

Olfactory Axons: A Remarkable Convergence

Dispatch

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Thousands of neurons expressing a given mouse odorant receptor project to a few glomeruli in the olfactory bulb. New observations on mice expressing small odorant receptor transgenes provide support for the idea that interdependence is involved in the maturation of this remarkable convergence.

The mammalian olfactory system allows the discrimination of many thousands of different odorants and provides information critical to a number of complex behaviors. The groundbreaking identification of odorant receptor genes by Buck and Axel [1], a little over a decade ago, has opened the way to the unraveling of the organization of the mammalian olfactory system. Odorant receptors are G-protein-coupled receptors that bind odorants and initiate a signal transduction cascade culminating in an action potential which is transmitted directly to the olfactory bulb in the central nervous system. There are over 1,000 different odorant receptor genes, yet each neuron appears to express only one allele of a single receptor gene [2,3].

Spatial restrictions play a role in the coding of olfactory information. The neurons expressing a given receptor are restricted to one of four partially overlapping zones in the olfactory epithelium, and within their zone these neurons appear to be randomly distributed amongst others expressing different odorant receptor genes. In their projections to the olfactory bulb, the thousands of neurons expressing a given receptor converge. The pattern of convergence is, at first approximation, quite simple: there are roughly 1,000 glomeruli and the neurons expressing a given receptor go to one of these thousand. The actual pattern observed in the bulb appears more complicated because the thousand-glomerulus array is actually repeated four times.

The left and right olfactory bulbs are spherical structures with glomeruli arranged on the entire surface; the medial and lateral surfaces of each olfactory bulb are mirror images of each other, and the entire left and right bulbs are mirror images of each other. There is a high degree of animal-to-animal consistency in the positions of the individual glomeruli. Exactly how each olfactory neuron projects to the correct glomerulus is currently unknown, but accumulating evidence from receptor-swap experiments suggests that the odorant receptor protein itself is instructive in axon guidance of primary olfactory neurons [4,5]. The neurons appear to use information from the zone where they reside and

the actual receptor that they express to find their targets in the olfactory bulb.

The stereotyped convergence in the olfactory bulb creates a spatial map of olfactory receptor responses: a map of 'olfactory receptor space' if you like. Different spatial activation patterns may correspond to distinct odorants. The spatial patterns are also stereotyped at the next level, the projections to cortical structures [6]. Again, in cortical regions examined, neurons connected to neurons expressing a given odorant receptor are found in stereotyped positions. It is not known if the specifics of the stereotyped map in the olfactory bulb are required for formation of the stereotyped maps in the cortex. Alternatively, it is possible particular neurons projecting to the cortex have their axon guidance processes specified irrespective of the exact location of the glomerulus they receive input from.

A number of mechanistic questions are raised by the organization of the peripheral olfactory system. First, what accounts for the observed expression patterns of odorant receptor genes; and, second, how does a neuron expressing a given receptor project to the correct glomerulus in the olfactory bulb? Starting with less than 10 kilobases of genomic DNA for each of two odorant receptor genes — *MOR23* and *M71* — and then making a set of deletions, Vassalli *et al.* [7] have now shown that small sequences are sufficient to drive expression in olfactory neurons. For all of the transgenes the authors describe, the genes were marked by inserting an internal ribosome entry site followed by sequences coding for the reporter fusion proteins tau-lacZ or tau-GFP, which allow the identification of cell bodies and axonal projections.

With respect to the control of odorant receptor gene expression, in certain aspects this work confirms what was shown previously by Qasba and Reed [8], but the present studies go further because Vassalli *et al.* [7] marked the projections of the neurons expressing the transgene and were able to observe convergence in the olfactory bulb of the transgene-expressing neurons. This convergence is similar to that observed earlier with larger transgenes [9–11], but is more exact, overlapping with the convergent projections of the neurons expressing the corresponding endogenous allele. Similar observations with small transgenes have been obtained by B. Shykind and R. Axel (unpublished data cited in [12]).

It appears that the mechanism that chooses an odorant receptor gene for expression has the ability to activate one piece of DNA at the expense of all other potentially expressed genes. The question of how two identical sequences can be differentially treated in a single nucleus was first raised by the discovery of monoallelic expression of odorant receptor genes [2]. The most striking thing about all the transgenes studied is that they are expressed in different cells to those expressing the corresponding endogenous

alleles ([7,8,9,11] and B. Shykind and R. Axel, personal communication). Moreover, in the cases where the transgenes have been analyzed in the homozygous state, they appear to show monoallelic expression ([7,9,11] and B. Shykind and R. Axel, personal communication).

The further delineation of the *cis* regulatory elements that control receptor gene expression should prove interesting and may provide a foundation for addressing the key question: how is just a single DNA molecule chosen for expression in a given olfactory neuron? The answer might lie in a model in which a single site in the nucleus of a particular olfactory neuron allows the binding and activation of one and only one odorant receptor gene. All other genes within a given neuron — including the second allele of the chosen gene or other copies introduced by transgenes — have lost in the competition for this singular site and are therefore transcriptionally silent.

A very intriguing observation deriving from the experiments with small transgenes pertains to the issue of how axon targeting is accomplished and how the resulting glomeruli are maintained. Recall that the convergent projections appear to be a function of the zone in which a neuron resides and the actual odorant receptor gene it expresses. In certain lines analyzed by Vassalli *et al.* [7], a significant fraction of neurons expressing the transgene lie outside the normal zone of expression of the corresponding endogenous gene. The authors found that these zonally ectopic neurons create glomeruli distinct from the glomeruli receiving input from neurons expressing an endogenous allele of the corresponding odorant receptor gene.

This result by itself is not surprising, given that receptor-swap experiments [4,5] and other prior experiments with transgenes [10,11] have yielded similar results. The striking observation is that some of the neurons expressing an endogenous allele corresponding to the transgenic allele project to the ectopic glomeruli. This apparent recruitment is observed in animals in which an endogenous receptor allele is marked with tau-GFP so that its projections can be compared to tau-lacZ-marked transgene-expressing neurons.

The observed recruitment is reminiscent of the interdependence model proposed a couple of years ago [10]. The interdependence model stipulates that the neurons expressing a given receptor independently find their respective ways to one of a few particular spots in the olfactory bulb and begin to form a glomerulus. Interdependence comes into play in the maintenance of the glomerular structure after its establishment; a certain number of input axons are required to maintain a glomerulus into adulthood.

The impetus for proposing the interdependence model came from analyses of mice carrying an *M12* odorant receptor transgene, in which the neurons expressing the transgene converged to a glomerulus distinct from the one receiving inputs from neurons expressing the endogenous *M12* gene. In the newborn transgenic mice, convergence of the transgene-expressing neurons was always observable, but strikingly, in adult animals, convergence was not always

observed — so the convergence at birth was not always maintained.

In these experiments, the probability of maintaining a given glomerulus was shown to be a function of the number of neurons expressing the transgene. Animals expressing the transgene in a relatively large number of neurons — approaching the number of neurons expressing an endogenous allele — always showed convergence, whereas when a relatively small number of neurons expressed the transgene, convergence was never seen. When an intermediate number of neurons expressed the transgene, convergence was sometimes present, sometimes not. Sometimes the left and right sides of the olfactory system in a single animal were drastically different: while equal numbers of cells expressing the transgene were present on both sides of the olfactory epithelium, convergence was present only on one side of the olfactory bulb. Thus there appears to be a stochastic component to the maintenance process.

In the case of the small transgenes analyzed by Vassalli *et al.* [7], the observed recruitment of the projections of neurons expressing an endogenous allele corresponding to the transgene might actually be due to the maintenance of projections of neurons that normally are present in an ectopic zone and thus project to ectopic portions of the olfactory bulb. In non-transgenic mice, these ectopic sites would not receive enough inputs to form and maintain a glomerulus, but in the transgenic mice the transgene-expressing neurons would provide the critical mass of inputs to form and maintain the ectopic glomeruli.

It will be interesting to examine older animals expressing small transgenes — especially those transgenic lines where a relatively low number of neurons express the transgene — to determine if the observed 'ectopic' glomeruli disappear over time. Vassalli *et al.* [7] also raise the possibility that homotypic interactions among axons expressing the same receptor might play a role in the mis-targeting of endogenous *MOR23*-expressing axons. Further experiments will be required to clarify the relative contributions of these mechanisms. The interactions between the axons of the primary neurons and the second order neurons in the forming glomeruli also must play a role in the establishment and maintenance of glomeruli. As the mechanisms emerge, it will be interesting to compare ways that the olfactory system uses the interactions of like neurons to help in establishing mature wiring patterns with the ways that similar interactions are used in other parts of the nervous system.

References

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