recognized as both rewarding and unrewarding depending on multiple contextual factors—that bulbar neuronal activity is not just modifiable, but intrinsically indeterminate and multistable, like a stimulus that can be either one thing or another depending on what surrounds it (Leopold and Logothetis, 1996).

Of course, any study that breaks new ground also raises as many new questions as it answers. Still to be understood, for instance, is the mechanism of mitral cell synchronization, which has somewhat different properties than that studied previously (Friedrich et al., 2004; Schoppa, 2006). While adrenergic feedback plays an undisputed role in shaping the number of SS emitted in response to particular odors, the way that this happens remains mysterious. It is also unclear whether, when coherently firing neurons are studied in larger ensembles, the observable patterns will become more complicated. Back at our choral concert, the introduction of a third voice adds further richness—atonality, for instance—to the information delivered in the music. Odors come with a richness of properties as well, above and beyond simple "reward-related" or not, which may be reflected only in the coherent firing of larger ensembles.

Also intriguing is the fact that coding odors in terms of their reward value does not necessarily imply more effective coding in terms of task performance. SS in trials in which the trained animal correctly identified an odor as rewarded (hits) did not differ from SS in trials in which the animal failed to respond to a rewarded odor (misses). This result (along with other well-thought-out controls performed by the authors, including contingency reversal tests) satisfactorily eliminates confounding nuisance variables such as reward-related motor behavior as explanations for the phenomenon, but begs the question of why, if bulbar neurons specifically signal that the proffered odor is reward related, the mouse fails to access the reward. It appears that representing the reward value of an odor may be necessary for correct task performance, but not sufficient; the generation of reward-relevant signals in OB is somehow independent of decision-making circuitry, which may sometimes fail to receive the message or fail to act on the message, depending on as-of-yet mysterious contextual variables.

But this is the job of high-quality research—not to simply add to the accretion of facts but to open up new vistas for study with results that surprise and challenge us. To add a new voice to the ongoing composition that changes the way the entirety is perceived. By revealing coding that is intrinsically "meaningful," Doucette et al. (2011) strike a new chord.

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Martinotti Cells: Community Organizers

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The specificity of connections made by inhibitory interneurons in the neocortex is not well understood. In this issue of *Neuron*, Fino and Yuste (2011) use an enhanced version of two-photon glutamate uncaging, which preserves inhibitory synaptic transmission, to demonstrate that somatostatin-positive interneurons form densely convergent connections onto pyramidal cells in layer 2/3 of mouse frontal cortex.

Like a sheaf of wiring diagrams that delineate the electrical circuitry of a building, the maps of synaptic connections between neurons are essential for a complete understanding of the inner workings of the brain. Recently developed optical and genetic tools have begun to clarify the connectivity of neural circuits with unprecedented spatial precision. Many questions still persist, particularly regarding the organization of inhibitory circuits in the neocortex, which remain enigmatic in part because of the diversity of interneuron types and methodological limitations.

Laser uncaging or photostimulation is a popular method of optically analyzing circuits. Caged compounds are molecules derived from neurotransmitters like

glutamate or GABA, which have inactivating chemical groups that can be rapidly photolyzed to convert the molecule into its bioactive form and allow binding to receptors on nearby neurons. Such uncaging is now increasingly achieved by two-photon excitation, which enables single-cell precision of neurotransmitter release and has led to exciting discoveries regarding excitatory circuits. Unfortunately, two-photon glutamate uncaging has not been as readily applied to the study of the organization of inhibitory neurons. This is because at the high (mM) concentrations necessary for two-photon uncaging, commonly used caged glutamate compounds such as MNI-glutamate strongly block GABAergic transmission. In this issue of *Neuron*, Fino and Yuste (2011) overcome this limitation by utilizing a new caged glutamate compound, RuBi-Glutamate, and demonstrate its effectiveness in a study on the connectivity properties of somatostatin-expressing interneurons.

RuBi-Glutamate was recently developed by Yuste and collaborators (Fino et al., 2009) and has the beneficial properties of a relatively high-absorption cross-section and a high-quantum efficiency of uncaging. This means that low concentrations of RuBi-Glutamate can be used with two-photon excitation to trigger spiking in presynaptic neurons and largely preserve postsynaptic GABAergic responses so that inhibitory connections can be detected. Armed with this new and improved version of two-photon glutamate uncaging, Fino and Yuste (2011) set out to study the pattern of connections formed by a specific subpopulation of neurons, somatostatin-positive interneurons, onto pyramidal neurons in layer 2/3 cortex. This was possible through the use of the GIN transgenic mouse line, which expresses GFP exclusively in somatostatin neurons (sGFPs; Oliva et al., 2000), of which about 80% were identified as Martinotti cells (McGarry et al., 2010). By using coronal brain slices, Fino and Yuste (2011) uncaged glutamate sequentially onto the somata of all sGFPs visible within a 600 × 800 μm field of view (which included all of layers 1–3) while simultaneously performing recordings of inhibitory postsynaptic currents in two or three pyramidal cells. This allowed them to determine whether each sGFP was connected or not to a given recorded pyramidal cell and thus to generate an input map for the pyramidal cell depicting all afferent connections from sGFPs. Moreover, by being able to visually identify and target all potentially connected sGFPs, they could accurately calculate the proportion of sGFPs that was actually connected to a given postsynaptic pyramidal cell and thus estimate the average connection probability of sGFPs onto pyramidal cells in layer 2/3 cortex.

Fino and Yuste (2011) analyzed more than 60 maps and observed a very high occurrence of connections made by sGFPs onto their neighboring pyramidal cells. Almost half of sGFPs within 400 μm and three quarters within 200 μm of a given pyramidal cell were connected. Interestingly, about a fifth of all of the pyramidal cells recorded had input connections from every single interneuron in the field (20 cells on average). The authors interpret this high convergence of interneurons onto a single pyramidal cell as also implying a high divergence of a single interneuron's connections to a neighboring population of pyramidal cells. While there is no direct evidence of this, it is a reasonable interpretation given the relatively small number of interneurons compared to pyramidal cells and the random selection of pyramidal cells by the experimenters.

To confirm the unexpectedly high degree of connectivity, the authors performed whole-cell patch clamp recordings of randomly selected pairs of sGFPs and pyramidal cells and observed a connectivity probability that closely agreed with that of their uncaging experiments. They also directly validated their method by intracellular electrical stimulation of the putatively presynaptic sGFPs identified by two-photon uncaging and detecting responses in the postsynaptic pyramidal cell, verifying that almost all (11 of 12) sGFPs were truly presynaptic to the pyramidal cell. It is worth noting that paired recordings were not performed to test the putatively unconnected sGFPs to determine false negatives, so the number of truly connected interneurons may actually have been underestimated in the uncaging experiments. The authors also demonstrated that the high density of interneuron connections to pyramidal cells was similar for adult and juvenile mice and therefore was not merely a transient pattern arising in immature brain circuits prior to undergoing synaptic pruning.

How unexpected is this high degree of connectivity from sGFPs onto pyramidal cells? It is certainly higher than estimations from previous studies using patch-clamp recordings of pairs or triplets of neurons (Thomson and Lamy, 2007). In layer 2/3 rat somatosensory cortex, the connection probability of somatostatin-containing interneurons onto pyramidal cells was 49% for intersomatic distance $\leq$ 50 μm (Kapfer et al., 2007), while in layer 2/3 rat visual cortex, the connection probability of adapting interneurons (which includes somatostatin-expressing neurons) onto a pyramidal neuron was 16% for an intersomatic distance of 40–50 μm (Yoshimura and Callaway, 2005). The high connection probability found in the present study may be due to methodological differences; for example, the capability of two-photon uncaging to stimulate interneurons at various depths may have provided a more accurate estimate of a connectivity probability that was previously underestimated.

Dense connections made by interneurons could contribute to the ubiquity of a common disynaptic inhibition motif, frequency-dependent disynaptic inhibition (FDDI), in which pyramidal cells inhibit each other via intermediate Martinotti cell activation. Martinotti cells, which were the majority of the interneurons recorded in the present study, are ubiquitously present in cortex. They regulate pyramidal cell activity via a significant axonal arbor in layer 1 that forms synapses onto apical dendrites of pyramidal cells, modulating dendritic spike generation and synaptic integration, as well as FDDI between pyramidal cells (Murayama et al., 2009). The probability of this disynaptic inhibition, around 20% in layer 5 frontal cortex of juvenile rats (Berger et al., 2009), suggests an underlying high degree of connectivity between Martinotti cells and pyramidal cells that is in line with the results in the present paper.

So what implications do the findings by Fino and Yuste (2011) have on the role of somatostatin-positive interneurons, such as Martinotti cells, in neocortical

function? It may be that Martinotti cells act as organizers of inhibitory activity across subnetworks of pyramidal cells. Pyramidal cells in layer 2/3 rat visual cortex tend to connect to each other preferentially and form fine-scale subnetworks (Yoshimura et al., 2005), while Martinotti cells connect to pyramidal cells from different subnetworks with equal probability (Yoshimura and Callaway, 2005). This is supported by experimental data from Fino and Yuste (2011), who compared the input maps for connected and unconnected pairs of pyramidal cells and discovered that the probability of receiving common inputs from neighboring sGFPs was not significantly higher for connected pyramidal cells than unconnected pyramidal cells. Thus, Martinotti cells have their own agenda and flaunt the subnetwork schema laid out by the pyramidal cells to indiscriminately connect to the pyramidal cells regardless of their subnetwork affiliation.

In light of this network topology, we can consider the implications of the highly convergent and (implied) highly divergent connections made by Martinotti interneurons onto pyramidal cells. If a single Martinotti cell is activated by high-frequency input from a pyramidal cell in subnetwork A, it will inhibit many pyramids in subnetwork A (feedback inhibition) but also equally as many in subnetwork B (lateral inhibition). This divergence may allow Martinotti cells to act as distributors of inhibition across all the pyramidal cell subnetworks within a local region and serve to decrease the gain of pyramidal cell output or facilitate synchronous activity. However, the inhibitory effect of a single Martinotti cell is modest (Kapfer et al., 2007; Silberberg and Markram, 2007), so convergent inputs from multiple Martinotti cells are probably necessary for effective inhibition of pyramidal cells. Therefore, when a small number of Martinotti cells are activated, network inhibition may not be triggered (or may only occur in few pyramidal cells), but when a large number of Martinotti cells are activated, for example if a subnetwork of pyramidal cells fires synchronously, the Martinotti cells will then cause strong convergent inhibition onto pyramidal cells across different subnetworks. Martinotti cells may thus be preferentially activated during synchronized excitatory activity in a local region, serving to balance excitation and prevent runaway cortical activity. Indeed, it has previously been shown that the recruitment of Martinotti cells increases supralinearly with the number of active pyramidal cells, effectively limiting cortical excitability during synchronous pyramidal cell activity (Kapfer et al., 2007). Also, the incidence of FDDI in a local (<150 μm) group of pyramidal cells increases exponentially as a function of the number of simultaneously activated pyramidal cells in layer 5 rat somatosensory cortex (Berger et al., 2010). These results are consistent with the concept of Martinotti cells acting together as strong effectors of inhibition.

An interesting parallel can be drawn between Martinotti interneurons and neurogliaform interneurons. Neurogliaform cells are ubiquitous in the cortex and have very dense axonal arborizations. Neurogliaform cells have the unique ability to induce long-lasting inhibition by producing an atypically slow $GABA_A$ response (Szabadics et al., 2007) as well as efficiently evoking $GABA_B$-receptor-mediated responses in postsynaptic neurons. A single neurogliaform cell is able to release a dense cloud of GABA, inducing volume transmission (Oláh et al., 2009). This dense cloud of inhibition allows the neurogliaform cells to nonsynaptically inhibit virtually all of the cells within its axonal field (<200 μm). Both Martinotti cells and neurogliaform cells similarly lead to a suppression of activity of nearly all cells in a local region. Martinotti cells would primarily suppress pyramidal cells, while neurogliaform cells would inhibit pyramidal and GABAergic neurons indiscriminately, yet both tend to have slow-onset responses (delays of tens to hundreds of milliseconds) and may share a general function of dynamically suppressing cortical excitability in a local region by increasing their inhibitory input in response to incoming excitatory activity.

The work of Fino and Yuste (2011) is a culmination of many technical advances by their research group and others and is a valuable stepping stone for future studies of neocortical circuit architecture. The high-efficiency RuBi-Glutamate caged compound largely preserves GABAergic transmission, enabling the mapping of inhibitory connections. The two-photon uncaging has single-cell precision and is high-throughput, due to automated cell identification and optimal path computation for sequential cell targeting. This enables the stimulation of up to 1000 neurons in less than 5 min (Nikolenko et al., 2007) so all of the potential presynaptic cells of a certain subtype can be probed, providing a comprehensive account of connectivity probability.

Exciting technological advances lie ahead. This technique would be made even more influential by the added functionality of measuring relative synaptic strengths. The net effect of a population of neurons depends heavily on the strengths of its connections and subtle gradients in the synaptic strength matrix can underlie starkly different network behaviors. Here, the binary results of Fino and Yuste (2011) demonstrate the maximum possible connectivity in the network because very weakly connected interneurons may not be able to induce inhibitory responses in postsynaptic pyramidal cells during physiological states in vivo. Thus, only a subset of the existing dense connections of interneurons to a given pyramidal cell may serve to modulate pyramidal cell output and it would be very useful to have this information embedded in our maps of synaptic connectivity.

Another useful extension of the present work would be to combine it with calcium imaging that would enable the rapid identification of all neurons postsynaptic to an interneuron targeted with glutamate uncaging. By categorizing the subtypes of these postsynaptic neurons, for example by post hoc immunostaining (Kerlin et al., 2010), this method could potentially provide a remarkably complete map of inhibitory connections. Furthermore, combined glutamate uncaging and calcium imaging would be useful for circuits with a high degree of recurrent connectivity such as layer 2/3 cortex as it would be advantageous to be able to rapidly examine bidirectionality of connections. The ever-increasing array of optical, genetic, and electrophysiological tools will allow comprehensive, high-resolution functional maps of synaptic connections for different cortical layers, cortical regions, and species to soon lie within our reach.

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