Signal Integration in Thalamus: Labeled Lines Go Cross-Eyed and Blurry

Benjamin K. Stafford[1,2,4] and Andrew D. Huberman[1,2,3,4,*]
[1]Department of Neurobiology
[2]Department of Ophthalmology
Stanford University School of Medicine, Stanford, CA 94305, USA
[3]BioX, Stanford University, Stanford, CA 94305, USA
[4]Salk Institute for Biological Studies, La Jolla, CA 92037, USA
*Correspondence: adh1@stanford.edu
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The brain uses sensory information from the periphery to create percepts. In this issue of *Neuron*, Rompani et al. (2017) show that visual signals are combined in unexpected ways that vastly expand the possible representations of the outside world.

The brain creates perceptions from incoming sensory information in two main ways. The first way is to maintain a given quality of information about the outside world in its pure, unadulterated form (sometimes called a "labeled line") as it is passed from one stage of neural processing to the next. The second way is to merge different lines of sensory information—through convergent synaptic wiring, in order to create new, often more elaborate perceptual representations. A classic example of this occurs in the visual system where the outputs of "spot detector" neurons in the visual thalamus converge onto cells in primary visual cortex (V1), thereby endowing V1 neurons with the property of orientation selectivity (Chapman et al., 1991). Where along the neuraxis sensory afferents get merged and the various integration strategies they employ at each processing station serve as bottlenecks to the types and range of perceptions the brain can create and thus is important to our experience of the world. In this issue of *Neuron*, Roska and co-workers (Rompani et al., 2017) reveal the surprising finding that, even at very early stations of subcortical sensory processing, many visual signals that were long thought to be kept separated from one another, actually get combined. The implications of these findings extend beyond the visual system and raise new models about brain structure, function, and change, in particular cortical processing and plasticity.

In general, the merging of labeled lines to create new sensory representations is thought to occur first and primarily within the neocortex. Neurons residing in subcortical areas like the thalamus, on the other hand, are thought to act largely as sensory relays by boosting or suppressing the strength sensory signals destined for the cortex but not fundamentally modifying the quality of information they convey. The problem with this idea, which has dominated neuroscience textbooks and models for decades, is that there are actually only a handful of concrete examples in which researchers have comprehensively mapped the input-output wiring diagrams of defined cell types within the deeper compartments of the mammalian brain. Thus, the current models could very well be incomplete or even wrong.

In this issue of *Neuron*, Rompani et al. (2017) addressed this gap in knowledge by carrying out a beautiful set of *trans*-synaptic labeling and circuit reconstruction experiments. The species and system they focused on was the mouse early visual pathway from the retina to the dorsal lateral geniculate nucleus (LGN), which serves as the primary and fastest route for visual information to reach the cortex. Mice, like their carnivore and primate counterparts, have ~30 types of retinal ganglion cells (RGCs) (Baden et al., 2016), the output neurons of the eye. Each of the 30 RGC types responds best to a particular portion and features within the visual world due to its specific dendritic shape and pattern of dendritic stratification in the retina. The size and shape of the RGC's dendritic tree determines the spatial extent over which it extracts visual information from the visual scene, whereas its stratification or "laminar" depth in the retina determines which presynaptic inputs are available to it and thus the quality of visual information it carries. As an entire group, the ~30 RGC types project to more than three dozen targets in the brain, and about half of those RGC types project to the LGN (Dhande et al., 2015). In an effort to determine where and how retinal output information is used by the brain, Rompani et al. (2017) sought to define the number and type of RGCs that synapse onto individual LGN relay neurons, using the structural properties of their dendrites described above to classify them into types.

At first this goal might seem straightforward to pursue but in fact it is far from trivial issue to resolve. The LGN contains both interneurons and relay cells, and the authors wanted to be sure they tracked the inputs to individual LGN relay cells, as opposed to groups of them. Thus, they made injections of an adeno-associated virus expressing GFP (AAV-GFP) into V1, which, due to the specific features of the viral serotype they used, infected the axons of cortical-projecting LGN relay neurons and caused the LGN cells to express GFP only within their nucleus. A short while later, they carried out a technically challenging second in vivo experiment on the same mice: a small region of the LGN was exposed and Rompani et al. (2017) injected, using a glass micropipette, one of the individual GFP+ relay neurons in the LGN. The

pipette they used contained a cocktail of one dye and three plasmids, each designed for a specific experimental purpose. The Alexa dye filled the impaled neuron and immediately allowed them to confirm that one (and only one) relay neuron was targeted and therefore also was infused with three cDNA plasmids. The first plasmid was designed to drive expression of Cre-dependent avian viral receptor TVA (which normally is not found in mammalian cells and on its own has no effect on the cell). The second plasmid expressed rabies virus glycoprotein (rabies G), a requisite component for rabies virus to pass retrogradely from one synapse to the next but that, on its own, also has no effect. The third plasmid drove expression of tdTomato, which would later allow the researchers to confirm that the plasmids were targeted to the individual Alexa/AAV-nuclear-GFP-expressing relay neuron and would also to help them recover its complete morphology. The retrograde AAV-nuclear GFP infection, combined with targeted injection of the above mentioned Alexa dye and three plasmid cocktail thus "primed" the LGN relay neuron to reveal all the presynaptic retinal inputs onto it, but only upon infection with a fourth and final ingredient: a rabies virus of the "EnvA" pseudotype lacking rabies glycoprotein but that binds TVA. The G-deleted and EnvA/TVA technology, initially brought to the field of neuroscience by Callaway and co-workers (Osakada and Callaway, 2013), limits rabies infection only to neurons that express the avian viral receptor TVA. To deliver this fourth ingredient, Rompani et al. (2017) bulk-injected the LGN with a G-deleted-EnvA-rabies virus that also expressed mCherry. Because EnvA can only bind and be expressed by neurons with TVA, in this way Rompani et al. (2017) ensured that the only neuron that could get infected was the individual relay neuron that was AAV-GFP back-labeled from V1 and targeted with Alexa dye/Cre-dependent TVA/tdTomato. The authors then waited for expression of the rabies virus and its various components in the LGN cell and the subsequent passage of G-deleted rabies/TdTomato to the RGCs that were presynaptic to it.

The number, type, and spatial distribution of RGC types shown to synapse on individual LGN neurons in these experi-

ments were incredibly informative. Rompani et al. (2017) knew that even RGCs of the same type can vary their dendritic arbor size according to location in the retina—a feature referred to as retinotopic-dependent dendritic scaling. While this feature is thought to be less prominent in non-foveated species such as mice, it sometimes still occurs (Bleckert et al., 2014). Thus, Rompani et al. (2017) were careful to make injections into defined regions of the LGN that get input only from a single retinal subarea. When they looked in the retina, indeed, they sometimes observed small groups of RGCs, all of which had relatively similar dendritic arbor sizes, shapes, and stratification patterns. In those retinas, there were at most 1–2 types of labeled RGCs, all closely positioned to one another, and whose dendritic trees overlapped and therefore extracted the same (or at least highly similar), types of visual information from restricted regions of visual space. These presynaptic clusters of RGCs were entirely consistent with the long-standing idea that LGN relay neurons receive information from small collections of RGCs and pass that information, that is qualitatively unaltered, to V1 (Figure 1). Rompani et al. (2017) called this pattern of wiring a "relay mode cluster" and determined, using statistical analyses, that the limited number and type of RGCs they observed in relay mode clusters were not due to chance nor did they relate to retinotopic location. The function of a LGN neuron whose presynaptic RGC inputs consisted of a relay mode cluster would essentially reflect the sum of its inputs and maintain the information those RGC encode, such as one direction of motion as it passed that information to V1 (Figure 1, green neurons in LGN and cortex).

The second pattern of RGC convergence observed was surprising: some LGN neurons received input from presynaptic clusters comprised of many different types of RGCs. Rompani et al. (2017) knew that the RGCs had to be of different types because from RGC to RGC they displayed marked variation in their patterns of dendritic stratification relative to the so-called ChAT-bands. The ChAT bands serve as fiduciary marks for the location and type of presynaptic interneurons that endow RGCs with their type-specific functional properties. Rompani et al. (2017) called

these mixed clusters of RGCs that project to individual LGN neurons "combination mode clusters." Convergent inputs of this sort onto individual LGN neurons no doubt produce mixing of the visual signals carried by each RGC type such as different directions of motion, luminance, and motion relative to background. The combination of these inputs would thus establish entirely novel receptive fields properties in the LGN neuron that it would in turn pass along to the cortex. A simplified version of one such possible integration scheme is shown in Figure 1 in which RGCs that each encode opposite directions of motion converge onto a single LGN relay neuron (Figure 1, schematized as gray cell in LGN projecting to gray neuron in cortex). This LGN neuron in turn would respond to both directions of motion and therefore be axial selective or orientation selective. The response properties of the LGN cell would also reflect the receptive field properties of the other RGC types from which it receives input.

The third general pattern of RGC convergence that Rompani et al. (2017) observed was especially surprising and indeed challenges another dogma in the field and one that is deeply engrained in the current textbooks. A central tenet of neurobiology is that neurons in the LGN are monocular; they receive synaptic drive from and therefore respond to light presented to one eye, or the other eye, but not both. Surprisingly, Rompani et al. (2017) found ample evidence for the fact that in mice, a large percentage of LGN neurons receive synaptic inputs from RGCs in *both* eyes. Moreover, combination mode clusters consisting of multiple RGC types were commonly observed projecting from the contralateral eye and synapsing onto LGN neurons that also received input from 1–2 RGC types in the retinotopically matched location of the ipsilateral eye. Rompani et al. (2017) termed these "binocular clusters."

The implications of these findings are several. First, the fact that there are some restricted sets of RGC types that converge their inputs onto LGN neurons confirms classic models and the idea that some visual signals will arrive in "pure" labeled line form into V1. Whether those are delivered to specific V1 layers is unclear but that would fit with our
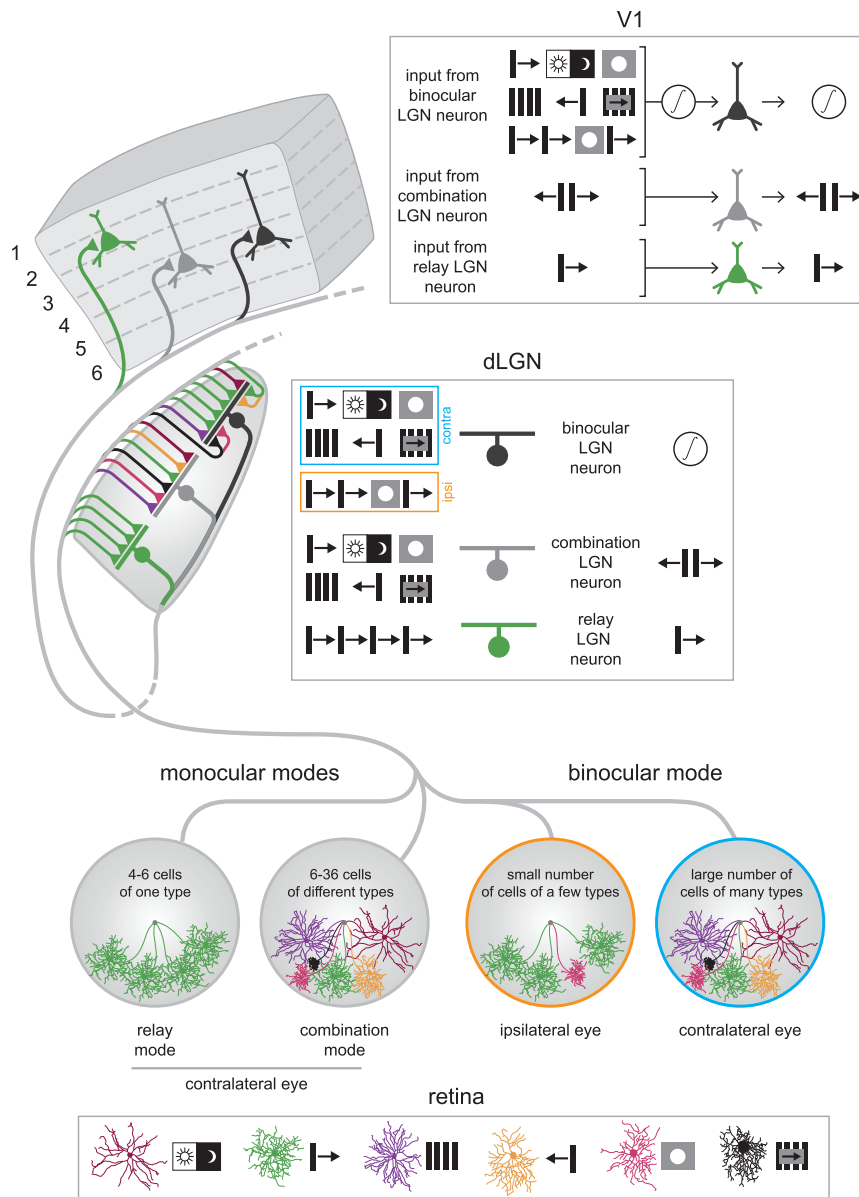
Figure 1. Specific Features of the Visual Scene Are Detected by Different Types of Retinal Ganglion Cells and Are Transmitted to the LGN in Three Different Ways
Information is transmitted by retinal ganglion cells (RGCs) to neurons in the LGN through one of three "modes": relay, combination, or binocular. In relay mode, a small number of RGCs of the same type transmit the same aspect of visual information to a corresponding relay neuron in the LGN (green neuron in LGN). In combination mode, a large number of RGCs comprised of many different types transmit a variety of visual information to a combination LGN neuron (gray neurons in LGN). In binocular mode, RGCs in both eyes converge their synapses on a single LGN neuron (black neuron in LGN). It is not entirely understood how V1 reads out the signals it receives from the three different types of LGN neurons, relay, combination, or binocular. However, the V1 neurons that receive each of these inputs alone or in combination must build their own receptive fields at least in part based on the incoming response properties of the afferents (thus, green and gray neurons in V1 represent relay and combination mode cells, respectively), and V1 neurons that receive input from binocular LGN neurons integrate retinal information from both eyes (black neuron in V1).

current understanding of visual pathway organization. Electrode recordings (Lien and Scanziani, 2013), *trans*-synaptic labeling and optical recordings (Cruz-Mar-

tín et al., 2014) support the idea that thalamic afferents deliver restricted types of visual information to different and specific layers of mouse V1 but that circuits

within V1 also may tune those inputs (Kondo and Ohki, 2016). The data from Rompani et al. (2017) also strongly support, however, the growing evidence that many of the more elaborate response properties observed in V1 such as direction and orientation selectivity (Cruz-Martín et al., 2014) as well as other response specializations, may be inherited from LGN neurons. As such, their findings motivate exploration of additional receptive field properties in LGN cells based on the observed patterns of RGC convergence. We know the mouse LGN harbors more than just center-surround neurons and includes both direction and orientation-tuned units (Piscopo et al., 2013), but the full extent of response properties should be revisited further based on the results of Rompani et al. (2017).

The other major implication of their work relates to the observed binocularity of LGN neurons. The mouse has long served as a model for ocular dominance plasticity under the assumption that the plastic changes occurring in the cortex after monocular deprivation were entirely cortical—a model that was grounded in data from the carnivore and primate, which, as far as we know, only contain monocular LGN cells. The data in Rompani et al. (2017) taken with the other recent findings from Howarth et al. (2014), who showed functionally that some LGN neurons in the mouse are binocular, suggest that some of the ocular dominance plasticity observed in mouse V1 may reflect shifts in retinal convergence and/or structural arrangements onto cells in the LGN. They also prompt exploration of the RGC convergence patterns to the LGN in other species. The region of the LGN into which Rompani et al. (2017) injected cells resided near the boundary of the eye-specific layers, so it is unlikely that all of the LGN territories contain binocular cells. Nonetheless, these findings and those of Howarth et al. (2014) do force re-exploration of several current models of mouse ocular dominance plasticity and possible re-evaluation of some of the underlying mechanisms as well.

More generally speaking, the results of Rompani et al. (2017) point to the importance of carrying out detailed mapping of cell-type-specific wiring patterns in the brain in order to understand sensory representations and processing. The authors

had the advantage that RGC stratification and dendritic extent are established criteria for 'typing'' RGCs and thus made it possible to infer the range of cell types and processing based on morphological criteria alone. Going forward it will be important to link these results with analysis of the response patterns of the RGCs and LGN neurons they synapse with, and ideally with those of V1 neurons too. In the meantime, the results of Rompani et al. (2017) point to the ways in which as brain scientists we might want to expand our thinking about perceptual processing: the cortex is the seat of perception but it apparently it gets delivered a much richer palate of sensory information to build those perceptions than was previously thought.

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A Butterfly Effect on Neural Stem Cells

Pierre Vanderhaeghen[1,2,*]
[1]Université Libre de Bruxelles (ULB), Institute for Interdisciplinary Research (IRIBHM), and ULB Institute of Neuroscience (UNI), 808 Route de Lennik, B-1070 Brussels, Belgium
[2]WELBIO, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium
*Correspondence: pierre.vanderhaeghen@ulb.ac.be
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Adult neural stem cells originate from the embryonic brain, but the underlying mechanisms remain poorly known. In this issue of *Neuron*, Falk et al. (2017) reveal how the timely control of cleavage plane orientation during division of embryonic neural progenitors has a specific and long-lasting impact on adult neurogenesis.

According to the butterfly effect, the properties of a hurricane could be influenced by the flapping of the wings of a distant butterfly several weeks earlier. This metaphor was originally used by Edward Lorenz in the context of non-linear models of weather prediction. It was meant not only to illustrate how seemingly small perturbations in inital conditions can lead to largely different results but also, and importantly, to point out that some chains of events are sometimes so hard or even impossible to untangle that they lead to the high unpredictability that often characterizes non-linear systems found in nature. Butterfly effects can be applied to neural development, where seemingly subtle cellular events, despite the inherent unpredictability of their direct impact, can have important and long-lasting conse-

quences on adult brain complexity and function. In this issue of *Neuron*, Falk et al. (2017) reveal such a striking effect, focusing on the mechanisms that control the embryonic generation of adult neural stem cells (aNSCs) and on a fascinating and rapidly evolving concept of developmental biology, the impact of cell division orientation on subsequent fate acquisition.

In the mammalian brain, while the vast majority of neurons are generated in utero, aNSCs can be found in specific neurogenic niches, such as the subependymal zone (SEZ) in the mammalian forebrain (Kriegstein and Alvarez-Buylla, 2009), where they generate several types of interneurons that will populate the olfactory bulb (OB) throughout much of adulthood. aNSCs share many features with radial glial cells (RGCs), the main em-

bryonic progenitor type of the vertebrate brain, including specialized apical and basal processes and expression of specific genes (Kriegstein and Alvarez-Buylla, 2009), but they also display a distinctive quiescent behavior, like many adult somatic stem cells, which has to be activated by various intrinsic and extrinsic cues to lead to neurogenesis and differentiation.

While our knowledge on aNSCs has accumulated at an impressive pace, the mechanisms underlying their embryonic origin have remained relatively scarce. Viral lineage-tracing experiments have shown that SEZ aNSCs originate in the embryo from ventricular progenitors (Fuentealba et al., 2015) within the lateral ganglionic eminence (LGE), a region of the ventral telencephalon that contains a particularly high diversity of progenitors