

# The yin and yang of cortical layer 1

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**Distinct populations of layer 1 inhibitory neurons inhibit or disinhibit layer 5 pyramidal cells. A massive patch-clamp recording effort, tapping up to eight cells simultaneously, maps their influences on the cortical network.**

Layer 1 (L1) of the neocortex stands apart from the other five cortical layers. It is immediately recognizable because of the sparseness of neurons. Those cells that do lie in L1 turn out to be almost entirely inhibitory neurons that fall into two to four classes<sup>1</sup>. L1 is of interest because it receives long-range axons from the thalamus and other cortical areas that carry feedback information<sup>2</sup> vital for cognitive and attentional processes<sup>3</sup>. Because of their strategic location among the tuft dendrites of L2/3 and L5 pyramidal neurons, L1 inhibitory neurons are also ideally positioned to shape the firing of the main excitatory neurons of the cortex<sup>4</sup>. This might have unexpectedly large and unusual consequences because this region of the pyramidal neuron has the ability to fire local dendritic action potentials<sup>5</sup>. Indeed, one class of L1 neurons has been identified as mediating a powerful suppression of dendritic Ca<sup>2+</sup> activity in L2/3 and L5 pyramidal neurons<sup>6,7</sup>.

In a *tour de force* of scientific enquiry, Jiang *et al.*<sup>8</sup> took on the function of L1 inhibitory neurons in the cortical network. Recording from up to eight locations simultaneously (including from the dendrites of pyramidal neurons) and testing nearly 15,000 connections, they systematically investigated the influence of L1 inhibitory neurons on pyramidal cell firing in slices of rat sensorimotor cortex. An effort on this scale was required because the interplay between the inhibitory neurons and pyramidal cells presents formidable experimental challenges. Recordings from L1 inhibitory neurons *in vivo* show that they fire tonically and can also participate in disinhibitory circuits<sup>9</sup>. It is thus likely that they interact not just with pyramidal neurons but also with different populations of inhibitory neurons, which substantially enhances the range of actions they may have on the network. Frustratingly, there are many different classes of interneurons with different firing properties and axonal terminations (Fig. 1). Moreover, the connection probabilities between these neurons is sometimes very low.

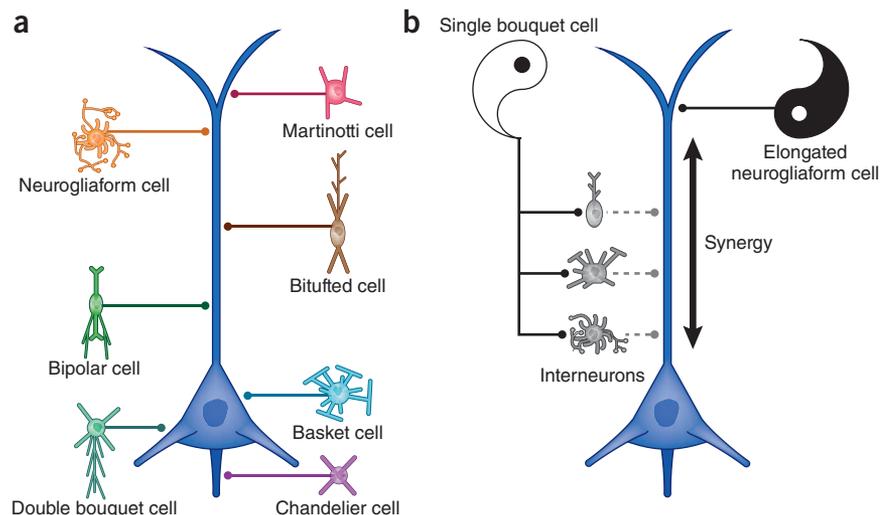
In the first part of their study, Jiang *et al.*<sup>8</sup> established the properties of the two main

classes of L1 inhibitory neurons, single bouquet cells (SBCs) and elongated neurogliaform cells (ENGs). Next they determined where on the soma, axon and dendritic tree of L5 pyramidal neurons the different inhibitory neurons of the cortical network connect (Fig. 1a). These data are invaluable for predicting the inhibitory neurons' influence on the firing properties of pyramidal neurons. Of particular interest in this regard is the influence on the special associative properties of the L5 pyramidal neurons. Previous work by one of the authors had established that the active intrinsic properties of L5 pyramidal neurons allow them to couple inputs arriving at the tuft and basal dendrites simultaneously<sup>10</sup>. This mechanism has been hypothesized to facilitate the association of top-down and bottom-up processes in the cortex<sup>10,11</sup>. The various inhibitory cell types, with specific influences on different parts of the dendritic tree, can affect this interaction either by blocking dendritic spikes directly or by altering the synergistic coupling between the somatic and dendritic compartments (Fig. 1b).

Crucially, the authors managed to show that SBCs and ENGs of L1 contact complementary sets of cortical interneurons and that they have competing influences on the associative

properties of L5 neurons. SBCs play the yin to ENG's yang. SBCs disinhibit L5 pyramidal neurons by downregulating the firing of interneurons that control the coupling of soma and dendrite in L5 cells. ENGs, through their direct effects on the L5 dendrites and electrical synapses with other inhibitory neurons, directly inhibit dendritic Ca<sup>2+</sup> spikes (Fig. 1b).

What could these separate circuits be achieving? Several possibilities are conceivable. The authors point out that L1 is ideally suited as the locus for an attentional mechanism. Attending to salient features in the environment is a vital function of the mammalian cortex. For instance, no matter how complex a visual scene is, it is possible to deliberately attend to say, all red, vertical or moving objects<sup>12</sup>. This is by no means a trivial computational task. However, L1 appears pivotal because this layer receives specifically attention-related cholinergic inputs from the basal forebrain<sup>9</sup> and also feedback input from higher cortical areas that are known to be crucial for attention. Inhibition of the top-down/bottom-up coupling would be an ideal mechanism for limiting attention to certain cortical areas or groups of cells (as in the 'searchlight' hypothesis<sup>13</sup>), possibly explaining how the cortex can effortlessly perform tasks such as targeted feature detection. Whatever the function



**Figure 1** Inhibitory neurons and their interaction with the active properties of pyramidal cells. (a) The menagerie of cortical inhibitory neurons, showing the region of the soma, axon or dendrites that each typically targets. (b) The connectivity of L1 inhibitory neurons (single bouquet cells and elongated neurogliaform cells) that define their yin-and-yang, disinhibitory-versus-inhibitory influences on the coupling between the tuft and basal regions of the L5 pyramidal neuron.

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of L1 inhibitory neurons, the presence of the two mechanisms in L1, one for inhibition and one for disinhibition, suggests that the cortex can be regulated in both directions. However, it is not clear from *in vitro* recordings whether one or both of these systems can be recruited *in vivo*.

In the final part of their study, therefore, Jiang *et al.*<sup>8</sup> tested the effects of L1 inhibitory neurons on dendritic spiking *in vivo*<sup>5</sup>. This required difficult blind dendritic recordings from L5 pyramidal neurons, again pioneered, a decade earlier, by one of the authors<sup>14</sup> and simultaneous somatic recording of L1 cells. These experiments demonstrated that the different L1 cortical circuits powerfully enhance or suppress dendritic Ca<sup>2+</sup> spikes in L5 pyramidal neurons in the functioning cortical network. The study by Jiang *et al.*<sup>8</sup> represents an extraordinary technical achievement

that simultaneously defines the local action of important classes of L1 inhibitory neurons. It now remains to be discovered through which L1-projecting pathways and under which circumstances the different circuits are activated. These experiments will perhaps be best approached using optogenetic techniques to suppress firing in specific populations of inhibitory neurons in behaving animals<sup>15</sup>. Understanding how local inhibitory mechanisms in L1 are activated by long-range connections and their influence on the local cortical circuit promises to bring decisive insights concerning the relationship between higher cognitive function and the precise details of neuronal firing.

#### COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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## UP states rise from the depths

Stuart Hughes & Vincenzo Crunelli

**A study optogenetically generating or suppressing activity in excitatory neocortical neurons *in vivo* finds that layer 5 pyramidal cells initiate and maintain widespread UP states, whereas layer 2/3 cells are subsidiary.**

Although the past 10 years have yielded considerable information about the cellular processes underlying the neocortical phenomena known as UP and DOWN states, neither the mechanisms that lead to the initiation and termination of UP states nor the neurons responsible for these transitions have been clearly established. UP states consist of step-like depolarizations accompanied by synaptic activity and firing, whereas DOWN states are periods of hyperpolarization and neuronal quiescence<sup>1</sup>. These states are reflected in the electroencephalograms of mammals during non-rapid eye movement sleep and under certain types of anesthesia as a slow (<1 Hz) rhythm<sup>1</sup>. Isolated UP states can also be observed in response to sensory stimulation<sup>2,3</sup> and direct thalamic activation<sup>3,4</sup>, suggesting that they represent a fundamental *modus operandi* of the neocortex. Several lines of evidence indicate that synchronized UP states most likely originate in infragranular cortical layers, and in particular layer 5 (refs. 3,5–8). However, whether UP state initiation is the exclusive remit of the infragranular cortex is questionable because other studies imply that these states may also be generated in layer 2/3 (refs. 7,9).

To distinguish between these possibilities, Beltramo *et al.*<sup>10</sup> used optogenetics to selectively depolarize or hyperpolarize excitatory neurons in either infragranular or supragranular (layer 2/3) neocortical layers. For infragranular neurons, this was achieved by stereotaxic injections of either adeno-associated virus–encoded channelrhodopsin2 (ChR2) or a combination of virally encoded halorhodopsin (eNpHR) and archaerhodopsin (Arch)<sup>11</sup>, respectively, into the Cre recombinase–bearing BAC transgenic mouse line Rbp4-Cre. In each case the transgene was expressed in ~14% of neurons, with ~95% of these being layer 5 pyramidal cells. For supragranular neurons, ChR2 or eNpHR plus Arch were selectively introduced via *in utero* electroporation, leading to expression in ~18% of layer 2/3 pyramidal cells.

Several previous results have implicated layer 5 as the source of synchronized UP states. First, current source density analysis of the slow rhythm in anesthetized cats reveals a dominant source in deep cortical layers<sup>3,5</sup>. Second, UP states in the rat and cat neocortex recorded through multiunit activity (MUA) *in vivo* or in cortical slices are first apparent, and most prominently expressed, in layer 5 before spreading to more superficial layers<sup>5–7</sup>. Third, it has been known for some time that layer 5 possesses effective machinery for initiating and transmitting low-frequency activity

across the neocortex<sup>8</sup>. A prevailing idea is that synchronized UP states are triggered by activity in a relatively small number of layer 5 pyramidal cells that possess sufficiently widespread connections to then engage a more extensive mosaic of cortical circuitry. Such activity could arise either through the summation of coincident, spontaneous miniature excitatory postsynaptic potentials<sup>5</sup> or through the presence of a subgroup of neurons that exhibit spontaneous, intrinsic oscillations<sup>9,12</sup>. The latter idea was initially prompted by theoretical work<sup>12</sup>, but subsequently a subset of layer 5 pyramidal cells that show spontaneous, intrinsic membrane fluctuations at <1 Hz<sup>9</sup> was identified in cortical slices. Interestingly, however, the same study showed that a subgroup of layer 2/3 pyramidal neurons can also display spontaneous activity at <1 Hz<sup>9</sup>. Together with the finding that synchronized UP states can sometimes be independently observed in superficial layers in neocortical slices in which connections between infragranular and supragranular layers have been severed<sup>7</sup>, this highlights the need to establish more clearly how and where UP states originate in the intact brain.

When Beltramo *et al.*<sup>10</sup> delivered light stimulation to the neocortex of urethane-anesthetized mice that express ChR2 in layer 5 pyramidal cells, the light provoked robust UP states in ChR2-positive cells that were largely indistinguishable from those that occurred spontaneously.

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