Cell Reports

Supplemental Information

In Vivo Monosynaptic Excitatory Transmission between Layer 2 Cortical Pyramidal Neurons

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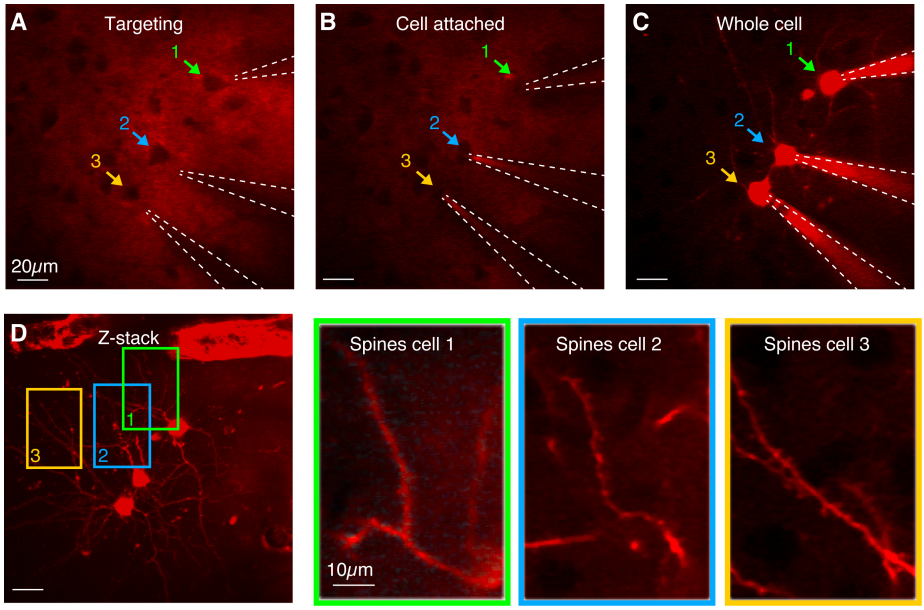

Figure S1. In vivo two-photon targeted triple whole-cell patch clamp recording procedure. Related to Figure 1.

(A) Targeting: first, three neurons in a wild-type mouse with pyramidal shaped somata were targeted using their shadow images. Arrows and numbers indicate the cell identity, depth of recording from pial surface 220 μm.

(B) Cell attached: next we formed a gigaseal between the glass micropipette and the cell membrane on all cells.

(C) Whole-cell: finally, the cell membranes were ruptured on each cell with negative pressure to go to whole-cell configuration.

(D) An example z stack maximum projection image of the recorded cells in (A–C) taken after termination of recording and slow removal of patch pipettes. Right boxes show example dendritic segments from single images taken from z stack showing spines with cell labeling color code related to (D).

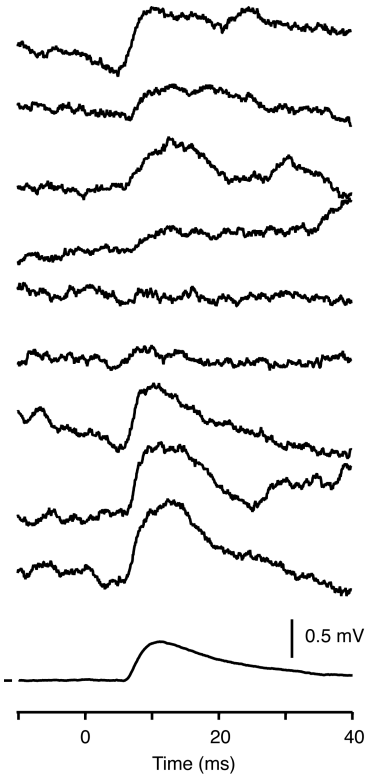

Figure S2. Example monosynaptic connection with a long latency. Related to Figure 2.

Top: nine representative single trial uEPSPs. Bottom: averaged synaptic response to a single presynaptic AP with a latency of 6.21 ms. V_m mark shows –57 mV.

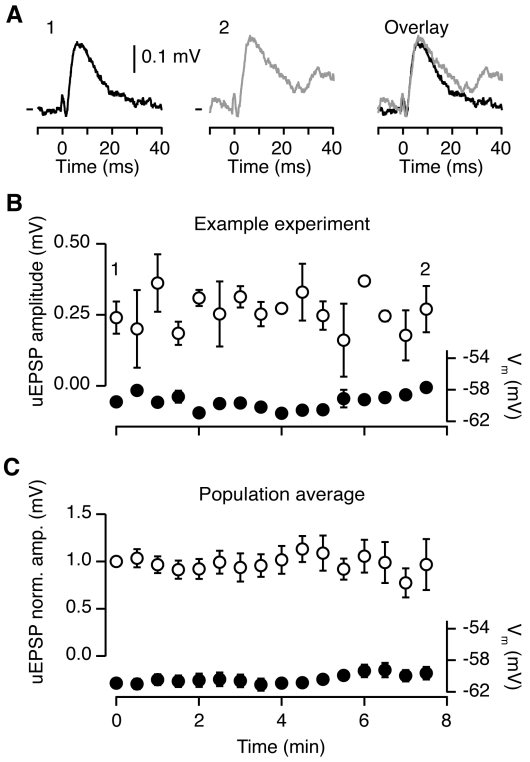

Figure S3. Stability of $_u$EPSP amplitude and membrane potential across time. Related to Figure 3.

(A) Left, black trace shows a $_u$EPSP average response to single APs delivered between 0–30 s from the start of stimulation during an example recording, V_m mark, –59.1 mV; middle, grey trace shows the $_u$EPSP average from the mean response during the last 30 s block of stimulation (420–480 s), V_m mark, –58.7 mV; right, overlay of the two responses with no adjustment of the V_m.

(B) Open circles show mean amplitude ± SEM of an example connection across 8 minutes of recording with each circle representing the average response to single downstate APs during a 30 s period. Filled circles below show the mean V_m for the same time periods. Example is the same as in (A) with the numbers 1 and 2 highlighting the example average responses shown in (A).

(C) The population average (n = 59 connections) of the downstate uEPSP amplitude and V_m across time. Amplitude is normalized to the mean uEPSP response during the first 30 s period. Filled circles show the corresponding population average V_m.

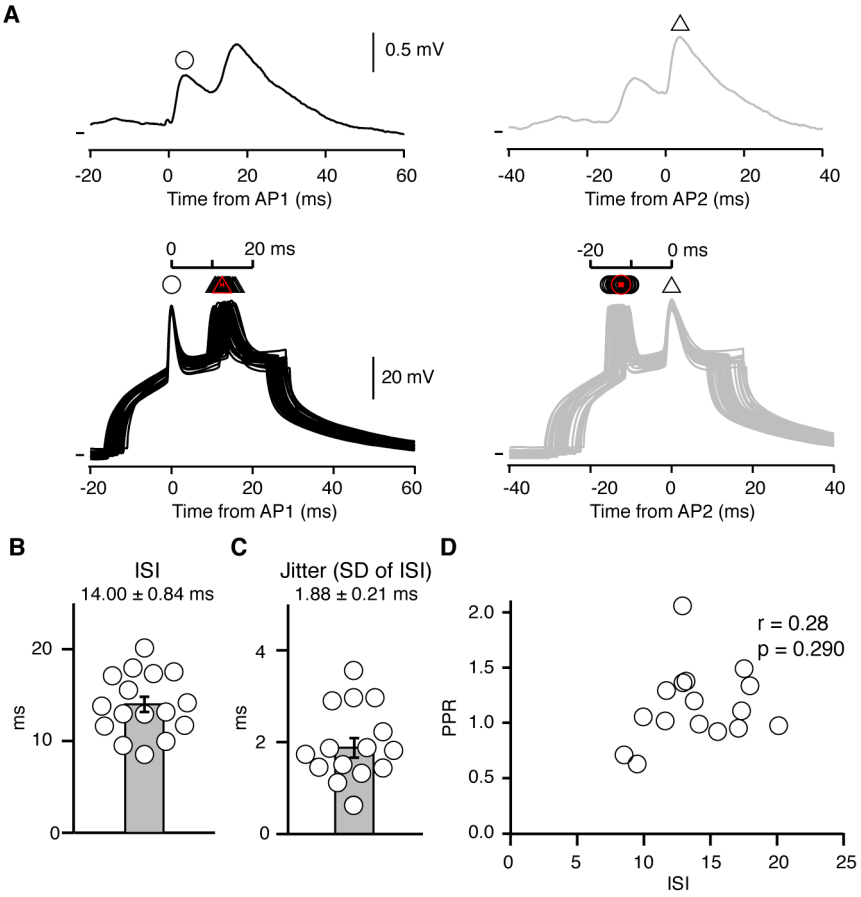

Figure S4. Jitter of presynaptic action potentials during measurement of paired pulse ratio. Related to Figure 4.

(A) Top, an example averaged postsynaptic response to, bottom, a doublet of presynaptic APs (n = 35 trials) during a paired pulse ratio experiment. Left shows average response triggered from AP1 (black) and right shows average response triggered from AP2 (grey). Open circles show the overlaid times of AP1, open triangles show the time of AP2. Red symbols show corresponding mean with SEM. Top left and right V_m mark indicates –62.5 mV. Bottom left and right V_m mark indicates –60.0 mV.

(B) Plot of the inter-spike interval (ISI) across 16 connections with each circle representing an individual connection and the bar showing the mean ± SEM.

(C) The jitter of the ISI across 16 connections as measured as the standard deviation of the ISI with each circle representing an individual connection and the bar the mean ± SEM.

(D) The paired pulse ratio (PPR) is not significantly correlated to the ISIs used in this dataset.

Table S1. Table of cell numbers, animal age, trials, kinetics, amplitude, reliability and PPR values of the connections identified in this study. Related to Figures 1 to 4.

	Mean	SEM	n	Median	Max	Min
Age (P)	22.32	0.20	188	22	30	18
Distance (µm)	41.01	0.74	878	36.07	160.62	11.88
Depth (µm)	-177.13	0.89	878	-176.65	-111.41	-256.77
n trials	62.86	7.71	59	50	279	9
V_m at latency (mV)	-60.35	0.63	59	-60.16	-50.14	-70.72
Latency (ms)	1.47	0.14	59	1.24	6.21	0.06
Rise time (ms)	1.97	0.13	59	1.62	5.64	0.79
Peak time (ms)	6.66	0.31	59	6.10	14.90	3.35
Half width (ms)	15.52	0.97	59	13.87	39.93	2.42
Decay time (ms)	16.22	1.24	59	14.09	49.75	2.43
Amplitude (mV)	0.43	0.07	59	0.27	2.59	0.05
Bidirectional amp (mV)	0.53	0.18	14	0.32	2.42	0.06
Unidirectional amp (mV)	0.40	0.07	45	0.26	2.59	0.05
CV	0.74	0.06	59	0.62	1.86	0.11
Failure rate (%)	21.91	0.30	59	18.03	65.52	0
CV without failure	0.42	0.03	59	0.40	1.11	0.07
Amplitude uEPSP1 (mV)	0.78	0.20	16	0.44	2.59	0.11
Amplitude uEPSP2 (mV)	0.77	0.16	16	0.52	2.39	0.11
Paired pulse ratio	1.15	0.09	16	1.08	2.06	0.63