63 treatments), to wild, free-ranging rats and scored each treatment for attraction and three behavioural responses. Nine treatments (taken from five compounds) statistically outperformed the current standard rat attractant, peanut butter. Attraction to treatments was highest at the two lowest concentrations (0.1 and 0.01 μg g⁻¹) and a statistically significant relationship of increasing attraction with decreasing treatment concentration was identified. Our study identified five compounds not previously associated with behavioural responses by rats that elicit equivalent or more intense behavioural responses than those obtained with peanut butter. Moreover, attraction to treatments was driven by a concentration-dependent relationship not previously reported. This is the first study to identify isopentanol, 1-hexanol, acetoin, isobutyl acetate and 2-methylbutyl acetate as possible semiochemicals/cues for rats. More broadly, our findings provide important guidance to researchers in the ongoing search for mammalian semiochemicals and cues.

Olfaction is the oldest and frequently the most important sense for animals1. Through sensing, processing, translating and interpreting thousands of different chemical signals in their environment, animals can regulate social and physiological behaviours, like mate-finding and reproduction, and locating food2–4. For most mammals, and especially rodents, olfaction is their primary sense, but despite its importance it remains one of the least understood senses5,6. For example, the mechanisms by which odours induce innate behavioural responses in mammals remains largely unknown6 and to-date only a small number of mammalian semiochemicals (a chemical substance that transmits a signal) have been formally characterised7.

Odouriferous products, be they urine, faeces, gland secretions, body odours, and foods, are complex natural mixtures. Australian brushtail possum cloacal secretions, for instance, contain >100 compounds across a range of different chemical classes8. This complexity has led to suggestions that mammalian olfaction may be primed to identify and interpret complex mixtures7,9–11. For instance, although olfactory receptors have compound specific-ity, intra-species communication is commonly suggested to be driven by combinations or “bouquets” of odours that work interactively as a whole12,13. However, of the formally characterised semiochemicals or cues with known signalling roles in mammals, many are single compounds14.

Single compound semiochemicals and cues have been reported to elicit an array of behavioural responses in mammals like biting and chewing responses in canids and felids15, attraction, aversion and inter-male aggression in mice6,16–18 and aversion, anxiety, mother-young development primers and female attraction in rats9–11,20. In some cases the behavioural responses to the single compound was as strong as for the complex mixture in which it was identified. Further, some olfactory sensory neurons can be triggered by single compounds, thus it is possible they elicit innate behavioural responses22. However, studies investigating olfactory-mediated behavioural responses to semiochemicals and cues are almost exclusively performed using laboratory-bred animals (e.g., Wistar rats or house mice) or captive animals held in pens, not free-ranging wild animals. This has important implications as the

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results obtained from wild, free-ranging animals are likely to provide more realistic “real world” outcomes as they discover, test and confirm animal responses across an enormous range of natural conditions. Thus, compounds can be presented to wild animals in a complex ‘odourscape’ of myriad competing olfactory signals and cues with different meaning or function. This means that any compound(s) that stand out from the ‘chemical cacophony’ and are detected by the target species may be important communicatory signals. Further, replication at the multi-population (site) scale is possible compared with finite captive (pen or laboratory) animals, thus allowing for broader, species-level inferences.

The search for semiochemicals and cues, whether single compounds or blends, is made difficult by a range of species- and compound-specific and environmental factors that have the capacity to significantly impact their identification. Firstly, an animal’s behavioural response to a compound is commonly concentration-dependent. For example, rats are attracted to carbon disulphide, dimethyl disulphide and dimethyl sulphide at 50 μg g⁻¹ but repelled at 100 μg g⁻¹. Secondly, an animal’s detection threshold to compounds is also compound-dependent. Rats have a detection threshold of 0.0001 ng g⁻¹ for 2,4,5-trimethylthiazoline, a predator odour that elicits fear in rats, but a threshold of just 1 μg g⁻¹ for some aliphatic esters; a difference of seven orders of magnitude. This heightened olfactory sensitivity to a compound(s) may provide important information about the behavioural relevance of that compound to the study animal. Thirdly, a compound’s molecular weight, vapour pressure and a suite of environmental factors directly impact signal propagation. Advection and turbulence can lead to patchy odour-active spaces (the space in which the compound is at, or above, the animals detection threshold), while wind can dramatically decrease the concentration of the compound at or near its source. Lastly, in-field studies are often subject to a range of logistical constraints. For example, monitoring devices like camera-traps are expensive to buy while their bulk, weight and installation time means relatively low numbers of treatments can be assayed at the same time. Further, scoring videos for behaviours is time consuming, therefore costly. Thus, studies aiming to identify signals or cues using wild, free-ranging animals must cope with extraordinary chemical, environmental and animal variance, and ensure that large numbers of treatments are subjected to within and between site replication in a balanced assay design.

We devised a rapid, highly replicable, field-based assay using tracking tunnels that are used internationally to monitor rats and have previously been used assess the attractiveness of compounds on wild rats. This allowed us to overcome the enormous environmental, species- and compound-specific issues associated with presenting compounds to wild, free-ranging animals and ensured we could assay the large number of treatments we intended to present in a robust, balanced design. We used this bioassay to present nine compounds that we identified as having the potential to act as signals/cues for rats, each at seven different concentrations (63 treatments). The nine compounds were identified following rapid, in-field bioassays that presented a range of food products to rats, the chemical profiling of the foods using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME GC-MS) and the statistical interrogation of the GC-MS dataset using partial least squares regression.

Our study objectives were to: (1) quantify the attractiveness of each compound on wild, free-ranging rats. We hypothesised that several of the nine compounds would be attractive to rats and that attraction to those compounds may be as strong as for a complex mixture such as peanut butter, a product that is widely used to lure rats; (2) quantify a range of behavioural responses to the compounds that may provide information about the compounds’ importance to rats. We hypothesised that attractive compounds were also likely to elicit behaviours such as biting and urine marking; (3) identify any concentration-dependent relationships between the treatments and each behavioural response. Given the olfactory sensitivity of rats to behaviourally important compounds and that concentration-dependent responses to some compounds has been reported in rats and other species, we hypothesised that a relationship between one or more of the behavioural responses and the treatment concentration was likely.

**Results**

**Trial eliminations – attraction.** Forty-two of the 63 treatments presented during Phase One (n = 5 per treatment) received a confirmed rat visit. Treatment I7 was the most attractive treatment with an attraction rate of 0.80, while A6, B6, I6 and C7 were the next most attractive treatments, each with an attraction rate of 0.60. These top five most attractive treatments were all presented at the two lowest concentrations: 0.1 and 0.01. These top five most attractive treatments were all presented at the two lowest concentrations of 0.1 and 0.01.

In Phase Two, the 27 treatments carried forward from Phase One were presented (n = 5 per treatment for Phase Two and thus n = 10 in total for the 27 compound-based treatments presented in Phase One and Two). Treatment F7 was the most attractive treatment after Phase Two, with an attraction rate of 0.50 (n = 10). Treatment I7 was the second-most attractive, with an attraction rate of 0.40 (n = 10). The mean attraction rates for the control and peanut butter standard were 0.13 (n = 70) and 0.16 (n = 70), respectively.

Sixteen treatments were more attractive than peanut butter after the 10 trials, nine of which were significantly more attractive (P < 0.01): A6, B3, B6, C6, C7, F7, I7, I2, I6 and I7. Of the nine significantly more attractive treatments, seven were presented at the two lowest concentrations of 0.1 and 0.01 μg g⁻¹. The nine significantly more attractive treatments were derived from five compounds: isopentanol (A); 1-hexanol (B); acetoin (C); isobutyl acetate (F) and 2-methylbutyl acetate (I). A trial-by-trial breakdown of the results is provided in Supplementary Information S1.
Biting, marking and investigation. Twelve of the 27 treatments presented in Phase Two received a biting event. Seven of the nine treatments that were significantly more attractive than peanut butter (A6, B6, C7, F7, I6 and I7) received a biting event and these were all presented at the two lowest concentrations (0.1 and 0.01 μg g⁻¹). The two statistically significant treatments that did not receive a biting event were B3 and I2 (presented at 100 and 1000 μg g⁻¹, respectively). Peanut butter had the highest biting rate (0.73), but only statistically outperformed three of the nine statistically significant treatments: I2 and B3 that received no biting (P = 0.006 for both treatments) and F7 that received only one biting event (P = 0.02). The control received no biting events. A range of different biting responses were recorded (Fig. 2).

Marking on or in tracking tunnels was recorded for 18 of the 27 treatments (Fig. 2). Treatment I2 (presented at 1000 μg g⁻¹) was the only one of the nine significantly attractive treatments not to receive a marking event. Six of the nine significantly attractive treatments scored a higher marking rate than peanut butter (PB marking rate = 0.36), with four presented at the two lowest concentrations receiving significantly more marking events than peanut butter (A6, C6, C7, P = 0.04; I6, P < 0.001). The control received no marking events.

Treatment A2 had the highest mean investigation score (195, SE ± 22.66). Conversely B5 (presented at 1 μg g⁻¹) scored the lowest mean score (23, SE ± 4.5), with the control receiving a higher mean score (110, SE ± 22.96). No statistically significant difference between treatments, peanut butter and the control was identified (H = 21.814 df = 17, P = 0.19). We only used assays with a minimum of two observations in our Kruskal Wallis analysis as the inclusion of a single observation would provide no variance. The data and R code for each test for each behavioural response are provided in Supplementary information S1.

Concentration-dependent relationships. Attraction to treatments was highest at the two lowest concentrations, with nearly half of all recorded visits occurring with treatments presented at 0.1 and 0.01 μg g⁻¹. The lowest recorded attraction rate (0.09) was for treatments presented at 1 μg g⁻¹. A statistically significant relationship between treatment concentration and attraction was identified (X²[GLMM] = 6.24, df = 1, P = 0.013).

Biting was only recorded at three concentrations: 0.01, 0.1 and 1000 μg g⁻¹, therefore we did not perform a GLMM for biting.

Marking on or in tracking tunnels was recorded at each of the seven concentrations. Marking was highest at the two lowest concentrations, with 53% and 44% of all visits to treatments at 0.1 and 0.01 μg g⁻¹, respectively, receiving some faecal and/or urine marking but no statistically significant relationship between treatment concentration and marking behaviour was identified (X²[GLMM] = 1.90, df = 1, P = 0.17).

Investigation was highest for treatments presented at 0.1 μg g⁻¹ and 100 μg g⁻¹ (mean score for both was 134) while treatments presented at 1 μg g⁻¹ scored the lowest for investigation (mean score 88). No statistically significant relationship between treatment concentration and investigation was identified (X²[GLMM] = 0.0, df = 1, P = 0.998). The data and R code for all models are provided in Supplementary information S1.

Discussion

Our study demonstrates that wild, free-ranging rats can detect and respond to a suite of different single compounds, and that when the compounds are presented at optimal concentrations they can elicit levels of behavioural responses that outperform a complex mixture like peanut butter. Furthermore, some behavioural responses to our compounds appear to be concentration-dependent, with higher levels of attraction and marking rates recorded at lower concentrations and a statistically significant negative relationship between attraction and compound concentration identified.
Rats were able to respond to a range of different single compounds amid the cacophony of olfactory noise found within and between sites, and commonly responded to the compounds with complex behaviours like urine marking and/or biting. This strongly suggests that isopentanol, 1-hexanol, acetoin, isobutyl acetate and 2-methylbutyl acetate may be important communicatory signals or cues for rats. The high levels of behavioural responses to compounds presented at low concentrations further supports this assertion. As detailed in the introduction, an animal’s olfactory sensitivity to an odorant can provide important information about that odorant’s evolutionary and behavioural importance to the focal animal. For instance, Norway rats *Rattus norvegicus* are several orders of magnitude more sensitive to 2,4,5-trimethylthiazoline, a predator odorant known to elicit fear in rats, than non-human primates for which it elicits no fear response. Moreover, urine/faecal marking is a common response by rats to orientate and attract conspecifics to objects of importance. We hypothesise, therefore, that isopentanol, 1-hexanol, acetoin, isobutyl acetate and 2-methylbutyl acetate may be behaviourally important semiochemicals or cues for rats given, (1) the level of attraction to the compounds across multiple independent populations, (2) the ability of rats to discern the compounds above each site’s olfactory noise and, (3) the low concentrations at which they elicited a behavioural response. To the best of our knowledge, none of the five compounds have been previously described as semiochemicals or cues for rats.

Some compounds elicited higher levels of urine marking than recorded for the peanut butter standard (e.g., compound A, C, and I for urine marking). This also raises the possibility that one or more of the five attractive compounds that we originally identified in foods may be pheromonal in nature as pheromones are commonly exaptation’s of compounds that originally had other uses, such as those derived from foods. Intriguingly, three of the five top performing compounds (isopentanol, 1-hexanol and acetoin) have been reported in rat urine, although they have not been formally characterised as pheromones.

Low attraction rates to higher concentrations may be due to the animal’s olfactory receptors interpreting the compounds differently or becoming fatigued through their saturation and ultimately leading to avoidance and/or repellence. Thus, the perceived aroma of the compounds may have changed with increasing concentration. A human example is 4-methyl-4-sulfanylpentan-2-one that typically occurs in wines made with Sauvignon blanc grapes. Below 5 ng L\(^{-1}\) humans discern this compound as having an aroma of passionfruit, but it smells of cat urine at ca. 5 ng L\(^{-1}\) or above. Moreover, some studies have shown that behavioural responses to odours are mediated by sensory neuron and glomeruli activation patterns that change dramatically depending on the concentration of the odour presented. We hypothesise that these factors, allied with the olfactory sensitivity of...
rats to behaviourally important compounds, may explain the concentration-dependent relationship for attraction we report in this study.

Concentration-dependent attraction to compounds has been demonstrated for nematodes, fruit flies, humans and rabbits. Those studies showed an initial increase in attraction to an olfactory stimulus with decreasing concentration until a peak response was achieved, after which attraction or the behavioural response began to decrease with increasing concentration, creating a curve akin to a normally distributed response. This normally distributed outcome is also demonstrated by who measured the frequency of penile erections in Norway rats in response to a blend of compounds. An initial increase in the frequency of erections was recorded with decreasing concentration until peak response was obtained, after which the frequency declined. It is possible, therefore, we may not have identified peak attraction in rats, as our study did not present the compounds at concentrations lower than (the concentration with the highest attraction rate). We suggest future studies should present the same compounds at concentrations lower than those used in this study as this will allow for the elucidation of the rat's peak concentration response and may, therefore, help pin-point the optimal concentrations for attraction and the behavioural responses for each of these compounds.

Our study demonstrates that five single compounds can act as signals/cues for wild, free-ranging rats and that they can elicit behavioural responses such as attraction, urine/faecal marking and biting that outperform more complex mixtures like peanut butter. To the best of our knowledge, this is the first study to identify isopentanol, 1-hexanol, acetoin, isobutyl acetate and 2-methylbutyl acetate as signals/cues for rats and to demonstrate a statistically significant relationship between attraction to compounds and their concentration.

Given the importance of olfaction to mammals (Solomon et al., 2007) and the concentration-dependent relationship for attraction identified in this study, we suggest bioassays assessing behavioural responses of mammals to semiochemicals should initially consider presenting compounds at low concentrations, such as or lower. That said, the physical properties of individual compounds, such as vapour pressure, and the animal's detection threshold for that compound, may demand that a broad spectrum of concentrations are at least initially considered. Indeed, a broad-spectrum approach may ensure the subject animal's peak response to a compound is found. Nonetheless, our findings suggest a bioassay that initially focuses on lower concentrations may prove a more fruitful approach to identifying behaviourally important semiochemicals, with higher concentrations trialled if attraction rates at low concentrations are poor. Further, given that behaviourally important compounds are likely to be discriminated at very low concentrations, consideration should be given to presenting compounds at concentrations lower than those presented during this study. Lastly, the identification of attraction to more than half of the compounds identified using partial least squares regression and detailed in our previous work provides demonstrable support for its use of our reductive statistical approach to identify single compounds that may act as cues or signals. This study provides evidence of the usefulness of our statistical response-guided approach to the identification of signals and cues for mammals, despite some authors suggesting it is not possible to simplify mammalian signal complexity using such a strategy.

Materials and Methods

**Treatment preparation and presentation.** Treatments were prepared by serially diluting an initial stock solution of each compound in medium chain triglyceride oil (MCT) in 2 mL microtubes (Supplementary Table S2 for dilution procedure). We used MCT as prior GC-MS analysis identified it as having the lowest volatile profile when compared with two traditional carrier media, propylene glycol and glycerine. Microtubes (2 mL) were subjected to mixing using a Chiltern MT19 vortex mixer for 20 seconds. One gram of the final treatment was pipetted into a 1.7 mL microtube for in-field presentation. Th microtube lids were sealed with Parafilm® and each treatment was placed in an individual, labelled zippered plastic bag and stored overnight at 4°C. The 63 treatments (nine compounds at seven concentrations – see Trial Design for further detail) were prepared 24 hours prior to each trial.

Treatments were presented to wild, free-ranging rats in pre-conditioned (washed with rainwater and left to air-dry outdoors for 2 weeks) tracking tunnels. Treatments were secured to the inside wall of tunnels using a...
cable tie. Tracking cards, with non-drying ink applied to the centre of the card, were placed in each tunnel to quantify visits and allow for the identification of the species visiting the treatments (Fig. 3). Only four rodent species are present in New Zealand and of those, only three (Rattus rattus, Rattus norvegicus and Mus musculus) were present in the trial locations, thus allowing for accurate track discrimination.

Tracking tunnels were installed along a single spatially stratified transect that followed walking tracks for ease of accessibility, speed of tracking tunnel installation and safety during in-field work. Tracking tunnels were installed between 2 and 15 m (into the forest) from pathways and fixed to the ground using metal mat pins. Each tracking tunnel was constructed and deployed by the same operative wearing a new pair of single use gloves to avoid human scent transmission to tracking tunnels or microtubes, and in-field cross-contamination between tunnels from unpacking and handling treatments. Tracking tunnels were cleaned after each trial using tap water, rinsed with rainwater and left to air-dry outdoors. Tracking tunnels were assigned to individual, concentration-specific treatments for the duration of the trials. Tunnels that received rat interactions, such as extensive chewing and/or urine marking or those where the treatment was spilt due to rat interactions with the microtube were, however, not used in future trials and were replaced with new, pre-conditioned tunnels.

**Trial design.** For Phase One, each of the nine compounds was presented at seven different concentrations, decreasing logarithmically from 10,000 to 0.01 μg g⁻¹. This was because (1) the concentration range covered those previously trialled for rats and other vertebrate pest species⁶¹⁻⁶⁴, (2) the relationship between odour intensity and concentration can be modelled by a log-linear relationship⁶⁵, and (3) the approach allowed us to investigate the impact of concentration on the behavioural responses of rats to treatments.

The nine trial compounds were provided letter codes from A to I while the seven concentrations were coded from 1 to 7, thus providing a unique identifier for each of the 63 treatments. The nine compounds and their associated codes were: isopentanol (A); 1-hexanol (B); acetoin (C); isopentanoic acid (D); 2,3-dimethylpyrazine (E); isobutyl acetate (F); isopentyl acetate (G); tetramethylpyrazine (H) and 2-methylbutyl acetate (I). The seven concentration-specific treatments for the duration of the trials. Tunnels that received rat interactions, such as extensive chewing and/or urine marking or those where the treatment was spilt due to rat interactions with the microtube were, however, not used in future trials and were replaced with new, pre-conditioned tunnels.

Response variables. Each treatment was scored for four behavioural responses: attraction, marking, biting and investigation. This is important as behaviour-specific responses to compounds may provide useful information regarding the behavioural importance of the compound to the focal species. Rats, for example, are known to deposit urine and faecal scent marks on or near foods or objects of interest that convey important information to conspecifics about the items presence and location⁴⁵,⁴⁶. The area tracked with footprints on or in the tracking tunnel and hereafter termed ‘marking’, (2) the presence of chew or bite marks on the microtube and/or tracking tunnel and hereafter termed ‘biting’ and, (3) the area tracked with footprints on each tracking card that received a visit and termed ‘investigation’. This was measured using a 10 × 47 cm Perspex sheet with a grid made up of 1 × 1 cm squares. The number of squares with rat tracks provided an investigation score that was designed to identify treatments that generated a strong response by an individual or that elicited visits from multiple individuals.

We used the attraction rate to direct the elimination process detailed in the Trial Design section. Treatments that had a statistically significantly lower attraction rate than the most attractive treatment after Phase One trials were eliminated and not carried forward to Phase Two trials. This allowed for a rapid removal of unattractive treatments. For completeness, however, and to avoid the possibility of false negative outcomes we applied the following additional criteria to the selection of treatments to be presented in Phase Two (1) if a statistically weaker treatment received either a marking and/or biting event that treatment was carried forward to Phase Two and,
(2) if a compound had only one representative from all seven concentrations to be carried forward to Phase Two, the potential concentration-dependent relationship using data from visitation to each type of odor was carried forward. All statistical analyses were run using data only obtained from tracking tunnels that received a confirmed visit. This allowed us to investigate whether attraction, biting, marking and investigation of each type of odor was concentration-dependent and investigation models were run using data only obtained from tracking tunnels that confirmed a visit. P (as the response was binary). A Linear Mixed-Effects Model (LMM) was used for investigation (because the response was binary).

The model data were constructed by combining the data for all the treatments based on concentration. For example, the results for all nine treatments presented at 10,000 µg g⁻¹ across the 10 trials were combined. We did this for all seven concentrations. This allowed us to investigate whether attraction, biting, marking and investigation of each type of odor was concentration-dependent and investigation models were run using data only obtained from tracking tunnels that confirmed a visit. This allowed us to investigate whether attraction, biting, marking and investigation of each type of odor was concentration-dependent and investigation models were run using data only obtained from tracking tunnels that confirmed a visit. All statistical analyses were run in R, version 3.1.36, with package lme4 used for fixed-effects models and car used for Type 3 Wald tests.

References
1. Dusenbery, D. Sensory Ecology: How organisms acquire and respond to information. (W.H. Freeman and Company, 1992).

2. Ache, B. & Young, J. Olfaction: diverse species, conserved principles. Neuron 48, 417–430 (2005).

3. Lledo, P., Gheusi, G. & Vincent, J. Information processing in the mammalian olfactory system. Physiol. Rev. 85, 281–317 (2005).

4. Liberles, S. & Buck, L. A second class of chemosensory receptors in the olfactory epithelium. Nature 442, 645–650 (2006).

5. Campbell-Palmer, R. & Rosell, F. The importance of chemical communication studies to mammalian conservation biology: A review. Biol. Conserv. 144, 1919–1930 (2011).

6. Saravia, L. et al. Combinatorial effects of odorants on mouse behavior. Proc. Natl. Acad. Sci. 113, E3300–E3306 (2016).

7. Apps, P. Are mammalian olfactory signals hiding right under our noses? Naturwissenschaften 100, 487–506 (2013).

8. McLean, S., Davies, N. & Wiggins, N. Scent chemicals of the brushtail possum, Trichosurus vulpecula. C. R. Acad. Sci. Paris 349, 1318–39 (2012).

9. Evans, C., Mackintosh, J., Kennedy, J. & Robertson, S. Attempts to characterise and isolate aggression reducing olfactory signals from the urine of female mice Mus musculus. J. Physiol. Behav. 20, 129–134 (1978).

10. Albone, E. Mammalian semiochemistry: the investigation of chemical signals between mammals. (Wiley, 1984).

11. Albone, E., Blazquez, N., French, J., Long, S. & Perry, G. Mammalian semiochemistry: issues and futures, with some examples from a study of chemical signalling In: the Chemical Signals in Vertebrates 4: Ecology, Evolution, and Comparative Biology (eds Duvall, D., Muller-Schwarze, D. & Silverstein, R.) 27–36 (Springer Science & Business Media, 1986).

12. Wyatt, T.pheromones and animal behaviour: Chemical signals and signatures. 2nd Ed. (Cambridge University Press, 2014).

13. Apfelbach, R., Parsons, M., Soinil, H. & Novotny, M. Are single odorous components of a predator sufficient to elicit defensive behaviors in prey species? Front. Neurosci. 9 (2015).

14. Apps, P., Weldon, P. & Kramer, M. Chemical signals in terrestrial vertebrates: search for design features. Nat. Prod. Rep. 32, 1131–1153 (2015).

15. Nilsson, S. et al. Behavioral responses to mammalian blood odor and a blood odor component in four species of large carnivores. PLoS ONE 9, e112694 (2014).

16. Novotny, M., Harvey, S., Jemiolo, B. & Alberts, J. Synthetic pheromones that promote inter-male aggression in mice. Proc. Natl. Acad. Sci. USA 82, 2059–2061 (1985).

17. Li, Q. et al. Synchronous evolution of an odor biosynthesis pathway and behavioral response. Curr. Biol. 23, 11–20 (2013).

18. Sievert, T. & Laska, M. Behavioral Responses of CD-1 mice to six predator odor components. Chem. Senses 31, 399–406 (2016).

19. McGregor, I., Schrama, L., Ambermoon, P. & Dielenberg, R. Not all ‘predator odours’ are equal: cat odour but not 2,4,5-trimethylthiazoline (TMT; fox odour) elicits specific defensive behaviours in rats. Behav. Brain Res. 129, 1–16 (2002).

20. Catalani, A. et al. Maternal corticosterone influences behavior, stress response and corticosteroid receptors in the female rat. Pharmacol. Biochem. Behav. 73, 105–114 (2002).

21. Zhang, J.-X., Sun, L., Zhang, J.-H. & Feng, Z.-Y. Sex- and gonad-affecting scent compounds and 3 male pheromones in the rat. Chem. Senses 33, 611–621 (2008).

22. Nara, K., Saravia, L., Ye, X. & Buck, L. A large-scale analysis of odor coding in the olfactory epithelium. J. Neurosci. Off. J. Soc. Neurosci. 31, 9179–9191 (2011).

23. Wyatt, T. Pheromones and Animal Behaviour: Communication by Smell and Taste. (Cambridge University Press, 2003).

24. Hoef, J. & Cressie, N. Spatial Statistics: Analysis of field experiments. In Design and Analysis of Ecological Experiments (eds Scheiner, S. & Gurevitch, J.) 389–307 (Oxford University Press, 2001).

25. Segal, I. et al. Effects of physical attributes and chemical composition of novel foods on food selection by Norwegian rats (Rattus norvegicus). J. Pest Sci. 87, 99–106 (2014).

26. Malnic, B., Hirono, J., Sato, T. & Buck, L. Combinatorial receptor codes for odors. Cell 96, 713–723 (1999).

27. Veer, V., Gopalani, N., Kumar, S. & Prakash, S. Bioassay of three sulphur containing compounds as rat attractant admixed in cereal-based bait against Rattus rattus Linn. Indian J. Exp. Biol. 40, 941–944 (2002).

28. Muller-Schwarze, D. Chemical Ecology of Vertebrates. (Cambridge University Press, 2006).

29. Bradbury, J. & Vehrencamp, S. Principles of Animal Communication. (Sinauer Associates Inc., 2011).

30. Laska, M. et al. Detecting danger–or just another odorant? Olfactory sensitivity for the fox odor component 2,4,5-trimethylthiazoline in four species of mammals. Physiol. Behav. 84, 211–215 (2005).

31. Hernandez, L., Laska, M. & Rodriguez Luna, E. Olfactory sensitivity for aliphatic esters in spider monkeys (Ateles geoffroyi). Behav. Neurosci. 117, 1142–1149 (2003).
32. Hudson, R. From molecule to mind: the role of experience in shaping olfactory function. J. Comp. Physiol. [A] 185, 297–304 (1999).
33. Liman, E. Use it or lose it: molecular evolution of sensory signaling in primates. Pflug. Arch. Eur. J. Physiol. 453, 125–131 (2006).
34. Visser, J. Host Odor Perception in Phytophagous Insects. Annu. Rev. Entomol. 31, 121–144 (1986).
35. Mason, J. & Blom, F. Coyote Lure Ingredients: What Determines Success? Wildl. Control Technol. 26–30 (1998).
36. Lindsey, G., Mosher, S., Fancy, S. & Smucker, T. Population structure and movements of introduced rats in an Hawaiian rainforest. Proc. Conv. Biol. 5, 94–102 (1999).
37. Russell, J., Beaven, B., MacKay, J., Towns, D. & Clout, M. Testing island biosecurity systems for invasive rats. Wildl. Res. 35, 215–221 (2008).
38. Pender, R., Shiel, A., Balic-Murphy, L. & Mosher, S. Large-scale rodent control reduces pre- and post-dispersal seed predation of the endangered Hawaiian lobeliad, Cyanea superba subsp. superba (Campanulaceae). USDA Natl. Wildl. Res. Cent. - Staff Publ. (2013).
39. Gillies, C. & Williams, D. DOC tracking tunnel guide v2.5.2: Using tracking tunnels to monitor rodents and mustelids. Department of Conservation, Wellington. (2013).
40. Nelson, T. et al. Effectiveness of rodent control and monitoring techniques for a montane rainforest. Wildl. Soc. Bull. 30, 82–92 (2002).
41. Mason, J., Bean, N. & Galef, B. Attractiveness of carbon disulfide to wild rats. Proc. Thirteen. Verteb. Pest Conf. Paper 20 (1988).
42. Jackson, M., Hartley, S. & Linklater, W. Better food-based baits and lures for invasive rats Rattus spp. and the brushtail possum Trichosurus vulpecula: a bioassay on wild, free-ranging animals. J. Pest Sci. 89, 479–488 (2016).
43. Jackson, M., Linklater, W. & Keyzers, R. The development of semi-chemical lures for invasive rats: an integrated chemical image and response-guided approach. In Proceedings of the 27th Vertebrate Pest Conference (Eds Timms, R. M. & Baldwin, R. A.) (University of California Davis, 2016).
44. Hayden, S. et al. Ecological adaptation determines functional mammalian olfactory subgenomes. Genome Res. 20, 1–9 (2010).
45. Galef, B. Norway rat’s communication about food and feeding sites. Natl. Wildl. Res. Cent. Repel. Conf. 1995 Paper 18, 185–201 (1995).
46. Brown, R. Object-directed urine-marking by male rats (Rattus norvegicus). Behav. Biol. 15, 251–254 (1975).
47. Gould, S. Exaptation: A Crucial Tool for an Evolutionary Psychology. J. Soc. Issues 47, 43–65 (1991).
48. Takacs, S., Gries, R., Zhao, H. & Gries, G. The sex attractant pheromone of male brown rats: identification and field experiment. Angew. Chem. Int. Ed. 55, 6062–6066 (2016).
49. Serrano-Contreras, J., Garcia-Perez, I., Melendez-Camargo, M. & Zepeda, I. NMR-Based metabonomic analysis of physiological responses to starvation and refeeding in the rat. J. Proteome Res. 15, 3241–3254 (2016).
50. Zhang, Z. et al. Metabonomics approach to assessing the metabolism variation and endogenous metabolic interaction of ginsenosides in cold stress rats. J. Proteome Res. 15, 1842–1852 (2016).
51. Xu, F., Kida, L., Hyder, F. & Shulman, R. Assessment and discrimination of odor stimuli in rat olfactory bulb by dynamic functional MRI. Proc. Natl. Acad. Sci. USA 97, 10601–10606 (2000).
52. Dunlevy, J., Kalua, C., Keyzers, R. & Boss, P. From vine to vine: Grape-derived flavour and aroma in wine. (Springer, 2009).
53. Yoshida, K. et al. Odour concentration-dependent olfactory preference change in C. elegans. Nat. Commun. 3, 739 (2012).
54. Khurana, S. & Siddiqui, O. Olfactory responses of Drosophila larvae. Chem. Senses 38, 315–323 (2013).
55. Courgeaud, G., Langlois, D., Sicard, G. & Schaal, B. Newborn rabbit responsiveness to the mammary pheromone is concentration-dependent. Chem. Senses 29, 341–350 (2004).
56. Nielsen, B. L. et al. A mixture of odorant molecules potentially indicating oestrus in mammals elicits penile erections in male rats. Behav. Brain Res. 225, 584–589 (2011).
57. Burger, B. Mammalian semiochemicals. Top. Curr. Chem. 240, 231–278 (2005).
58. Jolley, S. & Jolley, I. Pen and field tests of odor attractants for the dingo. J. Wildl. Manag. 56, 452–456 (1992).
59. Kimball, B., Mason, J., Blom, F., Johnston, J. & Zemlicka, D. Development and testing of seven new synthetic coyote attractants. J. Agric. Food Chem. 48, 1892–1897 (2000).
60. Shumake, S., Hakim, A. & Gaddis, C. Carbon disulfide effects on pre-baited vs. non-pre-baited rats exposed to low dosage zinc phosphide rodenticide bait. Crop Prot. 21, 545–550 (2002).
61. Bean, N., Galef, B. & Mason, J. The effect of carbon disulfide on food consumption by house mice. J. Wildl. Manag. USA (1988).
62. Spurr, E. et al. Effect of concentration of anal gland scent lures on the capture rate of ferrets (Mustela furo) in winter and spring. N. Z. J. Zool. 31, 227–232 (2004).
63. Galef, B. G., Mason, J. R., Preti, G. & Bean, N. J. Carbon disulfide: a semiochemical mediating socially-induced diet choice in rats. Physiol. Behav. 42, 119–124 (1988).
64. Clapperton, B., Phillips, S. & Woolhouse, A. Field trials of slow-release synthetic lures for stoats (Mustela erminea) and ferrets (M. furo). N. Z. J. Zool. 21, 279–284 (1994).
65. Wu, C., Liu, J., Zhao, P., Pringer, M. & Schaubeger, G. Conversion of the chemical concentration of odorous mixtures into odour concentration and odour intensity: A comparison of methods. Atmos. Environ. 127, 283–292 (2016).
66. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2016).
67. Bates, D., Maechler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48 (2015).
68. Fox, J. & Weisberg, S. An (R) Companion to Applied Regression, Second Edition. (2011).

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Author Contributions
M.D.J. and W.I.L. designed the field study. R.A.K. and M.D.J. designed the lure production protocols. M.D.J. carried out the laboratory and field work, data analyses and drafted the manuscript. M.D.J., W.I.L., R.A.K. edited the manuscript.

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