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Cortical feed-forward networks for binding different streams of sensory information

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Different streams of sensory information are transmitted to the cortex where they are merged into a percept in a process often termed 'binding.' Using recordings from triplets of rat cortical layer 2/3 and layer 5 pyramidal neurons, we show that specific subnetworks within layer 5 receive input from different layer 2/3 subnetworks. This cortical microarchitecture may represent a mechanism that enables the main output of the cortex (layer 5) to bind different features of a sensory stimulus.

Over the last 50 years, the idea of the 'functional column' has provided a dominant influence on our understanding of mammalian cortical circuits[1,2]. More recent studies have indicated that neurons within a column are further organized into subnetworks. The over-representation of reciprocal connections and triplet patterns in networks of layer 5 (L5) pyramidal neurons[3,4] indicates that their connections are clustered in subnetworks. Similarly, synaptically connected layer 2/3 (L2/3) pyramidal neurons in visual cortex[5] and within barrels (but not septa) of the barrel cortex[6] form subnetworks that receive common inputs from within L2/3 and from layer 4. How these subnetworks in L2/3 and L5 interact with one another is currently unknown. To address this, we investigated communication between subnetworks in L2/3 and L5 using triple whole-cell recordings from pyramidal neurons in brain slices of rat somatosensory cortex (see **Supplementary Methods** online).

To investigate whether L5 subnetworks receive common inputs from L2/3 pyramidal neurons, we recorded from pairs of L5 pyramidal neurons and sequentially from different presynaptic L2/3 pyramidal neurons (**Fig. 1a**; all experiments were carried out in accordance with the guidelines approved by the Animal Ethics Committee of the Australian National University). The probability that both L5 neurons received input from the same L2/3 neuron was significantly higher if the L5 neurons were also connected to each other. Both L5 neurons received synaptic input from the same L2/3 neuron in 22.1% of recordings when the L5 neurons were synaptically connected (total of 68 pairs with 15 double and 12 single connections), whereas this was the case in only 2.1% of recordings when the L5 neurons were not connected to each other (total of 340 pairs with 7 double and 100 single connections). From this data it can be calculated that, compared with random connectivity, the probability that a L2/3 neuron makes a synaptic connection with two L5 neurons is fourfold higher (4.4 ± 1.0; $P = 0.001$) if the L5 neurons are synaptically connected with each other (**Fig. 1b**, left), whereas the probability that two L5 neurons receive input from the same L2/3 neuron is reduced, although not significantly (0.69 ± 0.26; $P = 0.1$), if they are not synaptically connected (**Fig. 1b**, right). These findings show that individual

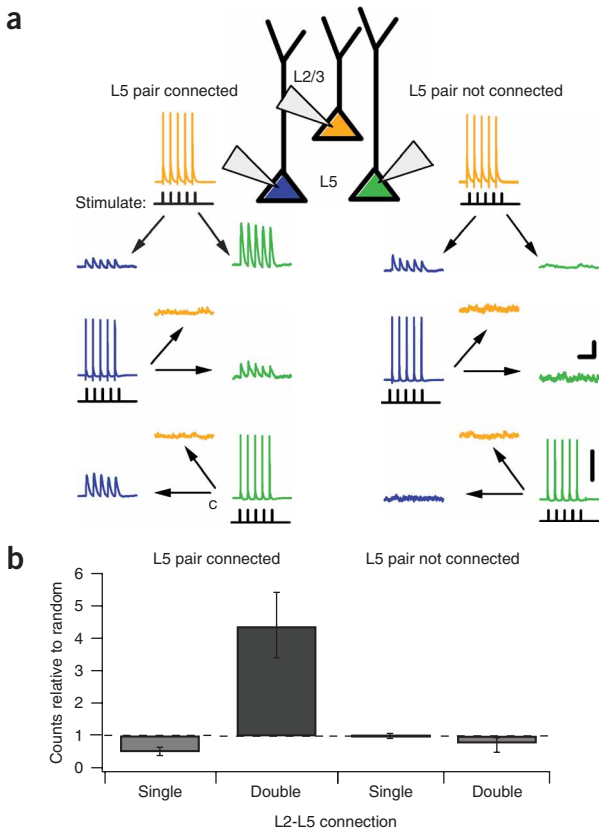

Figure 1 L2/3 neurons target the same L5 subnetwork. (**a**) Examples of triple recordings from connected (left panels) or unconnected L5 pairs (right panels). Inset shows recording scheme. Simultaneous recordings were made from two L5 neurons (blue and green) and one L2/3 neuron (orange). Black traces indicate presynaptic current injection used to stimulate action potentials. Scale bars: 100 ms, 1 mV for excitatory postsynaptic potential traces and 50 mV for action potential traces. (**b**) Numbers of connections between L2/3 neurons and one (single) or both (double) L5 neurons for connected (left panel) or unconnected (right panel) L5 pairs, shown relative to the expected counts for random network connectivity. Note the increase in double connections between L2/3 neurons and connected pairs of L5 neurons. Error bars represent s.d. (see **Supplementary Methods**).

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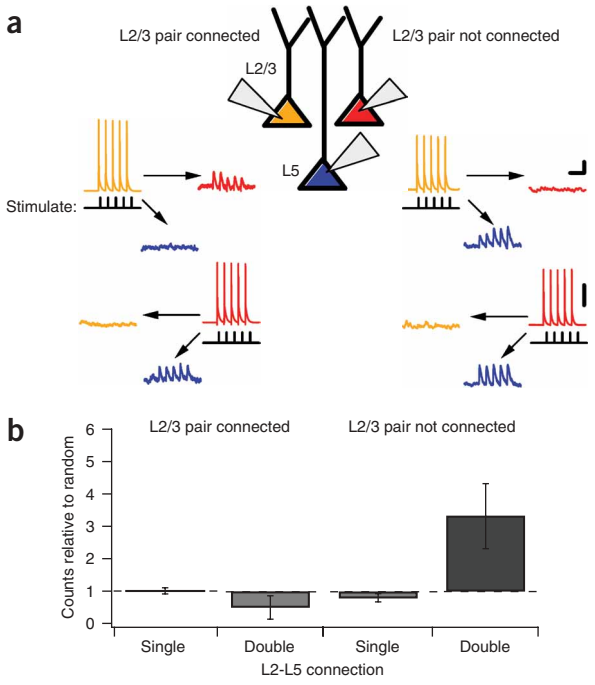

Figure 2 L5 neurons integrate inputs from different L2/3 subnetworks. (**a**) Examples of triple recordings from connected (left panels) or unconnected L2/3 pairs (right panels). Inset shows recording scheme. Simultaneous recordings were made from two L2/3 neurons (orange and red) and one L5 neuron (blue). Black traces indicate presynaptic current injection used to stimulate action potentials. Scale bars: 100 ms, 0.2 mV for excitatory postsynaptic potential traces and 50 mV for action potential traces. (**b**) Numbers of connections between one (single) or both (double) L2/3 neurons and the L5 neuron for connected (left panel) or unconnected (right) L2/3 pairs, shown relative to the expected counts for random network connectivity. Note the increase in double connections between unconnected L2/3 pairs and L5 neurons. Error bars represent s.d. (see **Supplementary Methods**).

L2/3 pyramidal neurons preferentially target L5 pyramidal neurons in the same L5 subnetwork.

To investigate whether L2/3 inputs onto L5 neurons originate from the same or different L2/3 subnetworks, we recorded from pairs of L2/3 pyramidal neurons and studied the connectivity of these neurons with L5 pyramidal neurons (**Fig. 2a**). The probability that the L5 neuron received input from both L2/3 neurons was significantly higher if the two L2/3 neurons were not connected to each other. Both L2/3 neurons connected to the same L5 neuron in 7.4% of recordings when the L2/3 neurons were not connected (total of 148 pairs with 11 double and 30 single connections), whereas this was the case in only 1.9% of recordings when the L2/3 neurons were connected (total of 106 pairs with 2 double and 35 single connections). Compared with random connectivity, the probability that the L5 neuron received input from both L2/3 neurons is therefore threefold higher (3.3 ± 1.0; $P = 0.006$) if the L2/3 neurons are not connected with each other (**Fig. 2b**, right), whereas this probability is halved (0.5 ± 0.3; $P = 0.05$) if the L2/3 neurons are connected (**Fig. 2b**, left). These findings indicate that L5 pyramidal neurons receive input preferentially from L2/3 pyramidal neurons located in different subnetworks.

The resulting connectivity scheme (**Supplementary Figure 1** online) includes connections between subnetworks in different layers, but not in a strict feed-forward manner. Recent work has indicated that subnetworks in L2/3 receive common inputs from L4 (ref. 5), and other studies have described the existence of specific subnetworks in L5 (ref. 3). We now show that L5 subnetworks share common inputs from individual L2/3 pyramidal neurons (**Fig. 1**). Moreover, we show that the output of individual L2/3 subnetworks is spread across different L5 subnetworks (**Fig. 2**), rather than simply being propagated from one subnetwork to the next. This enables individual L5 pyramidal neurons to integrate and bind information coming from different L2/3 subnetworks, which may encode different features of a stimulus. Consistent with this notion, several previous studies have indicated that neurons that code for the same orientation are connected to each other[7–10]. Furthermore, there is evidence that L5 neurons possess larger and more complex receptive fields than L2/3 or L4 neurons[11,12].

Connections between cortical subnetworks could evolve through Hebbian synaptic plasticity. Layer 2/3 neurons that receive similar inputs from L4 would be expected to be active at similar times, and would therefore be likely to connect to each other ('neurons that fire together wire together'). Synchronous activity in different L2/3 subnetworks that project to the same L5 subnetwork (**Supplementary Figure 1**), may be sufficiently powerful to trigger dendritic spikes and burst firing in L5 neurons[13]. Recent work indicates that burst firing and dendritic spikes in L5 neurons can lead to the induction of spike timing–dependent synaptic plasticity at both L2/3 to L5 and L5 to L5 connections[14,15]. In this way, Hebbian plasticity could lead to the described cortical connectivity scheme, and thereby may have an important role in binding information in the cortex.

In summary, we present data on the microarchitecture of cortical subnetworks, and propose that the convergence of information from different L2/3 subnetworks onto specific L5 subnetworks may represent a mechanism by which the main output pathway of the cortex, L5 pyramidal neurons, can bind different streams of sensory input.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

B.M.K. designed the experiments and did the calculations; B.M.K. and J.J.L. performed the experiments and data analysis; B.M.K., J.J.L. and G.J.S. jointly discussed the results and wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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