The intriguing results of Huang *et al.*[5] underscore the importance of proper ion channel expression and function in limiting the basal excitability of afferent pain fibers, but they also raise questions. First, is TMEM16C downregulated under conditions of chronic pain and, if so, is this partially responsible for the associated increase in neuronal excitability? Second, given that TMEM16C and SLACK are also present in tissues, such as the CNS[7,8] and heart, might a similar TMEM16C-SLACK–mediated regulation of cellular excitability occur more widely? Third, could this mechanism contribute to other pathophysiologies associated with altered SLACK function, such as epilepsy[9]? Fourth, does knocking down SLACK in mice reduce pain thresholds? At a mechanistic level, it remains unclear whether the interaction with TMEM16C results in an increased affinity of the SLACK channels for sodium ions or simply a greater sensitivity of the activation mechanism to this ionic agonist. Moreover, it remains to be determined whether the TMEM16C-mediated increase in membrane expression of SLACK is a result of effects on gene transcription, enhanced endoplasmic reticulum export or a stabilization of the protein complex at the plasma membrane. This last mechanism may be supported by observations that the internalization of SLACK channels is under dynamic second-messenger control[6]. Despite these open questions, the work of Huang *et al.*[5] is exciting and sets the stage for further exploration.

The functions of the TMEM16 (ANO) family of transmembrane signaling molecules are only incompletely understood. TMEM16A (ANO1), TMEM16B (ANO2) and TMEM16F (ANO6) have been described as small-conductance calcium-activated chloride channels[10–12] that help regulate chloride homeostasis. In contrast, most other members of this family do not appear to support chloride channel activity, despite a high degree of homology with those that do[4]. These chloride-impermeant members of the TMEM16 family may well mediate functions such as those described by Huang *et al.*[5]. Perhaps more intriguing is a scenario in which ANO proteins that are known to support a chloride conductance may also act in a manner that is reminiscent of TMEM16C; that is, by modulating other ion channels in a non-ionotropic manner. Irrespective of this possibility, the fact that TMEM16C acts as a modulator of K_{Na} channel function indicates a previously unrecognized role of the ANO family in regulating ion channel activity that demands a fresh look at this class of transmembrane molecules.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Cho, H. *et al. Nat. Neurosci.* **15**, 1015–1021 (2012).
2. Tian, Y., Schreiber, R. & Kunzelmann, K. *J. Cell Sci.* **125**, 4991–4998 (2012).
3. Duran, C., Qu, Z., Osunkoya, A.O., Cui, Y. & Hartzell, H.C. *Am. J. Physiol. Cell Physiol.* **302**, C482–C493 (2012).
4. Milenkovic, V.M., Brockmann, M., Stöhr, H., Weber, B.H. & Strauss, O. *BMC Evol. Biol.* **10**, 319 (2010).
5. Huang, F. *et al. Nat. Neurosci.* **16**, 1284–1290 (2013).
6. Nuwer, M.O., Picchione, K.E. & Bhattacharjee, A. *J. Neurosci.* **30**, 14165–14172 (2010).
7. Santi, C.M. *et al. J. Neurosci.* **26**, 5059–5068 (2006).
8. Charlesworth, G. *et al. Am. J. Hum. Genet.* **91**, 1041–1050 (2012).
9. Heron, S.E. *et al. Nat. Genet.* **44**, 1188–1190 (2012).
10. Yang, Y.D. *et al. Nature* **455**, 1210–1215 (2008).
11. Caputo, A. *et al. Science* **322**, 590–594 (2008).
12. Yang, H. *et al. Cell* **151**, 111–122 (2012).

A finely tuned cortical amplifier

Yunyun Han & Thomas Mrsic-Flogel

Three studies in visual and auditory cortex show that intracortical excitatory inputs amplify incoming sensory signals, as their sensory tuning is closely matched to that arriving from the sensory thalamus.

One of the longest-standing debates in neuroscience centers on how preference of individual neurons for sensory stimuli arise in the neocortex. In dedicated areas of sensory cortex, neurons are specialized to detect particular sensory features, such as the orientation and motion direction of edges in images, or the spectral content of sounds. Remarkably, anatomical studies have shown that a neuron in the cortex receives only a tiny fraction of its excitatory synapses from thalamic regions conveying information from the sensory organs[1], whereas the rest are largely provided by other cortical neurons embedded in a richly connected circuit. Our understanding of the relative contribution of thalamic and intracortical inputs in shaping neuronal response properties is far from complete. In this issue, three studies shed light, quite literally, on this question.

The establishment of sensory feature selectivity is perhaps best understood in primary visual cortex (V1). Classically, the selectivity for oriented bars or edges has been explained using Hubel and Wiesel's 1962 model, whereby 'simple' cells in cortical layer 4 first become selective for stimulus orientation or direction via the convergence of ON-center and OFF-center thalamic inputs whose receptive fields are offset in visual space, but are themselves not orientation or direction selective. In cat V1, each cortical column receives input from a limited subset of neurons in the lateral geniculate nucleus (LGN) of the thalamus, and the angle of apposition of ON and OFF domains determines the orientation preference of neurons in that column[2,3]. To isolate the contribution of thalamocortical input to cortical feature selectivity, previous studies have measured synaptic drive while silencing cortical activity by means of cooling or electric shocks[4,5]. These studies found that input from the thalamus is sufficiently tuned to establish the orientation selectivity of thalamorecipient cortical neurons.

What, then, is the contribution of intracortical connections to sensory responses of cortical neurons? Computational models informed by anatomy suggest that one role of intracortical connections is to amplify thalamocortical input (for example, ref. 6). This will depend on the strength and specificity by which cortical neurons connect to each other. We know that cortical connections are not randomly organized, as excitatory neurons with similar visual responses connect to each other preferentially (but not exclusively) in layer 2/3 of mouse and cat V1 (refs. 7,8). This suggests that neurons form partially overlapping subnetworks within which recurrent excitation may boost the responses more to preferred than to non-preferred stimuli.

The three new studies have now directly estimated the relative influence of feedforward and intracortical excitation to sensory evoked responses in layer 4 of mouse visual and auditory cortex[9–11]. These studies used *in vivo* voltage-clamp recordings in single neurons in combination with rapid and reversible silencing of cortical excitatory activity, which enabled them to isolate putative thalamocortical and intracortical components contributing

Yunyun Han and Thomas Mrsic-Flogel are at the Biozentrum, University of Basel, Basel, Switzerland.
e-mail: thomas.mrsic-flogel@unibas.ch

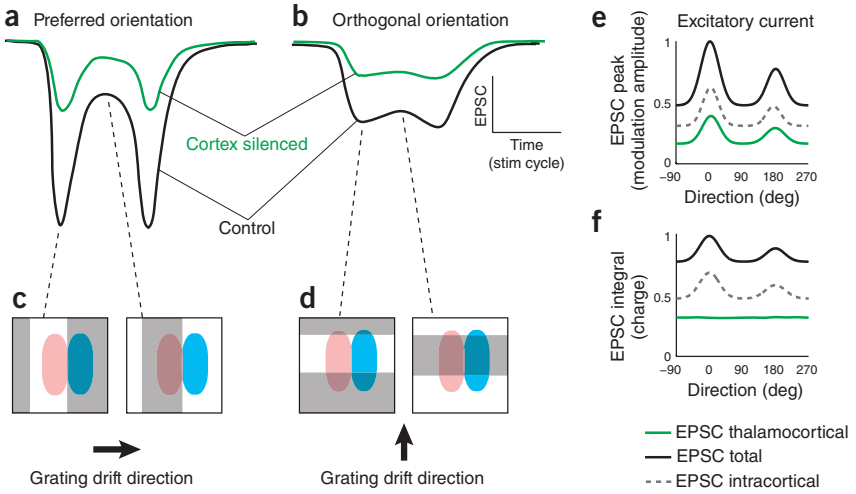

Figure 1 Dissecting the contributions of thalamocortical and intracortical excitation to total excitation in layer 4 neurons in mouse visual cortex. (**a**,**b**) Schematic excitatory postsynaptic current (EPSC) of a layer 4 cell when stimulated (stim) with a grating drifting at the preferred (**a**) or orthogonal (**b**) orientation. The black trace represents the EPSC under control conditions when cortical activity was left intact. The green trace represents the putative thalamocortical EPSC measured when cortical excitation was suppressed by optically activating channelrhodopsin-2 in parvalbumin-expressing interneurons. (**c**) When the light and dark bars of the preferred grating stimulus are aligned to the ON (red) and OFF (blue) subregions of the receptive field (that is, matched in phase), the evoked EPSC is maximal (left). When the light and dark bars of the preferred grating stimulus are out of phase with the ON and OFF subregions of the receptive field, the evoked EPSC is minimal (right). (**d**) When the orthogonal stimulus is shown, there is no substantial EPSC modulation by stimulus phase because light and dark bars of the grating are never aligned to the ON and OFF subregions of the receptive field. (**e**) Normalized EPSC amplitude (peak or stimulus-modulated component) of total, thalamocortical and intracortical inputs in response to different stimulus angles (EPSC intracortical = EPSC total – EPSC thalamocortical). (**f**) Normalized charge (integral of EPSC during each stimulus) of total, thalamocortical and intracortical EPSCs in response to different stimulus angles.

to the total excitation in response to a sensory stimulus. Silencing was achieved by optically activating a class of local inhibitory interneurons expressing parvalbumin that also expressed the light-sensitive cation channel channelrhodopsin-2. While stimulating the eye with drifting bars or gratings (**Fig. 1**), Ya-tang Li *et al.*[10] and Lien and Scanziani[11] found that the peak (or stimulus phase–modulated component) of thalamic excitation was orientation and direction tuned (**Fig. 1a,b,e**). This agrees with findings in cats[2–5] that thalamocortical inputs serve as the origin of cortical orientation selectivity. Importantly, however, the excitatory drive provided by intracortical circuitry was twice as strong and closely matched the orientation and direction tuning of thalamic excitation (**Fig. 1a,b,e**). Together, this implies that intracortical excitatory circuits amplify incoming feedforward signals proportionally by approximately threefold. In a separate study, Ling-yun Li *et al.*[9] made similar observations in auditory cortex, where excitation from thalamocortical and intracortical inputs was also matched in response to pure tones and frequency sweeps, thereby confirming that such amplification is likely a general cortical phenomenon.

These findings are interesting because they point to the functional specificity of intracortical excitatory inputs converging onto neurons in layer 4. Lien and Scanziani[11] report a key difference between intracortical and thalamocortical excitation. Unlike excitation provided by thalamocortical inputs, the net charge from intracortical inputs (integral of excitatory current during each stimulus) was tuned to orientation (**Fig. 1f**). This implies preferential connectivity between neurons responding to the same stimulus angle in layer 4, as previously observed in layer 2/3 (ref. 8). Notably, intracortical excitation was also strongly modulated by the stimulus phase at the preferred grating direction, but much less so for non-preferred stimuli (**Fig. 1a,b**). This suggests that iso-oriented neurons may also be wired with respect to their phase preference (that is, the relative position of ON and OFF subdomains). It is tempting to speculate that nearby neurons sharing the same orientation and phase preference receive common input from the thalamus, which may drive them to develop preferential recurrent connections during postnatal development, such that thalamocortical and intracortical signals become closely matched[12].

Thalamocortical synapses make up less than 10% of all of the synapses received by a layer 4 neuron in cat V1 (ref. 1). If the same is true in mouse V1, how could such a small portion of synapses add up to >30% of total excitation reported in these studies? One explanation is that thalamocortical inputs are more effective at driving cortical neurons because their synapses are stronger, more proximal to the soma, dendritically clustered or more synchronized in their activity (discussed in ref. 1). Another possibility is that there is a measurement bias toward thalamocortical excitation because of the spatial clamping error inherent in the voltage-clamp method. Owing to poor clamping of distal dendrites, which may predominantly receive intracortical inputs, a large portion of distal excitation may be underestimated. One way to address this issue is to directly record from dendrites of thalamorecipient neurons, which may also reveal any contribution of active dendritic processes to their orientation or direction selectivity.

Irrespective of methodological caveats, the function of intracortical excitatory connections in layer 4 is probably more diverse than simply boosting thalamocortical signals because this could, in principle, be achieved simply by strengthening thalamocortical synapses. Indeed, Ya-tang Li *et al.*[10] observed that receptive fields were slightly smaller when cortical excitation was blocked, suggesting that intracortical connections provide input from neighboring regions in visual space and may thereby contribute to the integration of local image features. More interestingly, sound-evoked excitation in primary auditory cortex was prolonged when intracortical excitation was present[9]. Allowing activity to persist longer not only makes it more robust to noise and more effective at driving postsynaptic targets, but may also enable the cortical circuit to integrate sensory information with contextual or behaviorally relevant inputs that are delayed in time.

Where does the orientation-tuned thalamocortical excitation come from in mouse V1? Mouse V1 does not contain a column-like orientation map, as nearby neurons exhibit a mixture of feature preferences. Thus, unlike in the cat, each 'column' in mouse V1 likely receives a more diverse repertoire of possible ON- and OFF-center thalamocortical inputs that can be combined in different ways to generate a range of orientation preferences. Individual neurons in layer 4 may therefore select only a subset of available inputs, whose receptive fields are offset in visual space, consistent with the original model by Hubel and Wiesel. Alternatively (and this may be specific to mouse visual system), Ya-tang Li *et al.*[10]

suggest that orientation or direction preference may originate not in the cortex, but in a population of orientation- or direction-tuned neurons upstream in mouse LGN, as reported previously[13–15]. Lien and Scanziani[11], however, did not find significant orientation-tuned responses in the LGN, and they argue that the spatial offset of ON and OFF subregions is sufficient to give rise to orientation selectivity without involving tuned thalamic neurons (**Fig. 1c,d**). Their argument is supported by the observation that the net thalamic excitatory charge was largely untuned (that is, the integral of excitatory current was the same irrespective of stimulus angle), whereas the grating phase–modulated component of thalamic excitation was much more selective (**Fig. 1a,b,e,f**). Although this argument may explain the emergence of orientation selectivity in the absence of orientation-tuned thalamocortical input, it does not, however, reveal how layer 4 neurons acquire directional selectivity, which likely depends on mechanisms not described here. There are two possibilities that could explain why orientation- or direction-selective thalamic neurons may not directly contribute their preference to layer 4 neurons in mouse V1: either thalamic neurons converging onto the same cortical neuron are not tuned to the same orientation or orientation-selective thalamic neurons provide input to other cortical cell types or layers, which were not examined in these studies. Resolving this issue merits further work, as it is important to establish whether the mechanisms giving rise to cortical feature selectivity are similar across mammalian species.

In summary, one function of the intracortical excitatory circuit may be to increase the gain and duration of sensory signals in layer 4. Several important questions remain. The effect of excitation on the spiking output of a layer 4 neuron is strongly influenced by intracortical inhibition, which was not examined in these studies. It will be important to determine whether inhibition in layer 4 simply acts as an additional gain control mechanism or whether it influences the integration of excitatory inputs in a more specific way; for example, to generate directional preference in visual cortex. Moreover, what is the function of local and long-range intracortical excitation in other cortical layers in the transformation of sensory signals, as these may have additional roles in contextual processing or the spatial integration of signals across sensory scenes? As the arsenal of available techniques grows, we are inching closer to uncovering the precise rules that govern the nature of intracortical excitatory interactions and the sensory computations they subserve.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. da Costa, N.M. & Martin, K.A. *J. Comp. Neurol.* **516**, 264–276 (2009).
2. Jin, J., Wang, Y., Swadlow, H.A. & Alonso, J.M. *Nat. Neurosci.* **14**, 232–238 (2011).
3. Reid, R.C. & Alonso, J.M. *Nature* **378**, 281–284 (1995).
4. Ferster, D., Chung, S. & Wheat, H. *Nature* **380**, 249–252 (1996).
5. Chung, S. & Ferster, D. *Neuron* **20**, 1177–1189 (1998).
6. Douglas, R.J., Koch, C., Mahowald, M., Martin, K.A. & Suarez, H.H. *Science* **269**, 981–985 (1995).
7. Gilbert, C.D. & Wiesel, T.N. *J. Neurosci.* **9**, 2432–2442 (1989).
8. Ko, H. *et al. Nature* **473**, 87–91 (2011).
9. Li, L., Li, Y., Zhou, M., Tao, H.W. & Zhang, L.I. *Nat. Neurosci.* **16**, 1179–1181 (2013).
10. Li, Y., Ibrahim, L.A., Liu, B., Zhang, L.I. & Tao, H.W. *Nat. Neurosci.* **16**, 1324–1330 (2013).
11. Lien, A.D. & Scanziani, M. *Nat. Neurosci.* **16**, 1315–1323 (2013).
12. Ko, H. *et al. Nature* **496**, 96–100 (2013).
13. Scholl, B., Tan, A.Y.Y., Corey, J. & Priebe, N.J. *J. Neurosci.* **33**, 10616–10624 (2013).
14. Piscopo, D.M., El-Danaf, R.N., Huberman, A.D. & Niell, C.M. *J. Neurosci.* **33**, 4642–4656 (2013).
15. Marshel, J.H., Kaye, A.P., Nauhaus, I. & Callaway, E.M. *Neuron* **76**, 713–720 (2012).

10,000 hours to perfection

Chris Miall

A study reports that a metabolic measure of synaptic activity in the motor cortex becomes dissociated from neural firing rates after extensive practice in a behavioral task, suggesting an increase in efficacy of synaptic inputs.

It is widely accepted that expertise takes practice—hence the '10,000-hour rule' that intense practice for up to 10 years distinguishes expert performers from the merely good[1]. But what is the neural consequence of such extended practice in a particular domain? How are knowledge that is learned and skills that are gradually refined reflected in neural activity? For obvious reasons, there are few studies of the effects of very long-term practice on architecture or function in the human brain. Perhaps the most extensive are longitudinal studies that last weeks or months—well short of 10,000 h. For example, the acquisition of juggling skills over 6 or 12 weeks leads to changes in the volume of gray matter in visual motion areas and to changes in the white matter linking these motion areas to parietal sensory motor regions[2]. More typically, studies report the effects of a few tens of hours of practice spread over several days. In this issue of *Nature Neuroscience*, Picard, Matsuzaka and Strick[3] report the neural outcome in the monkey of training that lasted months and years.

In a study that is out of the ordinary for its duration, as well as for its conclusions, the authors trained ten monkeys to each perform two of four sequential reaching tasks. The monkeys were required to reach to targets displayed on a touch screen for water rewards. The four tasks included two visually guided tasks, in which the target sequences were either randomly presented or required tracking across sets of three, and two internally generated tasks, in which the targets repeatedly appeared in the same position or in a sequence that the monkey held in short-term memory. All of these monkeys were trained for more than 7 months: three of them for more than 30 months and a fourth for more than 6 years. The authors argue that, although there were no gross differences in the kinematics of the movements, the monkeys learned a skill in the internally generated tasks, whereas they were only able to reactively follow the unpredictable targets presented in the visual guided tasks. At the end of these extended periods of behavioral training, each monkey performed one of the two trained tasks after administration of [^{14}C]2-deoxyglucose (2DG) in a terminal experiment. Uptake of the tracer was then examined post-mortem.

The 2DG technique can be used to assess metabolic energy consumption and is thought to represent a measure of presynaptic activity. It is a measure closely related to the most common signal recorded in functional magnetic resonance imaging (fMRI), the blood oxygen level–dependent (BOLD) signal[4]. This gave Picard *et al.*[3] a measure of the synaptic activation of the primary motor cortex across the two task categories (internally generated versus visually guided). It is striking that, across the five monkeys performing the internally generated tasks, there was very low 2DG uptake in

Chris Miall is in the School of Psychology, University of Birmingham, Birmingham, UK.
e-mail: r.c.miall@bham.ac.uk