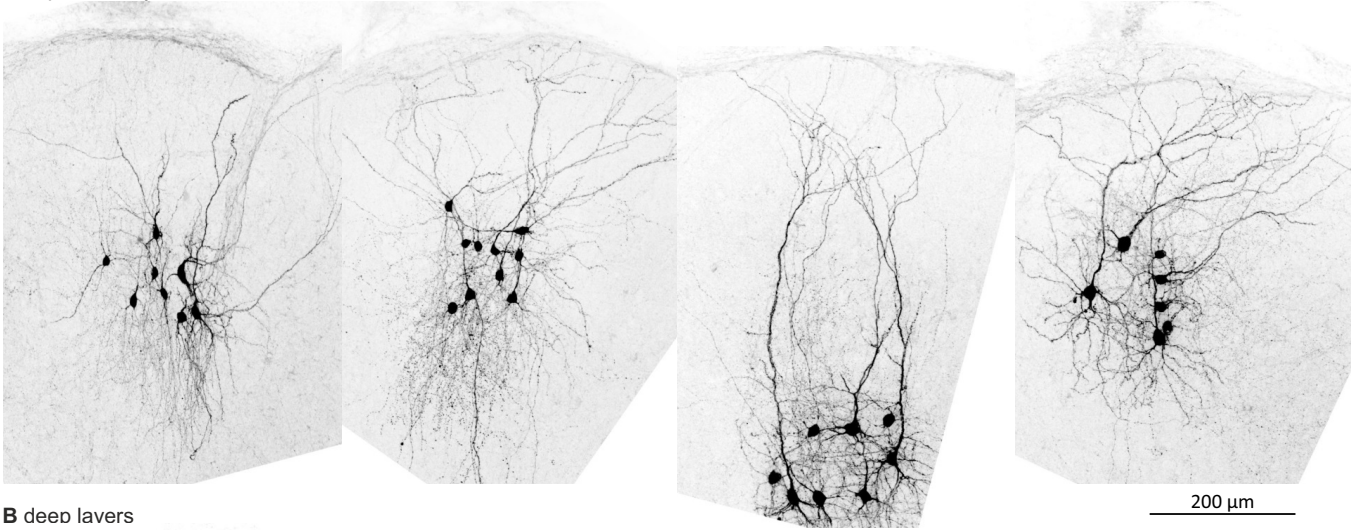
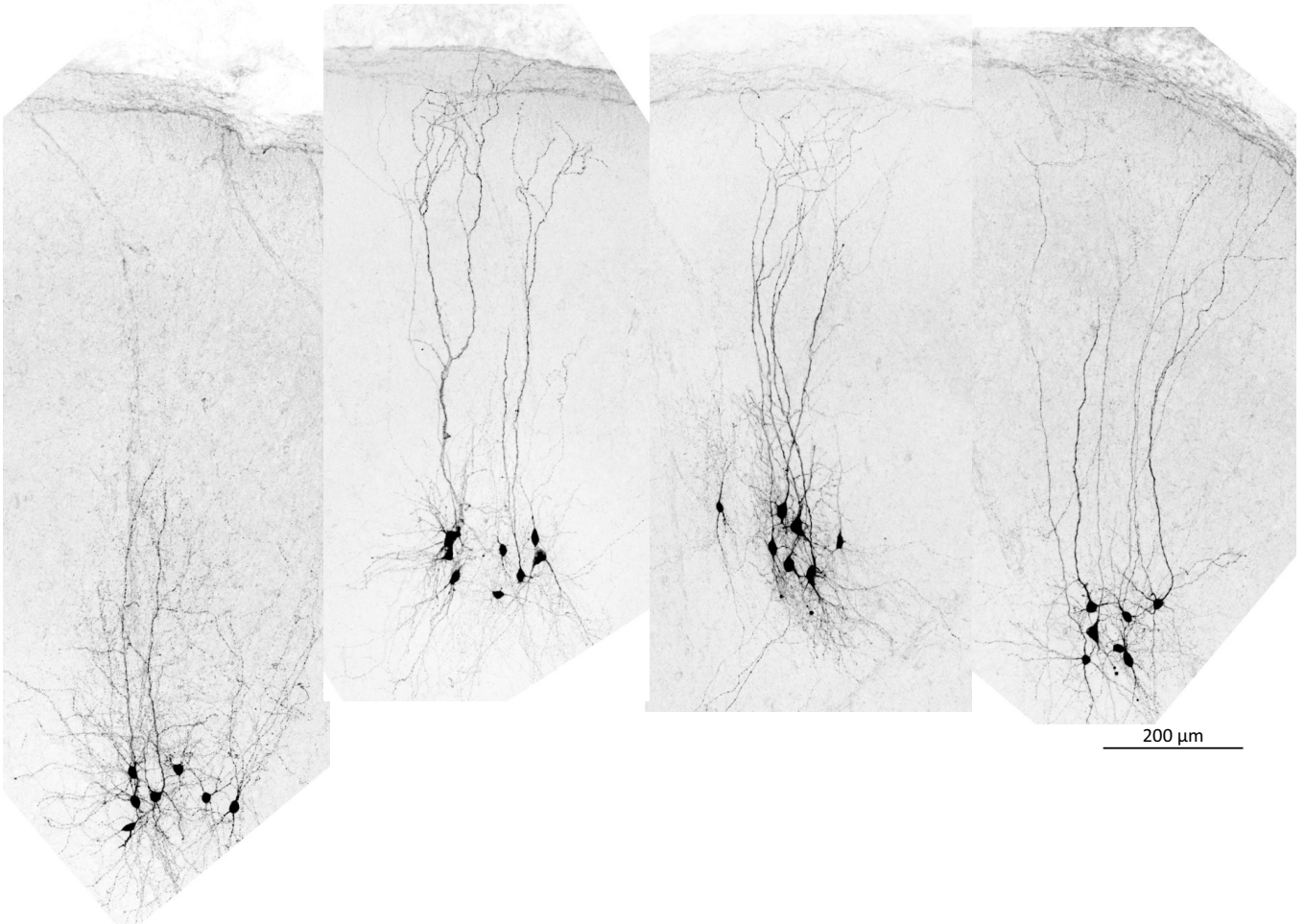


Supplement figure 1

A superficial layers

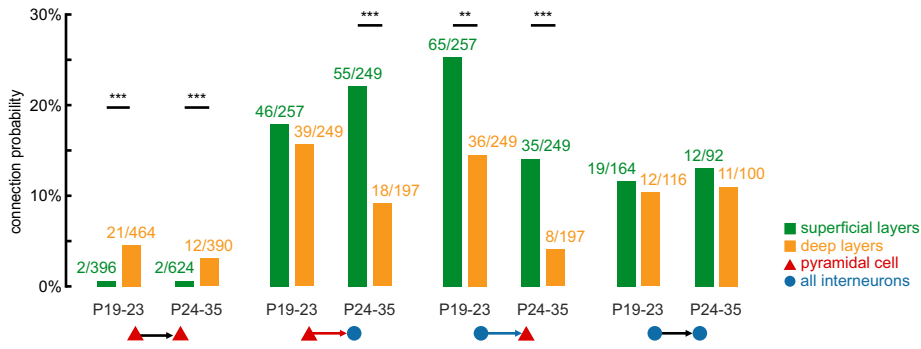


B deep layers



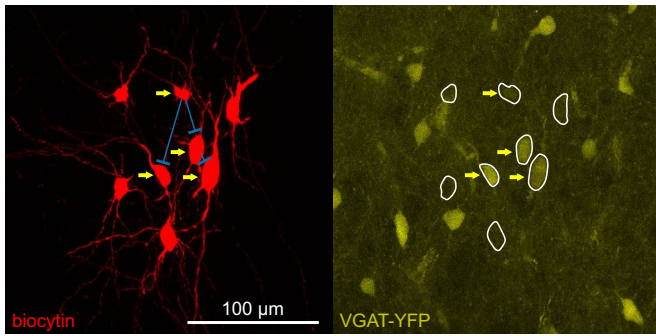
Representative images of recorded cells filled with biocytin. (A) Example of clusters from superficial layers. In a few experiments, such as in the second cluster, after recording from eight cells, individual pipettes could be retracted forming an outside-out patch and replaced while maintaining the recording configurations of the other seven cells. Additional cells could then be patched to start a new recording session with the remaining cells. (B) Examples of cell clusters in the deep layers. Note that in some cases the dendritic orientation that was used to determine the distance to the pia deviates from the perpendicular axis to the pia.

Supplement figure 2



Age-dependent connection probability. Connection probability is shown for different age groups (P19-23, P24-35), layers (green: superficial, orange: deep) and synaptic groups. Fisher's exact test was used for statistical comparison. *** $p < 0.001$, ** $p < 0.01$. Significant layer-specific differences exist within both age groups and all relevant synaptic groups, except in PC-IN connections of young animals.

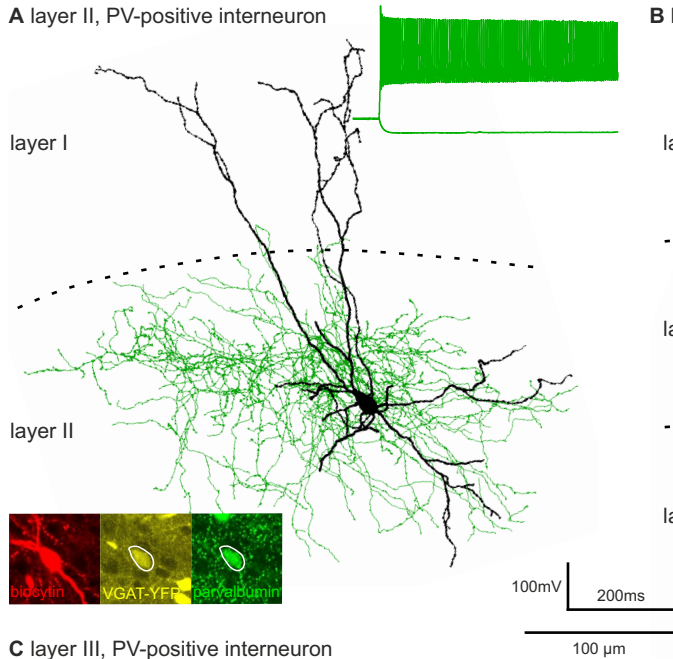
Supplement figure 3



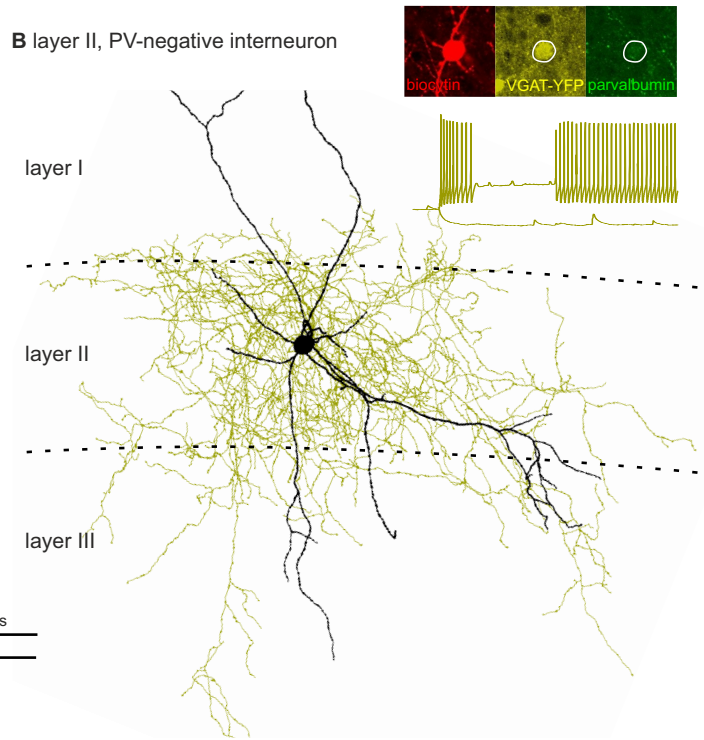
A cluster of eight cells with 4 YFP-positive cells (yellow arrows) in the deep layers filled with biocytin (red). This cluster contained, among other connections, three connections between interneurons (blue arrows). These represent three out of five observed deep IN-IN connections orientated towards deeper layers which resulted in the significant deviation in the orientation preference in this synaptic group.

Supplement figure 4

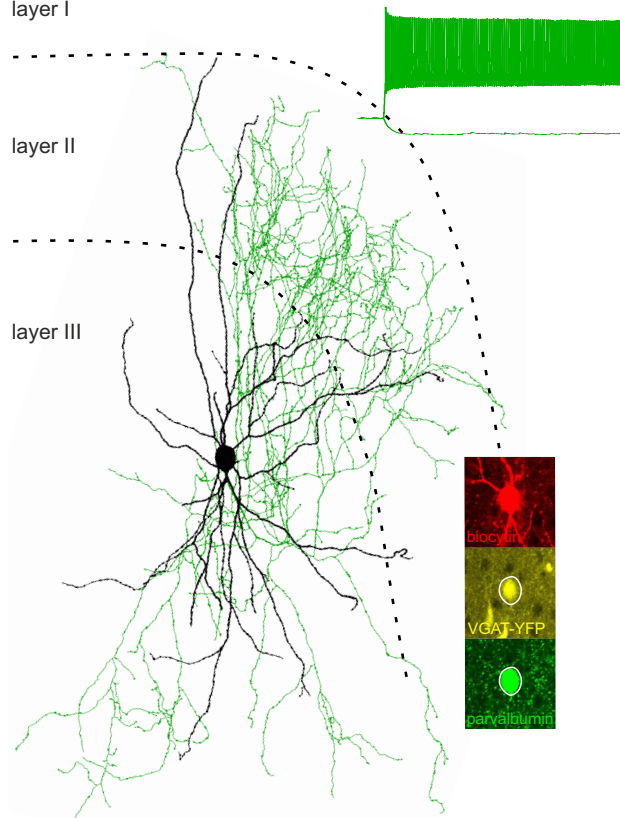
A layer II, PV-positive interneuron



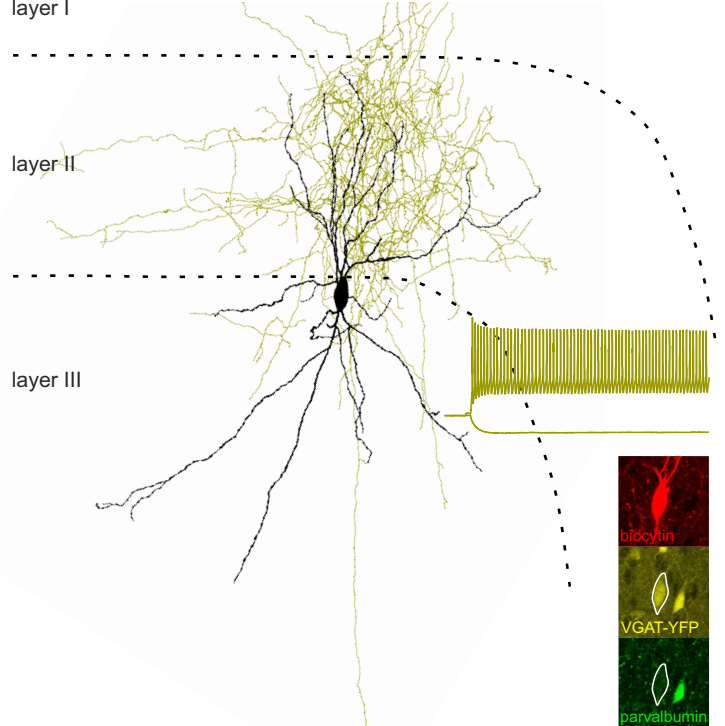
B layer II, PV-negative interneuron



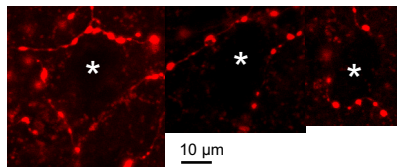
C layer III, PV-positive interneuron



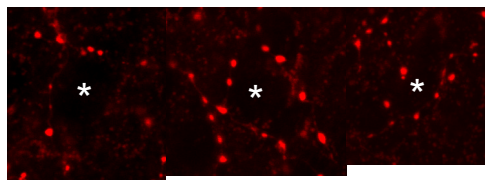
D layer III, PV-negative interneuron



E Higher magnification of axonal baskets from cell (A)

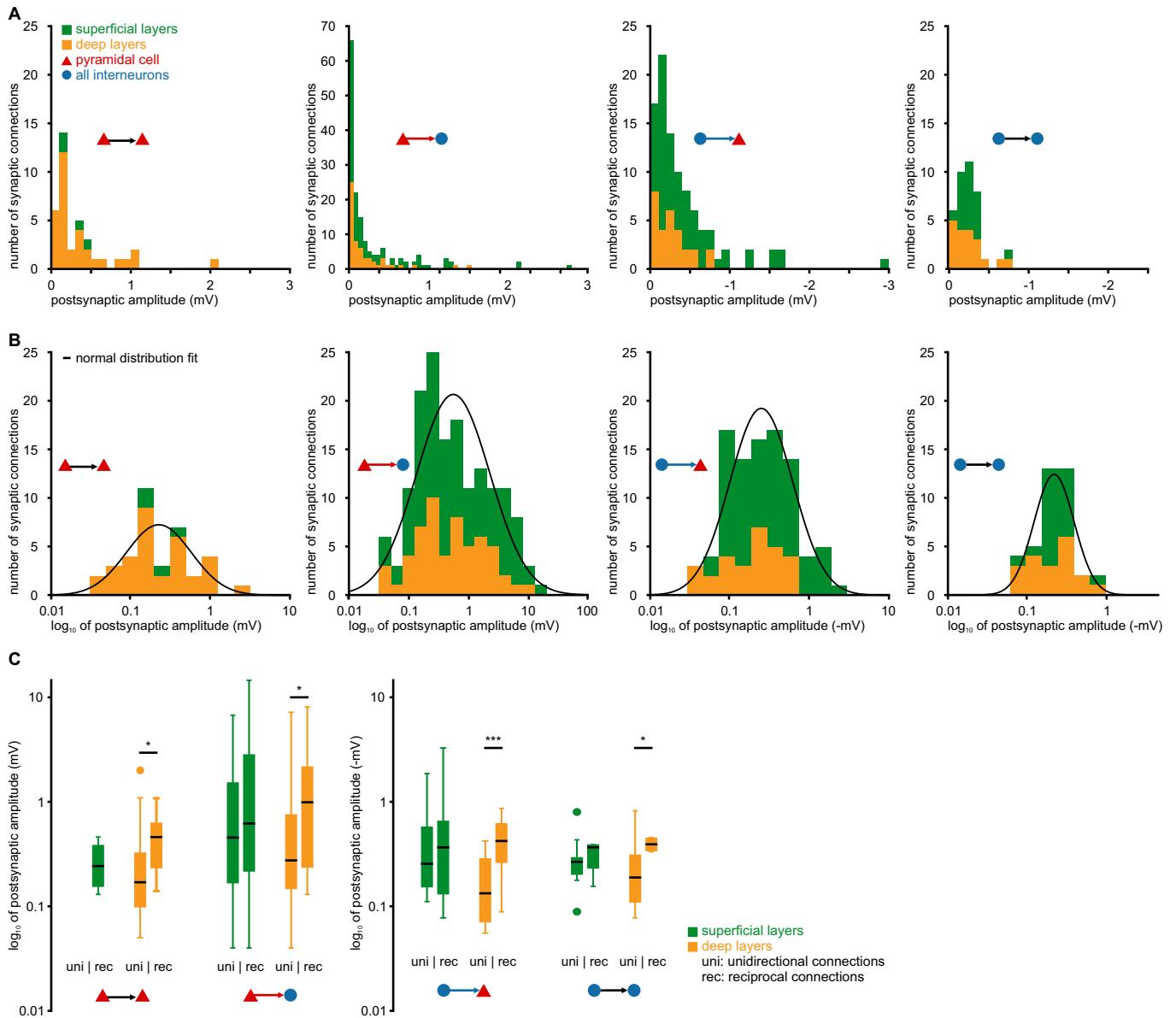


F Higher magnification of axonal baskets from cell (C)



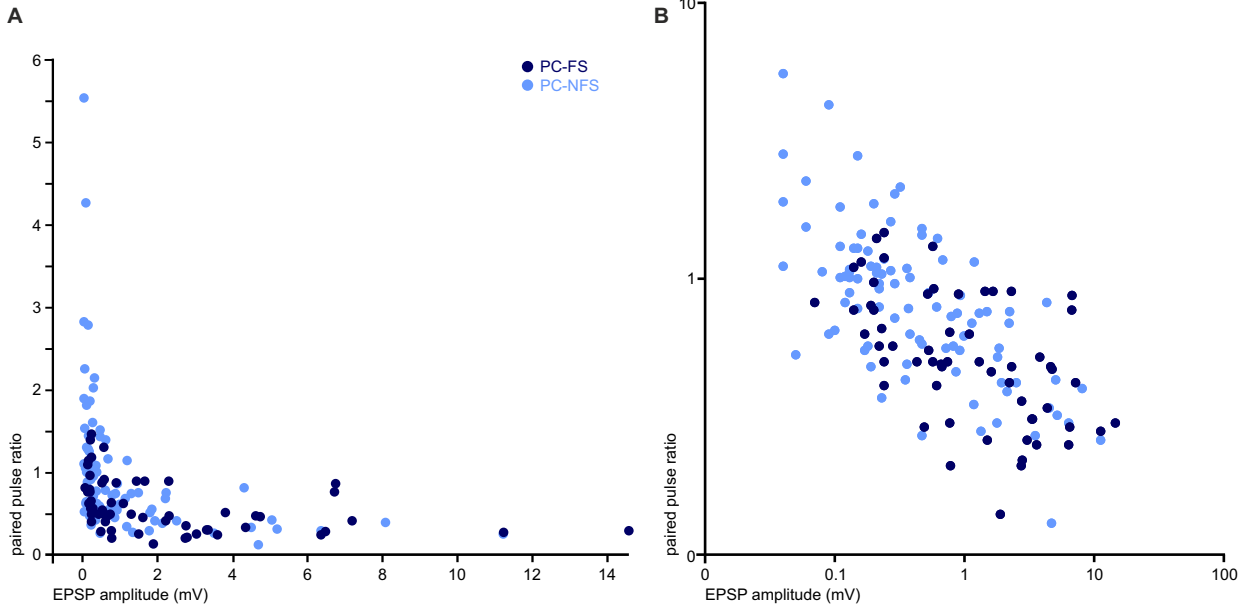
Morphological reconstructions of immunohistochemically identified interneurons. (A) Reconstruction of layer II parvalbumin-positive interneuron with axon colored in green. Fast spiking firing pattern is shown on the upper right side. Stainings in the lower left corner show the cell body positive for biocytin (red), VGAT-YFP (yellow) and PV (green). Dotted lines roughly depict layer borders. Respective scale bars apply to firing patterns, reconstructions and cell body stainings of panel A to D. (B) Reconstruction of layer II parvalbumin-negative cell with axons colored in yellow. (C) Layer III, parvalbumin-positive cell. (D) Layer III parvalbumin-negative cell. (E) Biocytin-labelled (red) presynaptic varicosities of parvalbumin-positive interneuron from panel A form axonal basket-like structures around somata of pyramidal cells (asterisk). (F) Axonal baskets of parvalbumin-positive interneuron from panel C.

Supplement figure 5



Log-normal distribution of postsynaptic amplitudes. (A) The postsynaptic amplitude distributions are shown for four synaptic groups. Each histogram furthermore distinguishes between superficial (green) and deep layers (orange). (B) The postsynaptic amplitude distributions are shown on a logarithmic scale for the amplitude. The black curve depicts a fitted normal distribution which in this case represents a log-normal distribution. (C) Box plots of postsynaptic amplitudes of unidirectional connections (uni) and reciprocal connections (rec) are shown on a logarithmic scale for each synaptic group and layer. Mann-Whitney U test was used for statistical comparison. * $p < 0.05$, *** $p < 0.001$.

Supplement figure 6



The relationship of short-term plasticity and amplitude of postsynaptic responses. A scatter diagram of the paired pulse ratio as a function of the postsynaptic amplitude for excitatory connections onto interneurons. PC-FS connections are plotted in dark blue and PC-NFS connections in light blue. (A) linear scale. (B) log₁₀ scale on both axes.

Supplement table 1

synaptic type	region	layer	connection probability										ventral vs dorsal			PC-FS vs PC-NFS			PC-PC vs PC-IN			
			found connections	tested connections	connection probability	standard deviation	p-value	proportion difference	95% confidence interval	p-value	proportion difference	95% confidence interval	p-value	proportion difference	95% confidence interval	p-value	proportion difference	95% confidence interval				
PC-PC	ventral	superficial	2	746	0.3%	0.2%	0.000	3.4%	1.7%	4.2%	0.293	0.5%	-0.6%	1.0%								
PC-PC	ventral	deep	19	524	3.6%	0.8%		0.6%	-2.1%	1.9%	0.716											
PC-PC	dorsal	superficial	2	274	0.7%	0.5%	0.009	3.5%	1.1%	4.7%												
PC-PC	dorsal	deep	14	330	4.2%	1.1%																
PC-FS	ventral	superficial	23	124	18.5%	3.5%	0.839	1.9%	-9.8%	7.6%	0.012	21.5%	3.8%	30.1%	0.456	3.3%	-8.3%	9.0%	0.000	18.3%	11.4%	21.6%
PC-FS	ventral	deep	10	60	16.7%	4.8%					0.818	1.7%	-10.6%	7.7%	0.265	5.7%	-13.1%	14.9%	0.000	13.0%	3.5%	17.7%
PC-FS	dorsal	superficial	14	35	40.0%	8.3%	0.007	25.0%	7.0%	33.8%					0.136	14.0%	-4.0%	22.9%	0.000	39.3%	23.0%	47.2%
PC-FS	dorsal	deep	12	80	15.0%	4.0%									0.823	2.4%	-6.7%	6.8%	0.000	10.8%	2.6%	14.7%
PC-NFS	ventral	superficial	37	243	15.2%	2.3%	0.215	4.3%	-1.9%	7.3%	0.023	10.7%	1.2%	15.4%					0.000	15.0%	10.4%	17.2%
PC-NFS	ventral	deep	24	219	11.0%	2.1%					0.693	1.7%	-6.4%	5.7%					0.000	7.3%	2.9%	9.5%
PC-NFS	dorsal	superficial	27	104	26.0%	4.3%	0.028	13.3%	2.4%	18.7%									0.000	25.2%	16.7%	29.4%
PC-NFS	dorsal	deep	11	87	12.6%	3.6%													0.008	8.4%	1.1%	12.0%
FS-PC	ventral	superficial	32	124	25.8%	3.9%	0.353	7.5%	-5.0%	13.6%	0.657	5.8%	-9.5%	13.3%	0.073	8.5%	-3.9%	14.6%				
FS-PC	ventral	deep	11	60	18.3%	5.0%					0.014	13.3%	2.4%	18.7%	0.111	8.3%	-8.2%	16.4%				
FS-PC	dorsal	superficial	7	35	20.0%	6.8%	0.033	15.0%	0.9%	21.9%					0.806	1.7%	-12.4%	8.6%				
FS-PC	dorsal	deep	4	80	5.0%	2.4%									0.539	3.0%	-3.7%	6.3%				
NFS-PC	ventral	superficial	42	243	17.3%	2.4%	0.030	7.2%	1.0%	10.3%	0.878	1.0%	-7.8%	5.3%								
NFS-PC	ventral	deep	22	219	10.0%	2.0%					0.670	2.0%	-5.0%	5.4%								
NFS-PC	dorsal	superficial	19	104	18.3%	3.8%	0.055	10.2%	0.9%	14.8%												
NFS-PC	dorsal	deep	7	87	8.0%	2.9%																

Connection probability, calculated by the number of found synaptic connections divided by the number of tested possible connections, is shown for different synaptic types, in the dorsal and ventral regions, and for the superficial and deep layers. The standard deviation is calculated by assuming a binomial distribution. Further columns show comparisons between layers, regions and cell types. P-values are calculated by the Fisher's exact test. We adjusted for multiple comparison (4 for excitatory connections, 3 for inhibitory connections) using the Bonferroni correction which determines that only p-values below 0.013 (excitatory connection) or below 0.017 (inhibitory connections) are significant at a level of $\alpha=0.05$, these values are highlighted in red. We calculated the proportion difference and its 95% confidence interval. To test for equivalence at a significance level of $\alpha=0.05$ the assumed range of inconsequential difference have to lie within the 95% confidence interval of the difference. One row is always tested against its corresponding row depending on comparison. Therefore, two rows would have the same statistics, allowing us to leave one of the rows empty which are indicated with the light grey filling. Rows which are not included in certain cell-specific comparisons are marked with the dark grey filling.

Supplement table 4

synaptic type	region	layer	half duration (ms)			statistics			superficial vs deep			ventral vs dorsal			PC-FS vs PC-NFS			PC-PC vs IN-PC										
			median	lower quartile	upper quartile	sample size	p-value	effect size r	median difference	95% confidence interval of median difference	p-value	effect size r	median difference	95% confidence interval of median difference	p-value	effect size r	median difference	95% confidence interval of median difference	p-value	effect size r	median difference	95% confidence interval of median difference						
PC-PC	ventral	superficial	15.6	10.7	20.5	2	0.842	0.06	-0.6	-16.1	12.7	1.000	0.39	-4.4	-13.6	4.8												
PC-PC	ventral	deep	16.4	11.2	22.9	17					0.941	0.02	0.7	-5.5	7.5													
PC-PC	dorsal	superficial	20.0	15.7	24.3	2	0.485	0.25	3.6	-13.0	15.6																	
PC-PC	dorsal	deep	14.5	12.7	19.2	10																						
PC-FS	ventral	superficial	10.2	5.5	13.8	23	0.274	0.19	2.0	-1.3	5.5	0.039	0.34	-3.6	-7.1	-0.1	0.077	0.24	-2.7	-6.2	0.3	0.240	0.26	5.4	-5.7	16.5		
PC-FS	ventral	deep	8.3	4.8	10.3	11						0.104	0.35	-2.4	-8.3	0.6	0.032	0.37	-4.0	-8.7	-0.3	0.001	0.58	9.0	3.0	14.8		
PC-FS	dorsal	superficial	13.4	11.5	17.4	14	0.176	0.27	2.6	-2.1	7.2						0.287	0.19	-2.8	-6.5	2.0	0.150	0.40	5.0	-5.0	14.9		
PC-FS	dorsal	deep	10.9	5.6	18.1	12											0.169	0.31	-3.5	-10.4	2.4	0.080	0.38	4.4	-0.5	9.4		
PC-NFS	ventral	superficial	11.5	8.8	16.5	33	0.891	0.02	0.4	-3.4	4.2	0.113	0.22	-3.1	-7.3	1.0						0.706	0.09	2.7	-11.1	13.8		
PC-NFS	ventral	deep	11.7	9.4	19.5	22						0.334	0.18	-3.3	-8.5	2.9						0.163	0.27	3.8	-0.9	9.1		
PC-NFS	dorsal	superficial	17.2	11.8	20.5	19	0.962	0.01	-0.2	-4.5	7.0											0.533	0.18	3.9	-11.6	16.6		
PC-NFS	dorsal	deep	16.8	13.9	19.6	9																0.968	0.02	0.1	-5.5	6.7		
FS-PC	ventral	superficial	24.0	20.8	28.5	18	0.740	0.09	-1.3	-23.7	10.6	0.378	0.18	-2.4	-11.7	3.5	0.728	0.06	-0.7	-3.4	5.4							
FS-PC	ventral	deep	25.0	16.8	46.6	3						1.000	0.22	-16.2	-24.4	5.4	0.937	0.05	3.3	-18.6	25.2							
FS-PC	dorsal	superficial	26.4	21.0	37.1	6	0.286	0.57	-14.8	-21.2	-2.5						0.470	0.17	3.7	-2.3	15.6							
FS-PC	dorsal	deep	41.2	41.2	41.2	1											0.400	0.63	11.3	3.2	15.5							
NFS-PC	ventral	superficial	24.5	18.4	27.2	21	0.416	0.15	-3.6	-11.6	6.0	0.825	0.04	1.2	-5.0	5.3												
NFS-PC	ventral	deep	27.3	19.1	35.8	10						0.635	0.15	-3.2	-19.6	10.1												
NFS-PC	dorsal	superficial	21.5	20.2	27.5	15	0.080	0.41	-8.1	-16.5	1.3																	
NFS-PC	dorsal	deep	29.9	27.4	34.3	4																						

Half duration of postsynaptic potentials are shown for different synaptic types, in the dorsal and ventral regions, and for the superficial and deep layers. Given the assumption of a non-parametric distribution, the median and the first and third quartiles are depicted. Further columns show comparisons between layers, regions and cell types. P-values are calculated by the Mann-Whitney U test. We adjusted for multiple comparison (4 for excitatory connections, 3 for inhibitory connections) using the Bonferroni correction which determines that only p-values below 0.013 (excitatory connection) or below 0.017 (inhibitory connections) are significant at a level of $\alpha=0.05$, these values are highlighted in red. Comparisons with an effect size $r > 0.3$ (medium effect) were highlighted in red. We calculated the median difference and its 95% confidence interval. To test for equivalence at a significance level of $\alpha=0.05$ the assumed range of inconsequential difference have to lie within the 95% confidence interval of the median difference. One row is always tested against its corresponding row depending on comparison. Therefore, two rows would have the same statistics, allowing us to leave on of the rows empty which are indicated with the light grey filling. Rows which are not included in certain cell-specific comparisons are marked with the dark grey filling.

