SUPPLEMENTARY MATERIAL

"An *in vivo* biosensor for neurotransmitter release and *in situ* receptor activity"

FIGURE CAPTIONS

Supplementary Figure 1. M1-CNiFER Dynamics in calcium-free media. These data demonstrate that the tonic M1-CNiFER response is dependent upon calcium in the media. **(a)** M1-CNiFERs respond with typical phasic and tonic components to 30 nM acetylcholine in the constant presence of calcium. (**b)** M1-CNiFERs respond with only the phasic component in calcium free media. Calcium returned to the media in the continued presence of 30 nM acetylcholine results in a response with phasic and tonic components, though possibly a slower phasic onset. (**c)** Calcium withdrawal from media during the M1-CNiFER tonic response abolishes the response.

Supplementary Figure 2. Repetitive stimulation of M1-CNiFERs with pulses of acetylcholine. Two second pulses of 300 nM acetylcholine pulses, with a 30 second interstimulus interval, were applied. Stabilization occurs at to an asymptotic value of 0.3-times the initial change with a time-constant or 70 s.

Supplementary Figure 3. **M1-CNiFERs respond to slowly-increasing concentrations of acetylcholine.** A linear gradient of acetylcholine from 0 to 10 nM was presented to M1-CNiFERs on a period of 1200 s; the gradient was calibrated by concurrent measurements of co-dissolved alexa-594. The M1-CNiFER response (black trace) to the slowly-increasing acetylcholine lacks a prominent phasic component as is characteristic of the response to a step of acetylcholine (Fig. 1b). The M1-CNiFER response to a bolus of 10 nM acetylcholine applied at the end of the experiment is largely unchanged from that applied prior to start of the gradient. Note that there is a small offset at the end of the experiment of possible technical origin; a similar level of drift is seen without the addition of acetylcholine (gray trace).

Supplementary Figure 4. Atropine, clozapine and olanzapine suppress receptor-mediated responses *in vitro*. In Flexstation™ 3 assay, the M1-CNiFER FRET response to 100 nM acetylcholine chloride is abolished by 100 nM atropine sulfate, 3 μM clozapine, and 3 μM olanzapine. All three effects are significant by t-test, $p < 0.001$, $n = 6$ for each group. Bars represent standard error.

Supplementary Figure 5. M1-CNiFER response to acetylcholine is maintained after brain implantation. M1-CNiFERs respond to intracortical 27 nl puffs of 1 mM acetylcholine chloride (cyan) but not PBS (grey). Time trace shows average response and standard error for 3 injections, same animal. Inset shows fractional change of 1/3 the area under the $\Delta R/R$ curve for 30 s after the stimulus as compared to that for 10 s before the stimulus, for either acetylcholine or PBS (3 animals, 3 trials each). Bars represent standard error.

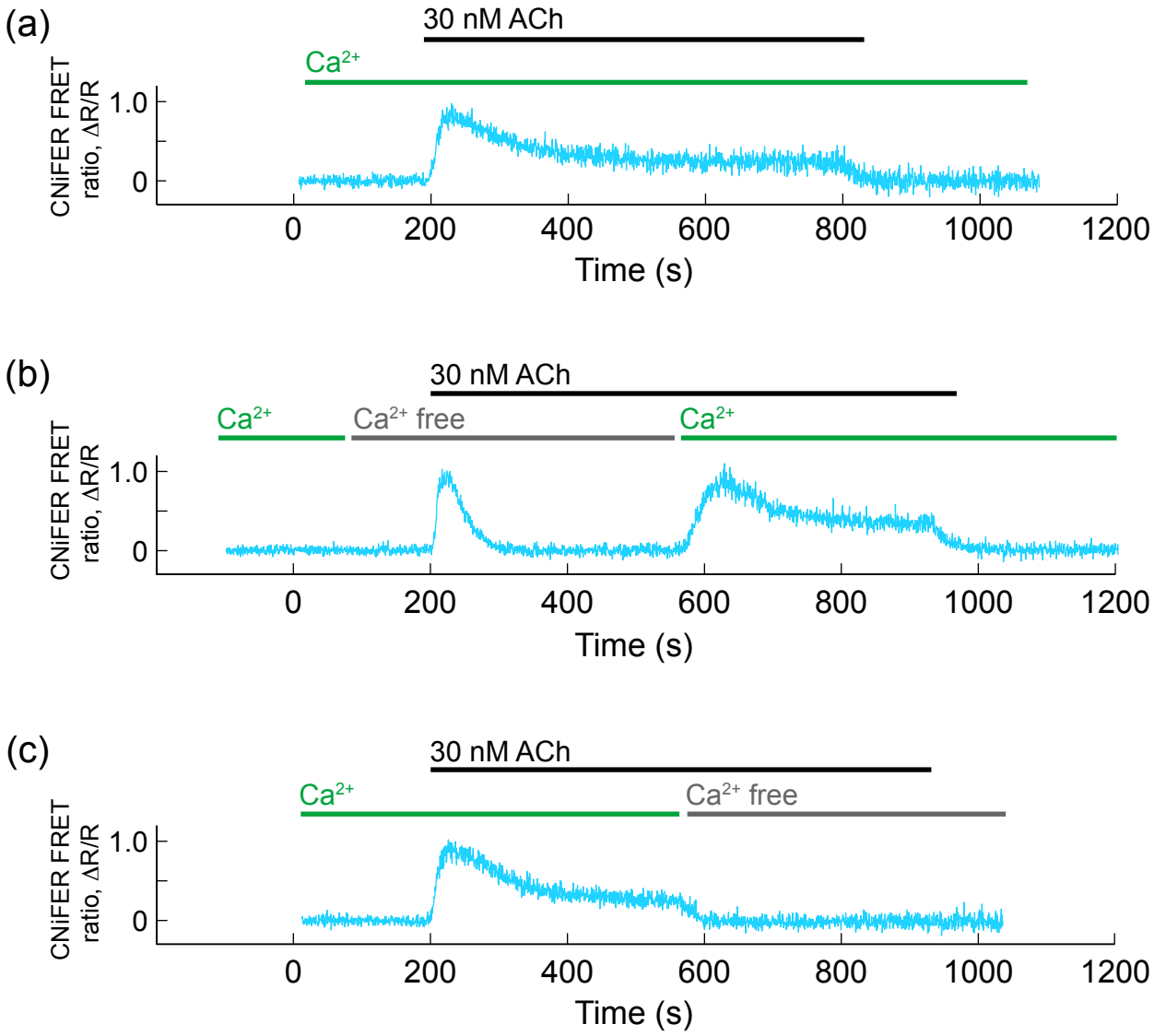
Supplementary Figure 6. Intact vasculature surrounding M1- and control-CNiFER sites in the frontal cortex of a live rat implanted two days earlier. Image is a z-projection spanning 140 μm from the cortical surface. Cerebrovasculature is visualized by 500 μl intravenous injection of 5 % (w/w) fluorescein dextran (yellow). Visibility of the fluorophore shows the vasculature is intact. M1-CNiFERs labeled in cyan (1,3), control-CNiFERs labeled in red (2).

Supplementary Figure 7. Immunostain against GFAP reveals no astrocytic scar around chronically implanted M1- and control-CNiFERs. In top image, mCherry and TN-XXL fluorescence are overlayed on a brightfield 3,3'-diaminobenzidine α-GFAP stain for astrocytes. Scale bar represents 200 μm. In bottom image, only TN-XXL fluorescence is overlayed to better visualize the morphology of CNiFER cells. Scale bar represents 50 μm. α-GFAP stain reveals cell bodies and long complex processes apparent at the surface of the brain and in deeper layers at ~ 1 mm (see white arrows). There is no astrocytic concentration (glial scar) along the implantation site. Functional data with *in vivo* two photon microscopy is typically acquired at depths of 50 to 200 μm below the pia, where few astrocytes in this section are present. Preparation was 2 d *in*

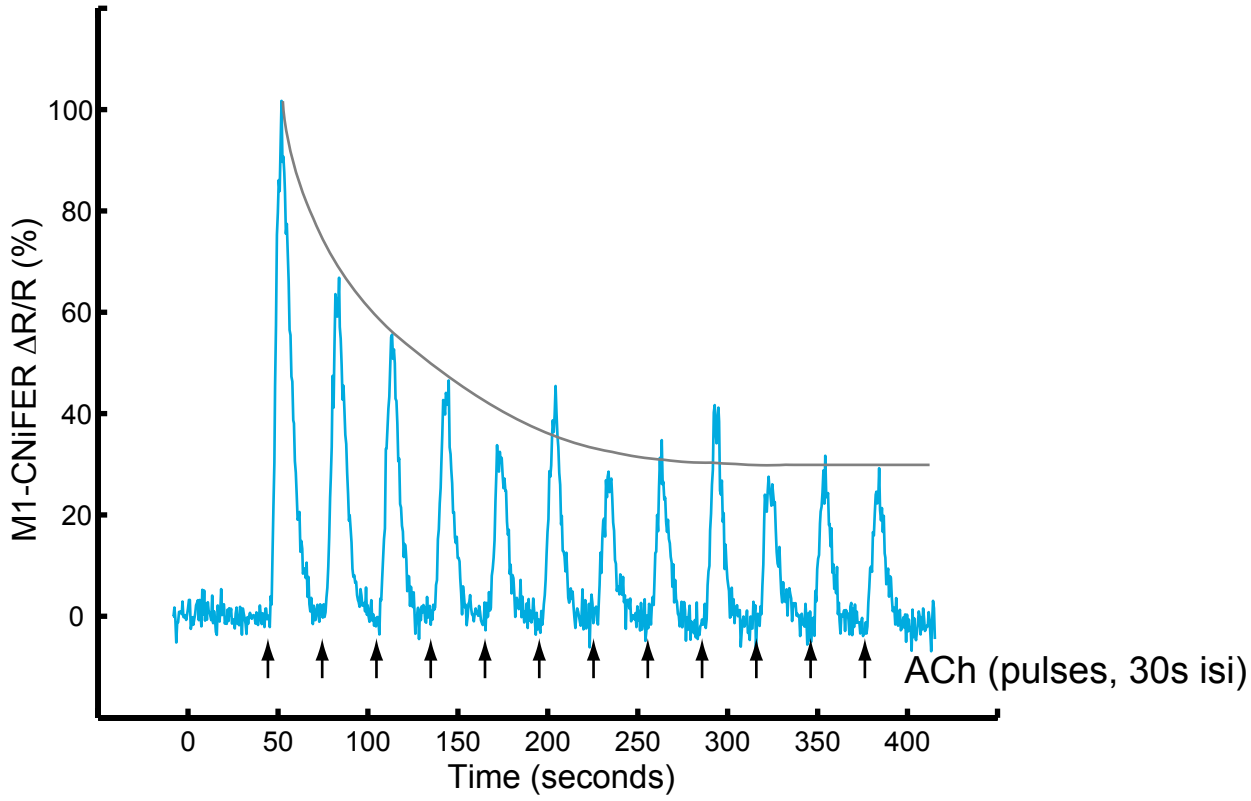
vivo; M1-CNiFER column extends to the cortical surface in a neighboring section (data not shown).

Supplementary Figure 8. Clozapine suppresses NBM-evoked activation of ECoG, consistent with suppression of M1-CNiFER response. (a) Spectral analysis of electrocorticogram reveals the hallmark of NBM-evoked cortical activation: a suppression of power in the δ-band. Intraperitoneal clozapine (5 mg/kg) abolishes this effect, consistent with its antimuscarinic properties and consistent with its effect on M1-CNiFERs. Pre-NBM spectra in blue, post-NBM spectra in green. **(b)** Summary graph showing statistical significance of NBM-evoked cortical activation as measured by minus one times the logarithm of the inverse power of delta band, -*log*[power in ECoG δ-band], and its modulation by clozapine. As expected, NBM stimulation significantly decreases power in the δ-band ($p = 0.005$, t-test). In the presence of clozapine, NBM stimulation mildly increases the power in the δ–band ($p = 0.04$, t-test). Furthermore, clozapine slows the baseline spontaneous electrocorticogram ($p = 0.008$, t-test). Bars represent standard error.

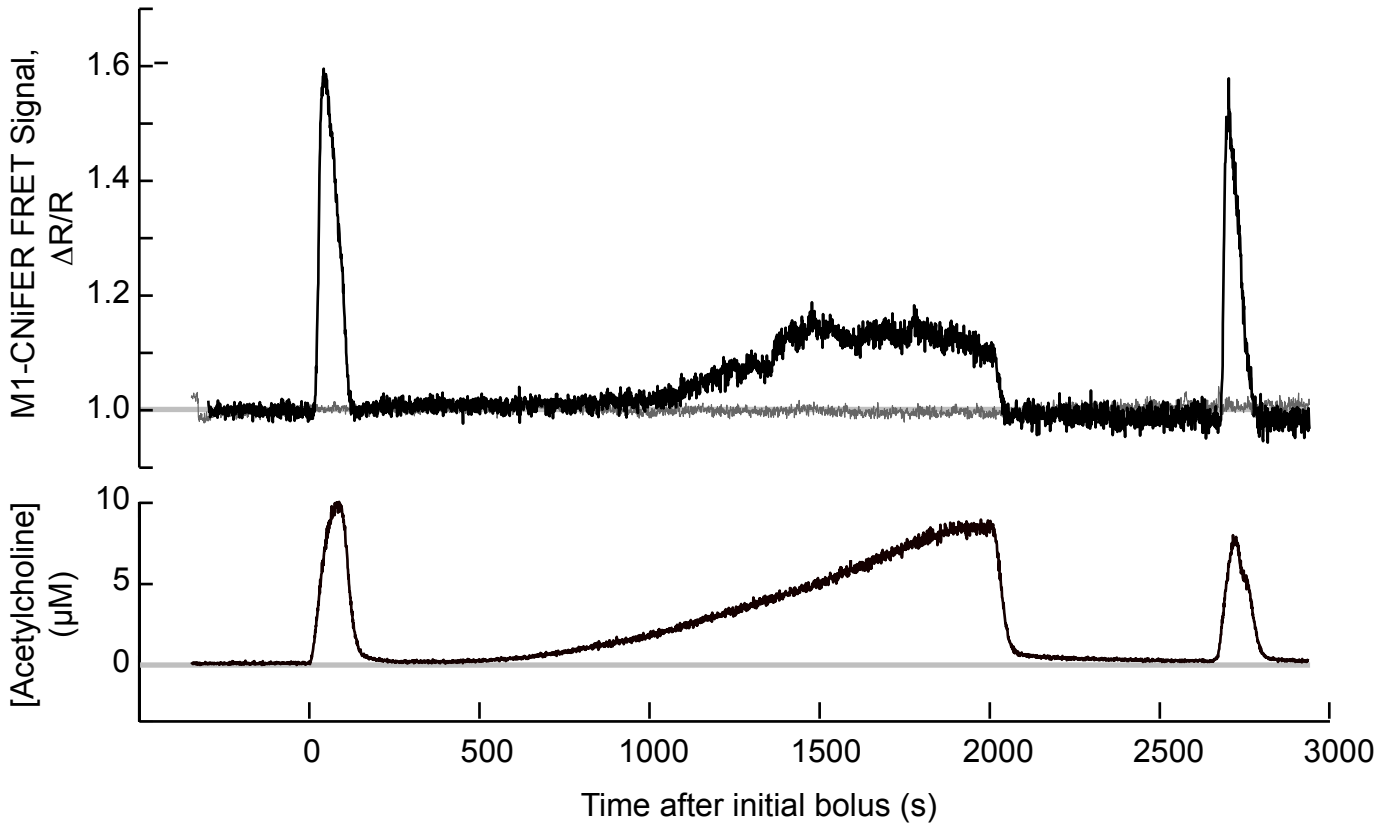
Supplementary Figure 9. Olanzapine suppression of NBM-evoked M1-CNiFER response is not activity dependent. Intraperitoneal olanzapine (3 mg/kg) suppresses the M1-CNiFER response to a single NBM stimulation delivered 20 minutes after injection of the antipsychotic. Black vertical dashed lines represent 600 μA NBM stimulations.



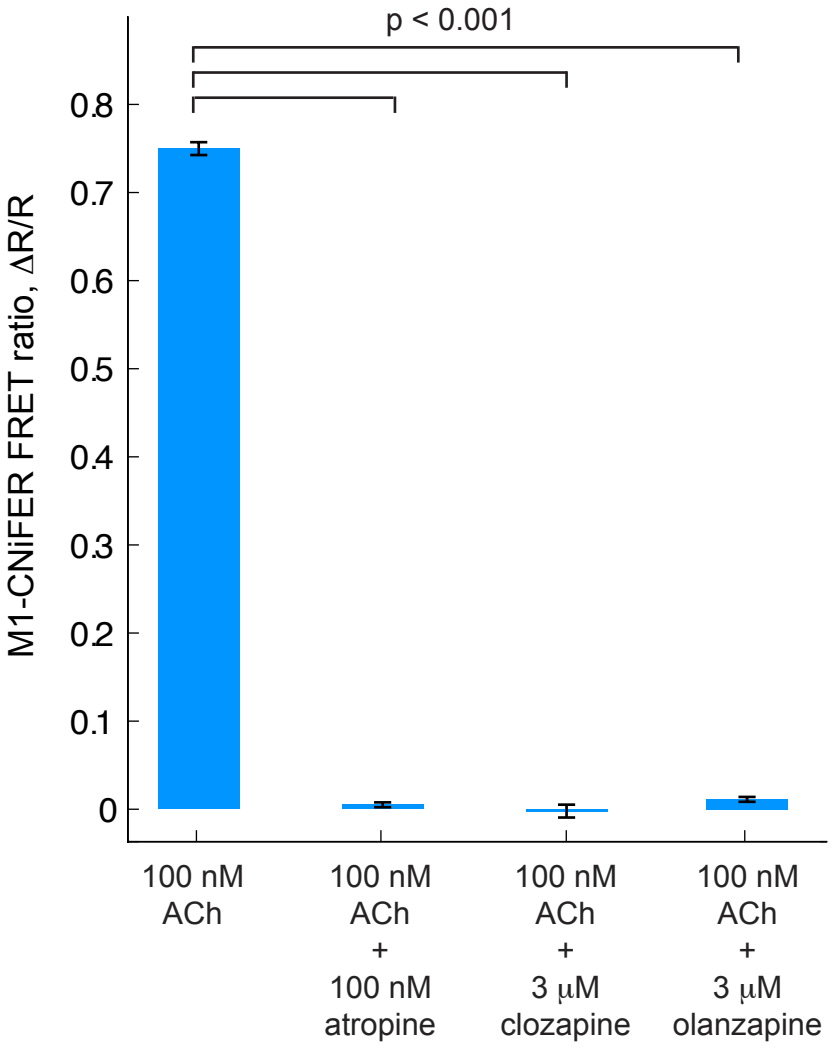
Supplemental figure 1. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



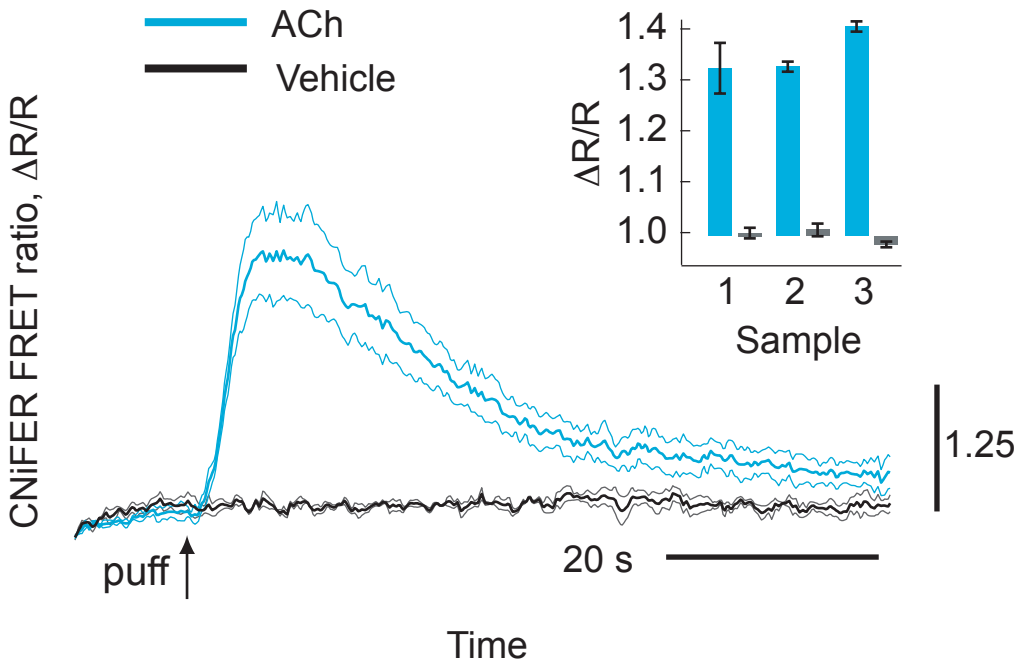
Supplemental figure 2. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



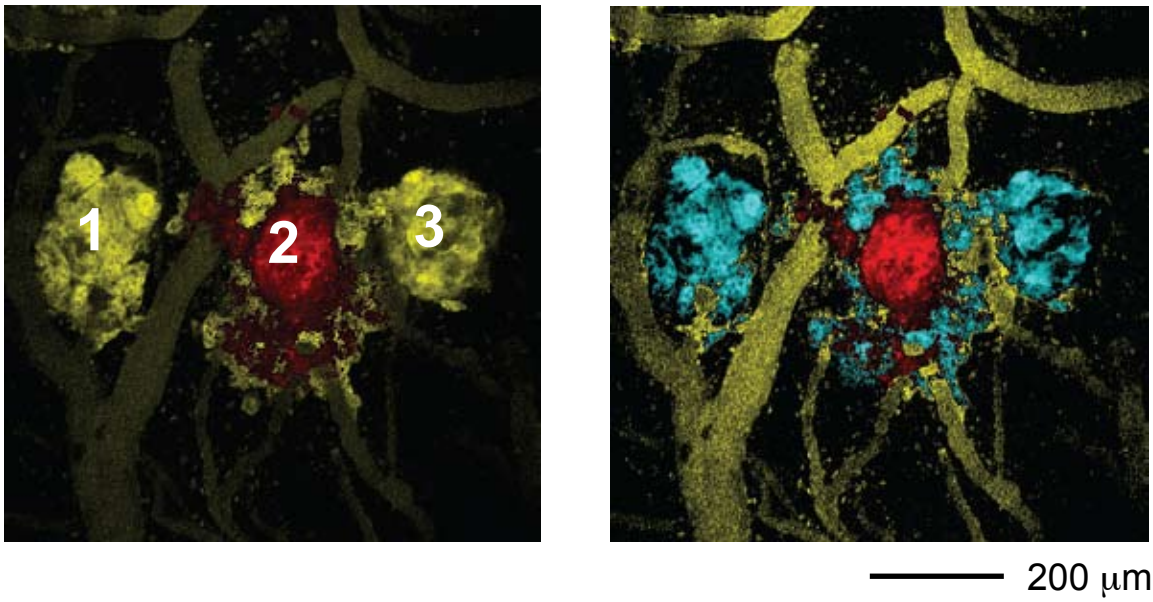
Supplemental figure 3. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



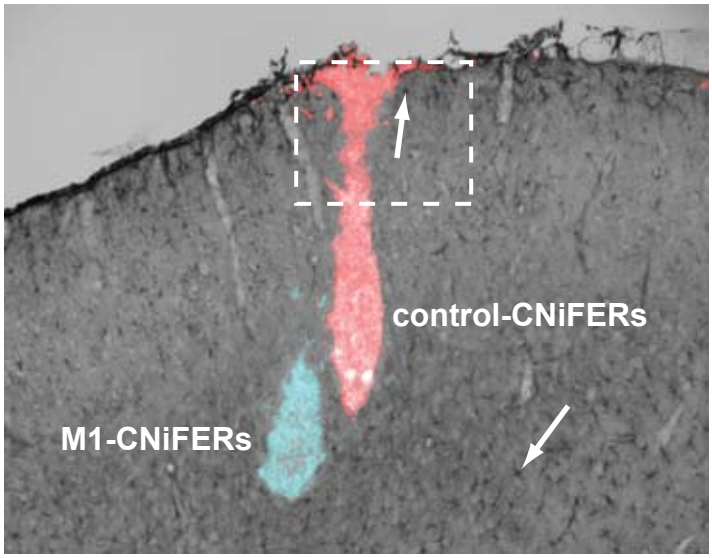
Supplemental figure 4. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



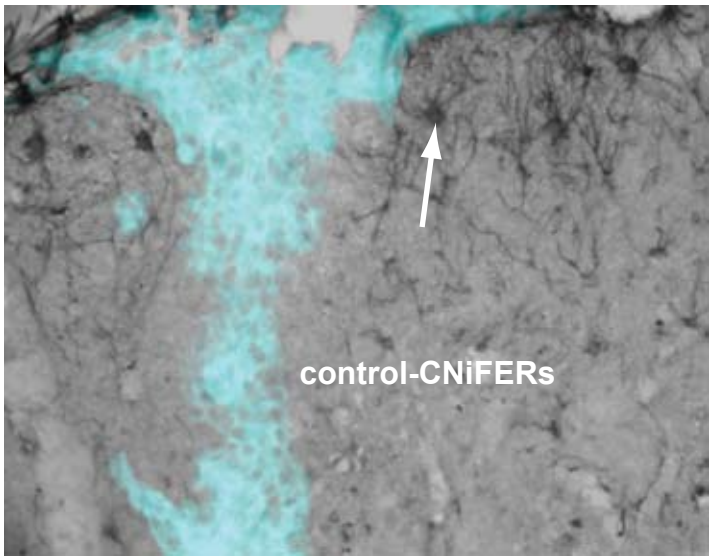
Supplemental figure 5. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



Supplemental figure 6 Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld

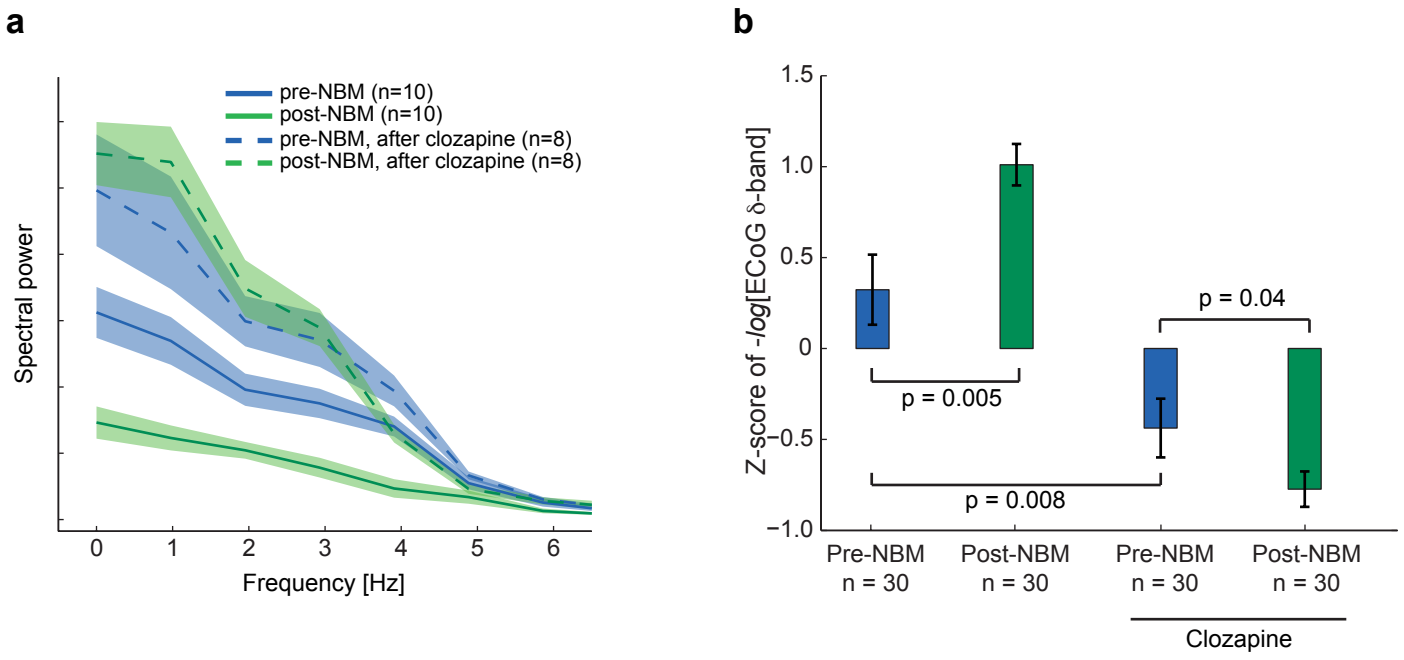


200 μm

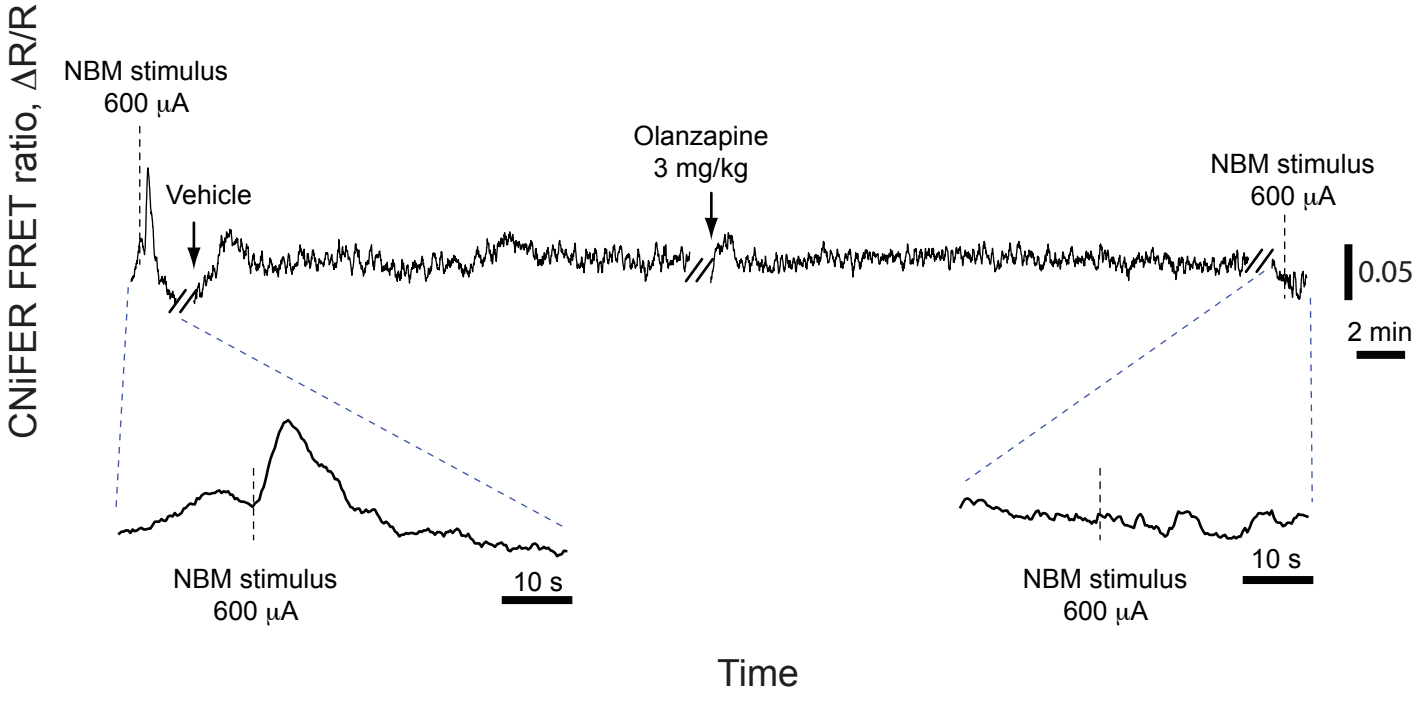


50 μm

Supplemental figure 7. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



Supplemental figure 8. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld



Supplemental figure 9. Q.-T. Nguyen*, L F. Schroeder*, M. Mank, A. Muller, P. W. Taylor, O. Griesbeck and D. Kleinfeld