Supplementary information

Intrinsic optical imaging

Intrinsic optical imaging was used to locate the D2 column of the barrel. The temporal muscle was partly removed and the skull thinned over a 3 mm by 3 mm window, centred 2.5 mm caudal and 5.5 mm lateral from bregma. An image of the blood vessel pattern was taken using illumination at 546 nm. Intrinsic imaging data was collected using a Dalsa CCD camera (type 1M60) in combination with an Imager 3001 and Imager 3001 lab interface (Optical Imaging, Rehoboth, Israel). The combination of a 135-mm top lens and a 50-mm bottom lens (NA 0.46) resulted in a final magnification of 2.7 times. During imaging, the exposed cortex was illuminated with a 630-nm interference filter and two light guides. Initial experiments were carried out to tune the stimulation protocol for optimal intrinsic signals. As a result, repetitive deflections (10 deflections) of a single whisker at a frequency of 5 Hz in the caudal direction were used here with effective amplitude of 5°. Data acquisition started 2 s before stimulus onset and total duration of data acquisition was 10 s. Data frames were captured at a frequency of 5 Hz with a binning of 3 by 3 pixels (312 × 308 pixels on brain, pixel size 15 μm^2). The intrinsic signal was averaged over 16 trials (15 s interstimulus time interval), which generated a spot roughly the size of a single column (diameter ~350 μm). Trials without whisker stimulation (average of 8 trials) were acquired for comparison.

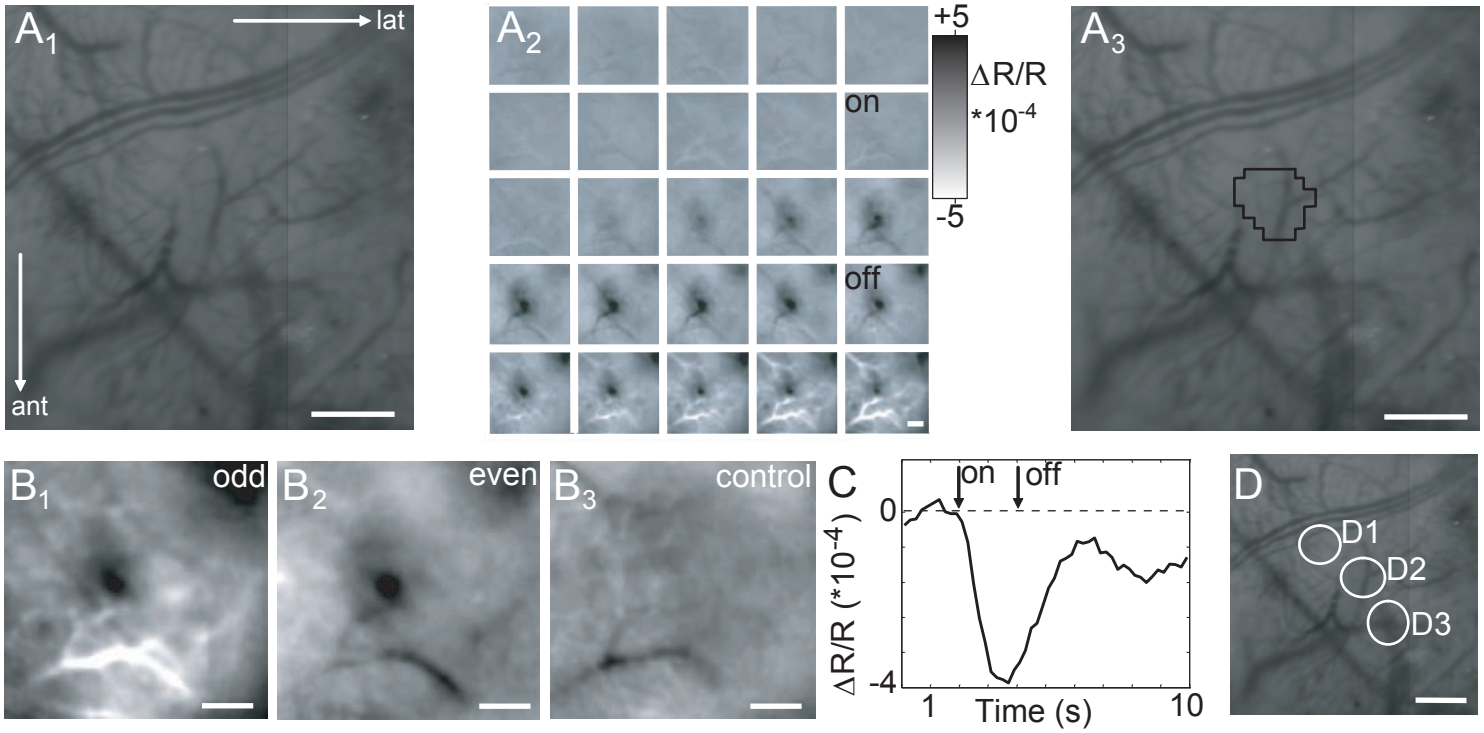
Data were analysed using Matlab (Mathworks) and custom-made software (H. Spors, Heidelberg, Germany). Off-line analysis included an additional 3 by 3 binning. To obtain values of relative reflectance change (ÄR/R), we averaged 10 frames before stimulus onset and divided the entire frame series by this average. Intrinsic optical signals were judged based on odd/even comparison, control trials, time course of region of interest and spatially segregated signals corresponding to different whiskers.

Table 1: Cell counts

Layer	Area (μm^2)	Thickness (µm)	Total cell density (*1000/mm^3)	Cell density GABA-postive neurones (*1000/mm^3)	Total number of cells per layer
L1		150			
L2/3	120000	600	52.6	8	3211
L4	120000	225	85.7	9.7	2052
L5A	120000	310	36.1	6.3	1109
L5B	120000	290	36.1	6.3	1037
L6	120000	275	42.5	5.5	1219
	total	1850			

Supplementary Table 1. **Cell counts**

Total number of cells is calculated by determining the volume of the particular layer (Area * Thickness) multiplied by the cell density for excitatory neurones (Total cell density – cell density GABA pos. neurones).

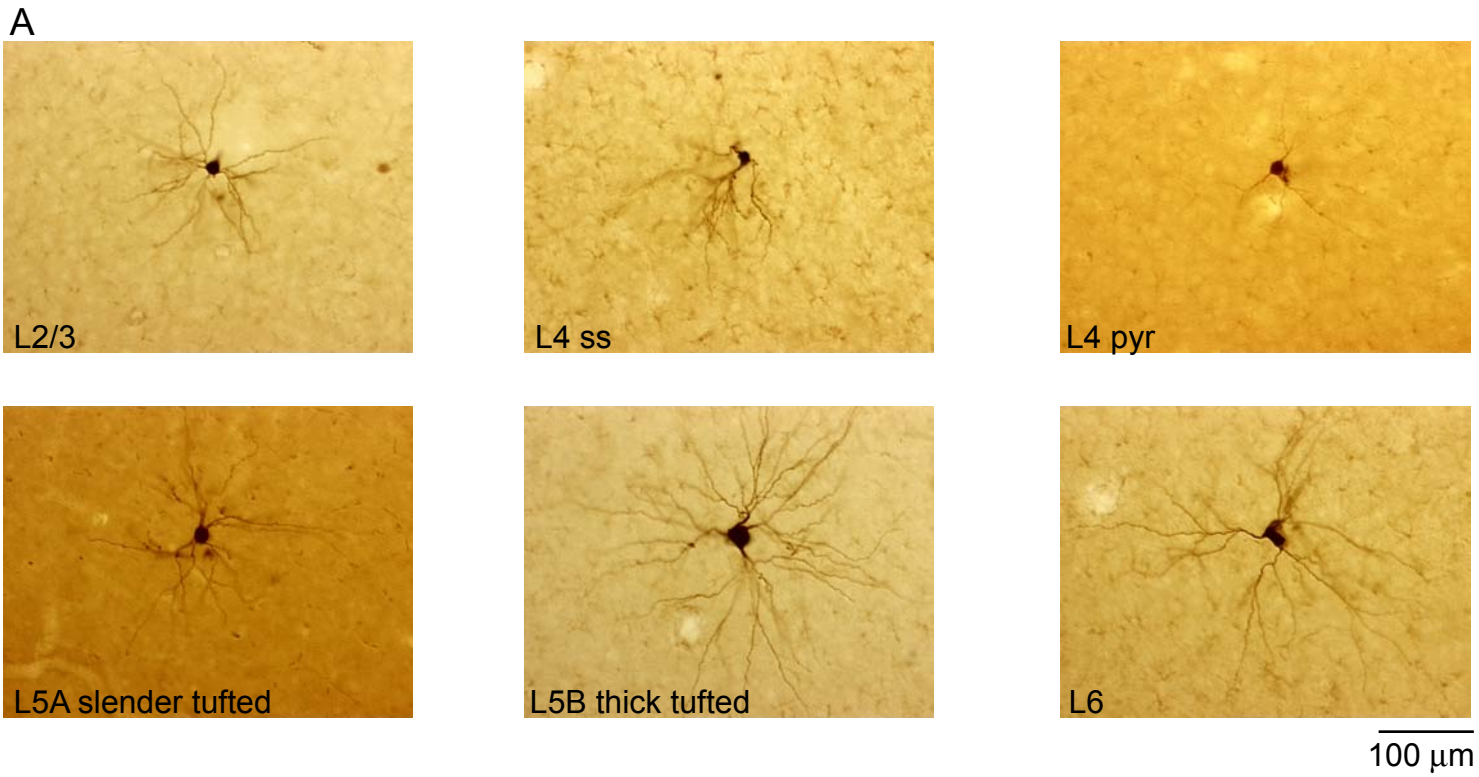


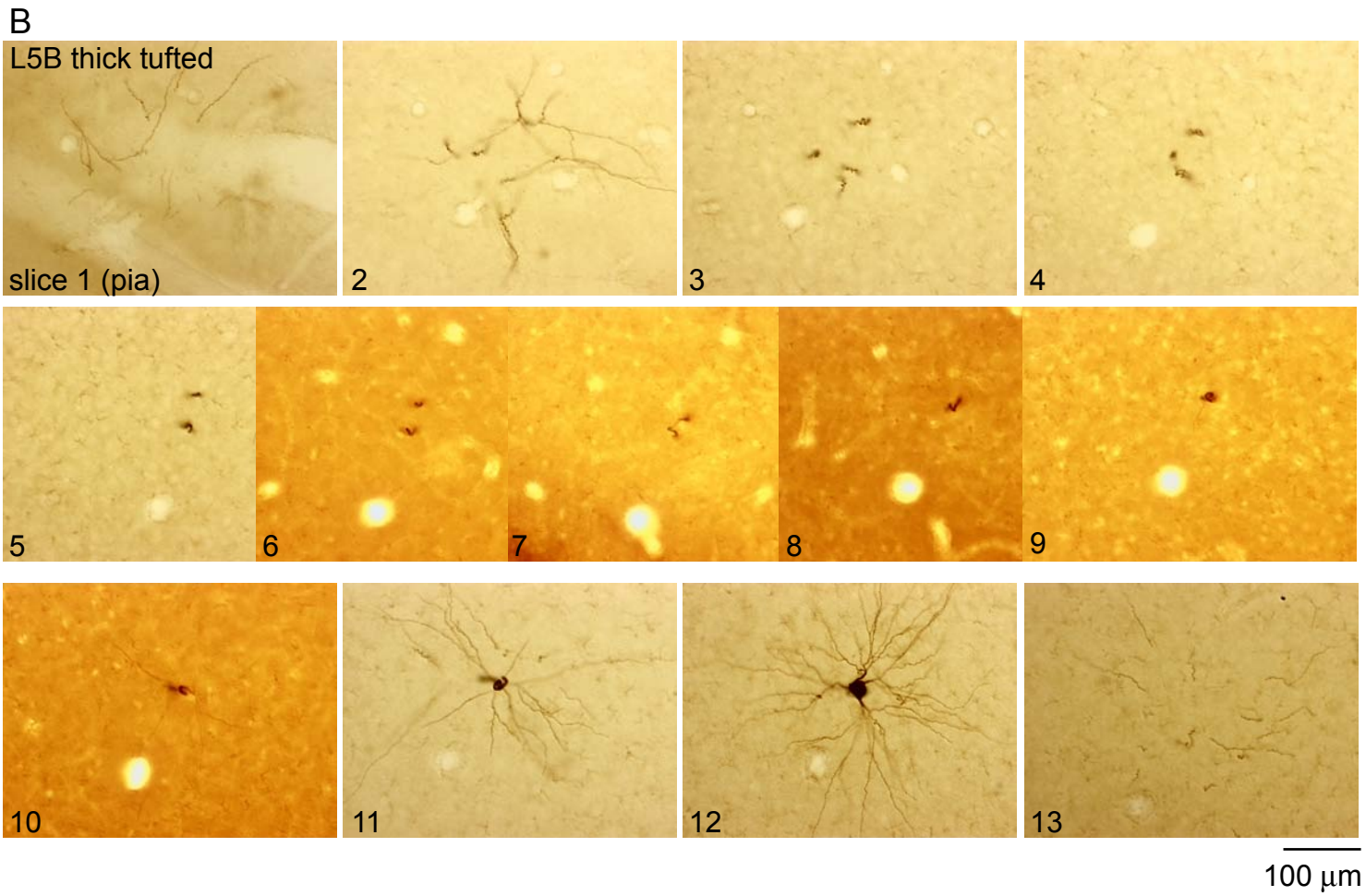
de Kock CPJ, Bruno RM, Spors H, Sakmann, B

Supplementary Figure 1

Supplementary Figure 1. Intrinsic optical imaging to target D2 column of barrel cortex

A1) Image of the blood vessel pattern covering the barrel cortex imaged through thinned skull. A2) Intrinsic optical imaging experiment (frame rate 5 Hz). After 2 s baseline, the D2 whisker is deflected 10 times at 5 Hz to activate the corresponding D2 column. This leads to a decreased reflection of light due to a change in the oxy-deoxy haemoglobin ratio, changes in blood volume and light scattering changes (illumination at 630 nm). A3) A region of interest is overlaid onto the blood vessel image to determine location for the craniotomy. B1-3) Control experiments illustrating that multiple trials result in activity at the same spot, whereas no activity is observed in the absence of the stimulus. (C) Time course of reflection of light (ĂR/R) for the region of interest after filtering in space. (D) Deflection of the D1 and D3 whiskers reveal the location of the D1 and D3 column, respectively.



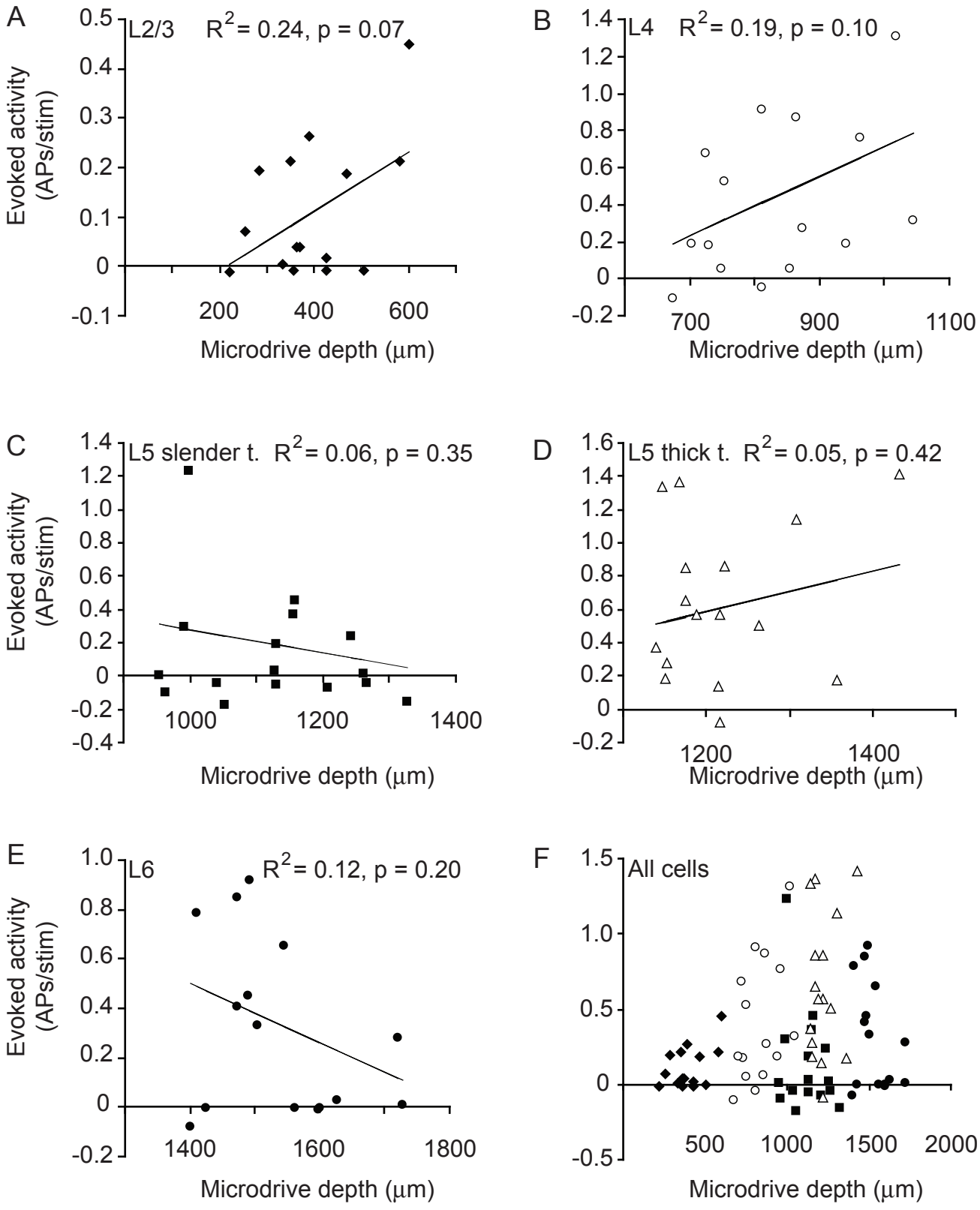


de Kock CPJ, Bruno RM, Spors H, Sakmann B

Supplementary Figure 2

Supplementary Figure 2. Photographs of biocytin-labelled cells

(A) Tangential view of sections containing the cell body of the representative examples shown in Fig. 1. (B) Stack of the L5 thick tufted cell shown in Fig. 1 showing photographs of all sections containing dendritic structures ranging from pia to L5B.

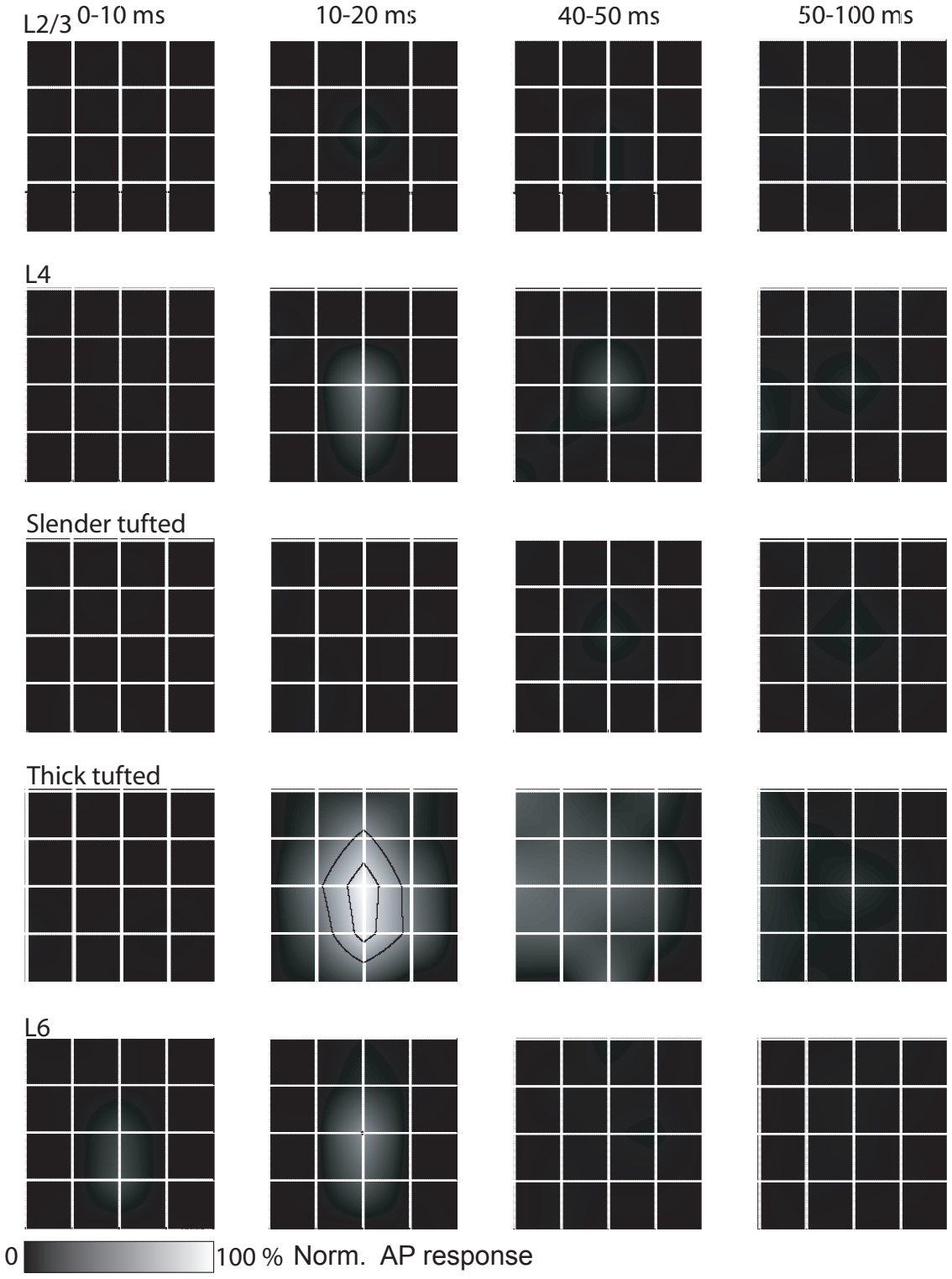


de Kock CPJ, Bruno RM, Spors H, Sakmann, B

Supplementary Figure 3

Supplementary Figure 3. Relationship of evoked AP activity and electrode position as estimated from micro drive depth

A-E) Evoked activity (APs/stim) is not correlated with recording depth for most layers or cell types. Note that in L2/3, there is a trend (P = 0.07) towards higher response amplitudes of cells located deeper in L2/3 (when omitting the data point at 600 µm though, no correlation is found (P = 0.48) between cell depth and response amplitude, average AP response per stimulus 0.09 ± 0.10). (F) Evoked activity *versus* recording depth for all cells. Overlap of cells classified as L4 and L5 slender tufted cells is most likely due to variability in brain size and location of the L4-L5 border from experiment to experiment.

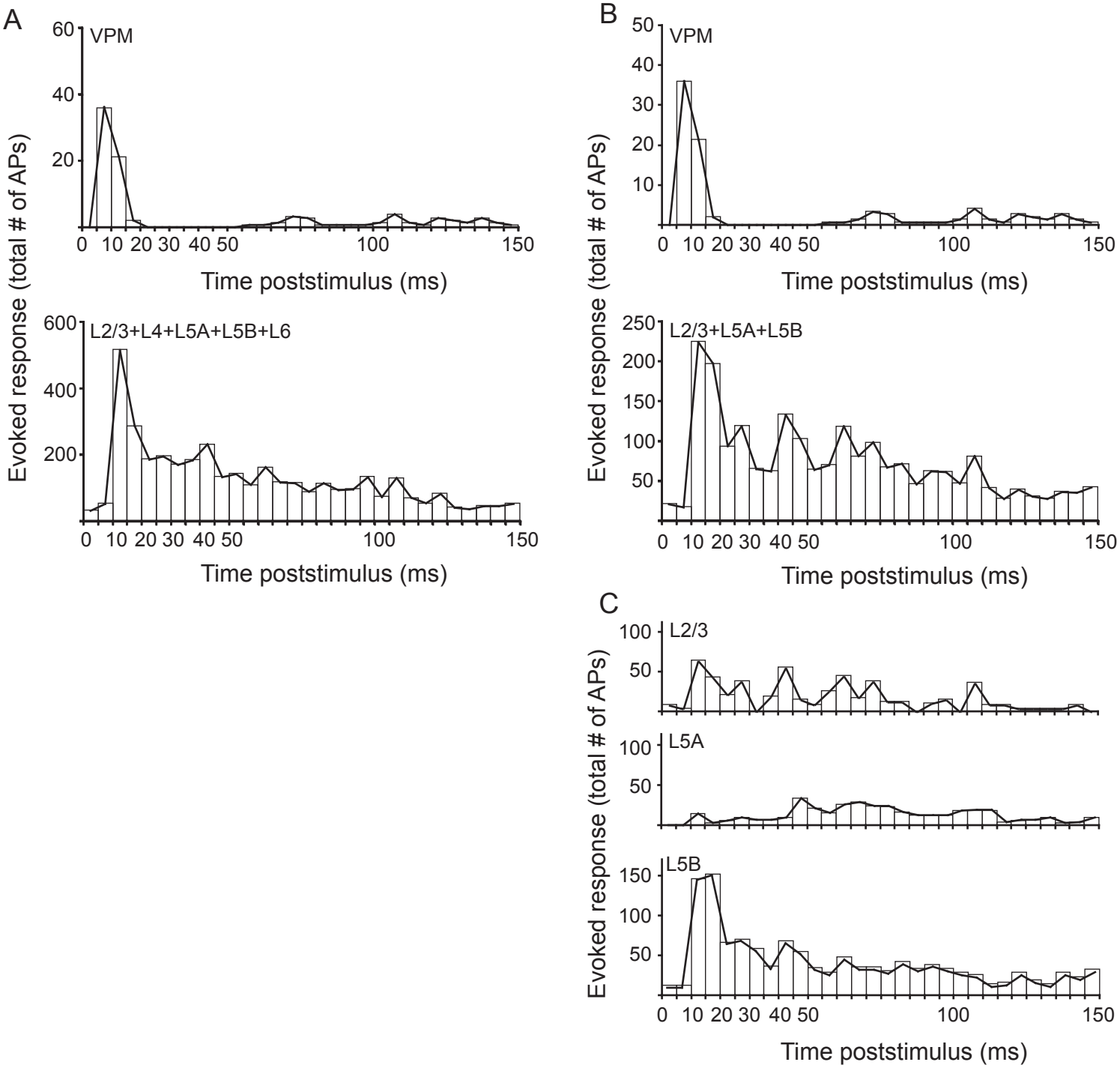


de Kock CPJ, Bruno RM, Spors H, Sakmann B

Supplementary Figure 4

Supplementary Figure 4. layer specific dynamics of suprathreshold RF structure

Same as in Fig. 6 (see Article), but responses were normalized to peak AP activity, which was in L5 thick tufted cells. Note that average evoked activity in other layers (AP/stimulus/cell) is much lower compared to L5 thick tufted cells.



de Kock CPJ, Bruno RM, Spors H, Sakmann B

Supplementary Figure 5

Supplementary Figure 5. Input-output AP budget

(A) Amplification of APs: average PSTHs illustrating the response in time after deflection of the principal whisker (at 5 ms bins). Compared to VPM, the sensory stimulus is represented by an increased number of APs in the cortical column. B,C) Desynchronization of APs: average PSTHs illustrating the response in time after deflection of the principal whisker (at 5 ms bins). Compared to VPM, the sensory stimulus is represented by an increased number of APs in the main output layers of the column that project to subcortical structures (B, L2/3, L5A and L5B, respectively) and (C) desynchronized AP firing in output layers is layer-specific.