The maps they are a-changin': plasticity in odor representation in interneurons

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Representations in excitatory neurons generally narrow as they are refined. Odor representations in interneurons, however, broaden with maturation and learning, as connections between interneurons and projection neurons expand.

Nobel prizes in literature, as in the sciences, go to those who capture universal truths. Bob Dylan released "The Times They Are a-Changin'" back in 1964, but its message seems to be more appropriate and timely than ever. In this issue of *Nature Neuroscience*, Quast *et al.* find unusual plasticity in odor representations in interneurons[1]. Plasticity in brain 'maps', spatial neural representations of the sensory world, has been studied for decades in the visual, auditory and somatosensory systems[2]. However, with few exceptions[3], the focus has largely been on representation in projection neurons.

For various reasons, interneurons were historically often thought to play second fiddle in neocortical circuits. Maps were often identified by tracing axonal projections, for example, from thalamus to sensory cortices. In addition, projection neurons in neocortex vastly outnumber interneurons. The long-recognized heterogeneity of interneurons and often seeming lack of spatial organization surely has not helped the case for investigating interneuron maps and their plasticity.

Yet interneurons reliably represent sensory information in a highly organized manner[4]. Furthermore, they are beginning to be recognized as the computational workhorses across brain regions and circuits[5]. Thus, understanding how sensory representations change in interneurons and how this is affected by development, maturation, learning and sensory deprivation indeed seems to be a timely task.

Quast *et al.*[1] cleverly leveraged several key features of the mouse olfactory bulb (OB) to address some of these questions (**Fig. 1a**). First, interneurons in the OB are certainly not fringe players: they make up 90% of the total neuronal population. Notably, the OB, which receives direct afferent input from olfactory receptor neurons in the nose, is a tightly layered structure. Granule cells (GCs), the interneurons that Quast *et al.*[1] studied, are neatly restricted to the core of the OB, allowing selective targeting using a combination of genetic and anatomically restrictive manipulations. Another crucial feature of the OB that Quast *et al.*[1] used to their advantage is that studying developmental plasticity is often inherently restricted to the very young animal in most of the brain. This presents considerable challenges for behavioral manipulations. However, GCs in the OB are among the very few neuronal cell types that are continuously generated throughout most of adult life, thereby allowing the study of neuronal maturation in the context of an adult animal[6]. Previous studies have shown that GC maturation and integration is strongly influenced by glutamatergic sensory input from projection neurons and neuromodulatory top-down activity, and learning odor–reward associations promotes survival of newborn cells, suggesting that contextual information is necessary for successful circuit integration.

Quast *et al.*[1] exploited this continuous GC neurogenesis by identifying two promoters that drive selectively expression in young or old granule cells, *Dlx5/6* and *Crhr1*, respectively (**Fig. 1a**). Targeting viruses that conditionally express the calcium indicator GCaMP6 into Dlx5/6-Cre animals allows the restriction of expression to GCs that are young and immature. When physiology experiments were performed 2 weeks after virus injection, these GCs had only just integrated into the OB network. Conversely, Cre expression in Crhr1-Cre animals is restricted to old, mature GCs. Thus, targeting the same viral construct to the GC layer resulted in GCaMP6 fluorescence selectively in mature, fully integrated GCs. Notably, the authors found that the Crhr1- and Dlx5/6- expressing populations are the same kind of cells, just captured at different points during maturation. This neat trick, using adult neurogenesis, stage-specific genetic markers and anatomical targeting, allowed the authors to follow GC populations during maturation or behavioral manipulations.

Using wide-field Ca^{2+} imaging in anesthetized mice, the authors could then assess the extent of activation of these targeted GCs. Consistent with previous work, they found that spatial odor representation in GCs was regionally restricted, albeit less so than in afferent inputs or projection neurons. (We use 'spatial representation' rather than 'map' for the olfactory system. Map, at least in its original, precise mathematical meaning, implies a continuous transformation from sensory space to geometric space in the brain. As the surface of the OB is a two-dimensional structure, this would imply that input space—olfactory sensory space—would have to be at most two-dimensional as well[7]. Although the dimensionality of olfactory sensory space is a hotly debated topic[8], it seems unlikely to be as low as two. Consistent with that, representation in the OB is generally not continuous[9].) Surprisingly, however, the authors found that maturation did not sharpen this representation (as is often seen in projection neurons), but instead broadened it, engaging GCs across a wider area of the OB (**Fig. 1b**). When Quast *et al.*[1] in turn reduced sensory experience by unilateral naris occlusion, they

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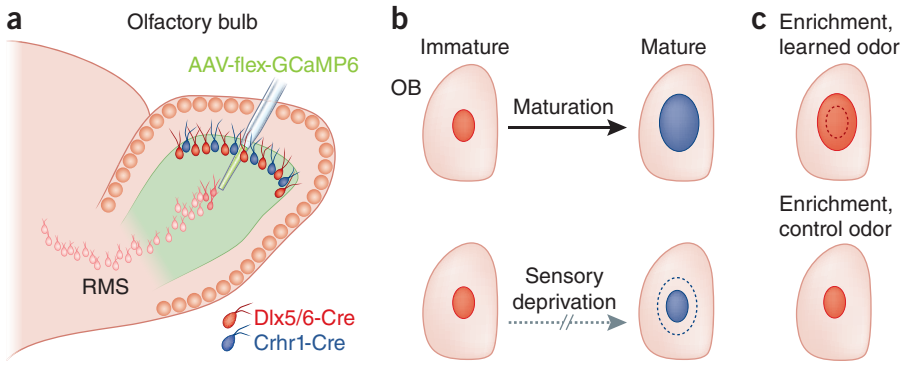

Figure 1 Odor representation in interneurons expands during maturation and learning. (**a**) Genetically targeted viral GCaMP6 expression restricted to two developmentally distinct OB granule cell populations. Dlx5/6-Cre drives GCaMP6 expression in young, immature GCs, whereas Crhr1 does so in old, mature ones. The GC layer is shown in green. RMS: rostral migratory stream. (**b**) Interneuron activity in the olfactory bulb (OB) assessed by wide-field Ca^{2+} imaging revealed an expansion of spatial odor representation over the course of GC maturation, subject to sensory stimulation. (**c**) Engaging animals in a go/no-go discrimination task broadened the representation in young, immature granule cells for learned odors, but not control odors.

observed less broadening: that is, old, mature GCs behaved almost like young, immature ones (**Fig. 1b**). When animals were engaged in a go/no-go discrimination task, representations were broad even in immature GCs (**Fig. 1c**). However, this broadening was restricted to learned odors, with novel odors still evoking narrow representations in young GCs (**Fig. 1c**).

How does this work? Although the detailed mechanisms behind these changes still await investigation, Quast *et al.*[1] provide some clues. In *in vitro* experiments, they found that mature GCs were indeed better integrated; that is, they made functional contacts with more projection neurons, in particular with mitral cells (MCs) that were further away. Although individual GC dendritic arbors are compact, stretching across only ~200 µm, their partners, MCs, have long lateral dendrites. Given that both MC and GC dendrites are both input and output structures, this enables GCs to influence (and be influenced by) MCs as far away as 1 mm in all directions. Quast *et al.*[1] showed that immature GCs could only be engaged by stimulating nearby MCs (substantially closer than 250 µm). In marked contrast, mature GCs were excited by MCs across all distances studied (up to 500 µm). Although GC spines, the site of MC–GC synapses, are indeed particularly plastic structures[10], the rules that establish and maintain these contacts have yet to be elucidated. One aspect might be geometry; for example, proximal, but not distal, parts of MC lateral dendrites may be close to the MC layer and in easy reach of immature GCs. Another key factor could be differential activity in proximal and distal lateral dendrites: action potentials propagating into MC lateral dendrites with an amplitude decreasing with distance could make proximal contacts easier to establish than distal ones. More generally, specific, yet-to-be-identified molecular cues might underlie this potential selective contacting.

Is there any computational benefit to this maturation? Inspired largely by a comparison with retinal circuits, GCs have long been thought to be involved in improving odor representation in projection neurons. Indeed, behavioral studies and manipulations of GCs suggest that GCs help to refine odor discrimination[11]. This is likely only a small part of the GC job description, as they have possible additional roles in integrating prior information or aiding identification of components in a mixture[12,13]. On a physiological level, although it has become clear that GCs are not implementing any retina-like spatial contrast enhancement[14], they are known to orchestrate activity of MCs, in particular on fast (gamma, 30–80 Hz) timescales[14], potentially synchronizing ensembles of MCs. A central unknown and a key challenge in the quest to understand the function of GCs is how GCs are connected to MCs: is it a random connection matrix, is connectivity distance-dependent, or are connections spatially clustered or structured in any particular way reflecting the statistics of odor activation?

Quast *et al.*[1] have provided key insights into how maturing GCs integrate into the projection neuron network, progressively connecting to more distal MCs. The authors demonstrate that this integration occurs during learning specifically for GCs activated by the learned odors. This process of integration might allow GCs to establish a specific connectivity with MCs and selectively boost synchronization for MCs that are engaged by a learned odor[15]. Recordings from projection neurons, as well as further recordings from GCs with higher temporal resolution, might help to challenge this hypothesis. It might well be that maturation not only alters the spatial integration of GCs in the network, but also changes their temporal response profiles to, for example, enable better or faster odor discrimination[11]—maybe in the way that Bob Dylan predicted: "The slow one now / Will later be fast."

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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