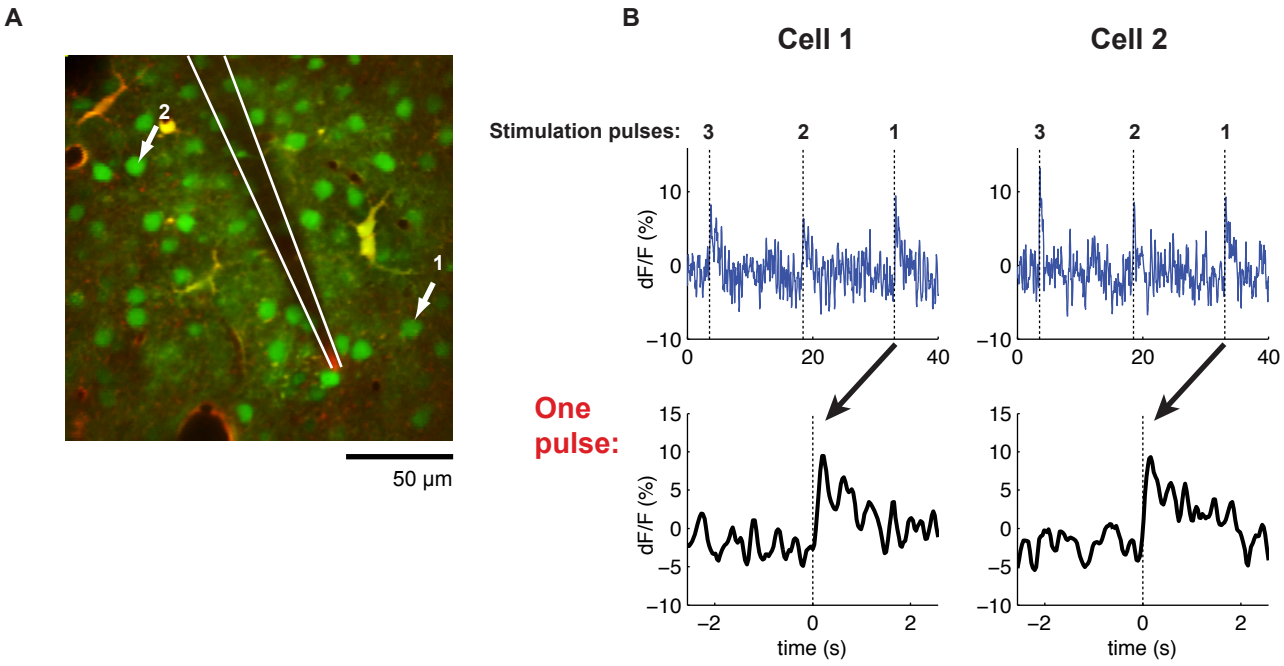
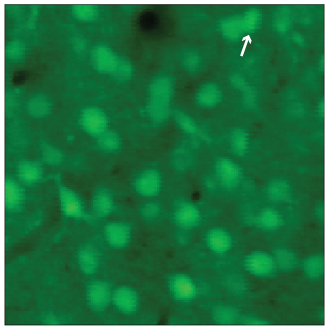
Supp Figs 1-10



Single neurons are reliable

A



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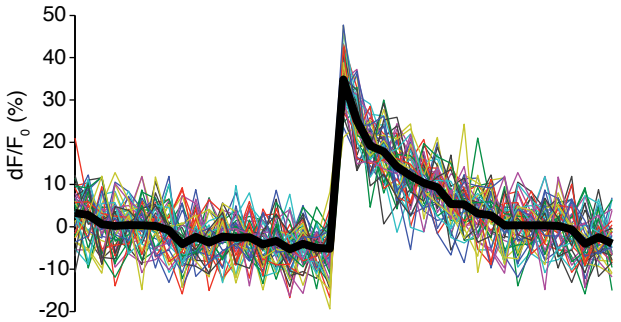

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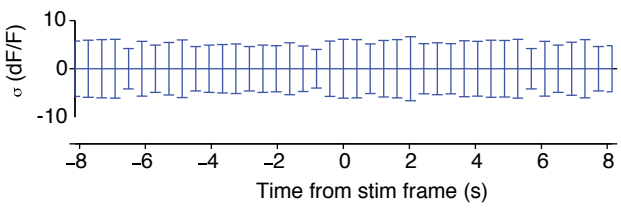
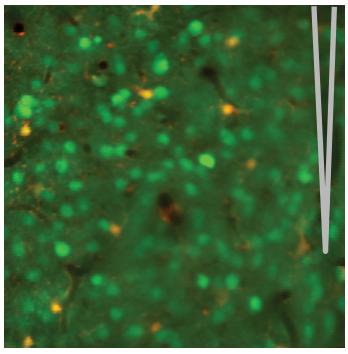
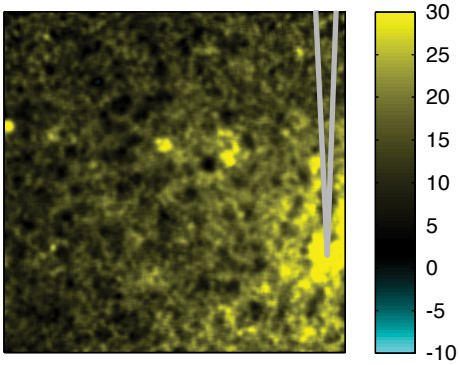


Patterns are reliable

D



E



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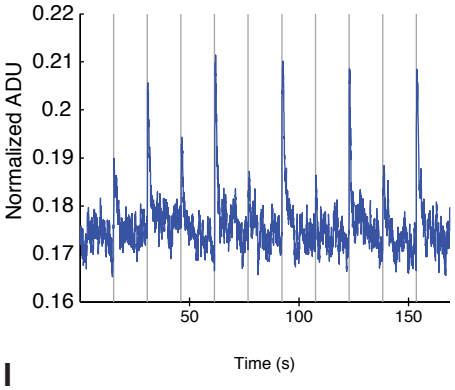

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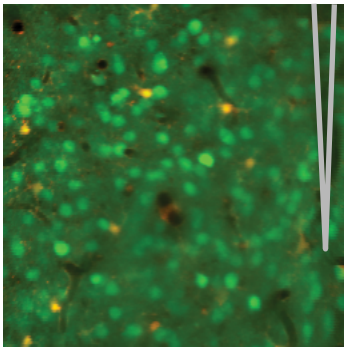
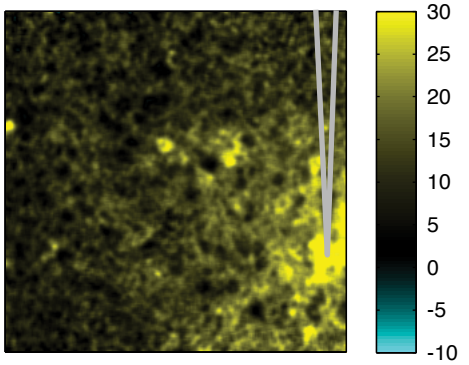


H



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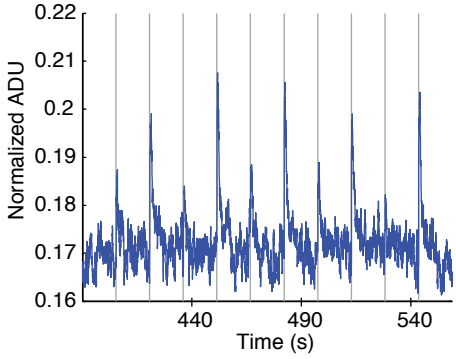
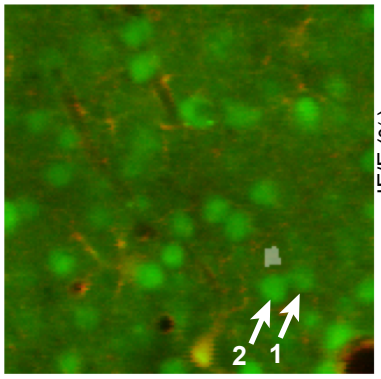
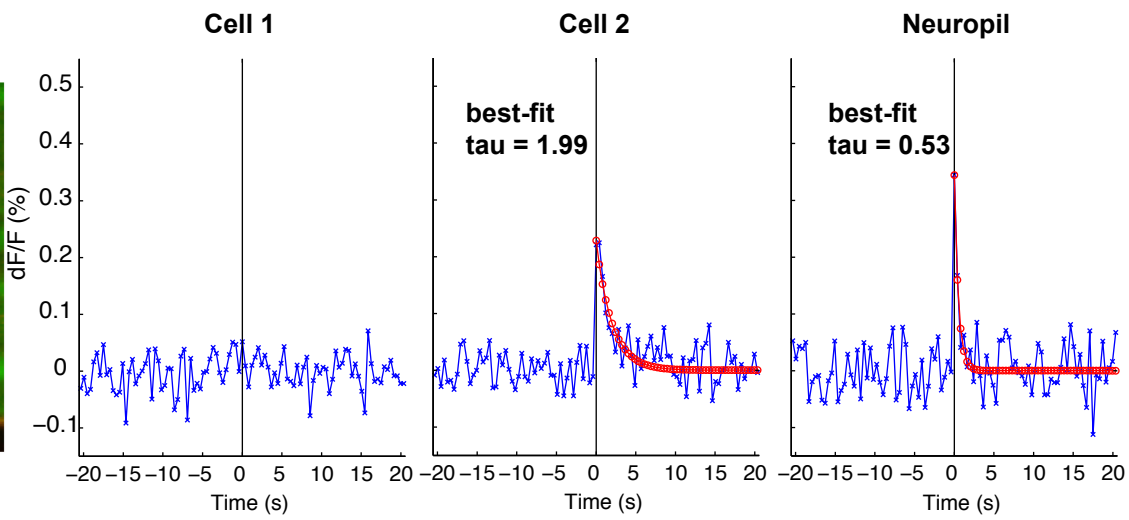


A

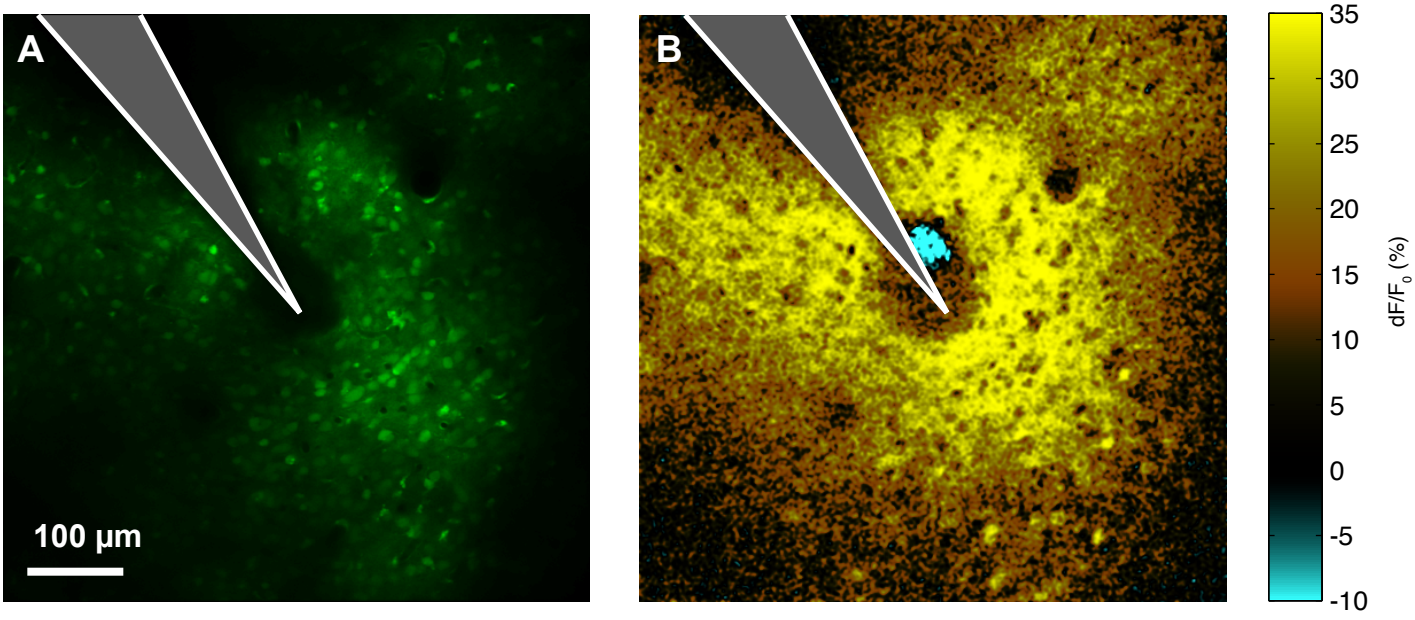

2
1

B

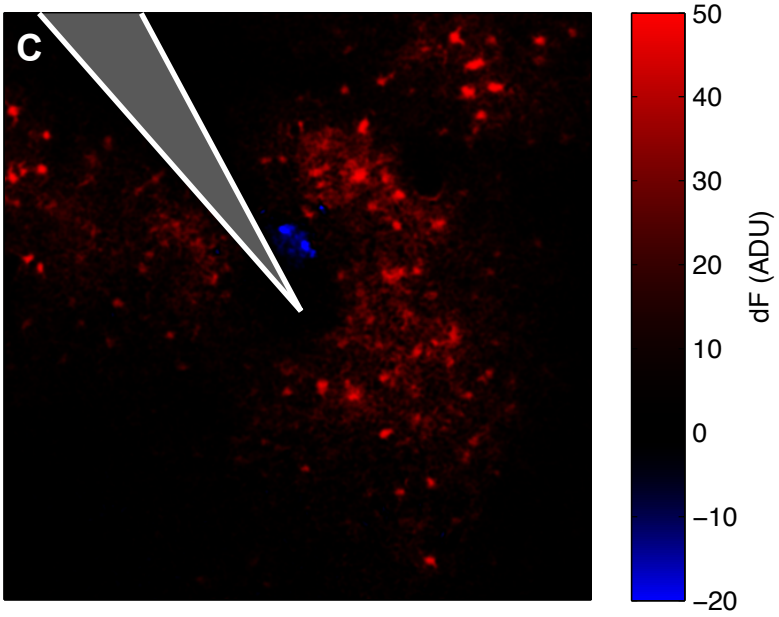

Cell 1
Cell 2
Neuropil
dF/F (%)
0.5
0.4
0.3
0.2
0.1
0
−0.1
best-fit
tau = 1.99
best-fit
tau = 0.53
−20 −15 −10 −5 0 5 10 15 20
Time (s)

stimulation: 50 µA

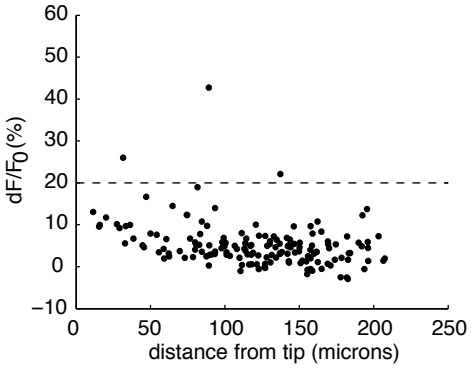
dF/F response map
neuropil and cells similar magnitude



dF response map
cells larger magnitude than neuropil

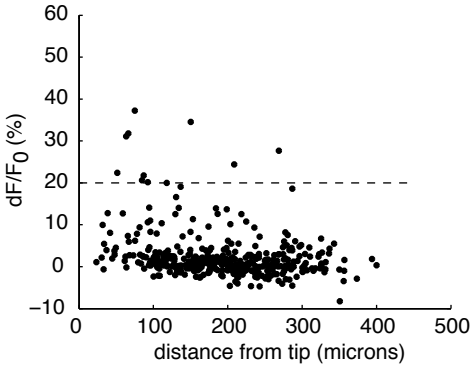


A



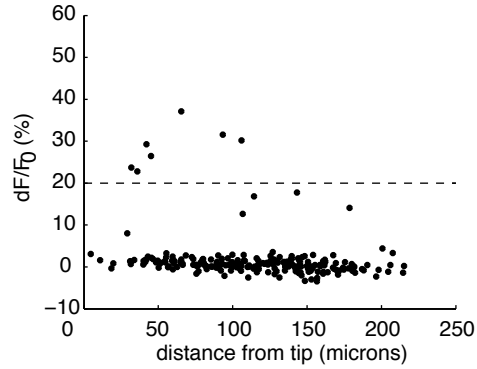
pipette
8 μA
100 ms train
average of 10 repetitions

B

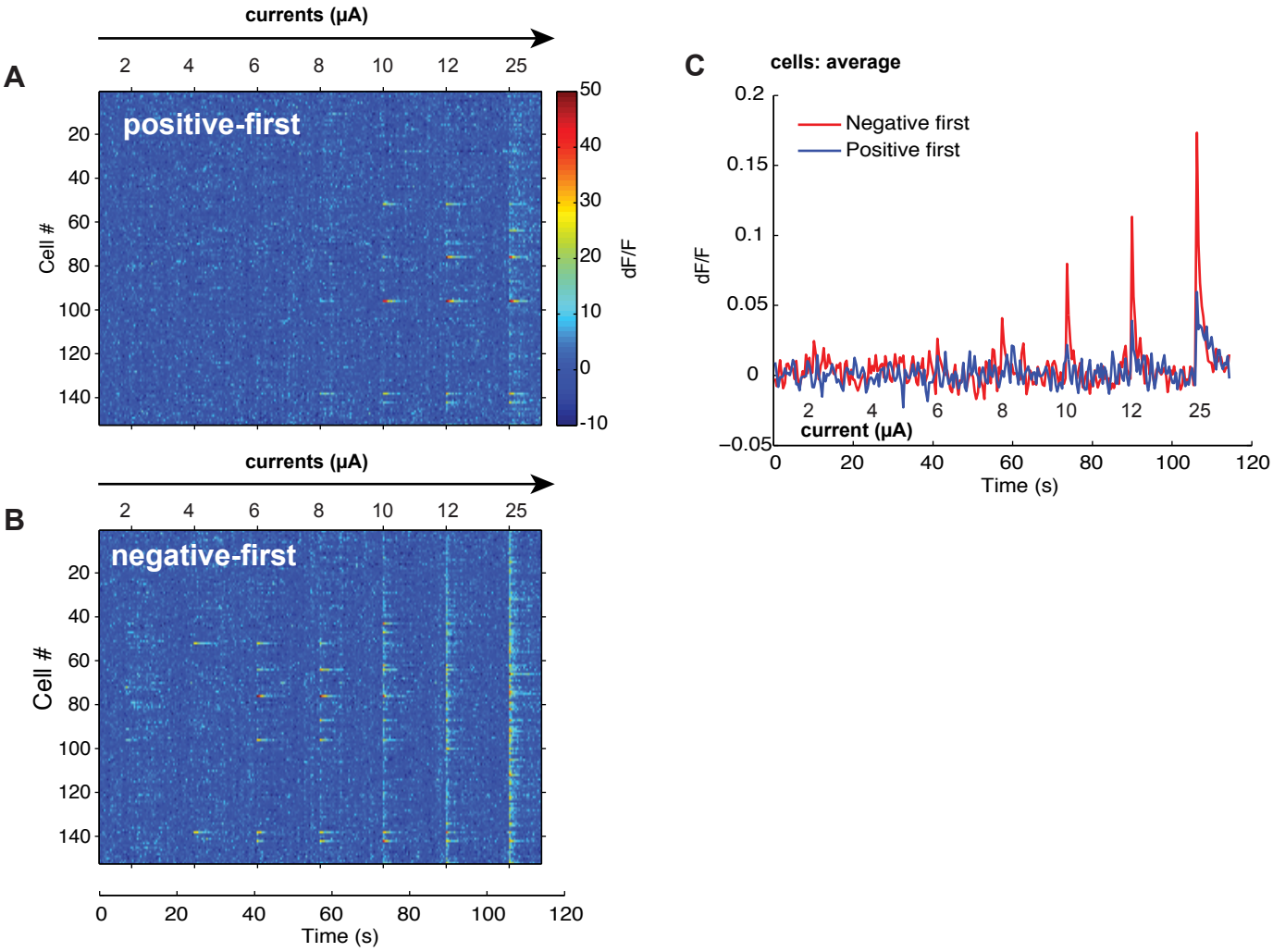


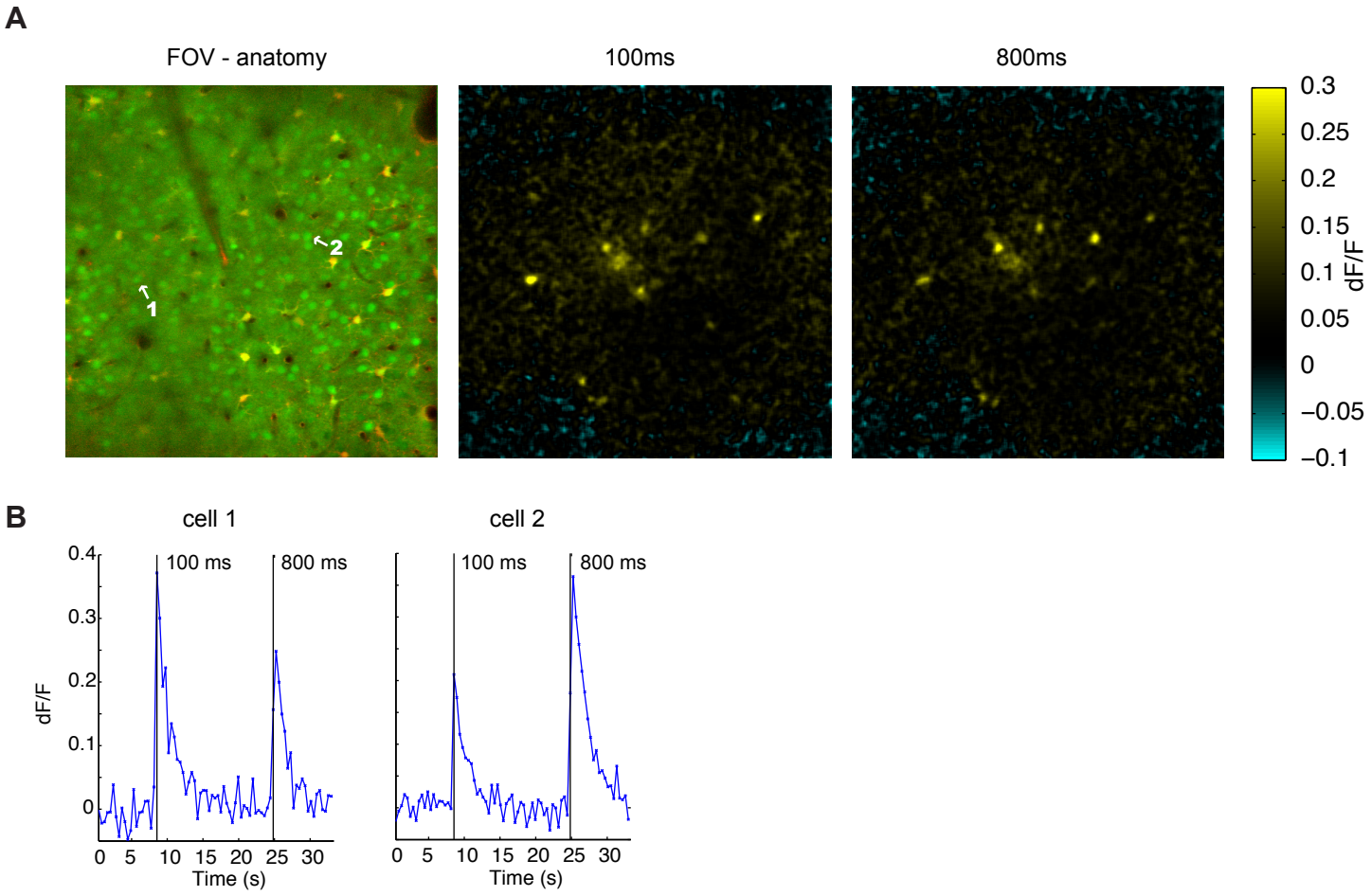
metal
10 μA
800 ms train
average of 20 repetitions

C



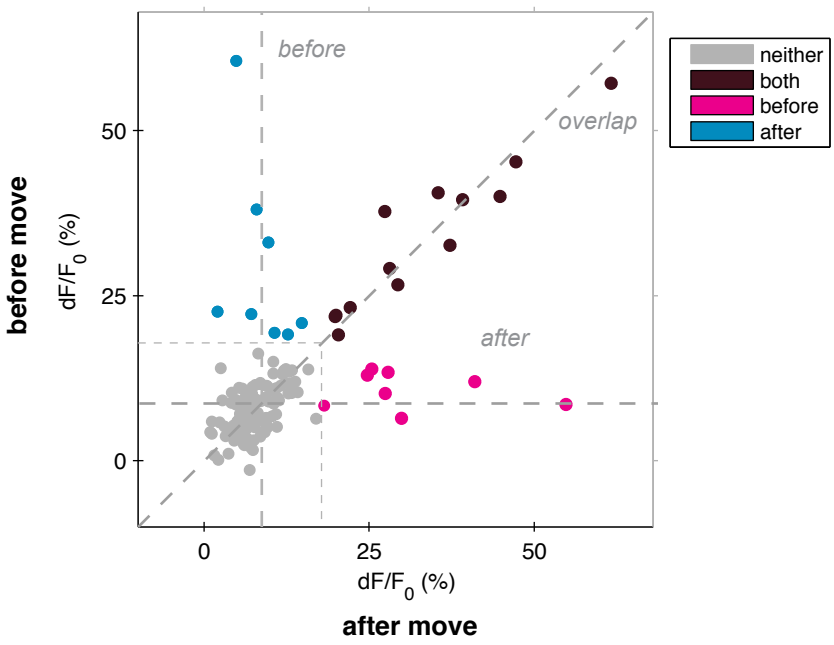
pipette
8 μA
100 ms train
average of **50** repetitions


A
currents (μA)
2
4
6
8
10
12
25
positive-first
Cell #
20
40
60
80
100
120
140
50
40
30
20
10
0
-10
dF/F
B
currents (μA)
negative-first
Cell #
0
20
40
60
80
100
120
Time (s)
C
cells: average
Negative first
Positive first
dF/F
0.2
0.15
0.1
0.05
0
−0.05
current (μA)
Time (s)

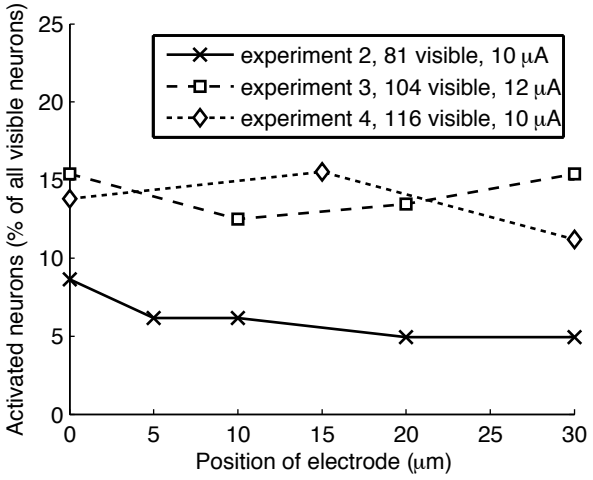

A
FOV - anatomy
100ms
800ms
1
2
0.3
0.25
0.2
0.15
0.1
0.05
0
−0.05
−0.1
dF/F
B
cell 1
cell 2
100 ms
800 ms
dF/F
0.4
0.3
0.2
0.1
0
0 5 10 15 20 25 30
Time (s)

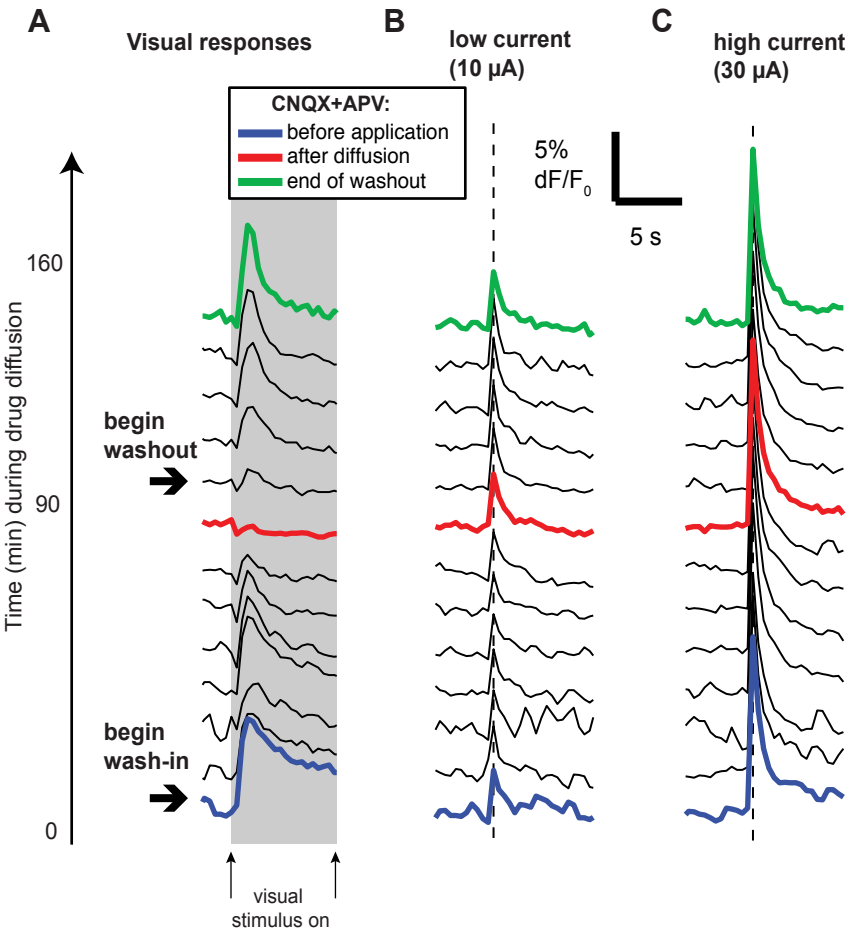
A

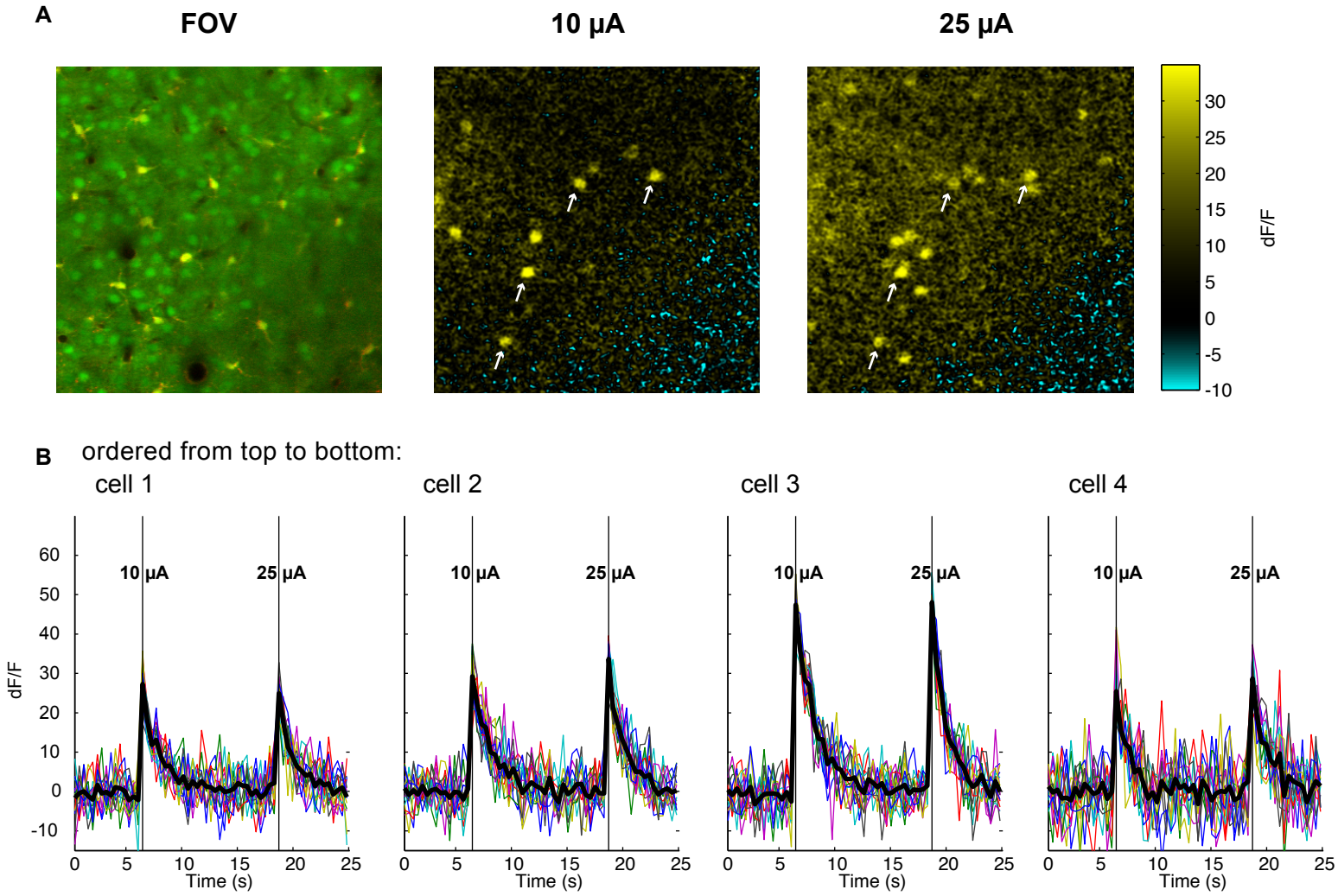
high current (25 µA):
effects of moving tip



B




A
Visual responses
B
low current
(10 µA)
C
high current
(30 µA)
CNQX+APV:
before application
after diffusion
end of washout
5%
dF/F0
5 s
Time (min) during drug diffusion
160
90
0
begin
washout
begin
wash-in
visual
stimulus on


A
FOV
10 µA
25 µA
30
25
20
15
10
5
0
-5
-10
dF/F
B
ordered from top to bottom:
cell 1
cell 2
cell 3
cell 4
10 µA
25 µA
dF/F
60
50
40
30
20
10
0
-10
0
5
10
15
20
25
Time (s)

Supplementary material

Histed, Bonin, and Reid

Contents:

[Supp Fig here]

Supplementary Figure 1: Responses **to single pulses can be detected.**
A, anatomical view with electrode (pipette). B, time courses of responses to trains of 3, 2, and 1 pulses from the two cells indicated by arrows in A. Bottom, enlarged view.

[Supp. Fig. here]

Supplementary Figure 2: Reliability of responses to stimulation trains.
A, field of view image; electrode (pipette) is ~100 μm to right of image. B, $\Delta F/F_0$ timecourses from 25 repetitions of the same train, from the cell indicated at the top right of A. Stimulation train was 25 pulses at 250 Hz (100ms total), 8μA. Heavy black line: mean. C, the standard deviation of each time point, showing that variability on stimulation frames is unchanged from baseline; remaining noise is largely shot noise.

D-I show that patterns of activated neurons area stationary over time (when tip position does not move). D, field of view image with electrode (pipette) position indicated at right. E, ΔF/F0 map, displaying average of 7 frames after each stimulation relative to a baseline of 200 frames before stimulation. F: time course of averaged cells and neuropil over field of view. Responses to ten stimulus trains, with low (6 μA) and high (12 μA) trains interleaved, but the map in E is computed from the 5 high current trains only. G-I, same conventions as D-F for 5 repetitions begun 3 min after D-F.

[Supp. Fig. here]

Supplementary Figure 3: Time constant of neuropil responses is shorter than somatic responses. A, field of view image; electrode (pipette) is ~50 μm to left of image. B. Typical timecourses of responses from cell and neuropil regions (neuropil region is shaded region above cells in A). Each timecourse is an average of response to 5 repetitions of a 35 μA, 25 pulse (100 ms) stimulus train. Best-fit exponential decay is plotted in red. Neuropil response is faster, and also typically homogenous (see Figs. 6,7).

[Supp. Fig. here]

Supplementary Figure 4: Responses to high currents. A, field of view image; electrode (tungsten) is indicated by gray outline; cat visual cortex B. $\Delta F/F_0$ map of response to a 50 μA train, 800 ms. Note large field of view. C. ΔF map for same data; this map emphasizes activated cells because baseline F_0 is lower for neuropil. ΔF calculated for two frames (1.5 s) after stimulation also to emphasize cells over neuropil (neuropil shows more rapid falloff). Black region around tip in (A) is a non-responsive region that developed during stimulation; it was strongly red fluorescent, suggesting deposition of tungsten which is autofluorescent. Such effects were not seen at threshold currents or with Pt-Ir electrodes or metal pipettes. Blue region in (B,C) corresponds to a likely gas bubble caused by stimulation. the bubble disappeared within seconds (as throughout the paper, the train was constant-current, and pulses were charge balanced)

Note that falloff of the signal at edges of images in (B,C) corresponds to falloff in staining: responses are also seen in the dim regions but are more noisy.

[Supp. Fig. here]

Supplementary Figure 5: Cell responses as a function of distance.
A, $\Delta F/F_0$ responses (y-axis) as function of distance from tip (x-axis), for individual cells. in an experiment in which an 8 μA, 100 ms train was applied through a pipette (10 repetitions). Dashed line indicate 20% threshold used in the paper. B, As in A, but data plotted for 10 μA, 800 ms train through a tungsten electrode (20 repetitions). C, Experiment using a pipette and a 100 ms, 8 μA train, but averaged multiple times (50 repetitions). Note low variability of non-activated cells.

[Supp. Fig. here]

Supplementary figure 6: Threshold is lower for anodal (negative-first) pulses.
A. average responses to cathodal (positive-first) pulses. Train length: 100 ms at 250 Hz. B, responses of the same cells to anodal (negative-first) pulses. Note that cells are activated with anodal currents as low as 4 μA, but cathodal currents first activate cells only at higher current. C: average across cells.
Currents of 2, 4, 6, 8, 10, 12, and 25 μA were used. In each plot current increases to the right.

These data are consistent with the idea that microstimulation at these currents primarily activates cells via their axons. It is known that axons have lower thresholds to anodal pulses (Tehovnik, 1996, Durand, 2000), while cathodal-first pulses may preferentially activate cell bodies (Ranck, 1975, Tehovnik, 1996).

[Supp. Fig. here]

Supplementary figure 7: Different length trains activate similar sets of neurons. Experiment in which 100 ms and 815 ms were delivered trains alternately (25 and 203 pulses, respectively). A, anatomy image; conventions as in Fig. 2. $\Delta F/F_0$ maps for the two train durations are shown at right. Stimulation current: 8 μA. B, average time courses of responses for two cells indicated by arrows in (A).

At this low, near-threshold current, though some neurons showed different levels of activation (examples plotted in B), these train lengths lead to similar sets of activated neurons.

[Supp. Fig. here]

Supplementary figure 8: Patterns of activated cells show greater overlap at higher currents.
A, Effects of moving the tip 15 μm on responses to high current stimulation. Same experiment as in Fig. 4C but but 25 μA trains instead of 10 μA (low and high current conditions were interleaved). This higher current produced greater overlap between the patterns of activation at the two locations (black data points). Note that a substantial number of cells were activated exclusively before or after moving the electrode (blue and pink data points).

B, Number of activated neurons at different tip positions for experiments shown in Fig. 4E. The number of activated cells is similar from one position to another, even though increasing distance of the tip from position 0 decreases the number of shared neurons (Fig. 4E).

[Supp. Fig. here]

Supplementary figure 9: Evolution of responses during wash-in and washout of CNQX/APV; constant effects of electrical stimulation throughout.
Responses to visual and electrical stimulation, for the experiment shown in Fig. 5A-C. The heavy blue, green, and red lines are the same as shown in Fig. 5C. In this experiment, wash-in took approx. 90 min.

A: visual responses for cells and neuropil averaged together. B: responses to 10 μA trains, same region as in A. C: responses to 25 μA trains. Trains were 100 ms in length (25 biphasic pulses, 0.2 ms each phase), and delivered through a glass pipette with the tip positioned in the field of view.

[Supp. Fig. here]

Supplementary figure 10: Larger currents tend to activate a superset of the cells activated at lower currents.

A, Field of view image, and ΔF/F0 pixel maps corresponding to two different currents. The two currents were interleaved.

The higher current activates more neurons, but the majority of neurons activated at 10 μA are also active at 25 μA. Increased neuropil activation is also seen (cf. Fig. 7).

B, Time courses of responses of four cells marked with arrows in A. Cells are ordered top to bottom so cell 1 is the topmost (and rightmost) indicated by an arrow, and 4 the lowest and leftmost. Heavy black lines, mean of 10 repetitions. Colored lines: individual trials. Vertical lines indicate frame on which 100 ms stimulation train was delivered. Scan rate: 2.5Hz.

These example neurons are typical of what we observed: peak ΔF/F0 does not increase with increasing current. Instead, there is a threshold current below which cells do not respond, and above which they respond strongly. This suggests an all-or-none effect of stimulation on individual neurons.

Supplementary movies

1 [stim-train-response-ratio.avi]:
Average response to a 100 ms (250 Hz) stimulation train at 10 μA. Glass pipette, mouse visual cortex. Positive fluorescence changes relative to baseline: red, negative responses (not seen): blue. Imaging rate: 2.5Hz.

2 [stim-train-response-raw.avi]:
Same data, shown as raw fluorescence values (in ADU, as acquired from photomultiplier tubes). Cells and neuropil and their response to the stimulation train are visible.