Research News

Sex and the single neuron: pheromones excite

Emily R. Liman

Pheromones are potent chemical signals used by animals across all phyla to control reproductive function and social behavior. Whereas there are few examples of the effects of pheromones on human behavior (fortunately?), in other species, such as laboratory mice, the effects of pheromones have been shown to be dramatic: a female mouse might abort a pregnancy if exposed to pheromones from a male mouse who is not her mate[1]. In these animals, ablation experiments have shown that the vomeronasal organ (VNO), a chemosensory organ, which lies at the rostral end of the nasal cavity, plays a key role in pheromone detection. However, direct evidence for transduction of pheromones by VNO sensory neurons has been lacking, and even basic questions about sensory transduction remained controversial. For example, it was not known whether sensory neurons depolarize and fire action potentials in response to sensory stimuli, similar to olfactory sensory neurons, or whether they hyperpolarize, similar to vertebrate photoreceptors[2]. The difficulty in detecting responses of VNO neurons to pheromones lies in the fact that the sensory cells are both heterogeneous in the receptors that they express and in their resulting ligand specificity. Thus identification of a cell that is responsive to a specific pheromone requires the ability to efficiently sample a large number of cells. This problem has now been overcome by two groups who have used different methodologies to record responses in populations of VNO neurons: in one case a 64-electrode multi-array[3] and in the other Ca^{2+} imaging[4].

Both groups developed methods to record from multiple cells, taking advantage of the unique anatomy of the VNO. The VNO is a tubular structure with a lumen down its length that communicates with the external environment and allows passage of chemical signals to the microvillous sensory endings of VNO neurons. In the approach taken by Leinders-Zufall *et al.*[4], the VNO was cut in tranverse section, so that cells across all layers as well and their processes remained intact. As such, Ca^{2+} transients in the cell body and in the dendrite and dendritic knob of sensory neurons could be imaged. In an alternative approach by Holy *et al.*[3] the VNO was cut longitudinally, so that the epithelial sheet could be flattened onto the surface of a 64-electrode microarray. In these experiments, electrical responses presumably originated from a subpopulation of neurons situated basally in the epithelium. Leinders-Zufall *et al.*[4] also developed methods for recording extracellular field potentials from the VNO in response to pheromones using an electro-vomeronasogram (EVG), a technique that allows a quick screening of potentially bioactive chemicals.

Using these techniques, both groups have greatly advanced our understanding of how VNO sensory neurons detect and discriminate among stimuli. Both groups first showed that they could detect sensory responses in VNO neurons. Holy *et al.*[3] recorded responses from the VNO sensory epithelium following the addition of mouse urine (diluted to prevent non-specific effects), which is known to contain pheromones. More than a third of the cells from which they recorded responded with trains of action potentials, indicating that the transduction process is depolarizing. Leinders-Zufall *et al.*[4] exposed the VNO to six chemicals that have been shown to be secreted in mouse urine and to act as pheromones. All six elicited a depolarizing response in the EVG, and each caused an increase in Ca^{2+} in a small fraction (0.5–3.0%) of cells. In addition, action potentials in responses to three of the chemicals were detected by loose-patch recording. Together, these data provide the first unequivocal evidence that VNO neurons depolarize in response to pheromones.

Both groups then deciphered the mechanism by which stimulus identity is encoded in the pattern of firing of the sensory neurons. Holy *et al.*[3] exposed the VNO epithelium to dilute urine from male and female mice and found that a small fraction of cells selectively responded to the urine of one or the other sex. Thus, identification of gender might be encoded in the pattern of responses of VNO neurons. Furthermore, Leinders-Zufall *et al.*[4] found that specific pheromones that are released by one or the other sex, were detected by non-overlapping populations of cells. For example, farnesene, a chemical released by male mice that accelerates puberty in female mice, when used at extremely low concentrations (10^{-10}–10^{-11} M) stimulated a population of cells that were distinct from the populations of cells that responded to the five other chemicals tested. This exquisite ligand specificity was maintained even when the concentration of the six chemicals were raised several orders of magnitude. The highly-tuned sensitivity of VNO neurons to specific pheromones, unlike the broadly-tuned response profile of olfactory neurons to general odorants, is consistent with the VNO function of detecting behaviorally relevant stimuli; presumably stimuli and receptors have co-evolved such that the detection of the signal has been optimized. Interestingly, the responsive cells were all located in an apical zone of the VNO that is associated with the expression of the G-protein alpha subunit, $Gi\alpha_2$ (Refs 5,6) and a family of putative pheromone receptors, the vomeronasal receptors (V1Rs) (Ref. 7). By contrast, more-basally situated cells express a different G-protein alpha subunit and a different family of putative pheromone receptors[8–10]. Thus, the data from Leinders-Zufall *et al.*[4] suggests that the V1R family of receptors might play a special role in the detection of reproductively relevant pheromones.

The ability to record electrophysiological responses from VNO sensory neurons has also allowed further progress in the understanding of the mechanism of sensory transduction in the vomeronasal system. Previous work has shown that sensory transduction probably involves a channel that is related to the transient receptor potential (TRP) channel, which underlies phototransduction in *Drosophila melanogaster*[11]. This suggests that VNO sensory transduction, similar to invertebrate phototransduction, might use a phospholipase C (PLC) signaling cascade[12]. Accordingly, Holy *et al.*[3] reported that responses to dilute urine

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are, in most cells, blocked by a PLC inhibitor. Leinders-Zufall *et al.*[4] showed that during transduction, internal Ca^{2+} is elevated as a result of the entry of extracellular Ca^{2+}, and that this influx is initiated at the dendritic knob. This process could occur if the channel mediating transduction is Ca^{2+} permeable or if depolarization of the knob causes the opening of voltage-activated Ca^{2+} channels. Finally, Holy *et al.*[3] showed that VNO sensory neurons do not adapt to a maintained sensory stimulus, a response property that might allow these cells to increase sensitivity via temporal integration.

These studies[3,4] will now allow sensory transduction in the VNO to be studied, not just with powerful molecular techniques that have been developed in recent years, but also with sensitive physiological techniques. The two approaches, together, promise to yield new insights into the nature of chemical communication in vertebrates.

References

1 Halpern, M. (1987) The organization and function of the vomeronasal system. *Annu. Rev. Neurosci.* 10, 325–362

2 Keverne, E.B. (1999) The vomeronasal organ. *Science* 286, 716–720

3 Holy, T.E. *et al.* (2000) Responses of vomeronasal neurons to natural stimuli. *Science* 289, 1569–1572

4 Leinders-Zufall, T. *et al.* (2000) Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* 405, 792–796

5 Halpern, M. *et al.* (1995) Differential localization of G proteins in the opossum vomeronasal system. *Brain Res.* 677, 157–161

6 Berghard, A. and Buck, L. (1996) Sensory transduction in vomeronasal neurons: Evidence for Gαo, Gαi, and adenylyl cyclase II as major components of a pheromone signalling cascade. *J. Neurosci.* 16, 909–918

7 Dulac, C. and Axel, R. (1995) A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83, 195–206

8 Herrada, G. and Dulac, C. (1997) A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell* 90, 763–773

9 Matsunami, H. and Buck, L.B. (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* 90, 775–784

10 Ryba, N.J. and Tirindelli, R. (1997) A new multigene family of putative pheromone receptors. *Neuron* 19, 371–379

11 Liman, E.R. *et al.* (1999) TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5791–5796

12 Ranganathan, R. *et al.* (1995) Signal transduction in Drosophila photoreceptors. *Annu. Rev. Neurosci.* 18, 283–317

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Meeting Report

Brain repair – new avenues to an eternal dream?

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Molecular and Cellular Mechanisms of Brain Repair, III Neurobiology Conference. Held at Villa Gualino, Turin, Italy; 11–14 October, 2000.

Adult mammalian CNS neurones show a limited capacity to regenerate after lesions. This might be ascribed to both intrinsic properties of CNS neurones and influences of the surrounding environment. The third neurobiology conference served as a platform for discussions between basic scientists involved in research on the molecular mechanisms of the neuronal cell body response to injury, and on strategies to promote CNS regeneration (Box 1).

The neuronal cell body response to lesions

CNS neurones react to axotomy in a stereotypic manner, termed the cell body response (CBR). As explained by T. Herdegen (Dept of Pharmacology, University of Kiel, Germany), activation of the c-JUN transcription factor and its upstream kinase c-JUN N-terminal kinase (JNK) plays a central role in the CBR. However, these molecules appear to be standby modules, which assist but do not ultimately decide on the outcome of the neurone with respect to death, survival, or regeneration[1]. Regeneration can be diverse among different neuronal populations. By contrast with a previous assumption, W. Tetzlaff (Dept of Zoology and Surgery, University of British Columbia, Vancouver, Canada) showed that a high proportion of rubrospinal neurones might survive cervical spinal cord lesions but become highly atrophic. The neurotrophin BDNF (brain-derived neurotrophic factor) not only reverses this atrophy but can even stimulate the regenerative capacities of rubrospinal neurones in chronic spinal cord lesion models. These findings suggest that there might be a silent potential of axotomized CNS neurones to regenerate when the appropriate growth factors are provided. However, adult cerebellar Purkinje cells, which also survive axotomy, lack any sign of axonal elongation independent of trophic factor treatment (P. Strata, Dept of Neuroscience, University of Turin, Italy and C. Sotelo, INSERM, Paris, France). F. Rossi (Dept of Neuroscience, University of Turin, Italy) reported that extrinsic myelin-associated cues appear to sufficiently suppress CBR in Purkinje cells that also limits their regenerative response. The retinal ganglion cells (RGC) belong to the type of CNS neurones that degenerate following axotomy[2]. M. Bähr (Dept of Neurology, University of Tübingen, Germany) summarized the molecular mechanisms of lesion-induced RGC death and presented several strategies that could interfere with these signal transduction cascades. Apoptosis is the major mechanism by which CNS neurones die after axonal lesion and this process is mediated by a family of cystein proteases termed caspases. The initiator caspases, caspase-8 and caspase-9, and the effector caspase-3 are of central importance, because inhibition of these enzymes proves highly neuroprotective in *in vivo* animal models. However, with respect to the clinical use of these factors that inhibit caspases, such as neurotrophins or peptide and protein caspase inhibitors, difficulties in administration [e.g. poor ability of crossing the blood–brain-barrier (BBB), intracellular mode of action, long-term availability] often represent major obstacles. One approach to overcome these difficulties is to use somatic gene therapy techniques, using adenoviral or adeno-associated viral vector systems, which are currently under development (L. Maffei, Dept of Neurophysiology CNR, Pisa, Italy,

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