Commentary

What the Frog's Nose Tells the Frog's Brain

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The olfactory system has the challenging task of responding to thousands of structurally dissimilar odors. In the last decade, advances in molecular biology and genomics have made it possible to obtain an understanding of the diversity of olfactory receptors (Mombaerts, 1999; Buck, 2000). In the mouse, for example, there are over 1,000 different olfactory receptor genes (Zhang and Firestein, 2002), and each olfactory receptor neuron (ORN) expresses only one of these olfactory receptors through a remarkable mechanism that includes feedback by the olfactory receptor protein excluding expression of all other olfactory receptor genes (Serizawa et al., 2003). Therefore, it is thought that each ORN will respond to a set of structurally related odors that stimulate the particular olfactory receptor expressed in that cell (Bozza et al., 2002). Thus, the information on odor stimuli that is sent to the brain is conveyed by the subsets of olfactory receptor neurons that express olfactory receptors stimulated by a particular odor (the combinatorial code hypothesis). Because one receptor is expressed per ORN, and because it is assumed that there is no processing of information within the epithelium, information content is determined entirely by the subset of odorant receptors stimulated by a particular odor.

A full test of the combinatorial code hypothesis must include a test of the responsiveness of receptor neurons to stimuli. Ideally, the responsiveness of the complete set of olfactory receptor neurons to a large number of behaviorally relevant stimuli should be determined. This, of course, is currently impossible. The number of biologically relevant stimuli for a rodent, for example, is in the thousands, and a complete study encompassing all subsets of olfactory receptor neurons would entail recording from thousands of neurons in order to parse the entire repertoire of >1,000 olfactory receptors.

The article by Manzini and Schild (2004) in this issue takes an interesting approach to this problem: They use stage 51–56 of tadpoles of *Xenopus*. Adults of this species express a particular subset of olfactory receptors (class I receptors) in a localized area of the olfactory epithelium (the lateral diventriculum) (Freitag et al., 1995; Mezler et al., 1999). Class I receptors have been proposed to respond to water-borne odorants. Thus, it is possible to simplify the ORN responsiveness sampling problem by studying the responsiveness of ORNs expressing this one class of olfactory receptors. In addition, previous work from these investigators shows that, in *Xenopus* tadpoles, these ORNs respond to amino acids, a subset of aqueous chemosensory stimuli that are known to be behaviorally relevant for amphibians and fish and well characterized chemically and physiologically (Caprio and Byrd, 1984). To further simplify the sampling problem, Manzini and Schild (2004) developed a slice preparation, which they can use to record odor-elicited calcium changes in a large number of ORNs in a reliable manner for prolonged periods of time.

Using this *Xenopus* tadpole slice preparation, Manzini and Schild (2004) present the most comprehensive study to date of the in situ responsiveness of olfactory receptor neurons. The authors recorded the responses of 283 ORNs to 19 amino acids, and they find that 204 of the 283 responses differed from each other. Thus, while some ORNs responded only to individual amino acids, several responded to a specific subset of amino acids. 36 of these response patterns occurred more than once. Presumably, if the authors had measured responses from a larger number of neurons all 204 individual patterns would occur more than once.

The large number of response patterns is surprising. Four class I olfactory receptors are known to be expressed in *Xenopus* lateral diventriculum (Freitag et al., 1995). The total number of class I receptors is likely to be larger, but is unlikely to be >100, based on genomic studies ranging from zebrafish to humans (Kratz et al., 2002). Considering that Manzini and Schild (2004) used a subset of water borne stimuli, how can such a large number of individual response patterns be explained within the context of the combinatorial code hypothesis—taking on account the finite number of olfactory receptors? The number of response classes seems to be too large to be compatible with the assumption that each ORN expresses a single receptor; but the authors make the interesting finding that there is a narrowing of ORN selectivity over developmental stages. This could suggest that changes in olfactory

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receptor expression may occur in individual ORNs throughout development.

A full understanding of the relationship of odor receptor expression to the information content of the input from the frog's nose to the brain will have to await further advances in olfactory receptor expression in single ORNs coupled with high throughput functional analysis of ORN odor responsiveness. In particular, feedback regulation of monoallelic receptor expression by olfactory receptor proteins might be faulty at early stages of development. In the mean time, the work of Manzini and Schild (2004, in this issue) clearly indicates that, throughout development, the information content of the input into the brain should not necessarily be assumed to be that implied by the combinatorial code hypothesis in its most parsimonious form. Interactions between ORNs in the epithelium, or between axons in axon bundles, and/or unexpected patterns of receptor expression in ORNs may lead to unexpected responsiveness of individual ORNs. To fully test the combinatorial code hypothesis, and to distinguish among the preceding and other possible interactions, it will be necessary to perform additional high throughput studies of ORN responsiveness in this and other species.

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