Figure S1: Histology following *in vivo* CaMPARI2 photoconversion. Related to Figure 1. Fluorescence of the green (left) and red (center) forms of CaMPARI2, as well as the merge (right), decreased as the distance from the virus injection and cannula insertion site increased following *in vivo* photoconversion.

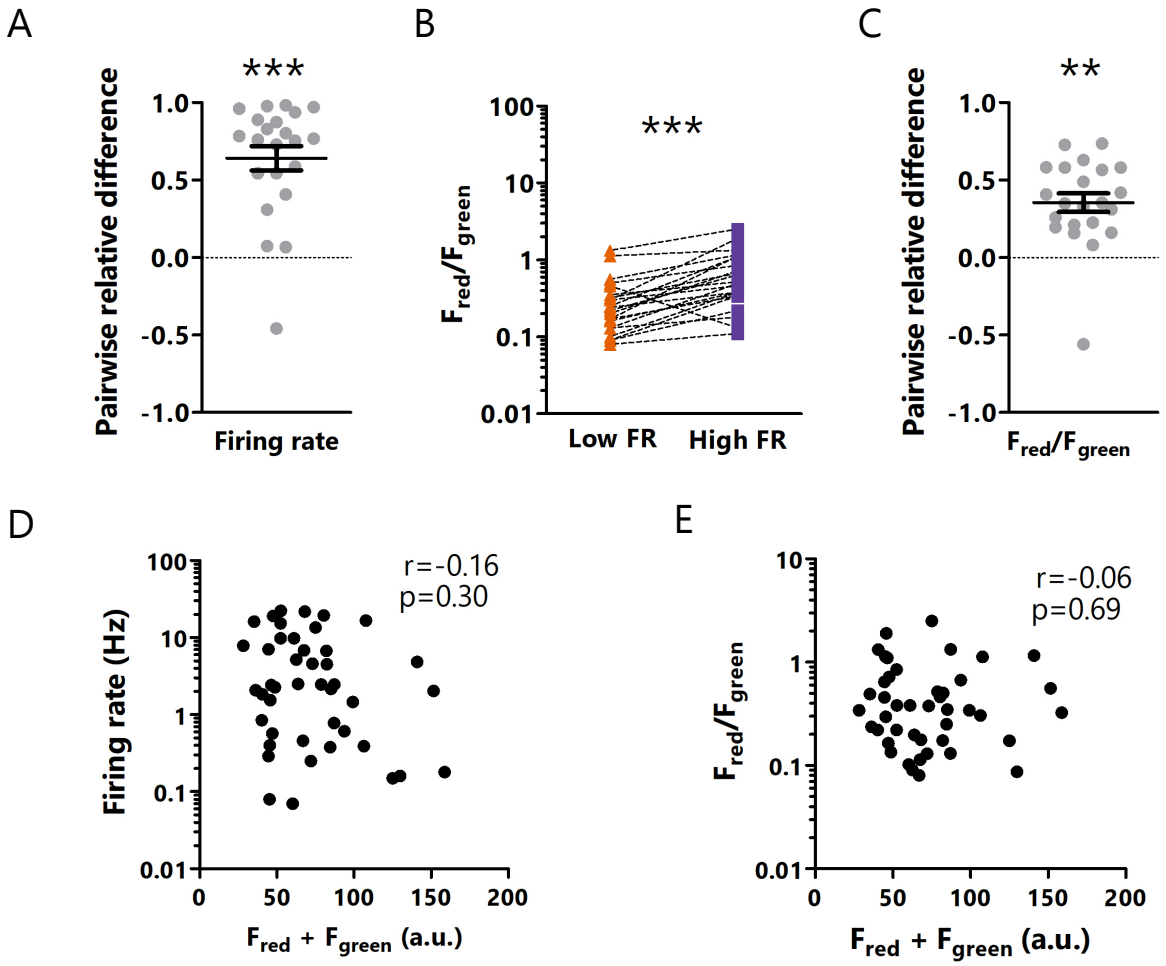

Figure S2: Photoconversion ratio primarily reflects differences in neuronal activity. Related to Figure 2. A: Pairwise relative difference in firing rate between high and low activity neurons as measured by red/green ratio, calculated by dividing the difference in firing rates by the sum of the firing rates for each pair. Values greater than 0 indicate that a higher value in the high activity (or more photoconverted) neuron. n = 22 pairs of cells from all layers from 11 animals, *** $p<0.001$, Wilcoxon signed-rank test. **B**: Comparison of red/green ratios following *in vivo* photoconversion between pairs of neurons sorted based on firing rate in active slice. n = 22 pairs of cells from all layers from 11 animals, *** $p<0.001$, paired t-test. **C**: Pairwise relative difference in red/green ration between high and low firing rate neurons. n = 22 pairs of cells from all layers from 11 animals, ** $p<0.01$, Wilcoxon signed-rank test. **D**: Correlation between *ex vivo* firing rate and total red + green fluorescence after *in vivo* photoconversion. Line represents linear best fit. r = Spearman's rank correlation coefficient. n = 44 cells from all layers from 11 animals. **E**: Correlation between red/green ratio and total red + green fluorescence after *in vivo* photoconversion. Line represents linear best fit. r = Spearman's rank correlation coefficient. n = 44 cells from all layers from 11 animals.

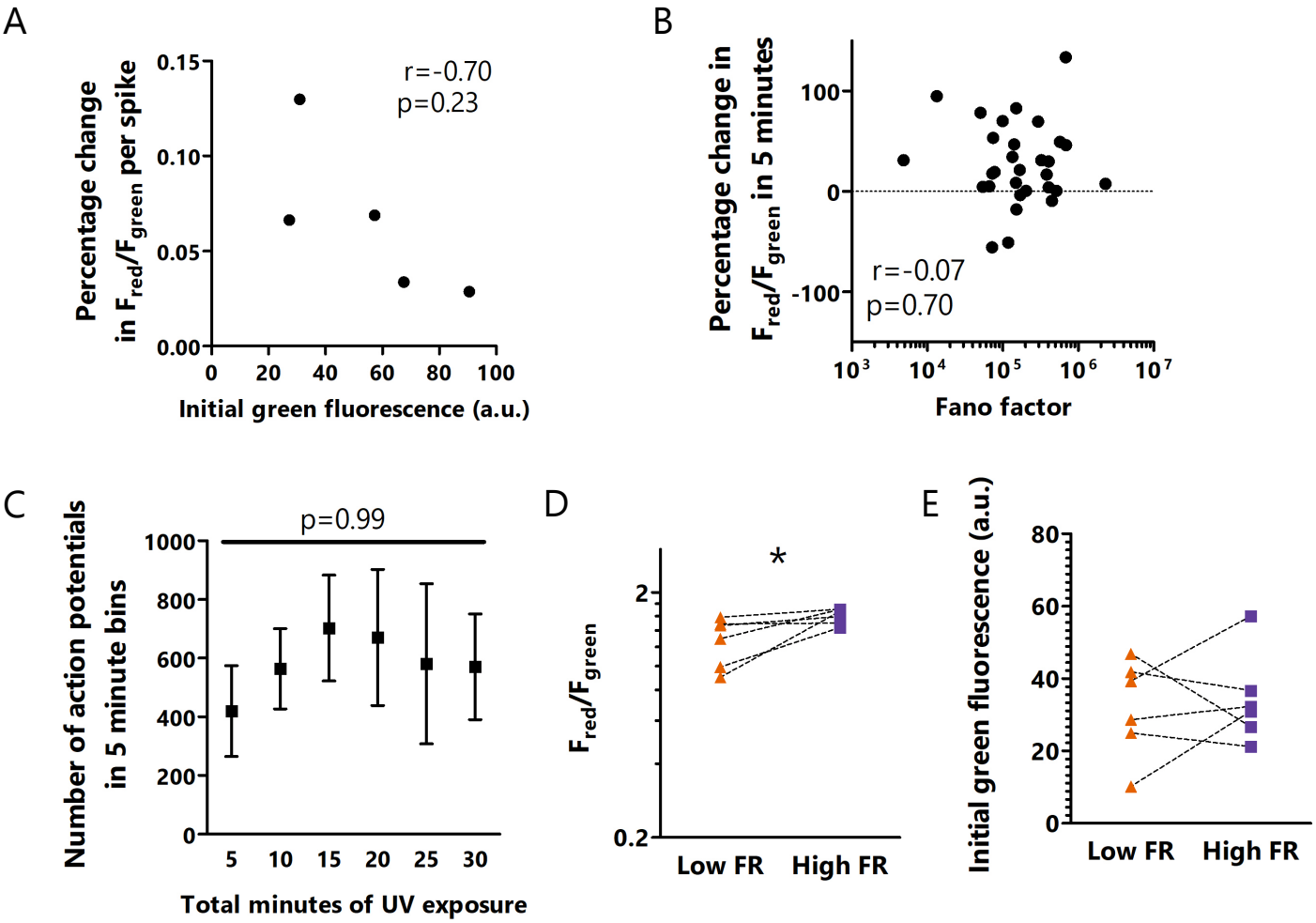

Figure S3: CaMPARI2 photoconversion rate is correlated with firing rates measured *ex vivo*. Related to Figure 3. A: Correlation between percentage change in red/green ratio per spike to initial green fluorescence. r = Spearman's rank correlation coefficient. **B**: Correlation between Fano factor and the percentage change in red/green ratio over 5-minute intervals during *ex vivo* photoconversion *ex vivo*. n = 5 cells from L4 from 4 animals, 6 5-minute windows per cell. r = Spearman's rank correlation coefficient. **C**: Comparison of mean number of action potentials in 5-minute windows during UV photoconversion *ex vivo*. p value calculated via 1-way ANOVA. **D**: Comparison of red/green ratios between pairs of neurons following 10 minutes of *ex vivo* photoconversion. **E**: Comparison of total green fluorescence before photoconversion between pairs of neurons sorted according to firing rate during 10 minutes of *ex vivo* photoconversion. A-C: n = 5 cells from 4 animals; D, E: n = 6 pairs of cells from 4 animals, Wilcoxon matched pairs signed-rank test. Neurons were sampled from layer 4, * $p<0.05$.

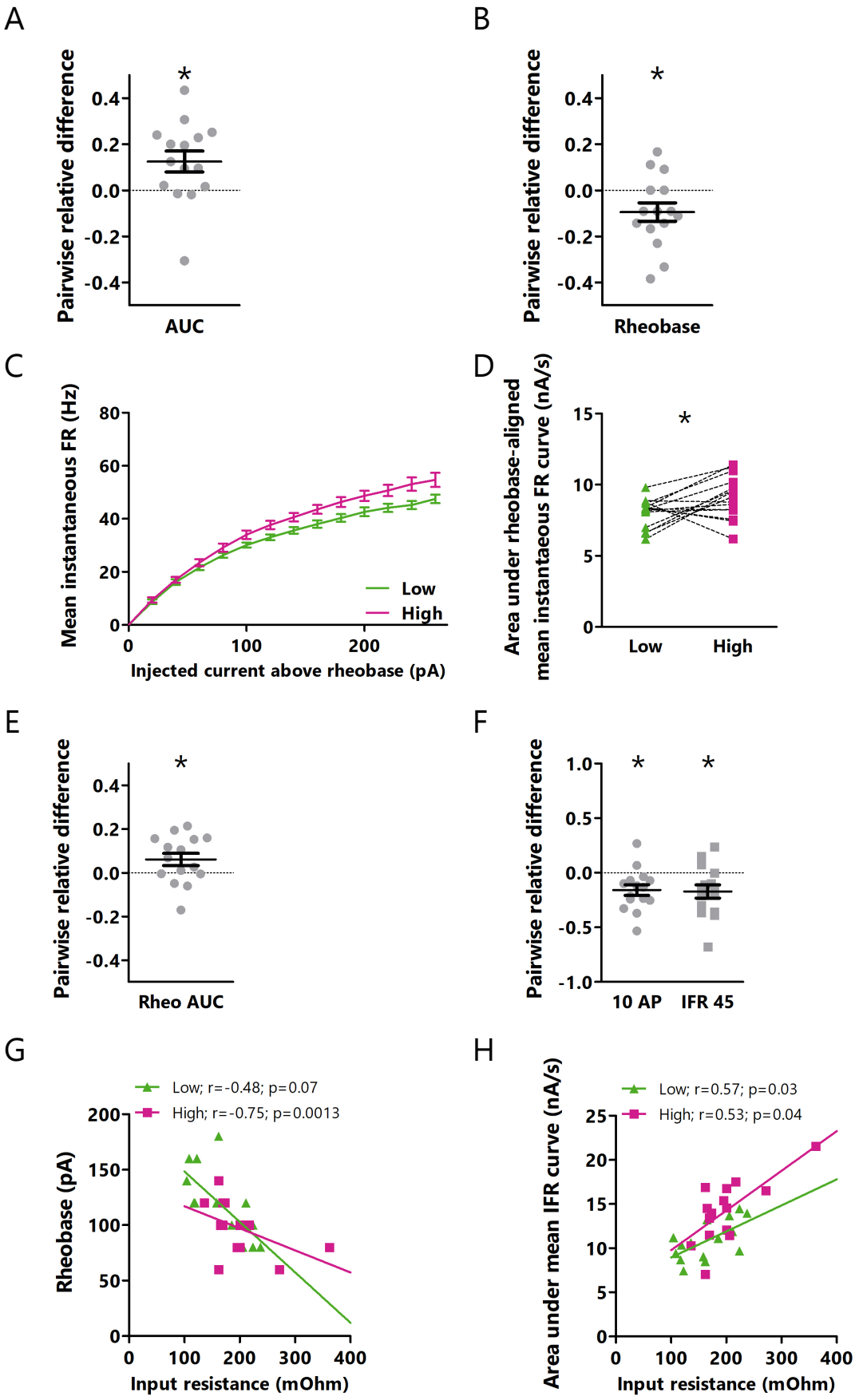
A
Pairwise relative difference
0.4
0.2
0.0
-0.2
-0.4
*
AUC
B
Pairwise relative difference
0.4
0.2
0.0
-0.2
-0.4
*
Rheobase
C
Mean instantaneous FR (Hz)
80
60
40
20
0
0
100
200
Injected current above rheobase (pA)
Low
High
D
Area under rheobase-aligned mean instantaneous FR curve (nA/s)
15
10
5
0
*
Low
High
E
Pairwise relative difference
0.4
0.2
0.0
-0.2
-0.4
*
Rheo AUC
F
Pairwise relative difference
1.0
0.5
0.0
-0.5
-1.0
*
*
10 AP
IFR 45
G
Low; r=-0.48; p=0.07
High; r=-0.75; p=0.0013
Rheobase (pA)
200
150
100
50
0
0
100
200
300
400
Input resistance (mOhm)
H
Low; r=0.57; p=0.03
High; r=0.53; p=0.04
Area under mean IFR curve (nA/s)
25
20
15
10
5
0
0
100
200
300
400
Input resistance (mOhm)

Figure S4: High and low activity neurons have rheobase-independent differences in intrinsic excitability. Related to Figure 5. A: Pairwise relative difference in the area under the F-I curve for high and low activity neurons. Values greater than 0 indicate that a higher value in the high activity (or more photoconverted) neuron. * $p<0.05$, one sample t-test. **B**: Pairwise relative difference in rheobase current for high and low activity neurons. * $p<0.05$, one sample t-test. **C**: Rheobase-subtracted mean instantaneous F-I curve for neuron pairs. **D**: Comparison of the area under the curves in S4C for each neuron pair. * $p<0.05$, paired t-test. **E**: Pairwise relative difference in spike frequency adaptation for high and low activity neurons. * $p<0.05$, one sample t-test. **F**: Pairwise relative difference in rheobase-adjusted area under F-I curve for high and low activity neurons. * $p<0.05$, one sample t-test. **G**: Correlation between input resistance and rheobase current for all neurons. r = Spearman's rank correlation coefficient. **H**: Correlation between input resistance and area under rheobase-adjusted firing rate curve for all neurons. r = Spearman's rank correlation coefficient. A through H: n = 15 pairs of cells from L4 from 7 animals.

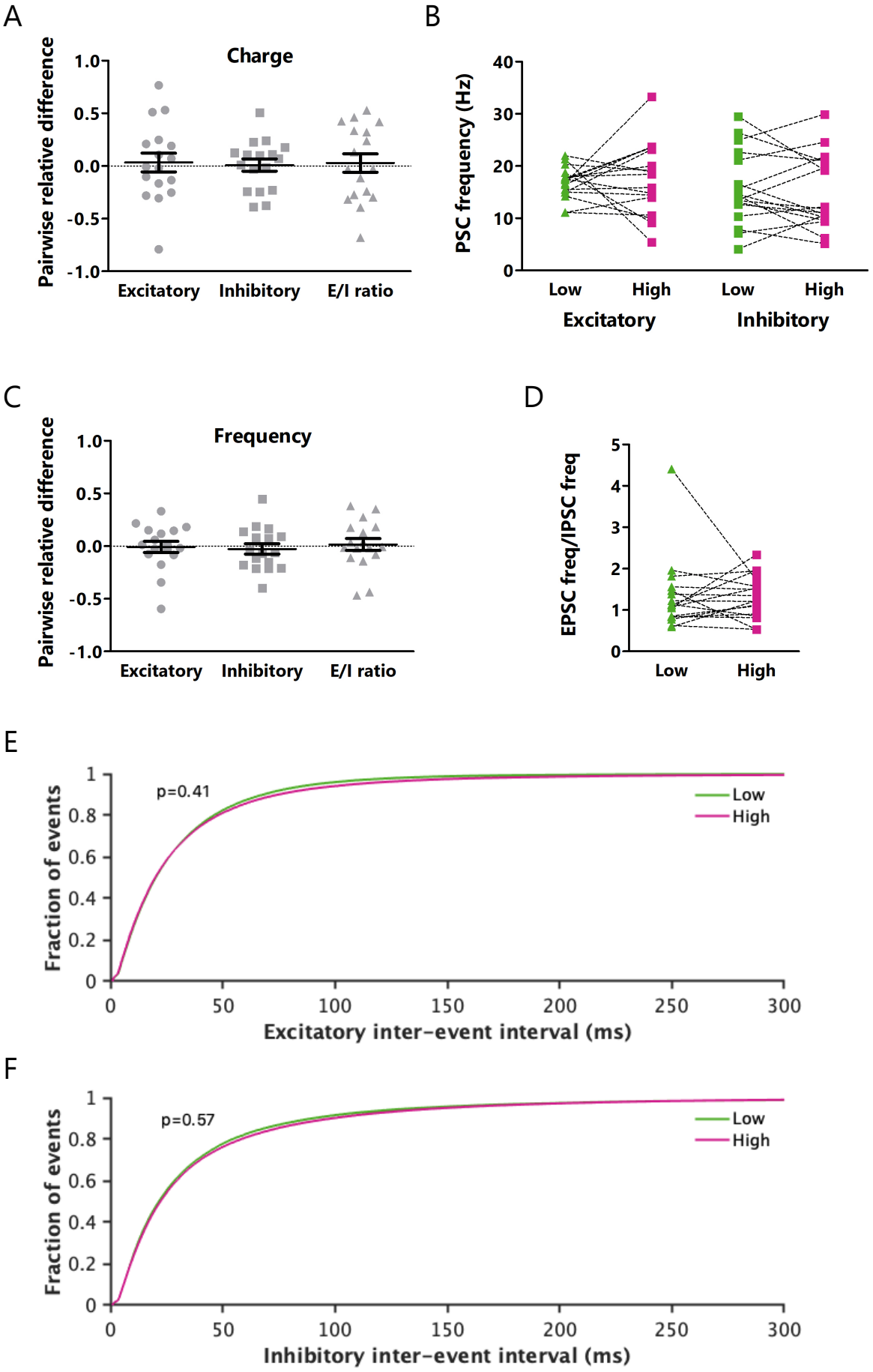
A
Charge
Pairwise relative difference
1.0
0.5
0.0
-0.5
-1.0
Excitatory
Inhibitory
E/I ratio
B
PSC frequency (Hz)
40
30
20
10
0
Low
High
Low
High
Excitatory
Inhibitory
C
Frequency
Pairwise relative difference
1.0
0.5
0.0
-0.5
-1.0
Excitatory
Inhibitory
E/I ratio
D
EPSC freq/IPSC freq
5
4
3
2
1
0
Low
High
E
p=0.41
Low
High
Fraction of events
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
Excitatory inter-event interval (ms)
F
p=0.57
Low
High
Fraction of events
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
Inhibitory inter-event interval (ms)

Figure S5: High and low activity neurons do not differ in the frequency of excitatory or inhibitory synaptic inputs. Related to Figure 7. A: Pairwise relative difference in excitatory charge, inhibitory charge, and ratio of excitatory to inhibitory charge for high and low activity neurons. Values greater than 0 indicate that a higher value in the high activity (or more photoconverted) neuron. No significant differences, one-sample t-test. **B**: Frequency of excitatory (left) or inhibitory (right) synaptic currents measured in pairs of neurons at their experimentally determined inhibitory (left) or excitatory (right) reversal potential. No significant differences, paired t-test. **C**: Pairwise relative difference in excitatory charge, inhibitory charge, and ratio of excitatory to inhibitory charge for high and low activity neurons. No significant differences, one-sample t-test. **D**: Ratio of frequency of excitatory to inhibitory currents for pairs of photoconverted neurons. No significant differences, paired t-test. **E**: Distribution of inter-event intervals of well-isolated excitatory synaptic events. Traces are truncated at 300 ms for clarity. 200 randomly selected events were analyzed from each cell for each condition. No significant differences, Kolmogorov-Smirnov test. **F**: Distribution of inter-event intervals of well-isolated inhibitory synaptic events. Traces are truncated at 300 ms for clarity. 200 randomly selected events were analyzed from each cell for each condition. No significant differences, Kolmogorov-Smirnov test. A through F: n = 17 pairs of cells from L4 from 11 animals.

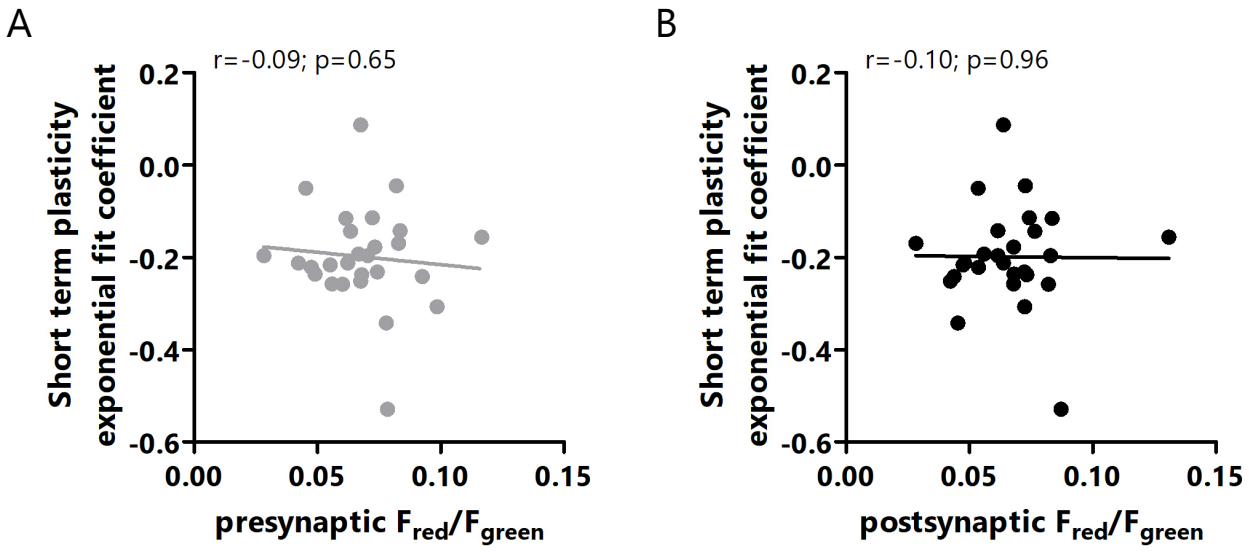

Figure S6: Additional measurements from pairs of neurons. Related to Figure 8. A: Correlation between the exponential decay coefficient of the amplitudes of five current pulses and the activity of the postsynaptic neuron. **B**: Correlation between the exponential decay coefficient of the amplitudes of five current pulses and the activity of the postsynaptic neuron. r = Spearman's rank correlation coefficient. n = 28 pairs of connected neurons from L4 from 13 animals out of 224 pairs tested from 16 animals. Each point is the average of 20 traces. r = Spearman's rank correlation coefficient.

	Parameter		Median	Mean	Std. Dev	95% CI		p value
Rheobase	Spike amplitude (mV)	Low	61.64	60.86	9.97	55.34	66.38	0.723
		High	59.90	59.93	8.53	55.20	64.65	
	AHP amplitude (mV)	Low	27.98	29.22	7.19	25.24	33.21	0.941
		High	28.30	29.05	6.75	25.31	32.79	
	Mean rise slope (V/s)	Low	152.90	150.20	23.35	137.30	163.10	0.719
		High	149.60	147.40	29.14	131.30	163.60	
	Mean decay slope (V/s) ^	Low	67.98	66.00	11.20	59.80	72.20	0.679
		High	67.10	65.67	7.42	61.56	69.78	
	Half width (ms) ^	Low	0.79	0.78	0.05	0.75	0.81	0.894
		High	0.78	0.79	0.06	0.75	0.83	
	Maximum rise slope (V/s)	Low	328.70	330.30	47.23	304.20	356.50	0.935
		High	329.10	329.30	49.84	301.70	356.90	
	Maximum decay slope (V/s) ^	Low	91.06	89.55	13.71	81.95	97.14	0.847
		High	90.73	90.42	11.56	84.02	96.83	
	Max rise/Max decay	Low	3.61	3.72	0.38	3.51	3.93	0.775
		High	3.55	3.67	0.50	3.39	3.95	
	Latency (ms) ^	Low	14.46	16.01	7.68	11.76	20.26	0.169
		High	10.47	12.59	6.08	9.22	15.95	
5 spikes	Spike amplitude (mV)	Low	55.56	57.70	8.61	52.93	62.47	0.678
		High	57.63	58.77	8.35	54.15	63.40	
	AHP amplitude (mV)	Low	29.78	30.34	6.56	26.71	33.97	0.680
		High	29.36	29.35	6.13	25.95	32.74	
	Mean rise slope (V/s)	Low	138.90	143.20	23.89	130.00	156.50	0.895
		High	151.80	142.20	26.00	127.80	156.60	
	Mean decay slope (V/s)	Low	63.94	60.20	12.03	53.53	66.86	0.804
		High	59.67	59.48	8.10	55.00	63.96	
	Half width (ms)	Low	0.81	0.82	0.08	0.77	0.86	0.797
		High	0.82	0.82	0.09	0.77	0.87	
	Maximum rise slope (V/s)	Low	317.20	314.30	52.78	285.00	343.50	0.835
		High	322.50	317.40	46.01	291.90	342.80	
	Maximum decay slope (V/s) ^	Low	89.42	84.57	15.88	75.78	93.37	0.978
		High	88.76	84.35	12.05	77.68	91.03	
	Max rise/Max decay	Low	3.83	3.77	0.58	3.45	4.10	0.891
		High	3.80	3.80	0.52	3.51	4.09	
	Latency (ms)	Low	6.95	6.97	2.25	5.72	8.22	0.830
		High	7.25	7.15	2.29	5.88	8.41	
	Input resistance (mΩ) ^	Low	161.80	165.30	46.58	139.50	191.10	0.079
		High	195.00	199.20	55.20	168.60	229.70	
	Capacitance (pF) ^	Low	75.59	78.25	11.56	71.85	84.65	0.083
		High	70.41	69.66	13.40	62.24	77.08	
	Resting membrane potential (mV)	Low	-66.70	-66.86	4.14	-69.15	-64.57	0.572
		High	-67.45	-67.40	3.58	-69.39	-65.42	
	Action potentail threshold (mV) ^	Low	-30.85	-31.47	2.82	-33.03	-29.91	0.208
		High	-32.83	-33.14	4.93	-35.87	-30.41	
	Sag percentage ^	Low	94.04	90.31	9.61	84.99	95.63	0.252
		High	94.67	93.73	4.04	91.49	95.97	

p values calculated via paired t-test except ^, which were calculated via Wilcoxon Signed Rank Sum test

Table S1: Additional electrophysiological properties of high and low activity neurons. Related to Figure 5. Values labeled "rheobase" are calculated for the first spike at rheobase, values labeled "5 spikes" are calculated for the first spike for the smallest current step to produce at least 5 action potentials. Passive properties are measured in voltage clamp with a -5mV voltage step.