CORRECTION NOTICE


The organization of two new cortical interneuronal circuits
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In the version of this supplementary file originally posted online, in Supplementary Figure 7a under SBC, the last 400 ms of trace 3 was a duplicate of trace 2; also, the corresponding legend referred to main-text Figure 7a,b instead of to Figure 8a,b. The errors have been corrected in this file as of 3 March 2013.
Supplementary Information for

The organization of two new cortical interneuronal circuits

Xiaolong Jiang¹,⁴, Guangfu Wang¹,⁴, Alice J. Lee¹,³, Ruth L. Stornetta⁴, & J. Julius Zhu¹,²

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Figure S1

Fig. S1. Axonal length density maps of L1-3 interneurons.
(a-i) Axonal length density maps of L1 interneurons (SBC: n=17; ENGC: n=15) and L2/3 interneurons (MaC: n=15; NGC: n=28; BTCs: n=19; BPC: n=15; BaC: n=15; DBC: n=19; ChC: n=15).

Figure S2

Fig. S2. SBCs and ENGCs form inhibitory circuits with different kinetics and GABA-R compositions.
(a) SBC- and ENGC-evoked uIPSPs in L2/3 interneurons from acute cortical slices before (black traces) and after (gray traces) bath application of 5 μM CGP35348, which blocks GABAA receptors, and 100 μM picrotoxin (PTX), which blocks GABAB receptors. Scale bars apply to all recording traces with 80 mV and 8 mV bars applied to traces with and without action potentials, respectively.
(b) The bar graphs show the amplitudes of SBC- and ENGC-evoked uIPSPs before and after bath application of CGP and PTX. Values for SBC-evoked uIPSPs (Ctrl: 0.41±0.10 mV; CGP: 0.48±0.09 mV; n=12, z=2.5; p=0.08; CGP+PTX: 0.00±0.00 mV; n=12, z=2.8; p=0.005) and ENGC-evoked uIPSPs (Ctrl: 1.93±0.42 mV; CGP: 1.49±0.33 mV; n=8, z=2.5; p=0.05; CGP+PTX: 0.00±0.00 mV; n=8, z=2.6; p=0.005). Asterisks indicate p<0.05 (Wilcoxon tests).
(c) The bar graphs show the latencies, rise times and decay time constants of PTX- and CGP35348-sensitive uIPSPs in L2/3 interneurons (SBC→MaC: n=1; SBC→NGCs: n=3; SBC→BTC: n=4; SBC→BPC: n=1; SBC→BaC: n=1; SBC→DBC: n=1; ENGC→MaC: n=1; ENGC→NGCs: n=3; ENGC→BTC: n=4). Values for the latencies (Ctrl: 1.5±0.2 ms; PTX: 1.8±0.3 ms; n=12 for SBC-evoked uIPSPs; Ctrl: 3.9±0.5 ms; PTX: 3.8±0.4 ms; CGP: 39.5±1.6 ms; n=8 for ENGC-evoked uIPSPs), rise times (Ctrl: 11.2±1.4 ms; PTX: 12.4±2.0 ms; n=12 for SBC-evoked uIPSPs; Ctrl: 20.3±2.5 ms; PTX: 25.1±5.1 ms; CGP: 116.3±26.5 ms; n=8 for ENGC-evoked uIPSPs), and decay time constants (Ctrl: 41.3±5.5 ms; PTX: 40.3±3.7 ms; n=12 for SBC-evoked uIPSPs; Ctrl: 154.3±6.1 ms; PTX: 115.9±9.1 ms; CGP: 262±22.1 ms; n=8 for ENGC-evoked uIPSPs). Note that CGP- and PTX-sensitive uIPSPs were calculated by digitally subtracting the evoked responses after including additional CGP and PTX in the bath solution, respectively.

Figure S3

Fig. S3. SBC→ and ENGC↔L2/3 interneuronal circuits differ in synaptic connectivity.
(a) SBC- and ENGC-evoked uIPSPs in L2/3 interneurons from acute cortical slices before (black traces) and after (gray traces) bath application of 5 μM CGP35348, which blocks GABAA receptors, and 100 μM picrotoxin (PTX), which blocks GABAB receptors. Scale bars apply to all recording traces with 80 mV and 8 mV bars applied to traces with and without action potentials, respectively.
(a) Reconstruction of L1 SBC (pink), L1 ENGC (green), L2/3 BTC (yellow) and L2/3 NGC (brown) recorded simultaneously from an acute cortical slice. The double colored dots indicate the putative synaptic contacts. The schematic drawing shows symbolically their synaptic connections.
(b) Single action potentials elicited in presynaptic L1-3 interneurons evoked uIPSPs in postsynaptic L1-3 interneurons.
(c) The bar graphs show the connectivity and strength of synapses formed between L1-3 interneurons located within the same column. Values for the connectivity (SBC→L2/3: 13.0%, n = 197 of 1510 tested connections; ENGC→L2/3: 22.1%, n = 126 of 570 tested connections; \( \chi^2 = 25.9; p < 0.0005 \); SBC→L2/3: 1.1%, n = 9 of 93 tested pairs; ENGC→L2/3: 62.5%, n = 55 of 88 tested ENGC→L2/3 pairs; \( \chi^2 = 78.8; p < 0.0005 \); Chi-squared tests), and strength (SBC→L2/3: 0.34±0.03 mV, \( n = 128 \); ENGC→L2/3: 0.54±0.07 mV, \( n = 80 \); U = 3,499; \( p < 0.005 \); Mann-Whitney Rank Sum test; L2/3→SBC: 0.17 mV, \( n = 1 \); SBC→L2/3 pair; L2/3→ENGC: 0.50±0.06 mV, \( n = 53 \) ENGC→L2/3 pairs).
(d) The schematic drawing shows symbolically inhibitory connections between SBC and BTC, and inhibitory and electric connections between ENGC and NGC recorded from an acute cortical slice.
(e) The recording traces show that single action potentials elicited in presynaptic SBC evoked uIPSPs in postsynaptic BTC, single action potentials elicited in presynaptic ENGC evoked spikelets and uIPSPs in postsynaptic NGC, and single action potentials elicited in NGC evoked spikelets in postsynaptic ENGC.
(f) The recording traces show that the depolarizing and hyperpolarizing current injections in SBC and BTC had no effect on the membrane potentials of BTC and SBC, respectively, and the current injections in ENGC and NGC induced small membrane depolarization and hyperpolarization in NGC and ENGC, respectively. Scale bars in a, e and f apply to all recording traces with 80 mV and 2 mV bars applied to traces with and without action potentials, respectively.
(g) The bar graphs show the connectivity and coupling coefficient of electric synapses formed between L1 and L2/3 interneurons. Values for the electric synapse connectivity (SBC→L2/3: 0.0%, \( n = 9 \) of 900 tested pairs; ENGC→L2/3: 9.1%, \( n = 7 \) of 79 tested pairs with intersomatic distance >150 μm; \( \chi^2 = 85.6; p < 0.0005 \); ENGC→L2/3: 66.7%, \( n = 14 \) of 21 tested pairs with intersomatic distance <150 μm; \( \chi^2 = 649.3; p < 0.0005 \); Chi-squared tests), and coupling coefficient (ENGC→L2/3: 3.30±0.59%, \( n = 7 \) pairs with intersomatic distance >150 μm; ENGC→L2/3: 6.22±0.91%, \( n = 14 \) pairs with intersomatic distance <150 μm; \( U = 77.0; p < 0.05 \); Mann-Whitney Rank Sum test). Asterisks in c and g indicate \( p < 0.05 \) (Chi-squared or Mann-Whitney Rank Sum tests).

Figure S4

(a) MaC (n = 12)  
NGC (n = 21)  
BTC (n = 21)  
BPC (n = 15)  
BaC (n = 15)  
DBC (n = 16)  
ChC (n = 15)  

(b) MaC→L5P (n = 16/21)  
BTC→L5P (n = 145/1,408)  
BPC→L5P (n = 8/136)  
BaC→L5P (n = 126/891)  
ChC→L5P (n = 4/84)  

Figure S5

(a) Figure S4  
(b) MaC→L5P (n = 16/21)  
NGC→L5P (n = 140/443)  
BTC→L5P (n = 145/1,408)  
BPC→L5P (n = 8/136)  
BaC→L5P (n = 126/891)  
D BC→L5P (n = 43/219)  

(b) MaC→L5P (n = 16/21)  
NGC→L5P (n = 140/443)  
BTC→L5P (n = 145/1,408)  
BPC→L5P (n = 8/136)  
BaC→L5P (n = 126/891)  
D BC→L5P (n = 43/219)  

Figure S4. L2/3 interneurons in SBC→ and ENGC→L2/3 interneuronal circuits differ in dendritic anatomy.
(a) L1 fractions of dendritic arborization of L2/3 interneurons targeted by SBCs and ENGCs (MaC→SBC: 6.5±5.1%, \( n = 5 \); MaC→ENGC: 45.5±5.6%, \( n = 7 \); U = 0.0; \( p < 0.005 \); NGC→SBC: 0.0±0.0%, \( n = 10 \); NGC→ENGC: 37.8±5.8%, \( n = 11 \); U = 0.0; \( p < 0.0001 \); BTC→SBC: 7.0±2.2%, \( n = 10 \); BTC→ENGC: 49.7±4.2%, \( n = 11 \); U = 0.0; \( p < 0.0001 \); BPC: 0.9±0.3%, \( n = 15 \); BaC: 4.3±2.1%, \( n = 15 \); DBC: 8.4±3.2%, \( n = 16 \); ChC: 6.0±1.9%, \( n = 15 \). Asterisks indicate \( p < 0.05 \) (Mann-Whitney Rank Sum tests).
(b) Axonal length density plots targeted by SBCs and ENGCs (MaC→SBC: \( n = 5 \); MaC→ENGC: \( n = 7 \); F = 0.4; \( p < 0.05 \); NGC→SBC: \( n = 10 \); NGC→ENGC: \( n = 11 \); F = 1.8; \( p < 0.05 \); BTC→SBC: \( n = 10 \); BTC→ENGC: \( n = 11 \); F = 2.7; \( p < 0.05 \); ANOVA tests). Mann-Whitney Rank Sum tests indicate that the soma of MaCs, NGCs and BTCs targeted by SBCs were located deeper in L2/3 than that of MaCs, NGCs and BTCs targeted by ENGCs (MaC→SBC: 161.8±19.9 μm; \( n = 5 \); MaC→ENGC: 76.0±8.3 μm; \( n = 7 \); U = 0.0; \( p < 0.05 \); NGC→SBC: 166.7±17.8 μm; \( n = 10 \); NGC→ENGC: 44.2±6.0 μm; \( n = 11 \); U = 0.0; \( p < 0.01 \); BTC→SBC: 161.3±22.4 μm; \( n = 10 \); BTC→ENGC: 66.3±5.0 μm; \( n = 11 \); U = 0.0; \( p < 0.01 \).

Figure S5. SBC→ and ENGC→L2/3 interneuronal circuits differentially innervate L5 pyramidal neurons.
(a) The bar graphs show the connectivity and strength of synapses formed between SBCs, ENGCs, L2/3 interneurons postsynaptic to SBCs, or L2/3 interneurons postsynaptic to ENGCs, and L5 pyramidal neurons within the same column. Values for the connectivity (SBC→L5P: 0.0%, \( n = 0 \) of 530 tested connections; ENGC→L5P: 20.4%, \( n = 53 \) of 259 tested connections; \( \chi^2 = 116.3; p < 0.0005 \); ENGC→L5P: 20.5%, \( n = 132 \) of 657 tested connections; \( \chi^2 = 8.8; p < 0.05 \); Chi-squared tests) and strength (ENGC→L5P: 0.22±0.03 mV, \( n = 19 \); SBC→L2/3→L5P: 0.30±0.07 mV, \( n = 15 \); ENGC→L2/3→L5P: 0.28±0.07 mV, \( n = 15 \); U = 115.0; \( p = 0.92 \); Mann-Whitney Rank Sum test). Note that ENGCs, but not L2/3 interneurons postsynaptic to ENGCs, form synapses on L5 pyramidal neurons located in both the same and neighboring columns (ENGC→L5P: 0.0%, \( n = 0 \); ENGC→L5P: 20.4%, \( n = 53 \); ENGC→L5P: 5.2%, \( n = 6 \); \( \chi^2 = 14.1; p < 0.05 \); ENGC→L5P: 7.1%, \( n = 1 \); ENGC→L5P: 15.8%, \( n = 9 \); \( p < 0.02 \); Mann-Whitney Rank Sum test).
The bar graphs show the connectivity and strength of synapses formed between L2/3 interneurons and L5 pyramidal neurons within the same column. Values for the strength (MaC→L5P: 0.24±0.03 mV, n=11; NGC→L5P: 0.35±0.03 mV, n=88; BPC→L5P: 0.17±0.04 mV, n=8; BaC→L5P: 0.47±0.05 mV, n=67; DBC→L5P: 0.47±0.05 mV, n=41; ChC→L5P: 0.29±0.07 mV, n=4; F=3.8; p<0.005; ANOVA test). Note that L2/3 interneurons form synapses on L5 pyramidal neurons located in the same columns, but not those in neighboring columns (MaC→L5P_same column: 7.3%, n=16/219; MaC→L5P_neighboring column: 0.0%, n=0/74; \( \chi^2 = 5.7; p < 0.05 \); NGC→L5P_same column: 31.6%, n=140/443; NGC→L5P_neighboring column: 0.0%, n=0/106; \( \chi^2 = 11.8; p < 0.005 \); BPC→L5P_same column: 10.3%, n=8/136; BPC→L5P_neighboring column: 0.0%, n=0/81; \( \chi^2 = 4.9; p < 0.05 \); BaC→L5P_same column: 14.1%, n=126/891; BaC→L5P_neighboring column: 0.0%, n=0/51; \( \chi^2 = 8.3; p < 0.05 \); DBC→L5P_same column: 19.6%, n=43/219; DBC→L5P_neighboring column: 0.0%, n=0/43; \( \chi^2 = 19.1; p < 0.005 \); ChC→L5P_same column: 4.8%, n=4/84; \( \chi^2 = 3.7; p < 0.05 \); Chi-squared tests).

Fig. S6. EM confirms the majority of LM-identified synaptic boutons.

(a) Reconstruction of L2/3 DBC (blue) and L5 pyramidal neuron (grey) recorded simultaneously from an acute cortical slice. The double colored dots indicate the putative synaptic contacts identified by LM.

(b) Single action potentials elicited in presynaptic DBC evoked uIPSPs in postsynaptic L5 pyramidal neuron. 80 mV and 4 mV bars apply to traces with and without action potentials, respectively. Note the average uIPSP trace (black), as well as superimposed individual uIPSP traces (gray).

(c) Four light microscopy (LM)-identified synaptic boutons were confirmed with electron microscopy (EM). Arrow heads in EM images indicate synaptic junctions established by the axon of DBC.

(d) The numbers of LM- and EM-identified synapses.

Fig. S7. Spikelet-like events are prevalent in ENGCs recorded in vivo.

(a) Spontaneous recording traces of the SBC and ENGC in figure 8a-b in expanded scales. Red arrows indicate the spikelets characteristic of electric synapses. Note the spikelets absent in SBC, but prevalent in ENGC. Scale bars apply to all recording traces.

(b) Percentages of L1 interneurons displayed the spikelets (SBCs: 0%, n=0/18; ENGCs: 87.5%, n=7/8; \( \chi^2 = 21.6 \)). Asterisk indicates p<0.05 (Chi-squared test).

Fig. S8. Schematic drawing shows key features of SBC↔ and ENGC↔L2/3—I→L5 pyramidal neuronal circuits. See the main text for the detailed architecture of the two cortical interneuronal circuits.
Nature Neuroscience: doi:10.1038/nn.3305

Table S1
Synaptic connectivity between L1 and L2/3 interneurons

<table>
<thead>
<tr>
<th>Cell connection pattern</th>
<th>L2/3s in same columns (Connected/total and %)</th>
<th>L2/3s in neighboring columns (Connected/total and %)</th>
<th>( \chi^2 ) and P value (Chi-squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBC→Mac</td>
<td>6/124 4.8% 0/79 0.0%</td>
<td>3.9 0.047</td>
<td></td>
</tr>
<tr>
<td>SBC→NGC</td>
<td>18/250 7.3% 0/69 0.0%</td>
<td>5.3 0.022</td>
<td></td>
</tr>
<tr>
<td>SBC→BTC</td>
<td>68/466 14.6% 0/76 0.0%</td>
<td>12.7 0.000</td>
<td></td>
</tr>
<tr>
<td>SBC→BPC</td>
<td>34/122 27.9% 0/19 0.0%</td>
<td>7.0 0.008</td>
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</tr>
<tr>
<td>SBC→BaC</td>
<td>29/305 9.5% 0/64 0.0%</td>
<td>6.6 0.012</td>
<td></td>
</tr>
<tr>
<td>SBC→DBC</td>
<td>7/82 8.5% 0/43 0.0%</td>
<td>3.9 0.048</td>
<td></td>
</tr>
<tr>
<td>SBC→ChC</td>
<td>13/75 17.3% 0/22 0.0%</td>
<td>4.4 0.036</td>
<td></td>
</tr>
</tbody>
</table>

Note that SBCs rarely form mutual inhibitory connections with L2/3 MaCs, NGCs and BTCs, even though synaptic connections formed between various types of L1-3 interneurons and L5 pyramidal neurons.

Note the Chi-squared tests show no difference between physiologically and light microscopically identified ChCs, DBCs, BPCs, BaCs, and NGCs.

Table S2
Correlation of physiology-, LM- and EM-identified inhibitory connections on L5 pyramidal neurons

<table>
<thead>
<tr>
<th>Cell connection</th>
<th>Connected pairs (LM/physiology)</th>
<th>No. of boutons in LM-identified pairs</th>
<th>Unconnected pairs (LM/physiology)</th>
<th>( \chi^2 ) and P value (Chi-squared)</th>
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</thead>
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<tr>
<td>ENGC→LSP</td>
<td>6/7</td>
<td>4.5±0.7  (n=6)</td>
<td>11/11</td>
<td>1.7 0.729</td>
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<tr>
<td>MaC→LSP</td>
<td>10/11</td>
<td>5.4±0.4  (n=10)</td>
<td>14/14</td>
<td>1.3 0.714</td>
</tr>
<tr>
<td>NGC→LSP</td>
<td>17/17</td>
<td>3.7±0.3  (n=17)</td>
<td>29/30</td>
<td>0.6 0.831</td>
</tr>
<tr>
<td>BTC→LSP</td>
<td>27/28</td>
<td>5.7±0.6  (n=27)</td>
<td>71/71</td>
<td>2.6 0.874</td>
</tr>
<tr>
<td>BPC→LSP</td>
<td>8/8</td>
<td>4.3±0.4  (n=8)</td>
<td>14/14</td>
<td>1.000</td>
</tr>
<tr>
<td>BaC→LSP</td>
<td>28/31</td>
<td>3.8±0.3  (n=26)</td>
<td>42/43</td>
<td>1.9 0.501</td>
</tr>
<tr>
<td>DBC→LSP</td>
<td>26/27</td>
<td>4.1±0.3  (n=26)</td>
<td>24/24</td>
<td>0.9 0.843</td>
</tr>
<tr>
<td>ChC→LSP</td>
<td>4/4</td>
<td>3.8±0.6  (n=4)</td>
<td>10/10</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table S3
Synaptic connectivity and strength between L1 and L2/3 interneurons in different cortical areas

<table>
<thead>
<tr>
<th>Presynaptic L2/3s in sensory cortex</th>
<th>L2/3s in motor cortex</th>
<th>( \chi^2 ) and P value (Chi-squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell type</td>
<td>(Connected/total and %)</td>
<td>(Connected/total and %)</td>
</tr>
<tr>
<td>SBC</td>
<td>110/181</td>
<td>13.7% 52/469 11.1% 1.9 0.171</td>
</tr>
<tr>
<td>ENGC</td>
<td>40/186</td>
<td>21.5% 16/77 20.8% 0.0 0.856</td>
</tr>
</tbody>
</table>

Table S4
Synaptic connectivity and strength between L1-3Is and L5 pyramidal neurons in different cortical areas

<table>
<thead>
<tr>
<th>Presynaptic LSPs in sensory cortex</th>
<th>LSPs in motor cortex</th>
<th>( \chi^2 ) and P value (Chi-squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell type</td>
<td>(Connected/total and %)</td>
<td>(Connected/total and %)</td>
</tr>
<tr>
<td>MaC</td>
<td>7/112</td>
<td>6.3% 5/83 6.0% 0.0 0.791</td>
</tr>
<tr>
<td>NGC</td>
<td>88/279</td>
<td>31.5% 30/99 30.3% 0.1 0.819</td>
</tr>
<tr>
<td>BTC</td>
<td>62/381</td>
<td>9.9% 30/191 9.4% 0.1 0.812</td>
</tr>
<tr>
<td>BPC</td>
<td>3/66</td>
<td>4.5% 1/26 3.9% 0.0 0.882</td>
</tr>
<tr>
<td>BaC</td>
<td>72/507</td>
<td>14.2% 31/238 13.0% 0.2 0.662</td>
</tr>
<tr>
<td>DBC</td>
<td>23/127</td>
<td>18.1% 13/63 20.6% 0.2 0.676</td>
</tr>
<tr>
<td>ChC</td>
<td>2/44</td>
<td>4.5% 1/19 5.3% 0.0 0.902</td>
</tr>
<tr>
<td>ENGC</td>
<td>13/72</td>
<td>18.1% 9/48 18.3% 0.0 0.965</td>
</tr>
</tbody>
</table>

Movie S1. 3D reconstruction reveals distinguished axonal anatomy of SBCs and ENGCs.
This movie shows the 3D structure of L1 SBC (pink) and L1 ENGC (green) recorded from an acute cortical slice.

Movie S2. 3D reconstruction reveals distinguished axonal anatomy of L2/3 interneurons.
This movie shows the 3D structure of L2/3 MaC (red), NGC (orange), BTC (yellow), BPC (dark green), BaC (cyan), DBC (blue) and ChC (purple) recorded from acute cortical slices.