Long-Range Intracortical Excitation Shapes Olfactory Processing

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Recurrent intracortical connections are believed to be especially dense in the piriform cortex. Two studies in this issue of *Neuron* report several interesting features of the long-range intracortical connections and reveal their physiological roles in shaping olfactory processing.

Odor molecules have drastically different physicochemical characteristics. How are neurons connected in the mammalian olfactory system to detect and discriminate vastly different odors? By the stage of the main olfactory bulb (MOB), the wiring appears to be largely based on the identity of odorant receptors (ORs; Figure 1). In mice, each olfactory sensory neuron (OSN) expresses only one OR gene out of the repertoire of over 1000, and OSNs expressing a common OR send convergent axonal projections to roughly 2 glomeruli in the MOB (Buck and Axel, 1991; Mombaerts et al., 1996). Each glomerulus is associated with a subset of 25–50 mitral/tufted cells, which receive primary excitatory input from isofunctional OSNs and respond selectively to the odor ligands of their related OR (Tan et al., 2010). An individual odorant evokes a stereotypical spatial activation pattern at the glomerular layer in the MOB (Rubin and Katz, 1999), which is then transmitted to the piriform cortex through the axons of mitral/tufted cells via the lateral olfactory tract (LOT). Surprisingly, individual odorants evoke sparsely and randomly distributed sets of neurons in the piriform cortex (Stettler and Axel, 2009).

The abrupt randomization of cortical activation patterns might be generated by divergent projections from the bulb to the cortex and/or associative connections within the cortex. Recent tracing studies reveal that the axonal terminals of individual mitral/tufted cells are diffusively distributed throughout the piriform cortex (Ghosh et al., 2011; Sosulski et al., 2011). Transsynaptic tracing and intracellular recordings show that individual pyramidal neurons (PNs) in the piriform integrate inputs from at least scores of glomeruli (Davison and Ehlers, 2011; Miyamichi et al., 2011). In addition to bulbar inputs, PNs in the olfactory cortical areas are believed to receive extensive recurrent intracortical connections (Haberly, 2001). However, the exact nature and physiological importance of intracortical associative connections have not been clearly established in the olfactory system. In this issue of *Neuron*, two elegant studies provide direct evidence for the presence and functional roles of long-range cortical excitation in the piriform cortex (Franks et al., 2011; Poo and Isaacson, 2011).

In the Franks et al. study, the authors used optogenetics to dissect intracortical connections in brain slices (Franks et al., 2011). By delivering genes with viral vectors, the authors expressed the light-sensitive channel Channelrhodopsin-2 in a focal cluster of neurons in the mouse anterior piriform cortex. These $ChR2^+$ neurons were activated by brief light pulses and their effects were examined by whole-cell recordings from $ChR2^-$ PNs at different distances from the center of viral infection. In a vast majority of recorded cells, light stimulations evoked large monosynaptic excitatory postsynaptic currents (EPSCs). Strikingly, the photocurrents produced by intracortical excitatory connections remained equally strong across millimeters in the piriform cortex. In contrast, photocurrents steeply decreased with increasing distance in the primary somatosensory and visual cortex, suggesting unique cortical circuits for olfactory processing.

By comparing the amplitude of photocurrents evoked by single $ChR2^+$ axonal inputs with that of quantal EPSCs, Franks et al. (2011) find that a recurrent axon forms only one functional synapse with a specific PN and its activation leads to the transmitter release from at most a single synaptic vesicle. Based on the saturated amplitude of overall photocurrents, the authors estimate that a PN receives ~20 activated inputs in response to the stimulation of ~8,000 $ChR2^+$ neurons. Extrapolating this data to the assumed overall number of 1 million PNs in the piriform, the authors speculate that individual PNs may receive recurrent excitatory inputs from over 2,000 cortical neurons. Because a substantial number of extended neuronal processes may be sectioned in slice preparations, this number might even be an underestimation.

In a neural network with extensive recurrent excitatory connections, odor-evoked activity of any single neuron could lead to continuous propagation of action potential firing and may even create epileptic overexcitation. Consistent with an earlier study showing the presence of global inhibition in the piriform (Poo and Isaacson, 2009), Franks et al. (2011) find that light stimulation also generates distant inhibitory responses. The strengths of inhibition scale with stimulus intensities and are often larger than those of excitation produced by intracortical recurrent connections. The inhibition is substantially blocked by glutamate receptor antagonists, suggesting that it is mainly produced by polysynaptic activation of local GABAergic neurons.

Do the intracortical connections play any role in processing incoming sensory signals from the bulb? Franks et al. (2011) show that these connections can either

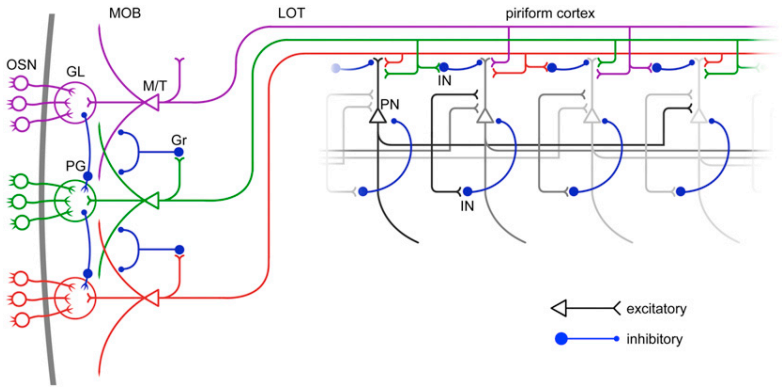

Figure 1. Highly Simplified Schematics Illustrating the Neuronal Wiring Patterns in the Olfactory System

The projection from the olfactory epithelium to the MOB conforms to the rule of "labeled-line" so that a given glomerulus receives converging input from OSNs expressing a specific OR and drives the excitation of an exclusive subset of mitral/tufted (M/T) cells. However, the projection of M/T cells to the piriform cortex becomes random and diffusive. Individual PNs integrate M/T inputs associated with scores of glomeruli. In addition, PNs extend long-range axonal outputs to excite other PNs and at the same time receive recurrent cortical inputs from thousands of PNs. GABAergic interneurons (INs) mediate both feed-forward and feedback inhibitions to stabilize the cortical circuit. GL, glomerular layer; Gr, granule cells; PG, periglomerular neurons.

increase or reduce the effects of bulbar inputs on the firing activity of PNs. In another study in this issue, Poo and Isaacson (2011) provide direct demonstration that intracortical excitatory connections enhance neuronal responses to odor stimuli.

Poo and Isaacson (2011) performed challenging in vivo whole-cell recordings from rat PNs and used an in vivo pharmacological approach to selectively silence intracortical connections. Functional $GABA_B$ receptors are expressed on the axonal terminals of cortical neurons in the piriform but are absent on those of mitral/tufted cells. Local application of baclofen, a $GABA_B$ receptor agonist, selectively abolished intracortical excitation but left the LOT-evoked excitation largely intact. Interestingly, a majority of odor-evoked EPSCs is blocked by baclofen application, suggesting that intracortical connections but not bulbar inputs determine the strength of odor responses of PNs.

Moreover, Poo and Isaacson (2011) find that the effect of baclofen on olfactory responses depends on the response selectivity of individual PNs. Baclofen mildly reduces odor-evoked EPSCs for selectively tuned neurons, but strongly blocks the excitatory responses and thus sharpens the tuning for broadly-tuned neurons. In contrast, baclofen exerts similarly effective blockade of odor-evoked inhibitory responses on all neurons irrespective of their excitatory tuning. Because all PNs appear to receive the same amount of excitation from LOT inputs, the authors suggest that intracortical excitations might contribute strongly to the odor-responses of broadly-tuned neurons but may have weak effects on selectively responsive neurons.

Together, these studies (Franks et al., 2011; Poo and Isaacson, 2011) offer several insights into our understanding of the circuit wiring scheme in the piriform cortex (Figure 1) and suggest important roles of recurrent intracortical connections in shaping cortical odor representation. Their results indicate that, although the chance of any pair of PNs forming synaptic connections is low, an individual PN might receive excitatory inputs from thousands of other PNs across a distance of millimeters. In addition, PNs can activate local GABAergic neurons to generate global inhibition to maintain the balance of cortical activity. In response to an odorant, the bulbar input might activate a small subset of PNs, which then recruits a larger population of PNs and interneurons to generate a complex pattern of excitation and inhibition in the piriform. Some PNs may receive stronger intracortical excitation than others and thus exhibit broader olfactory tuning.

Results from these two new studies suggest additional experiments to pin down the exact wiring pattern in the piriform cortex. Do all PNs play equal roles in intracortical association? Morphologically identified PNs exhibit highly diverse olfactory tunings in terms of both excitation and inhibition in an awake mouse (Zhan and Luo, 2010). Both new studies indicate heterogeneity among PNs in receiving intracortical excitation. In vivo recordings show that broadly tuned neurons tend to be more frequently activated by odor-elicited intracortical excitation (Poo and Isaacson, 2011). Consistently, individual PNs appear to receive quite variable amount of recurrent excitation in slice preparations (mean ± SD: 441 ± 334 pA; Franks et al., 2011). It remains unclear whether PNs uniformly extend long-range axonal terminals to synapse on thousands of other PNs, or whether some subtypes of PNs exert much stronger influence on other PNs. This may be tested by serially activating small subset of $ChR2^+$ neurons with focal illumination and mapping area-specific effects on a group of $ChR2^-$ neurons. Interneurons represent another major class of cortical neurons. Although the interneurons are labeled as a single type of inhibitory neurons in the diagram (Figure 1), they can be divided into various subtypes based on their morphological, neurochemical, and functional features. The roles of these different interneuron subtypes in signal processing remain to be investigated. The latest development of Cre-Driver mouse lines for various cortical interneurons should facilitate the probing of their precise roles in olfactory processing (Taniguchi et al., 2011).

Lastly, the new findings further raise questions on the exact function of intracortical connections in olfactory percept formation. Recurrent neural networks may be particularly suited for enhancing perception robustness and olfactory learning (Haberly, 2001). For example, the glomerular spatial activation patterns evoked by a given odorant may vary because of the noises associated with stimulus delivery and intrinsic circuit activity. Intracortical recurrent connections provide the function of pattern completion such that an identical population of cortical neurons is activated by an odorant despite variable LOT inputs (Barnes et al., 2008). However, inappropriate recurrent connections would impair perception precision and stability in an individual animal

as well as perception consistency across individuals. An overly strong capability of pattern completion would deny an animal its ability to discriminate different odorants. Excessive plasticity would lead an animal to perceive the same object differently after an unreasonably short period. Similarly, completely random connections would make it difficult to generate consistent perception across individuals, so that fruits may no longer consistently smell "fruity" to human individuals. The connections in the piriform cortex must be carefully carved to achieve a delicate balance among different behavioral needs. The wiring stochasticism in the piriform cortex highlights the needs and challenges of searching for logic in the neural circuits underlying animals' amazing sense of smell.

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MeCP2: Phosphorylated Locally, Acting Globally

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In this issue of *Neuron*, Greenberg and colleagues revise our understanding of how activity-dependent MeCP2 phosphorylation regulates distinct aspects of brain development and circuit function. The study also suggests a prominent role for MeCP2 in the regulation of global chromatin state in vivo.

MeCP2 (X-linked methyl-CpG-binding protein 2) is an abundant nuclear protein that binds methylated DNA and historically has been thought to act as a transcriptional repressor critical for normal neural development. Mutations in the gene encoding MeCP2 cause the Autism-spectrum disorder Rett Syndrome (RTT). In this issue of *Neuron*, Cohen, Greenberg, and colleagues demonstrate that activity-induced phosphorylation of MeCP2 at a single serine residue (S421) controls distinct aspects of synapse development and social behavior (Cohen et al., 2011). In contrast to prior studies implicating this phosphorylation event in the dynamic regulation of MeCP2 binding at specific promoters, the present study suggests that the primary function of MeCP2 in neurons is not to regulate transcription of specific genes but rather to regulate chromatin remodeling on a global scale.

DNA methylation is an epigenetic modification that plays an essential role in mammalian embryogenesis presumably through repressive effects on gene transcription. Functional studies have demonstrated that methylation of DNA can inhibit transcription by either blocking transcription factor access to target regions or by acting as homing sites for methyl-CpG binding domain proteins (MBDs) (Bird, 2002). Interest in DNA methylation and nervous system development took an unprecedented turn over a decade ago when Zoghbi and colleagues first identified independent mutations in the MBD and transcriptional repression domains of the human *MECP2* gene as disease-causing mutations leading to RTT (Amir et al., 1999). Rett syndrome is a progressive and debilitating neurodevelopmental disorder that predominantly affects young girls at an estimated 1–10,000–15,000 ratio. Mice that lack MeCP2 either globally or conditionally in the central nervous system develop symptoms similar to RTT (Chen et al., 2001; Guy et al., 2001). If MeCP2 functions as a transcriptional repressor, then the identification of genes dependent upon MeCP2 for proper transcriptional regulation should provide insight into the pathophysiology of RTT. Numerous groups attempted to answer this question by examining global transcriptional profiles from forebrain, hypothalamus, or