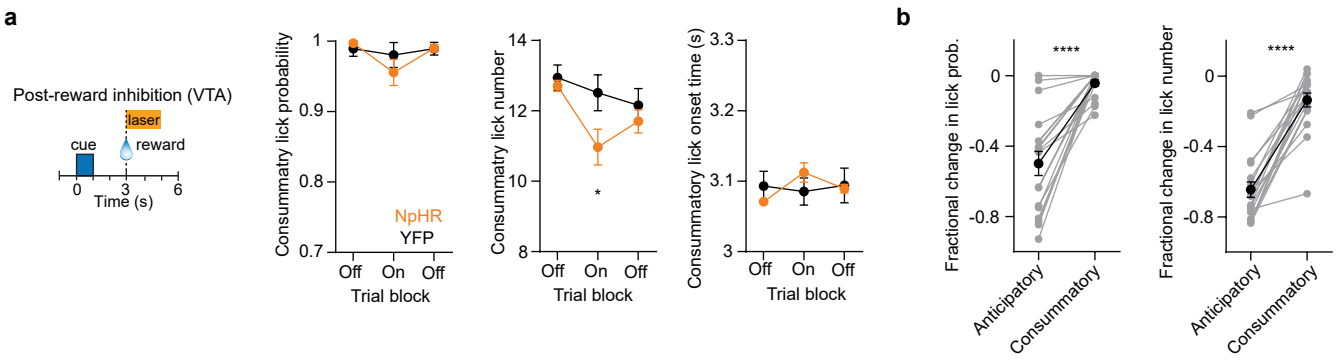
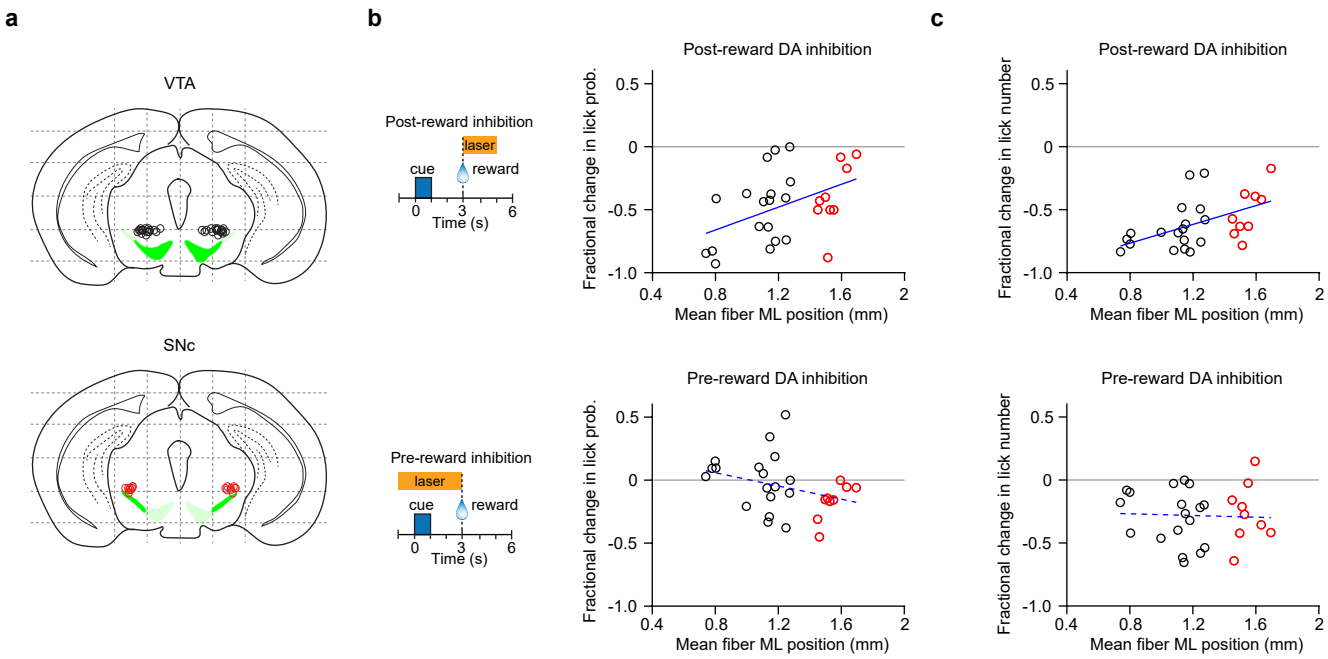


Supplementary Figure 1.

Effect of post-reward VTA DA neuron inhibition on reward consumption.

a. (Far left) Trial structure of Pavlovian conditioning task with post-reward VTA DA inhibition. (Left center) Reward consummatory licking probability in each of the three trial blocks (n = 18 eNpHR3.0$^+$ and 14 YFP$^+$ mice, two-way RM ANOVA, group effect: $F_{1,30}$ = 0.1, P = 0.71, trial block effect: $F_{2,60}$ = 5.6, P = 0.006). (Right center) Consummatory lick number in each of the three trial blocks (two-way RM ANOVA, group effect: $F_{1,30}$ = 2.3, P = 0.14, trial block effect: $F_{2,60}$ = 10.6, P = 0.0001). Post-hoc Sidak's test: *P = 0.02. (Far right) Consummatory lick onset time in each of the three trial blocks (two-way RM ANOVA, group effect: $F_{1,30}$ < 0.0001, P = 0.99, trial block effect: $F_{2,60}$ = 2.6, P = 0.08). Data are expressed as mean ± SEM.

b. (Left) Fractional change in the probability of anticipatory versus consummatory licking during post-reward VTA DA inhibition (n = 18 eNpHR3.0$^+$ mice, two-sided paired t-test, t_{17} = 6.5, ****P < 0.0001). (Right) Fractional change in the number of anticipatory versus consummatory licks (two-sided paired t-test, t_{17} = 9.7, ****P < 0.0001). Darker shaded symbols represent mean ± SEM.



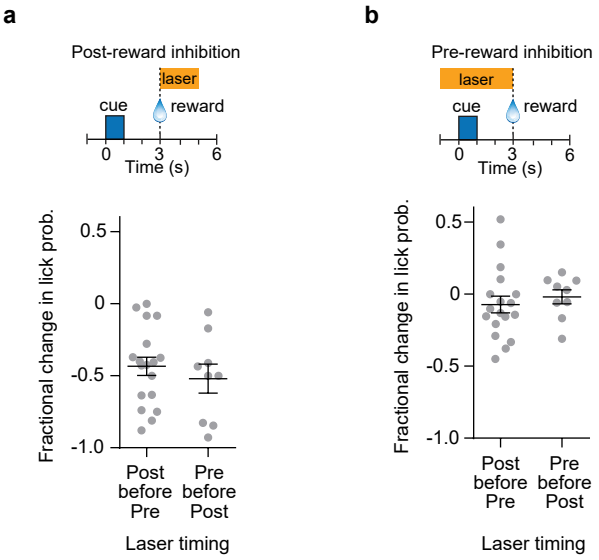
Supplementary Figure 2.

Optical fiber position in the VTA/SNc and correlation with behavioral changes.

a. Histologically determined optical fiber tracks for the 18 eNpHR3.0$^+$ mice targeting the VTA (top, black open circles), and 9 eNpHR3.0$^+$ mice targeting the SNc (bottom, red open circles) for behavioral experiments in Fig. 1. Grid lines are spaced 1 mm apart.

b. Change in anticipatory lick probability versus mean value of optical fiber ML position. The mean value of the position was calculated as the average of the absolute value of the left and right fiber ML position. Data from VTA (black circles) and SNc (red circles) experiments were combined (total of 27 eNpHR3.0$^+$ mice). (Top) Fractional change in lick probability during post-reward inhibition (Pearson R = 0.44, *P = 0.02). (Bottom) Fractional change in lick probability during pre-reward inhibition (Pearson R = -0.33, P = 0.1). Blue line represents best line fit to the data.

c. Change in anticipatory lick number versus mean value of optical fiber ML position. Data from VTA (black circles) and SNc (red circles) experiments were combined (total of 27 eNpHR3.0$^+$ mice). (Top) Fractional change in lick number during post-reward inhibition (Pearson R = 0.52, P = 0.006). (Bottom) Fractional change in lick number during pre-reward inhibition (Pearson R = -0.04, P = 0.84).

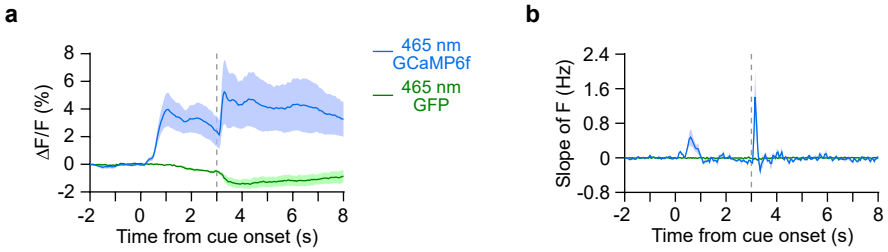


Supplementary Figure 3.

Optogenetic inhibition effects do not depend on the order of pre- and post-reward test sessions.

a. (Top) Schematic of the post-reward inhibition experiment. (Bottom) Fractional change in anticipatory lick probability for post-reward sessions that were administered before or after pre-reward. There is no significant difference (n = 18 post before pre, 9 pre before post mice, experiments combined from VTA and SNc eNpHR3.0^{+} mice in Fig. 1, two-sided unpaired t-test, t_{25} = 0.7, P = 0.46).

b. (Top) Schematic of the pre-reward inhibition experiment. (Bottom) Fractional change in anticipatory lick probability for pre-reward sessions that were administered before or after pre-reward. There is no significant difference (n = 18 post before pre, 9 pre before post mice, experiments combined from VTA and SNc eNpHR3.0^{+} mice in Fig. 1, two-sided unpaired t-test, t_{25} = 0.6, P = 0.55). Data are expressed as mean ± SEM.

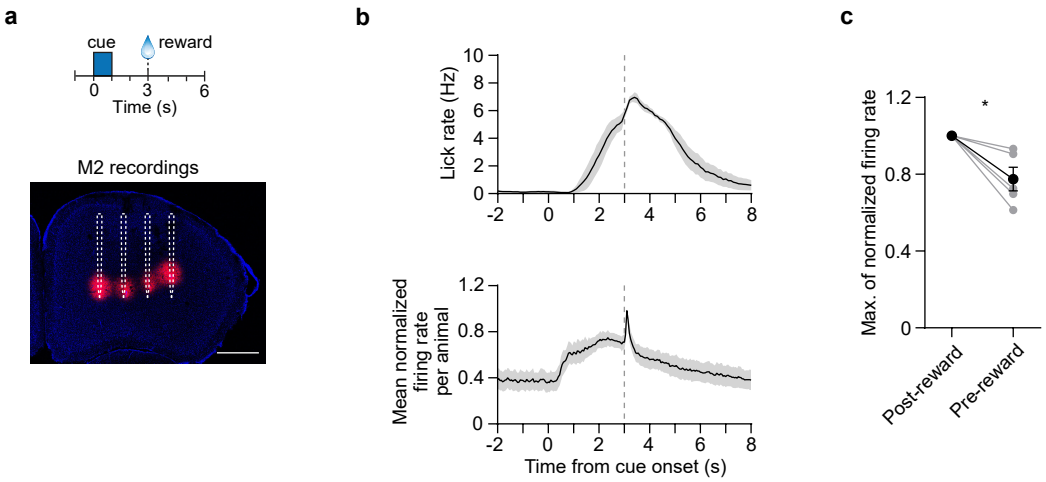


Supplementary Figure 4.

Comparison of photometry signals from GCaMP6f and GFP expressing mice.

a. Mean fractional change in photometry signal as a function of time. Blue lines represent data collected from GCaMP6f expressing DAT-Cre mice (n = 6, data also shown in Fig. 3), and green lines represent data collected from GFP expressing mice (n = 3). The excitation wavelength was the same for both groups (465 nm).

b. Mean slope of the photometry signal as a function of time. Data are expressed as mean ± SEM.



Supplementary Figure 5.

Electrophysiological recordings of M2 activity during behavior.

a. (Top) Trial structure of the Pavlovian reward conditioning task used during the recordings. (Bottom) Histologically reconstructed position of a four shaft, 256 electrode silicon microprobe in M2. The red signal is fluorescence from a dye coated on the probe. Dashed white lines represent the approximate area sampled by the electrodes.

b. (Top) Mean lick rate as a function of time (n = 5 C57Bl/6J mice). (Bottom) Mean normalized firing rate per animal as a function of time.

c. Maximum value of the mean normalized firing rate per animal in the pre-reward (0 – 3 s) and post-reward (3 -5 s) period (n = 5 mice, two-sided paired t-test, $t_4 = 3.6$, $^*P = 0.02$). Data are expressed as mean ± SEM.