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Supplemental Information

Rapid Integration of Artificial Sensory Feedback during Operant Conditioning of Motor Cortex Neurons

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Supplemental information

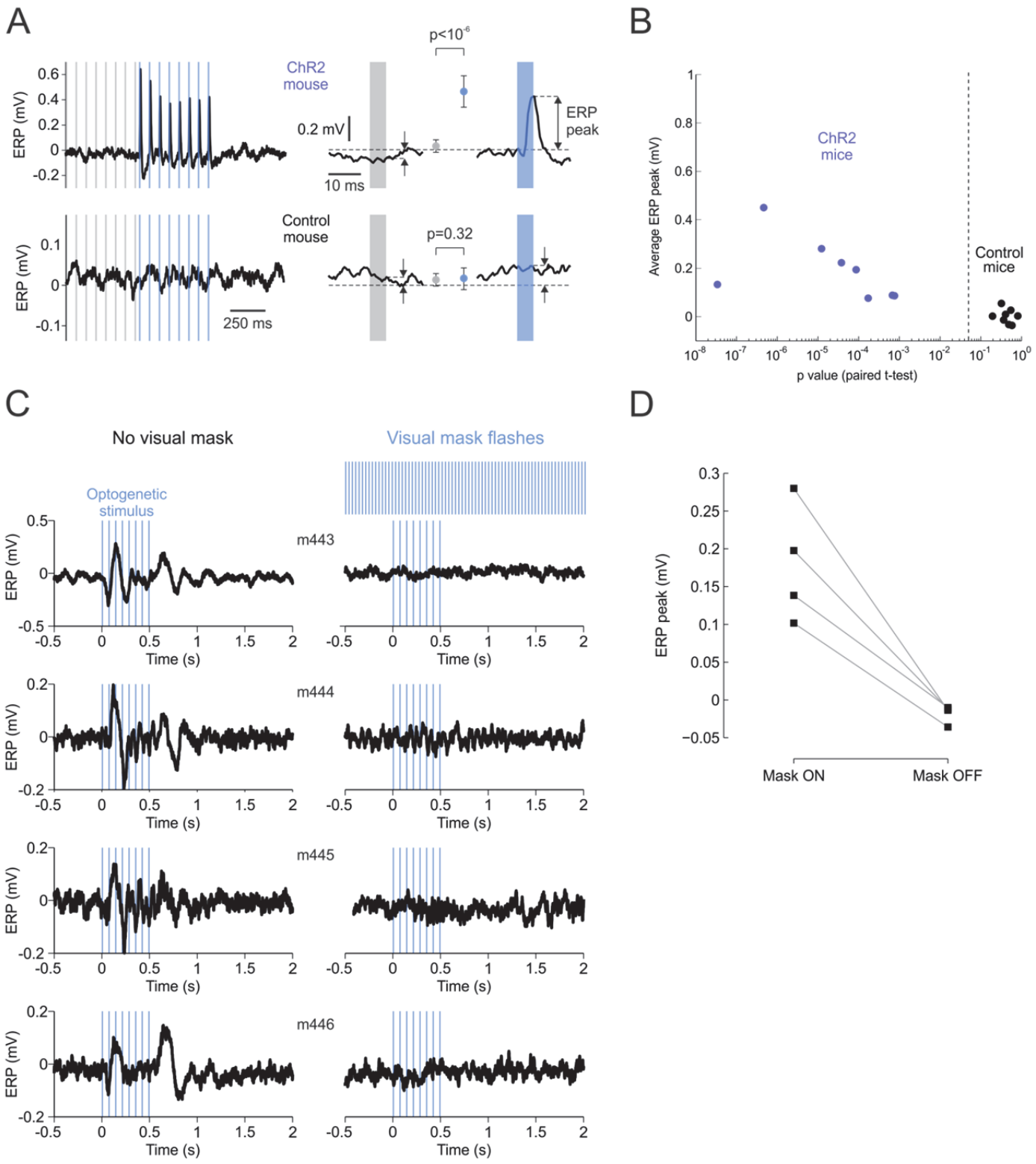

Figure S1. Related to Figure 1. Mouse classification and efficacy of the visual mask.

(A) Average event related potentials (ERPs) measured differentially between the S1 and V1 cortical surface electrodes (left panels) and example mean ERP peaks (right panels) evoked by optical pulses (blue) and during time-matched baseline periods (grey) are shown for one ChR2 and one control mouse. Mean (± s.e.m.) peak

size was compared between stimulation (blue data points) and baseline periods (grey data points) for each mouse (paired t-test).

(B) Dashed black line denotes the p=0.05 (paired t-test) classification criterion.

(C) Left panels: with the mask turned off, visually evoked potentials were apparent at the onset and offset of the optogenetic stimulus for 4 tested control mice. Right panels: these neural responses were supressed when a visual mask consisting of continuous ≈30 Hz blue light pulses illuminating the experimental setup was turned on.

(D) A comparison of visually evoked ERP peaks for the 4 control mice shown in (C) between the mask ON and OFF test conditions depicts the consistent efficacy of the visual mask.

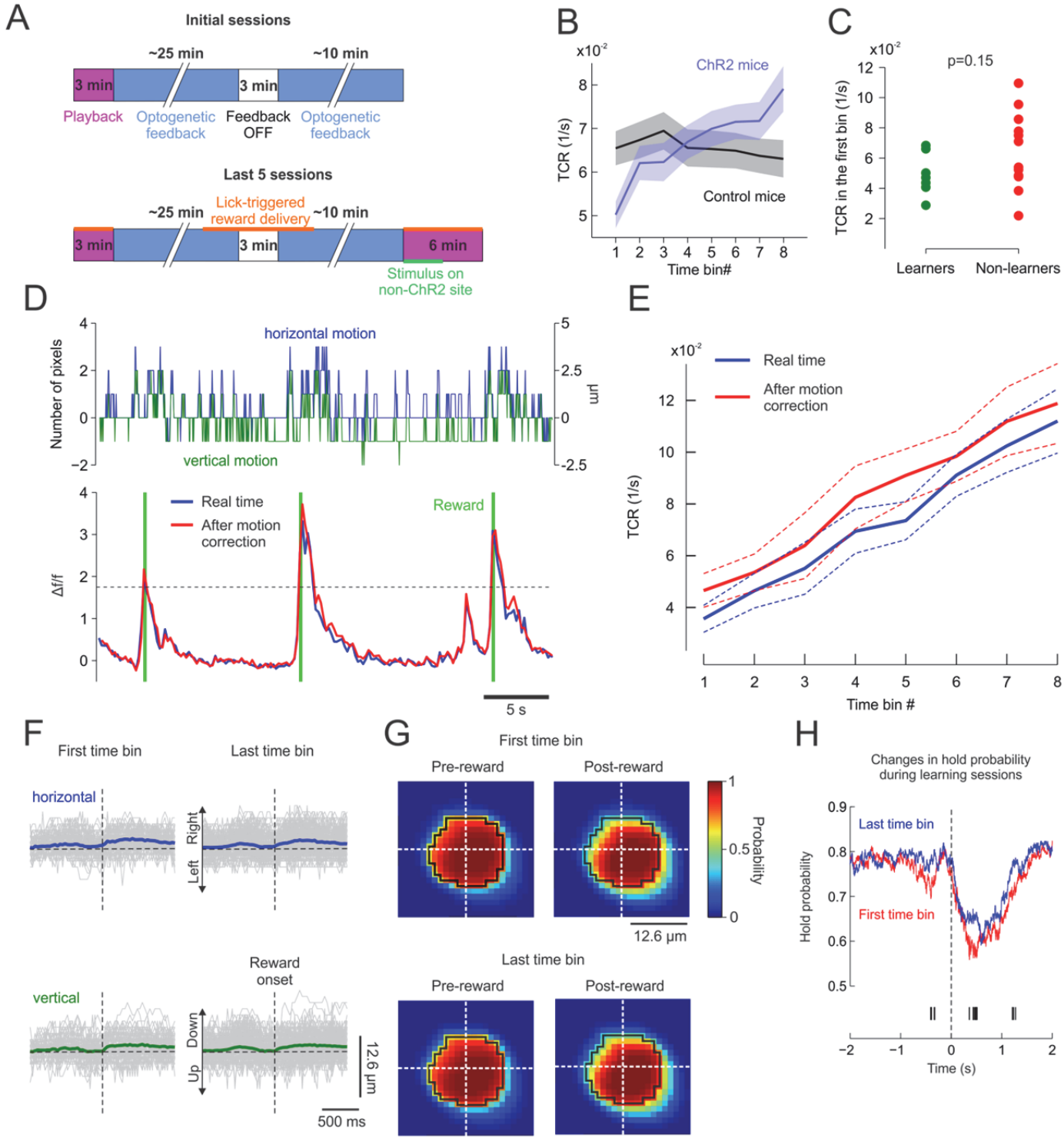

Figure S2. Related to Figure 2. Effects of initial levels of reward reinforcement, lateral image displacements and forepaw movements.

(A) Experiment timeline.
(B) Non-normalized population mean (± s.e.m.) TCRs of ChR2 (8 mice, 127 sessions) and control (11 mice, 158 sessions) animals. Because controls lacked optogenetic feedback a wider range of baseline conditioned activity levels (i.e. TCR in the first time bin) was explored in that group to test for the effects of initial reward reinforcement on learning. Control mice therefore on average started a session with slightly higher reward levels.
(C) No significant effect of baseline TCR was however observed as it did not differ between learners (n=7) and non-learners (n=12) (two-tailed non-paired t-test, p=0.15, t(17)=1.513).
(D) Example activity trace of a conditioned neuron (bottom) extracted from the two-photon images in real-time, and after offline correction for the measured vertical and horizontal image motion artefacts (top).
(E) Mean (± s.e.m.) TCR for the 33 learning sessions in ChR2 mice recorded during conditioning in real-time and simulated offline with the motion corrected images. Higher reward rates obtained after motion correction indicate that image movements were detrimental and not assistive to threshold crossings.
(F) Individual (grey) and mean horizontal and vertical image motion (green and blue) aligned to reward onset for all threshold crossings across the 33 learning sessions in the first and last time bins. Both horizontal and vertical motion were not significantly different between the last and first time bins in the 1 s interval both before and after reward onset (t-test, p>0.05). Motion artefacts were therefore not a contributing factor to reward rate increases during learning.
(G) Average conditioned neuron contour (black) and probability of any image pixel to be included in that contour computed for all threshold crossings across the 33 learning sessions in a 1 s period before and after reward delivery. Image motions preferentially occurred post-reward and did not overtly differ between the first and last time bins of conditioning.
(H) Hold probabilities, aligned to threshold crossings, in the first and last time bins across 33 learning sessions. Ticks delineate time points when the two probabilities were different (p<0.01, two-tailed bootstrap test). Because touch sensor releases preceding threshold crossings decreased over time, it is unlikely that learning was mediated by more frequent forepaw movements.

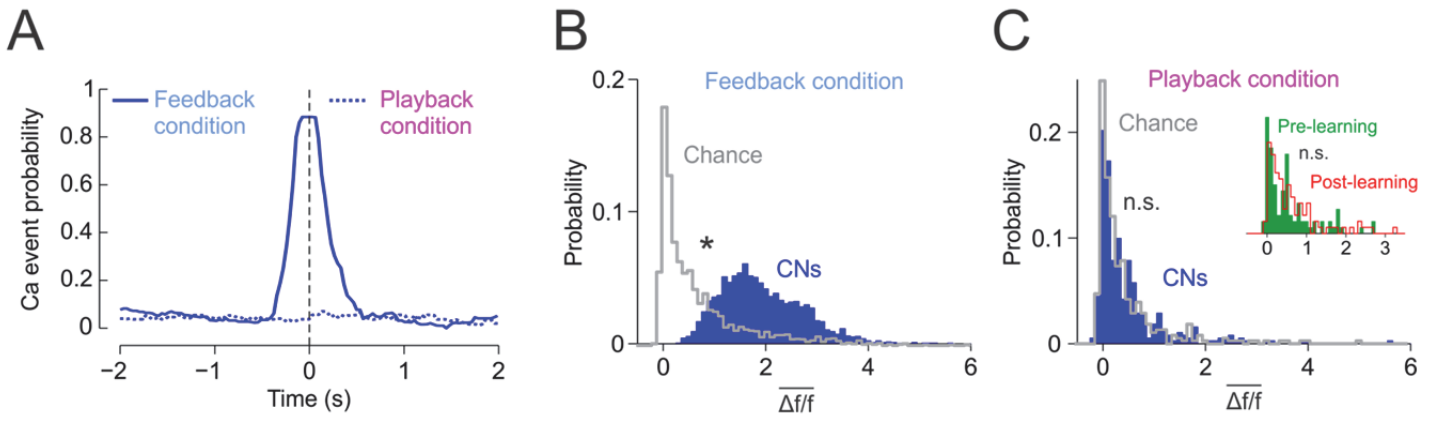

Figure S3. Related to Figure 2. S1 stimulation alone does not evoke activity in the conditioned M1 neurons.

(A) Ca event probability of CNs aligned to threshold crossings in the feedback and playback conditions of the same sessions. The direct effect of S1 activation on the conditioned M1 neurons can be measured at times of

threshold crossings in the playback condition. These instances provide maximal optogenetic stimulations not paired to the CN's activity, but in otherwise identical experimental settings.
(B) Distribution of mean $\Delta f/f_0$ values of CNs (n=2440 threshold crossings in 33 learning sessions) in the -0.5 s and 0.5 s relative to threshold crossings compared to time-matched baseline periods (chance) in the feedback condition. *: p≈0, k-s=0.634, two-sample Kolmogorov-Smirnov goodness-of-fit test.
(C) Same data as in (B) for the playback condition (n=382 threshold crossings), n.s.: p=0.13, k-s=0.0838. Inset: pre- and post-learning distributions of the same data in 11 learning sessions for which the playback condition was tested both at the start (i.e. pre-learning: n=96 threshold crossings) and end (post-learning: n=97 threshold crossings) of the session and thus involving data from the same neuron, n.s.: p=0.87, k-s=0.0842.

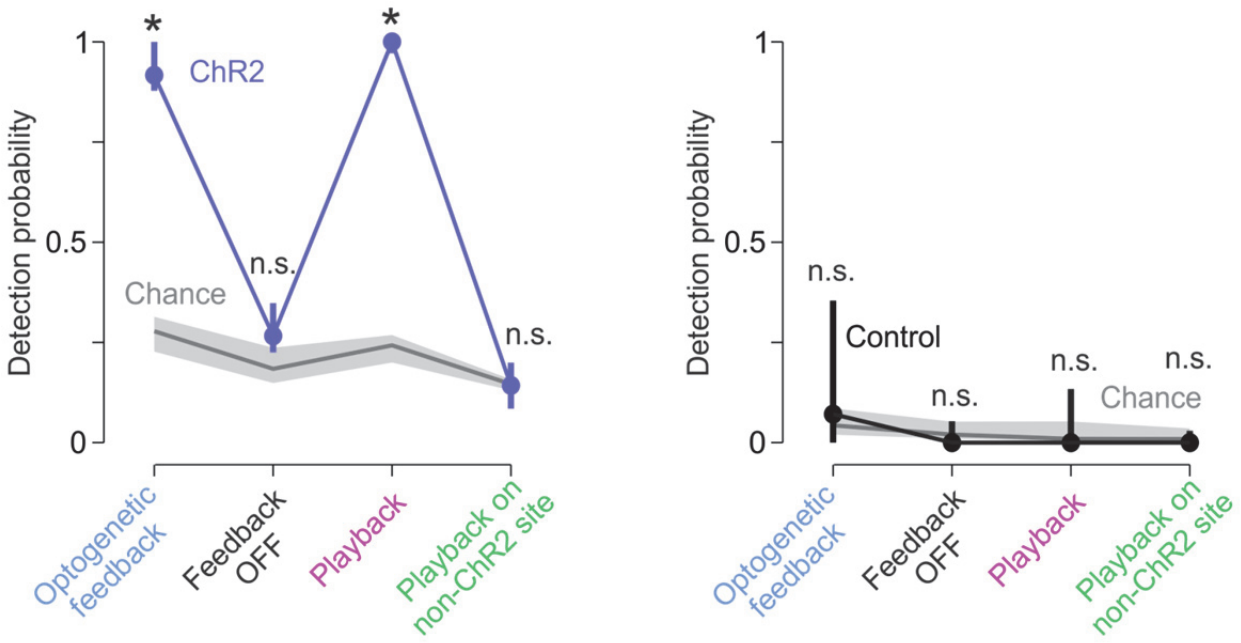

Figure S4. Related to Figure 2. Comparison of detection probability to simulated chance levels.

Same data as in Figure 2E compared to simulated chance levels (see Methods for details). *: $p<10^{-11}$, n.s.: p>0.05, Kruskal-Wallis test.

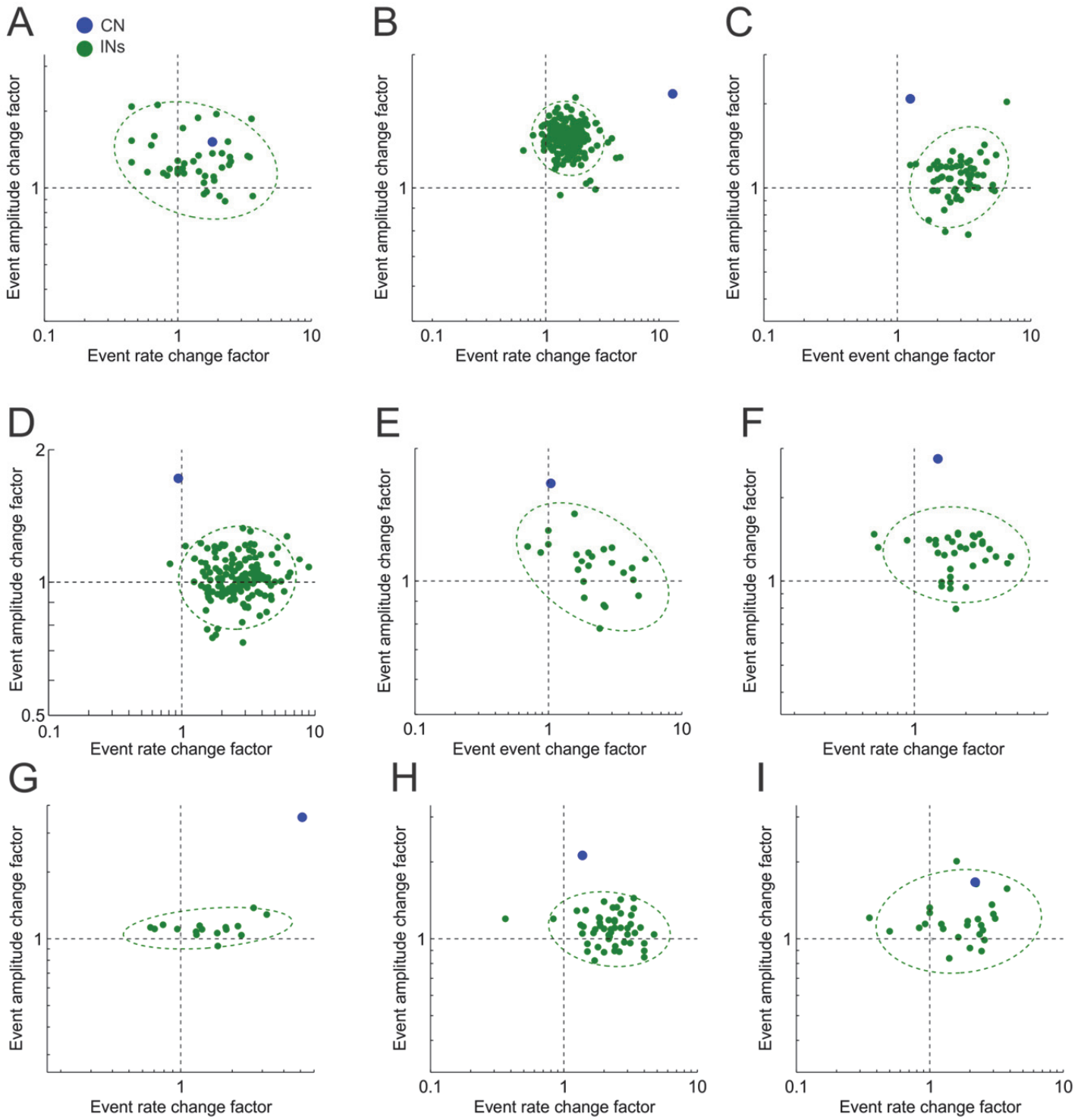

Figure S5. Related to Figure 3. Activity changes of CNs and INs during learning.

(A-I) Event rate and event amplitude changes between the first and last time bins of CNs and INs in 9 example learning sessions. Dotted contours are 95% confidence ellipses.

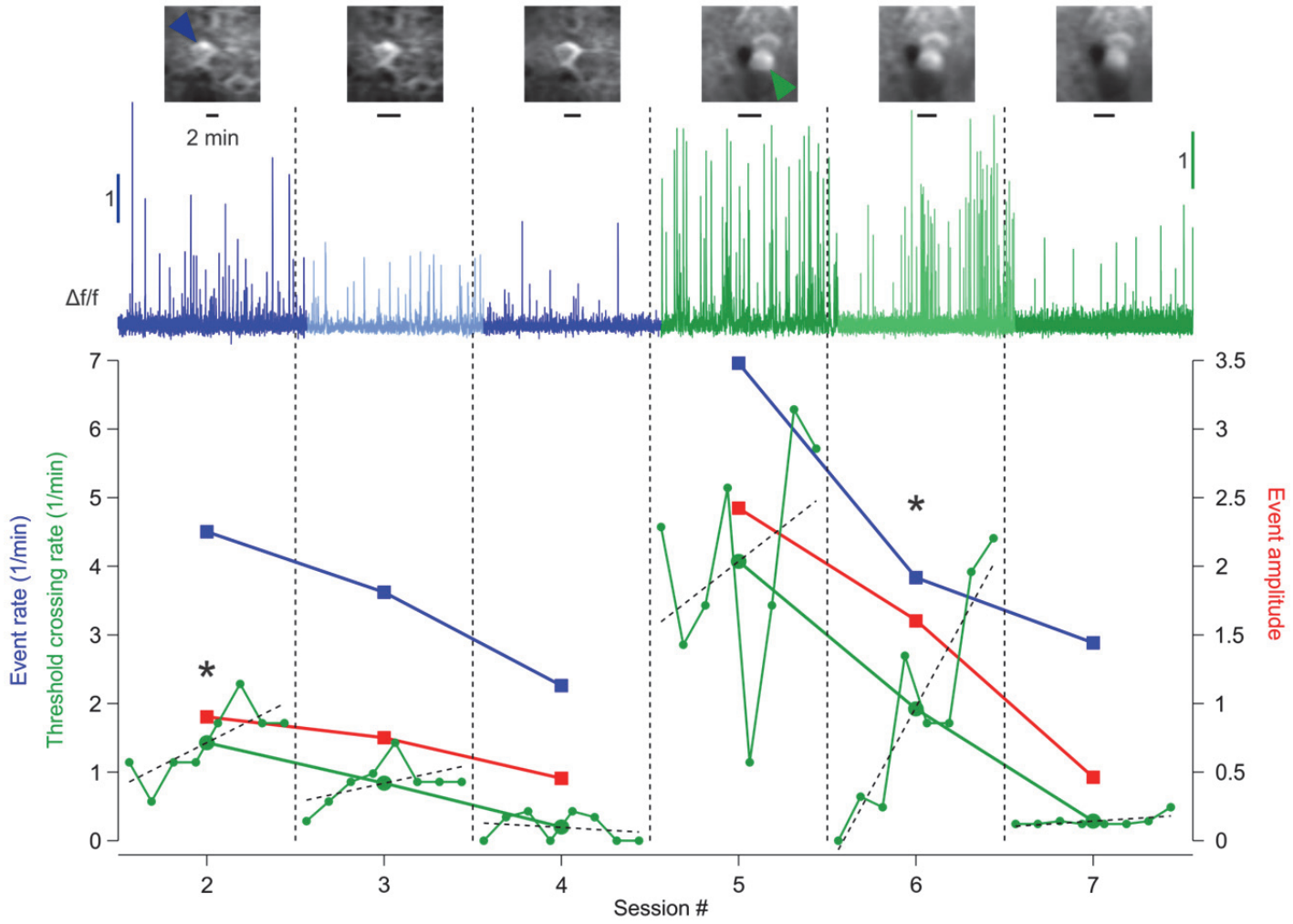

Figure S6. Related to Figure 4. "Dropping-out" of conditioned neurons.

Two example neurons conditioned under optogenetic feedback in the same animal over 4 and 3 consecutive days, respectively. Within session changes in threshold crossing rate (small green circles) and their linear fits (dashed black lines) are shown for sessions #2 through #7. Cross session averages of reward rate (big green cricles), event rate (blue squares) and event amplitude (red squares) together with the calcium dependent activity traces (top) depict the flexible day to day activity of the conditioned neurons. Insets are session average two-photon images zoomed in on the conditioned neurons (arrowheads). *: learning session. The mouse learned to increase TCR on the second day of training, still attempted to do the same on the third day, albeit less successfully, after which point the conditioned neuron became inactive preventing further learning to take place. We were then obliged to switch to a different, active neuron, on the following training day where a non-significant increase in TCR was observed. The second day of conditioning, significant learning occurred throughout the session. Once again, the neuron became relatively inactive thereafter.

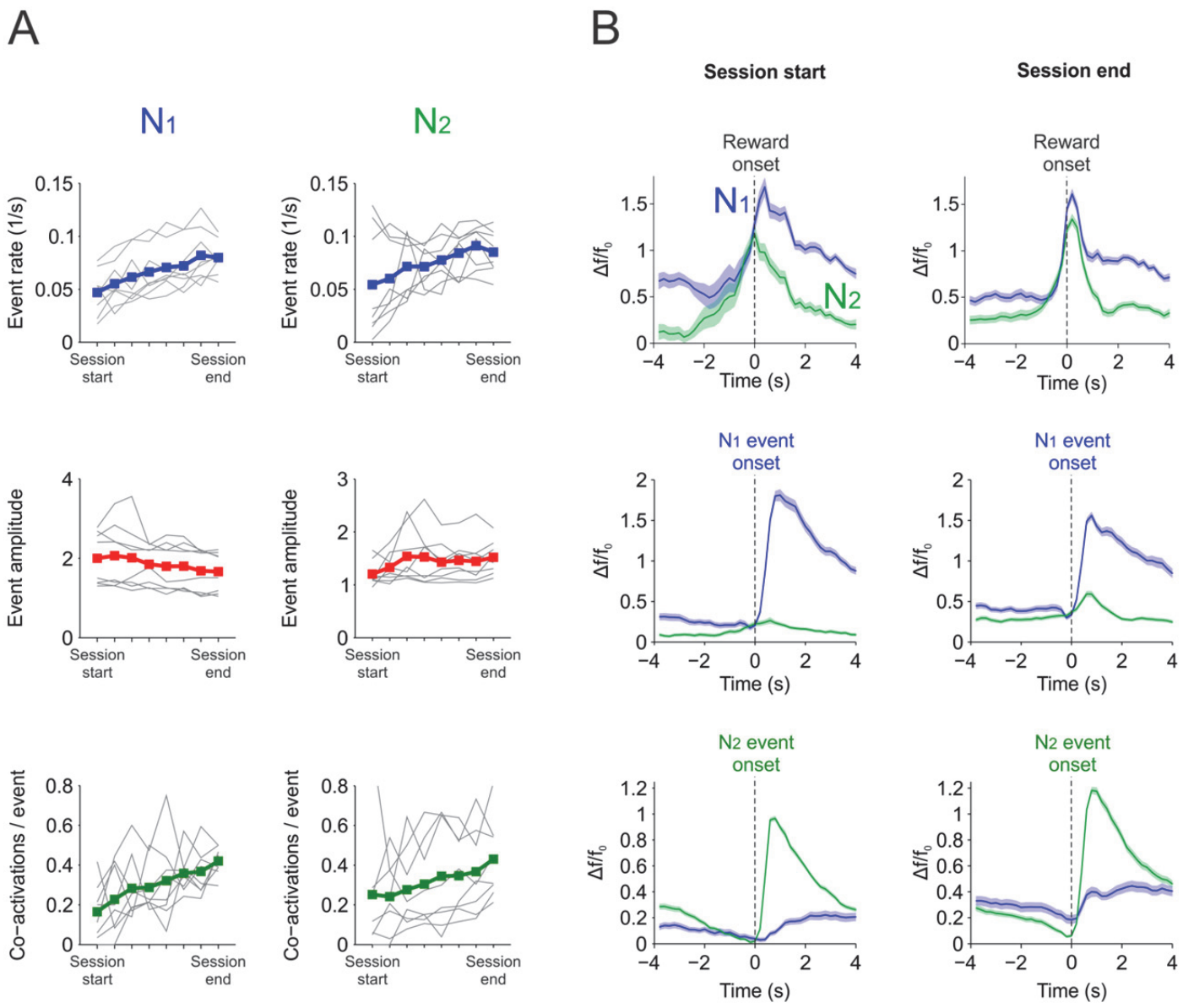

Figure S7. Related to Figure 5. Increased co-activation of neuron 1 (N_1) and neuron 2 (N2) guides learning during multi-neuron conditioning.

(A) Within session changes of average Ca event rate (blue), event amplitude (red) and number of co-activations per Ca event (green) for N_1 (left panels) and N_2 (right panels) during the multi-neuron conditioning experiment (n=8 sessions, 2 mice). Gray lines depict data from individual sessions. Significant increases in event rates (N_1: p=0.0046, N_2: p=0.25, Kruskal-Wallis test) and amplitudes (N_1: p=0.21, N_2: p=0.02, Kruskal-Wallis test) between session start and end were observed, but also in the co-activations/event ratio (N_1: p=0.0033, N_2: p=0.035).
(B) Comparison of mean (± s.e.m.) N_1 and N_2 activity between session start (left panels) and session end (right panels) aligned on reward onset (top panels), all N_1 event onsets (middle panels) and all N_2 event onsets (lower panels).

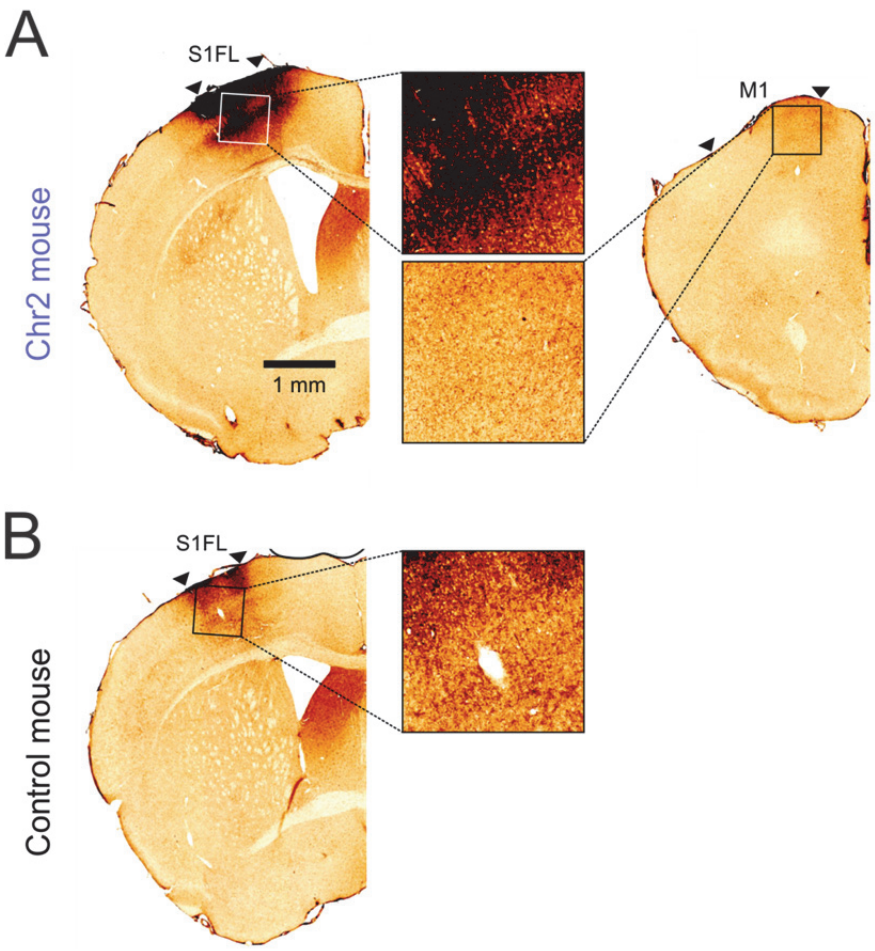

Figure S8. Related to STAR Methods. Histological verification of ChR2 expression in representative ChR2 and homozygous control mice. Coronal sections immunostained against ChR2 (DAB reaction).

(A) ChR2 expression in a ChR2 mouse at the injection site (S1FL) corresponding to the Cre-virus dilution 1:1000 and at the imaged M1 site.
(B) Reduced ChR2 expression in S1FL in a homozygous control mouse that received a Cre-virus dilution 1:1000.

Movie S1. Related to Figure 5. Multi-neuron conditioning.

The head-restrained mouse under the two-photon microscope was required to coactivate two neurons (green and blue) while keeping a third one inactive (red) to bring the ensemble activity (i.e. the rate of the optogenetic stimulus) above reward threshold. The calcium fluorescence of each neuron controlled the joint angle of a robotic arm and the arm's distance to target was proportional to the feedback rate. The blue light mask pulses (1.5 ms) as well as the optogenetic stimulus pulses (5 ms) were undersampled by the mouse video acquisition rate (120 Hz). The undersampled light flashes apparent in the mouse video emanate from the blue light mask. In reality, the mask produced a perceptually stable illumination of the setup.