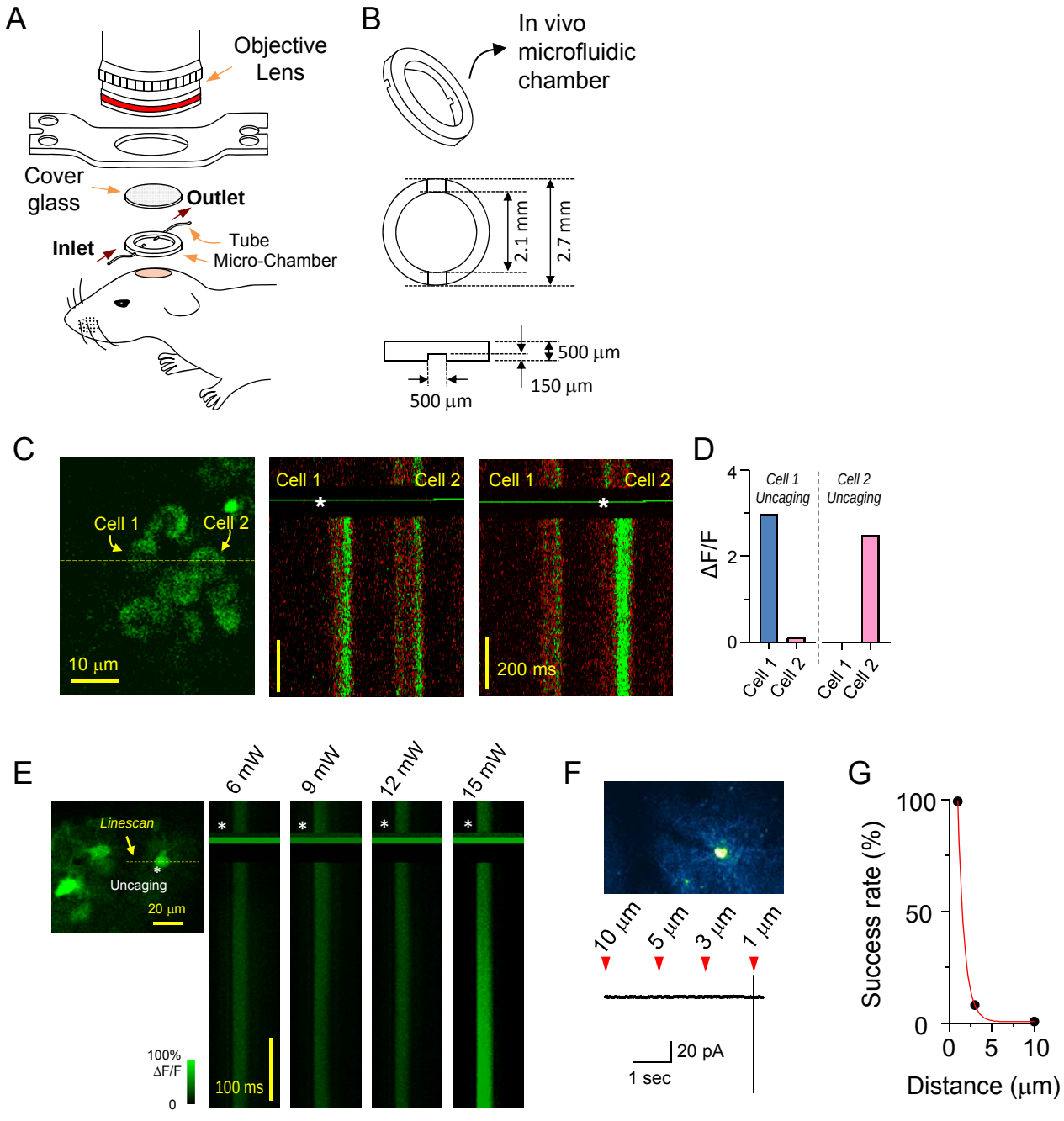
A
Objective
Lens
Cover
glass
Outlet
Inlet
Tube
Micro-Chamber
B
In vivo
microfluidic
chamber
2.1 mm
2.7 mm
500 µm
150 µm
500 µm
C
Cell 1
Cell 2
10 µm
Cell 1
Cell 2
Cell 1
Cell 2
200 ms
D
ΔF/F
Cell 1
Uncaging
Cell 2
Uncaging
Cell 1
Cell 2
Cell 1
Cell 2
E
6 mW
9 mW
12 mW
15 mW
Linescan
Uncaging
20 µm
100%
ΔF/F
0
100 ms
F
10 µm
5 µm
3 µm
1 µm
20 pA
1 sec
G
Success rate (%)
Distance (µm)

Fig. S1. Verification of *in vivo* two-photon photolysis and imaging. (A) Schematic design of *in vivo* imaging and uncaging system. After craniotomy at primary somatosensory cortex, a custom-designed thin, small micro-chamber was implanted. Thin tubes were connected in both sides of chamber, so that caged compounds and pharmacological drugs were perfused through the tubes. A small leak between the micro-chamber and skull was sealed with kwik sil^{TM} (World Precision Instruments, Inc). Coverglass was placed on the top of the micro-chamber to cover the micro-chamber and a head plate was fixed by dental cement. (B) Design of microfluidic chamber. A detail measurement of micro-chamber was shown in the top and side views. (C) Measurement of Ca^{2+} transients from two neurons when either neuron was activated separately. Cell 1 and Cell 2 were activated one by one and Ca^{2+} response was observed only from a neuron we activated by glutamate uncaging. (D) Bar graph of fluorescence change in neuron 1 and 2 when they were activated by glutamate photolysis. (E) All-or-none fashion of Ca^{2+} transients. Uncaging laser power was gradually increased targeting onto the same neuron. (F) Representative recording showing cell firing only when the neuron was activated within 1 μm distance from the cell body. (G) Success rate of distance-dependent AP trigger (n=11).

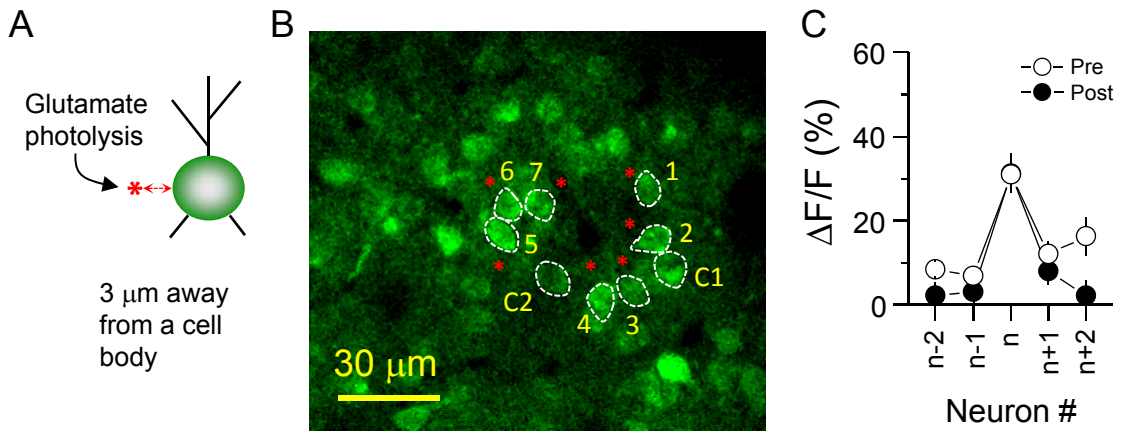

Fig. S2. Glutamate spillover does not cause the magnitude of Ca^{2+} responses. (A) Schematic diagram of glutamate photolysis. Glutamate was photoreleased 3 μm away from the cell body. (B) The location of uncaging spots is marked as a red asterisk. (3 μm away from the cell body). Glutamate was photoreleased sequentially (from neuron #1 to #7) three times in a same order and then no further uncaging was given for two minutes. These 3 x sequential activations were repeated 15 times for a total of 45 (3x15) sequential activations. (C) Fluorescence change was monitored after photoreleasing glutamate 3 μm away from the cell body. Repetitive spike trains total 45 times did not cause any significant changes in Ca^{2+} magnitude in neighboring neurons by spillover.

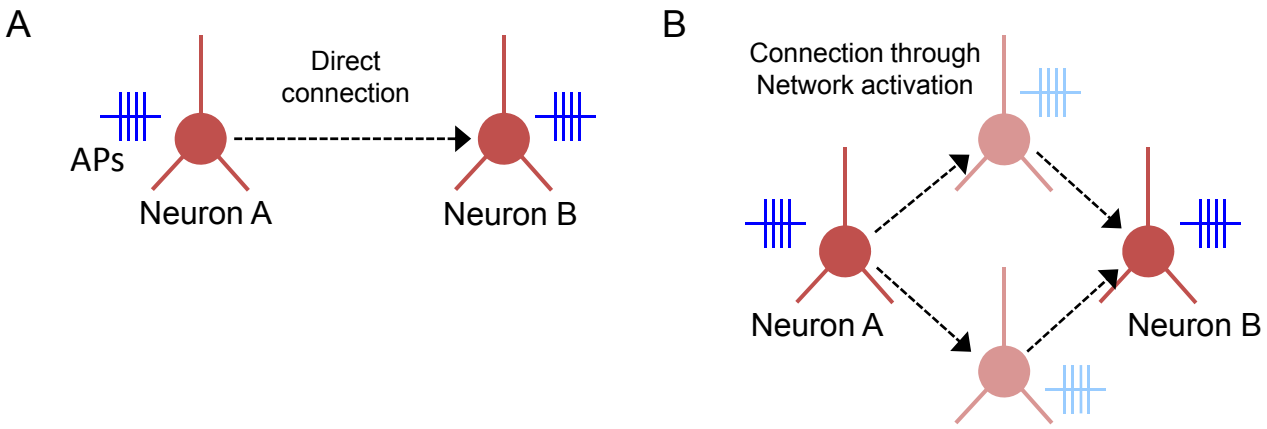

Fig. S3. Potential cellular mechanisms of functional circuit reorganization. (A) Circuit reorganization via making direct monosynaptic connection among neurons. In this example, neuron A and B are connected weakly, so APs are not able to be triggered from Neuron B by activating neuron A. When neurons are activated together many times, the direct monosynaptic connection becomes stronger to fire together or new strong connections are made among them. (B) Neurons become functionally connected through indirect network activation although they are not directly connected.

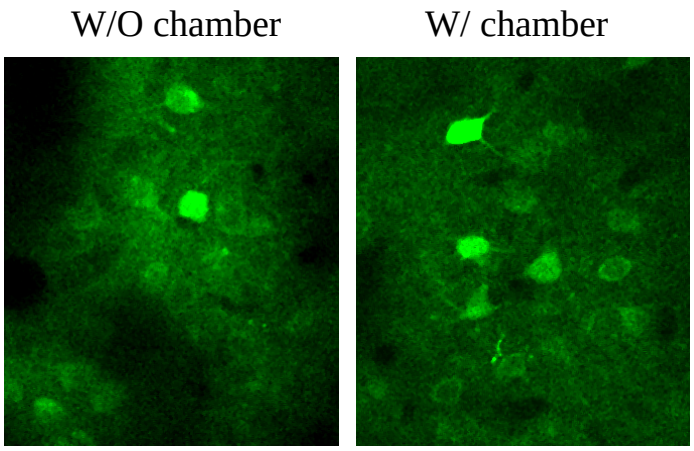

Fig. S4. Comparison images with and without *in vivo* micro-chamber. Images were taken in the exactly same conditions under the same microscope. Glass window directly pressed the brain surface when the chamber was not used.

Table 1

Test

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-3	1.11	2.90	22.65	3.48	0.0000309725	82
n-2	5.38	2.64	23.73	3.72	0.0000513298	98
n-1	4.48	2.29	25.79	3.98	0.0000008466	114
n	39.04	7.62	35.34	6.74	0.6319491696	130
n+1	4.54	2.15	29.71	3.90	0.0000000132	114
n+2	3.37	2.04	27.47	3.03	0.0000000043	98
n+3	4.34	1.06	23.68	2.99	0.0000028346	82

Table 2

Control

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	1.80	3.14	1.57	3.01	0.957893351	29
n-1	1.80	3.02	0.38	3.23	0.751561911	29
n	28.38	7.09	34.48	5.29	0.49942796	29
n+1	0.75	4.47	4.22	3.38	0.543559891	29
n+2	1.74	3.40	7.04	2.53	0.229147869	29

Table 3

	1ms			20ms			100ms		
	Average	SEM	n	Average	SEM	n	Average	SEM	n
n-2	26.73301	3.171684	25	21.30267	3.905207	10	8.164401	1.677979	10
n-1	32.47889	2.966215	30	25.67951	3.657282	15	10.28649	0.963994	15
n	39.69671	1.758103	35	34.84388	4.609949	20	36.12847	1.602566	20
n+1	33.64517	4.002061	30	24.00496	3.275077	15	11.0895	2.419325	15
n+2	26.22248	3.431657	25	21.8628	3.723536	10	11.77161	2.452496	10

Table 4

200ms Interval

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	9.51	7.07	-1.15	3.30	0.19	10
n-1	6.17	3.91	4.89	5.10	0.84	15
n	41.25	9.45	45.33	8.36	0.74	20
n+1	-2.78	4.05	1.77	3.89	0.42	15
n+2	-3.44	3.57	8.63	4.79	0.05	10

Table 5 CPP

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	-2.79	3.75	0.47	4.67	0.59	10
n-1	0.45	3.55	0.84	3.10	0.93	15
n	32.77	6.60	36.58	6.97	0.69	20
n+1	6.27	2.61	-0.34	2.24	0.06	15
n+2	9.26	3.29	5.11	3.90	0.42	10

Table 6

	KN-62			KN-93			KN-92		
	Average	SEM	n	Avergae	SEM	n	Average	SEM	n
n-2	3.987902	2.876836	10	7.560611	1.293438	15	25.19503	6.196556	10
n-1	7.788826	1.330061	15	5.594001	1.223317	20	28.20104	5.775818	15
n	31.99761	1.36506	20	30.49602	0.789613	25	31.04638	4.875743	20
n+1	6.012459	2.730284	15	5.916957	1.379058	20	28.54631	6.783979	15
n+2	4.938112	2.718362	10	6.367751	1.352577	15	25.19794	6.767128	10

Table 7 3 cells

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	-3.65	5.48	2.26	1.88	0.35	5
n-1	2.91	2.42	7.97	2.26	0.14	10
n	25.03	3.99	24.56	6.63	0.95	15
n+1	1.84	2.40	8.91	2.23	0.05	10
n+2	3.49	2.75	-4.98	3.04	0.07	5

Table 8 Gabazine

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	6.01	1.85	25.96	3.21	0.000087240	10
n-1	5.59	0.98	28.71	3.09	0.000001844	15
n	28.51	4.46	30.76	4.66	0.728605333	20
n+1	5.33	2.13	28.44	3.92	0.000036348	15
n+2	5.42	3.68	24.78	4.39	0.003446303	10

Table 9 Muscimol

	Pre		Post			
	Average	SEM	Average	SEM	p-Value	n
n-2	2.04	1.33	3.22	1.45	0.55393	14
n-1	3.77	1.08	5.98	1.73	0.28668	19
n	26.77	2.34	27.91	2.34	0.7327	24
n+1	4.74	1.84	3.83	1.22	0.6823	19
n+2	5.08	0.98	2.85	1.88	0.30519	14

Average and SEM in all supplementary tables represent ΔF/F (%)