# Pattern, and not magnitude of neural activity determines dendritic spine stability in awake mice

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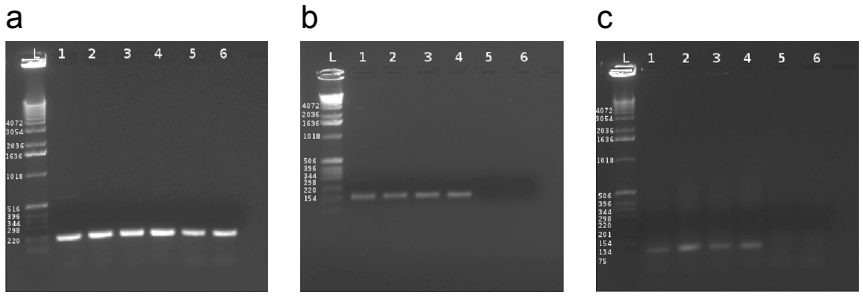
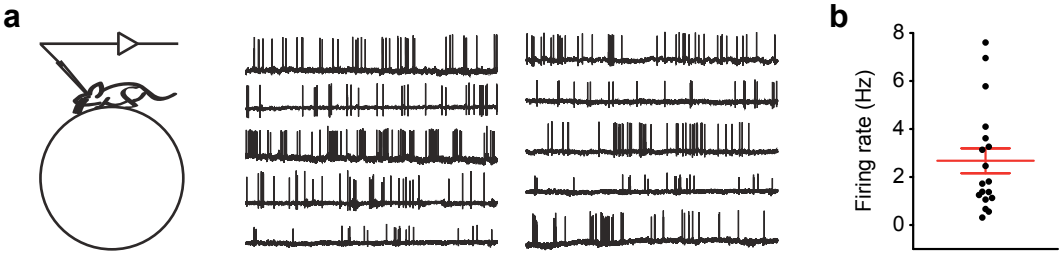
## Supplementary Information

Supplementary Figure 1



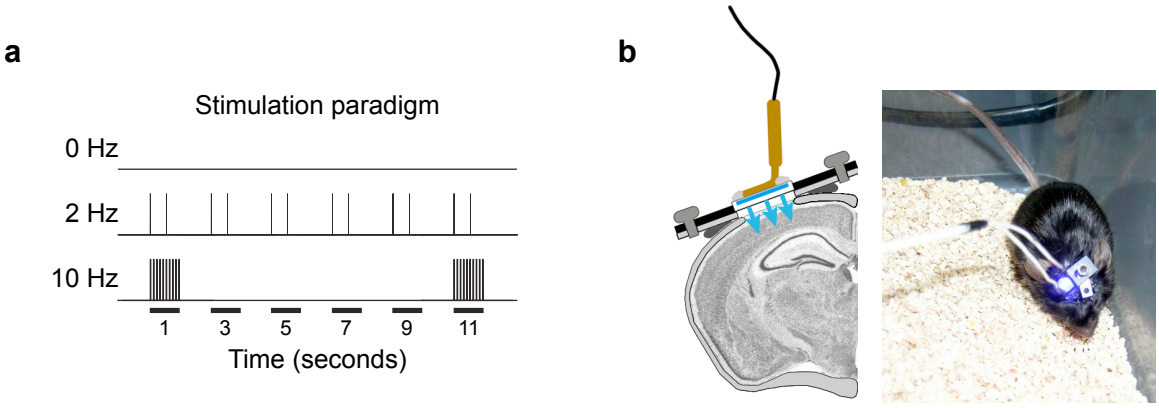

**Supplementary Figure 1. GAPDH, GFP and ChR2 expression in transgenic and control brains.** Layer 5 GFP positive cells were laser captured from 4 double transgenic brains (Lanes 1-4). Layer 5 cells were also captured from 2 wild type control brains (Lanes 5 and 6). RNA was isolated, amplified and converted to cDNA. PCR was performed for the housekeeping gene GAPDH (**a**), and the transgenic genes GFP (**b**) and ChR2 (**c**). The numbers on the left indicate the size of the ladder bands. cDNA amplicon sizes: GAPDH, 211 base pairs; eGFP, 150 base pairs; ChR2, 94 base pairs. There is a similar level of GAPDH cDNA in all samples. However, the eGFP and ChR2 transgenes are only present in the transgenic brains, lanes 1-4.

Supplementary Figure 2




**Supplementary Figure 2. Spontaneous firing rates of layer 5 neurons in awake mice.** (**a**) Cartoon of a mouse on a floating Styrofoam ball with a patch pipette inserted into layer 5 of somatosensory cortex. Recordings from awake mice maneuvering on this ball permitted accurate measures of spontaneous firing rates. Example traces of 10 isolated layer 5 neurons from awake mice are shown. Each trace is 20 seconds in duration. (**b**) Plot of mean firing rates for all 18 recorded neurons from 5 mice. Red bars show the mean and standard error of this distribution (2.4 ± 0.5 Hz).

Supplementary Figure 3




**Supplementary Figure 3. Optical stimulation paradigm.** (**a**) Schematic showing the pattern of light stimulation in control mice (0 Hz), 2 Hz, and 10 Hz groups. Note that the pattern differs but the total number of spikes is the same between the 2 Hz and 10 Hz groups (average 1 Hz). (**b, left**) Schematic showing a cross section through one hemisphere of a mouse brain. A blue LED embedded in a removable head mount is secured via two screws to a headbar attached to the skull via dental acrylic. A flexible wire tethers the diode to a controller. A small piece of coverglass replaces a similarly sized piece of skull to permit high optical access to the underlying cortex. (**b, right**) Photograph of a mouse tethered to the head-mounted blue LED.