system by silencing T cells. Thus, in cancer settings, application of PD-L1 would most likely promote cancer growth and decrease overall survival. Considering these factors together, the implications of modulating the checkpoint pathway in cancer pain are still only poorly understood and deserve detailed analyses with respect to cancer growth, cancer–nerve interactions and neuro-immune interactions in the tumor milieu.

In summary, the groundbreaking work by Chen *et al.*[5] identifies PD-L1 as an endogenous analgesic mediator acting via PD-1. The results highlight endogenous controls that limit the excitability of the pain pathway and thus put a brake on the actions of nociceptive sensitizers that trigger chronic pain. The results hold strong translational potential and will stimulate further work on precise molecular and cellular mechanisms and the transferability to human subjects, with a word of caution regarding the necessity of testing implications for potential adverse effects, particularly with respect to cancer, immune function and infection, in patients with chronic pain.

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Direction selectivity starts early

Qi Fang & Huizhong W Tao

Disruption of retinal direction selectivity reveals both peripheral and central computations contributing to direction selectivity in mouse visual cortex. These mechanisms work together to better encode motion directions and speeds.

The ability to detect the direction and speed of moving objects, such as prey and predators, is critical for animal survival. It relies on direction-selective neurons in the visual system, particularly those in the visual cortex. These neurons respond preferentially to motion in a particular direction. Although neuronal direction selectivity (DS) has long been thought to emerge in the visual cortex, recent genetic identification of several types of direction-selective ganglion cells (DSGCs) in the retina and discovery of their projections to the dorsal lateral geniculate nucleus (dLGN), the visual thalamus that provides input to the cortex, raise the possibility that DS generated peripherally may be relayed to the cortex. Indeed, imaging of visually evoked calcium responses in dLGN axon terminals has revealed direction-selective inputs to the cortex, especially cortical layer 1 (refs. 1–3), with thalamic DS likely deriving directly from peripherally generated DS[1,4–6]. However, because most retinal ganglion cells (RGCs) or dLGN neurons are not direction selective[5,6] and dozens of thalamic neurons converge onto a cortical cell[7], it is difficult to predict how much peripheral DS can contribute to cortical DS or whether cortical DS can be explained entirely by peripheral DS.

In this issue of *Nature Neuroscience*, Hillier *et al.*[8] address this question by using a combination of state-of-the-art genetic, electrophysiological and *in vivo* imaging approaches that has rarely been achieved in a single study. With a series of well-designed experiments, the authors identified two functionally distinct forms of DS, retina-dependent and retina-independent, that contribute to the establishment of cortical DS. The retina-dependent form mainly processes posterior motion and higher motion speeds, while the retina-independent form processes more evenly distributed directions and speeds. These two mechanisms working together can compute a wider range of motion information.

The authors took advantage of two genetic mouse models to disrupt retinal DS *in vivo*. In the first set of experiments, they used *FRMD7^{tm}* mice. In this mutant line, with partial loss of function of the *Frmd7* gene, starburst amacrine cells (SACs) that specifically express FRMD7 fail to provide asymmetric inhibition to RGCs they synapse on, which is critical for generating DS responses in RGCs[9]. This results in a loss of horizontal DS[10], which the authors exploited to study the retinal contribution to the horizontal DS of cortical neurons. To confirm that retinal DS was affected, they performed two-photon imaging-guided patch-clamp recording in isolated retinas of *Drd4-GFP* mice, in which a subset of ON-OFF DSGCs that prefer posterior motion are labeled with GFP[11]. ON-OFF RGCs respond to both increments and decrements of light intensity. In *Drd4-GFP;FRMD7^{tm}* mutant retinas, the GFP-labeled neurons lost DS and responded similarly to all testing directions.

In a second set of experiments, the authors crossed *Chat-Cre* with *loxP-STOP-loxP-DTR* mice to selectively express the diphtheria toxin receptor (DTR) in SACs. Injection of diphtheria toxin into both eyes killed SACs. They tested the effect of SAC ablation 7 days after injections in *Hb9-GFP* mice, in which ON-OFF DSGCs preferring superior motion are labeled with GFP[12]. With imaging-guided patch-clamp recording, they demonstrated that GFP-labeled neurons completely lost superior DS and exhibited greatly increased responses to other directions.

The authors further examined DS of RGCs at the population level by using high-density microelectrode arrays to record motion-evoked spike responses. Results from a dataset of more than 1,000 RGCs demonstrated that diphtheria toxin-induced SAC ablation nearly abolished retinal DS and that the overall visual responses were elevated. Note that DS of some RGCs does not depend on asymmetric inhibition[9]. Therefore, it is not surprising that a small subset of RGCs remained direction-selective after SAC ablation. The ablation did not affect other properties such as the proportion of ON-OFF cells or orientation-selective cells in the retina.

To test whether retinal DS is indeed affected by SAC ablation *in vivo*, the authors performed

Qi Fang and Huizhong W. Tao are at the Zilkha Neurogenetic Institute, Department of Physiology and Neuroscience, Neuroscience Graduate Program, Keck School of Medicine, University of Southern California, Los Angeles, California, USA.
e-mail: htao@usc.edu

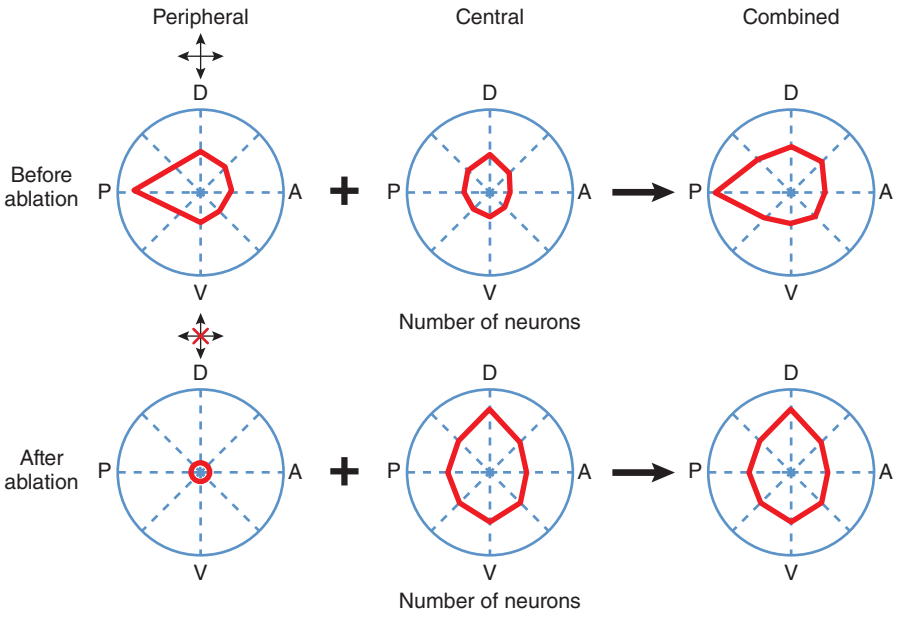

Figure 1 Two forms of direction selectivity contributing to the cortical selectivity. The first form relies on peripheral (retinal) circuits (left). The second relies on central circuits downstream of the retina (middle). The radial axis represents the number of direction-selective neurons preferring each of the eight motion directions. Red loop in each plot depicts the number of neurons preferring each direction. A, anterior; P, posterior; D, dorsal; V, ventral.

two-photon calcium imaging of retinal axons in the outer shell of the dLGN, the preferential target of DSGCs[1,5]. As expected, none of the imaged axon terminals were direction-selective in SAC-ablated mice. Furthermore, the optokinetic reflex, a DSGC-dependent behavior, was lost. The series of experiments together demonstrated loss of retinal DS both *ex vivo* in the isolated retina and *in vivo* in the intact retina. These two mouse models thus allowed the authors to specifically eradicate peripheral DS throughout development or shortly after drug delivery.

What are the functional outcomes after the disruption of retinal DS? Would the cortical selectivity be reduced or disappear? If not, would the distribution of preferred directions in the cortical cell population be changed? To answer these outstanding questions, the authors imaged visually evoked calcium responses of layer 2/3 neurons in the primary visual cortex. In control mice, about half of the neurons were direction-selective when a relatively stringent criterion was used for defining DS cells. Notably, the preferred directions of these neurons were not evenly distributed but displayed a prominent bias toward posterior motion, with more than 25% of the neurons preferring posterior motion. This number would have been 12.5% if they were equally distributed. However, in both mouse models with disruption of retinal DS, the posterior preference was no longer dominant. Instead, the balance was tilted toward vertical directions, in particular toward the dorsal direction. Notably, the proportion of direction-selective neurons remained the same, indicating that the overall level of cortical selectivity was unaffected after disrupting retinal DS.

From the above results, it can be concluded that there are two forms of DS contributing to the cortical selectivity: a retina-dependent form with a strong bias toward posterior motion and a retina-independent form with more evenly distributed preferred directions, slightly biased toward vertical directions (**Fig. 1**). The former is attributed to DS generated in RGCs, while the latter is attributed to DS generated by circuits downstream of the retina, in the dLGN or in thalamocortical or cortical circuits. Since the overall selectivity level was not changed after disrupting retinal DS, we can conclude that there is a compensatory increase of selectivity generated in central circuits (**Fig. 1**).

This insight, that there may be two distinct forms of motion processing present in the cortex, was further substantiated by experiments testing the effect of motion speed. In the retina, the firing rates of DSGCs neurons increase with motion speed. Hillier *et al.*[8] found this reflected by a progressive elevation of direction-selective cortical cell responses with increasing speed when they analyzed the posterior component in control mice. However, the response level remained relatively constant across testing speeds in *FRMD7^{tm}* mice, in which retinal horizontal DS is abolished. This suggests that the retina-dependent form of DS computes motion preferentially at high speeds, while the retina-independent form handles more evenly distributed speeds. Therefore, the two functionally distinct computations work in concert to better process motion information across different directions and speeds.

This *tour de force* study leads to two intriguing questions for future investigations. First, although the study points to the presence of both periphery-dependent and periphery-independent forms of DS, the exact contribution of each form to the cortical selectivity in the normal condition is not fully characterized. The injection of diphtheria toxin into the eye likely leads to a gradual loss of amacrine cells and therefore a gradual loss of retinal DS over subsequent days. Since SAC ablation leads to an increase in the overall response level in RGCs, but not in cortical cells, this suggests that a homeostatic change has occurred in central circuits, possibly through increasing inhibition[13]. Such enhanced inhibition is expected to sharpen the selectivity of existing weakly tuned neurons[14], which, besides other possible changes, may directly contribute to the enhancement of periphery-independent selectivity (**Fig. 1**). The development of tools to acutely disrupt retinal DS is thus necessary for dissecting the contribution of central circuits to cortical DS in the normal condition. Second, the results of Hillier *et al.*[8] implicate a strong influence of retinal DS, which has a heavy bias toward posterior motion. This is the most frequently experienced motion direction as rodents with laterally positioned eyes run forward. Such posterior bias has not, however, been observed previously in a multielectrode-array study of RGC spikes[15] or in a calcium-imaging study of thalamic axon terminals in the cortex[2]. Hillier *et al.* argue that there might be more posterior- than dorsal-motion-preferring DSGCs, as GFP-labeled RGCs in the *Drd4-GFP* retina outnumbered those in the *Hb9-GFP* retina[8]. However, there is no evidence that all dorsal-motion-preferring DSGCs are labeled in the *Hb9-GFP* mouse. If no posterior bias is present in retinal outputs, such bias in cortical cells would indicate that central circuits can in fact strongly shape DS in the cortex in the normal condition. With more and more DSGC subtypes being characterized, the results of Hillier *et al.*[8] highlight the extent to which these peripheral neurons may shape central visual processing. Development of new tools and more experiments will be needed to further resolve this issue.

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The thalamic paradox

László Acsády

Most thalamic research has focused on sensory transmission. Now three independent groups reveal the thalamus to be critical in behaviors linked to frontal cortex and the maintenance of persistent cortical activity during delays.

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Before the nineteenth century, few if any scientists attributed major functions to the cortex. Cortex was mainly regarded as a rind around the more important parts of the brain. According to the leading theories of the era, the highest sensory functions were located in the thalamus and the highest motor functions in the striatum[1]. This view changed abruptly at the end of the nineteenth century as a result of the famous experiments by Fritsch and Hitzig, who discovered that electrical activation of the cortex induces movements[2]. The importance of cortex was soon demonstrated in all major cognitive functions, and cortical computations continue to fascinate us today, perhaps more than ever. However, in this corticocentric view of the brain, the thalamus was relegated to subserving an elementary function: namely, providing accurate topographical sensory information to the cortex. All other higher-order processing was (and is) attributed mainly to hierarchically organized cortico-cortical connections (**Fig. 1**). This view prevails even though all cortical regions are known to have strong bidirectional connections with the thalamus[3]. In addition, it is also clear that only a minority of the thalamus receives subcortical sensory inputs[4]. Thus, largely because of a historical bias, the role of the thalamus in cognitive functions has not been explored in depth, and as a consequence there is a substantial gap in our knowledge concerning the nonsensory functions of the thalamus. I call this chasm between the basic anatomical data and the lack of cognitive studies the thalamic paradox.

This is now about to change. As with the cortex in the nineteenth century, critical experiments were needed to launch a new way of thinking about thalamus in the twenty-first century. The simultaneous publication of two papers in *Nature*[5,6] and one in *Nature Neuroscience*[7] about interactions between thalamus and frontal cortex in different behavioral situations in mouse marks the beginning of a new era in thalamic research. Whereas we may have thought sensory transmission to be the rule in thalamocortical function, now it seems equally likely that it is the exception, a highly specialized form of thalamocortical activity. As shown in these three landmark papers, the rules of operation are qualitatively distinct in other parts of thalamic circuits.

The new data unequivocally demonstrate the importance of thalamus in frontocortical functions. In addition, they show the conceptual differences in thalamocortical interactions in sensory and frontal territories. In sensory transmission, there is a clear one-way drive of cortical activity by the thalamus to accurately transfer transient sensory events to the cortex[8]. According to the new studies, however, in the frontal cortex, there is a continuous reverberation of activity between the cortex and thalamus. The data show a mutual interdependence of cortical and thalamic activity that persistently maintains information in the cortex. The three papers tell the same story of sustained interactions, and yet there are significant differences among them. This indicates that, depending on the task and the actual circuit in question, the thalamocortical interplay in frontal cortex may take many different forms and support cortical functions in various ways.

All three papers revolve around persistence of frontal cortical activity during behavior. Persistent activity is widely regarded as the neuronal correlate of the internal representation of an environmental variable[9], decision-making[10], preparation of a motor act[11] or working memory[12]: in brief, something we need to keep in mind before we act. Persistent activity frequently manifests as a sequential activation of well-defined cell populations (called synfire chains) that tiles the period during decision-making and is known to require recurrent synaptic connectivity.

The behavioral and decision variables in the three studies were different, yet the three papers together make a very strong case that the thalamus acts to maintain persistent activity in the frontal cortex. Guo *et al.*[5] asked mice to lick left or right depending on the location of an object they sensed with their whiskers (directional licking task). Bolkan *et al.*[7] asked mice to remember which way they turned in a T-maze and to choose the opposite arm after a delay (spatial, delayed nonmatch-to-sample task). Finally, Schmitt *et al.*[6] asked mice to keep in mind a rule (attend to vision or attend to audition) during a delay period (two-alternative forced-choice task). In all three cases, precisely timed optogenetic inhibition of the relevant thalamic territories perturbed both the sequential cortical activity that tiled the delay period as well as task performance. This clearly demonstrates that frontal cortex and cortico-cortical connections alone are not sufficient to perform persistent delay activity.

So, what was actually represented in the thalamus during the delay periods while the mice were deciding what to choose, and what was the impact of the thalamus on cortical activity? This is where the three studies diverge. The strongest interdependence of cortical and thalamic activity was observed by Guo *et al.*[5]. Thalamocortical neurons displayed directional responses (lick left, lick right) that tiled the delay period (~1 s) between sample and choice. Notably, this activity was identical to that of cortical pyramidal cells. Blocking thalamic activity resulted in a marked drop in cortical firing and the loss of directional specificity. Likewise, blocking cortical activity abolished thalamic

László Acsády is in the Laboratory of Thalamus Research, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.
e-mail: acsady@koki.hu