

Supplementary Information for

Inhibition of impulsive action by projection-defined prefrontal pyramidal neurons

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Figs. S1 to S7

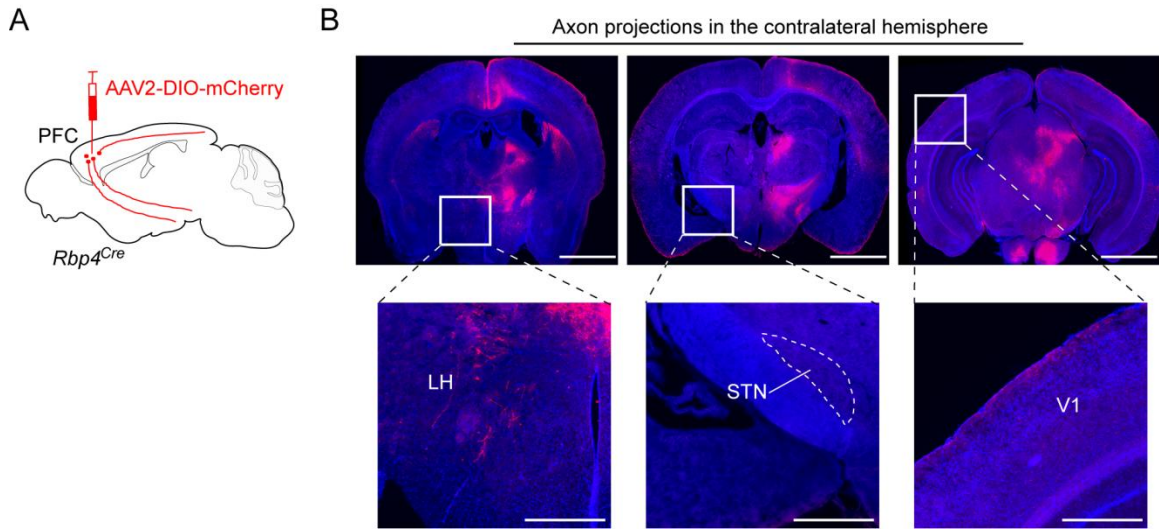


Fig. S1 (related to Fig. 1)

dmPFC layer 5 neurons project to contralateral V1 and LH but not STN. (A) Virus injection for tracing axonal projections from dmPFC layer 5 neurons, same as Fig. 1A. (B) Fluorescence images of axonal projections in the contralateral hemisphere. Bottom row shows enlarged view of the regions in white boxes (top row). Red, mCherry; blue, DAPI. Top row, scale bar, 2 mm. Bottom row, scale bar, 500 μ m.

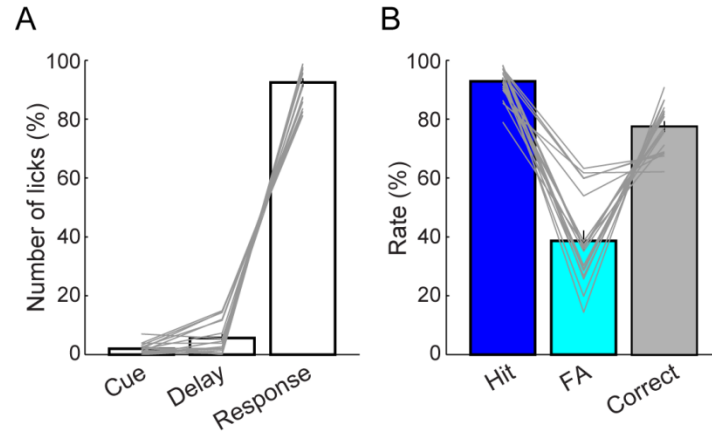


Fig. S2 (related to Fig. 2)

Performance of Go/No-Go task after training. (A) Percentages of licks during cue, delay and response periods. Each line represents one mouse (n = 18 mice). Bar, population average. (B) Performance quantified by Hit, FA, and Correct rate. Each line represents one mouse (n = 18 mice). All error bars indicate \pm SEM.

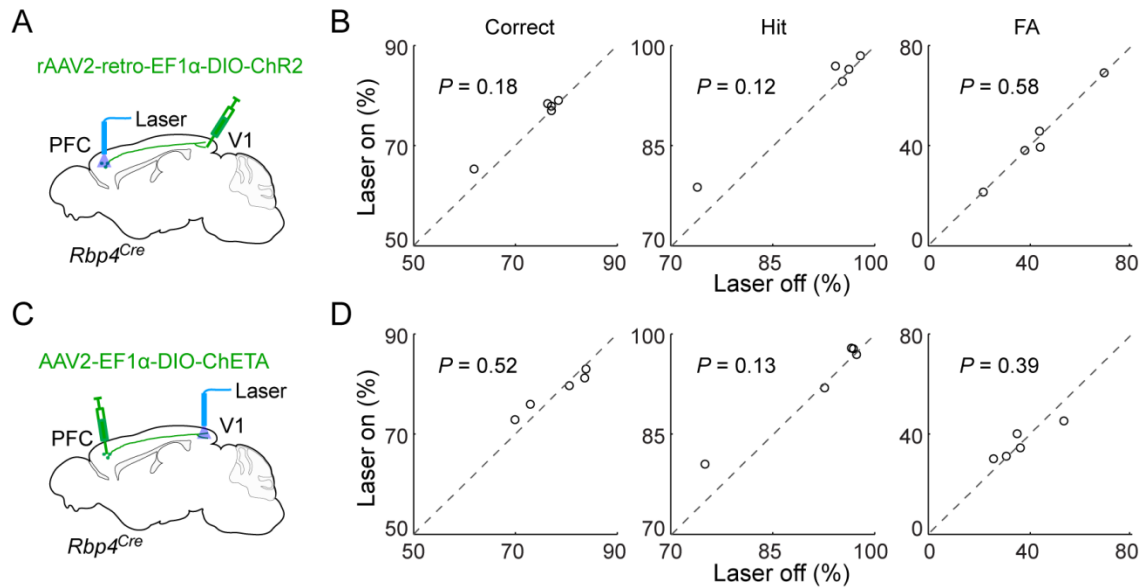


Fig. S3 (related to Figs. 5 and 6)

Activating PFC \rightarrow V1 neurons or PFC \rightarrow V1 axons has no significant effects on performance.

(A) Schematic of optogenetic activation of PFC \rightarrow V1 neurons. (B) Activation of PFC \rightarrow V1 neurons (10 Hz) has no significant effects on task performance (Correct rate: $P = 0.18$, Hit rate: $P = 0.12$, FA rate: $P = 0.58$, bootstrap). Each circle represents one mouse ($n = 5$ mice).

(C) Schematic of optogenetic activation of dmPFC axon terminals in V1. (D) Activation of PFC \rightarrow V1 axons (10 Hz) has no significant effects (Correct rate: $P = 0.52$, Hit rate: $P = 0.13$, FA rate: $P = 0.39$, $n = 5$ mice).

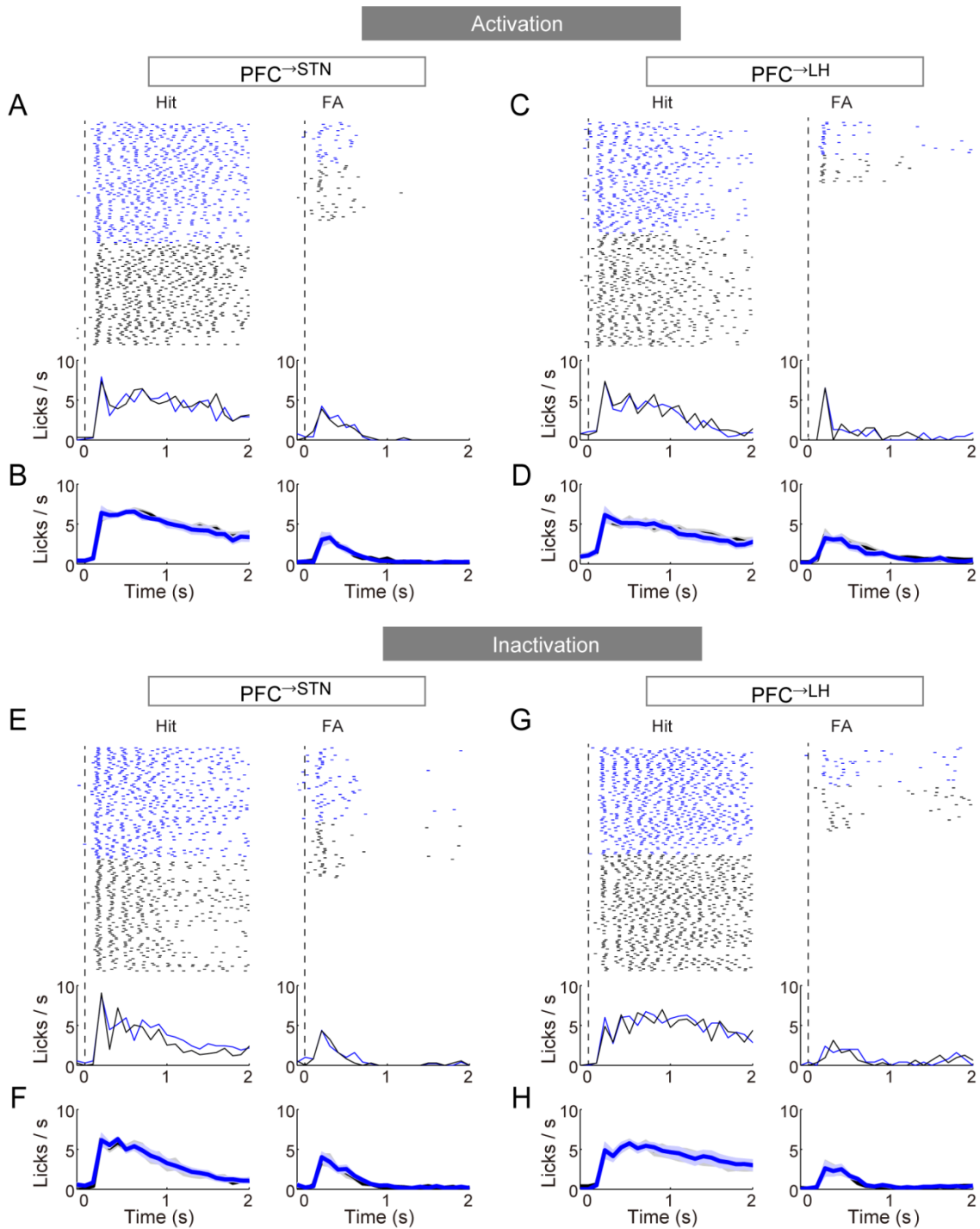


Fig. S4 (related to Fig. 5)

Optogenetic manipulation of $\text{PFC}^{\rightarrow\text{STN}}$ or $\text{PFC}^{\rightarrow\text{LH}}$ neurons has no significant effect on the licking movement. (A) An example session with $\text{PFC}^{\rightarrow\text{STN}}$ neuron activation. Left, Hit trials; right, FA trials. Blue, laser on trials; black, laser off trials. Each tick indicates one lick. Vertical dashed line indicates the start of response window. (B) Population average

of lick rate with (blue) or without (gray) activation of PFC^{→STN} neurons (n = 6 mice). (C-D) Similar to (A-B) but for PFC^{→LH} neuron activation (n = 9 mice). (E-F) Similar to (A-B) but for PFC^{→STN} neuron inactivation (n = 6 mice). (G-H) Similar to (E-F) but for PFC^{→LH} neuron inactivation (n = 7 mice).

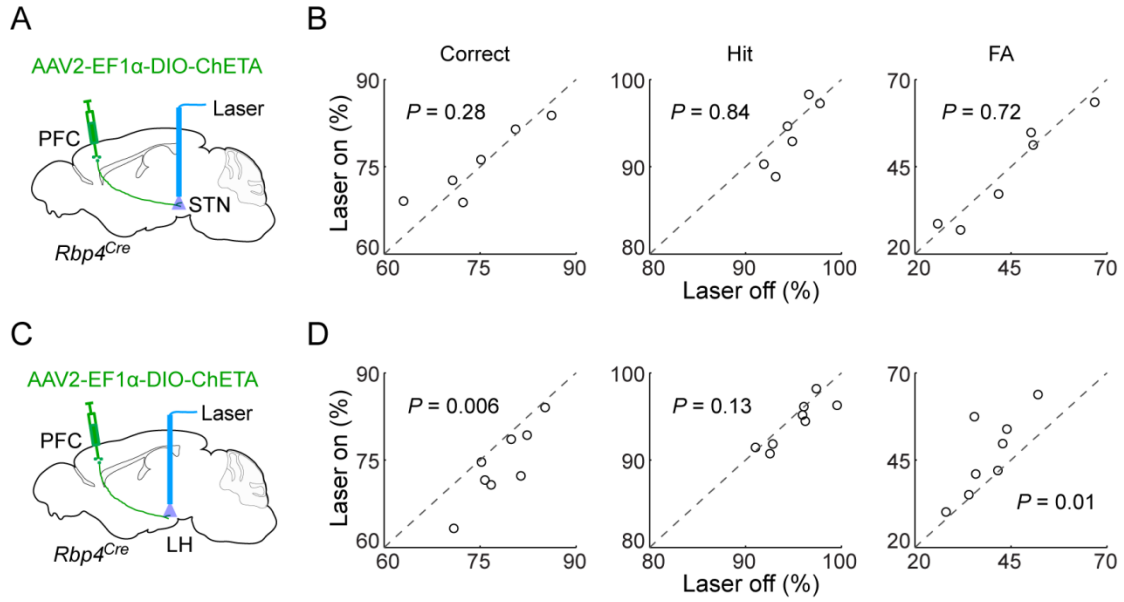


Fig. S5 (related to Fig. 6)

Effect of activating PFC \rightarrow STN axons or PFC \rightarrow LH axons. (A) Schematic of optogenetic activation of dmPFC axon terminals in the STN. (B) Activation of dmPFC axon terminals in the STN at 10 Hz has no significant effects on task performance (Correct rate: $P = 0.28$, Hit rate: $P = 0.84$, FA rate: $P = 0.72$, bootstrap, $n = 6$ mice). (C) Schematic of optogenetic activation of dmPFC axon terminals in the LH. (D) Activation of dmPFC axon terminals in the LH at 10 Hz caused a significant behavioral impairment due to a strong increase in FA rate (Correct rate: $P = 0.006$, Hit rate: $P = 0.13$, FA rate: $P = 0.01$, $n = 8$ mice).

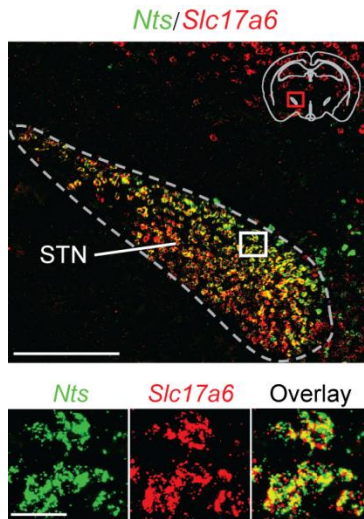


Fig. S6 (related to Fig. 6)

STN glutamatergic neurons express *Nts*. Top, fluorescence image showing double FISH of *Nts* and *Slc17a6* in the STN (red box in the coronal diagram). Scale bar, 500 μm .

Bottom, enlarged view of the region in white box (top). *Slc17a6*, gene encoding vesicular glutamate transporter 2. Scale bar, 20 μm .

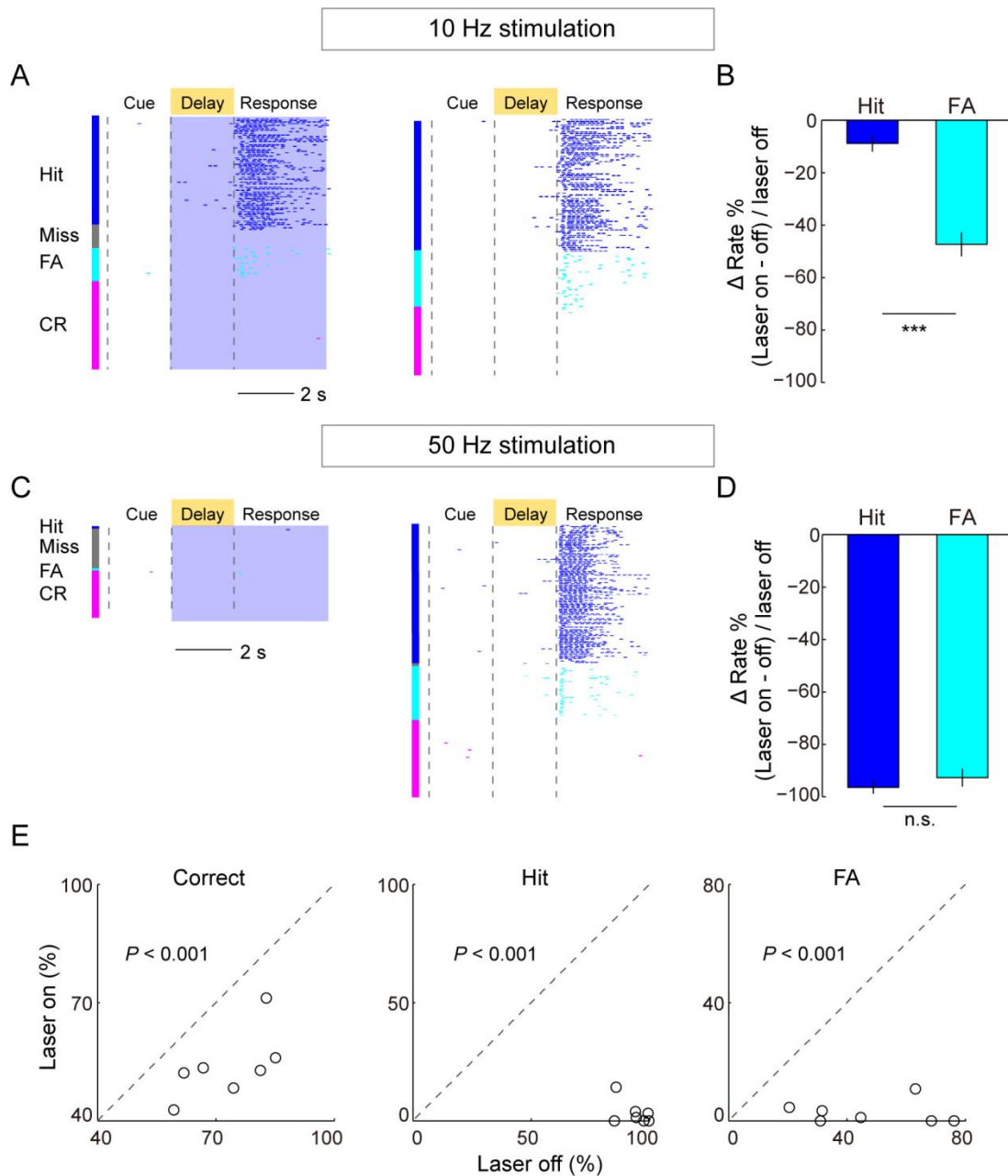


Fig. S7 (related to Fig. 6)

Effects of activating STN neurons at different frequencies. (A) An example session with STN activation at 10 Hz. Left, laser on trials; right, laser off trials. Blue shading, laser stimulation. Each tick indicates one lick. (B) STN activation at 10 Hz caused a much larger reduction in FA rate than in Hit rate ($P < 0.001$, bootstrap, $n = 7$ mice). (C-D) Similar to (A-B) for STN activation at 50 Hz, which caused similar reduction in Hit and FA rates ($P = 0.28$, $n = 7$ mice). n.s., not significant. (E) Activation of STN at 50 Hz

severely impaired task performance (Correct, Hit and FA rates: all $P < 0.001$, $n = 7$ mice).
All error bars indicate \pm SEM.