Supplementary Figures

A Bottom-up Reward Pathway Mediated by Somatostatin Neurons in the Medial Septum Complex Underlying Appetitive Learning

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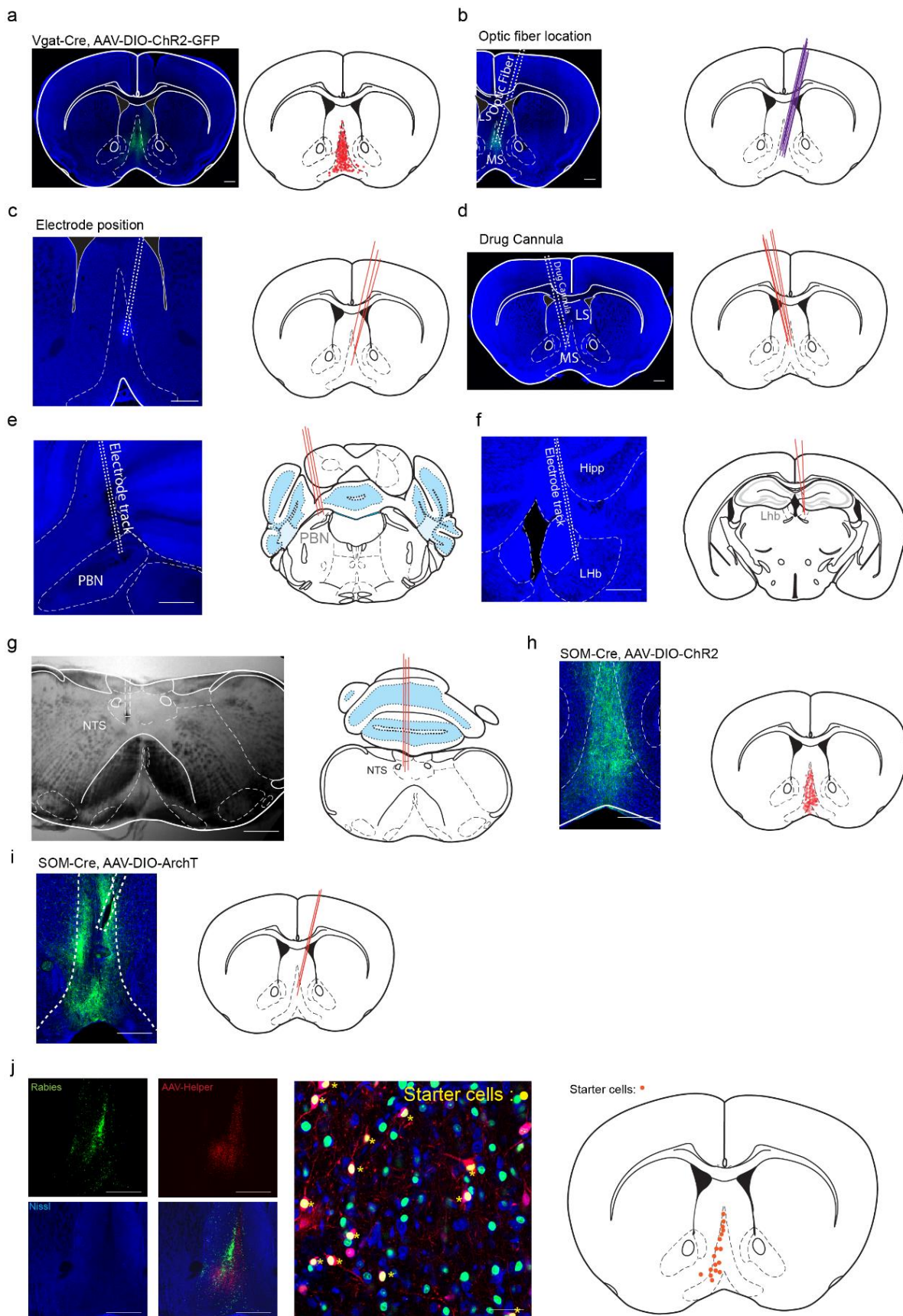
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a Vgat-Cre, AAV-DIO-ChR2-GFP

b Optic fiber location

Optic Fiber
LS
MS

c Electrode position

d Drug Cannula

Drug Cannula
LS
MS

e Electrode track
PBN
PBN

f Electrode track
Hipp
LHb
Lhb

g NTS
NTS

h SOM-Cre, AAV-DIO-ChR2

i SOM-Cre, AAV-DIO-ArchT

j Rabies   AAV-Helper

Nissl

Starter cells

Starter cells:

Supplementary Figure 1 | Post hoc histological verifications of viral expression and placements of electrodes and optic fibers.

a, An example coronal section showing the expression of ChR2-EYFP in a Vgat-Cre mouse. Right, superimposed ChR2-EYFP expressing cell locations across animals. Each small dot represents one cell (n = 7 mice). **b**, An example coronal section showing the track of the optic fiber implanted a Vgat-Cre mouse expressing ChR2-EYFP. Right, superimposed tracks of optic fibers across experiments (n = 7 mice). **c**, An example coronal section showing the track of the optrode electrode in MS. Right, superimposed tracks of electrodes (n = 3 mice). Each line represents one track. **d**, An example coronal section showing the track of drug cannula in MS. Right, superimposed tracks of cannulas (n = 5 mice). **e**, An example coronal section showing the track of the electrode in PBN. Right, superimposed tracks of electrodes (n = 3 mice). **f**, An example coronal section showing the track of electrode in LHb. Right, superimposed tracks of electrodes (n = 2 mice). **g**, An example bright field image showing the track of electrode in NTS. Right, superimposed tracks of electrodes (n = 3 mice). **h**, An example coronal section showing the expression of ChR2-EYFP in a SOM-Cre mouse. Right, superimposed ChR2-EYFP expressing cell locations (n = 3 mice). **i**, An example coronal section showing the track of optic fiber implanted in a SOM-Cre mouse with ChR2-EYFP expression in MS. Right, superimposed tracks of electrodes (n = 3 mice). **j**, Images of pseudo-yped rabies virus expression (n= 3 mice). Yellow stars indicate starter cells which were transduced by both AAV-helper and pseudo-typed rabies virus. Right, superimposed starter cell locations. Scale bar: 500 μm in all panels.
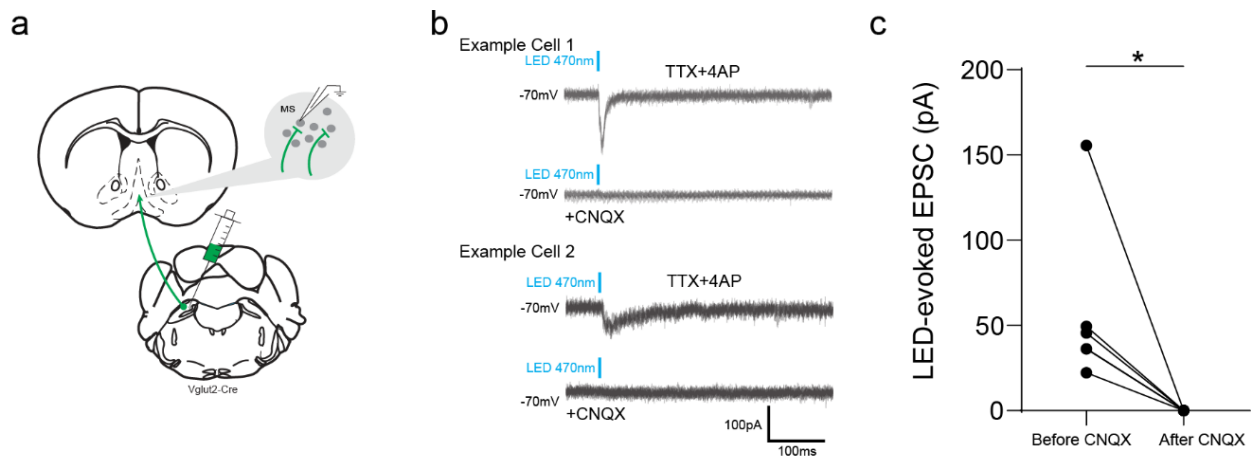
Supplementary Figure 2 | AMPA-receptor mediated synaptic currents of the excitatory PBN-MS projection.

a, Illustration of the experimental scheme. AAV-DIO-ChR2 was injected into PBN while in vitro brain slice recording was performed in MS. **b**, Two example neurons exhibited light-evoked inward synaptic currents recorded at -70mV in the presence of TTX and 4AP. Perfusion of CNQX completely blocked the currents. **c**, Amplitudes of light-evoked synaptic currents before and after CNQX application (n = 5). *, p = 0.0002, ratio paired t test, two-sided. Source data are provided as a Source Data file.
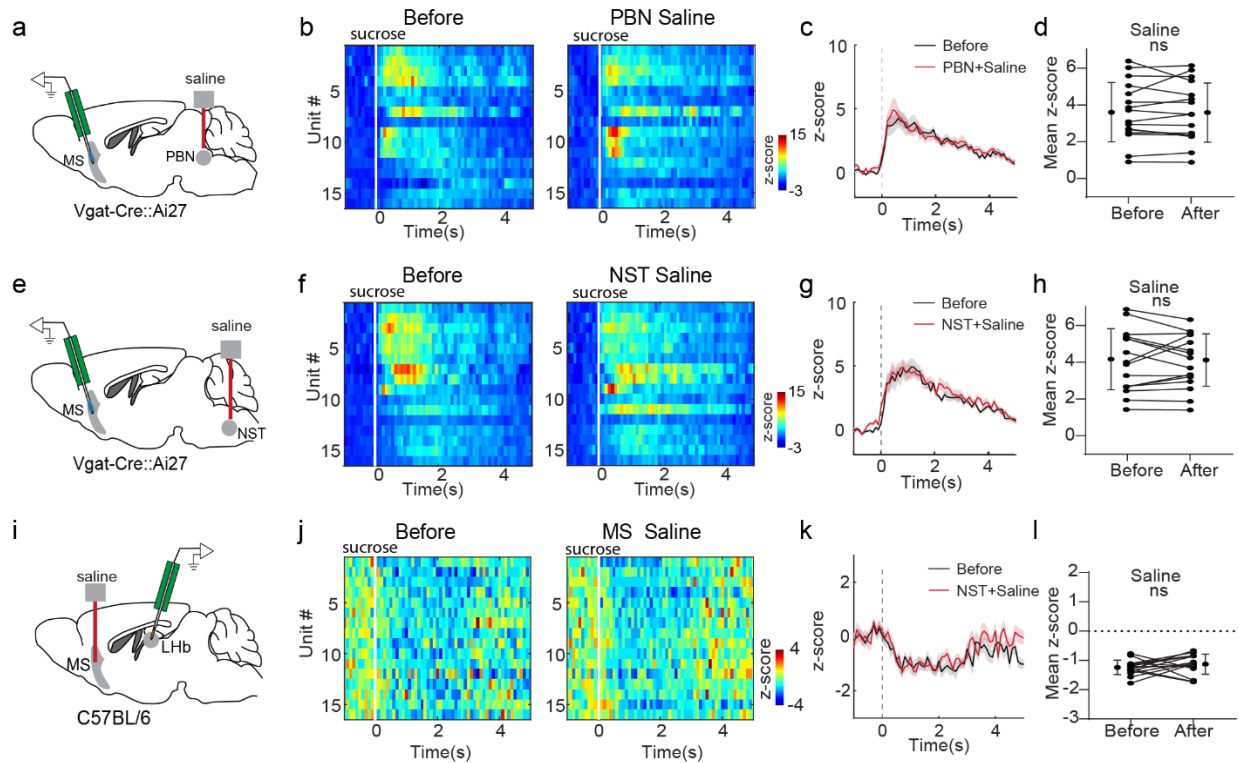
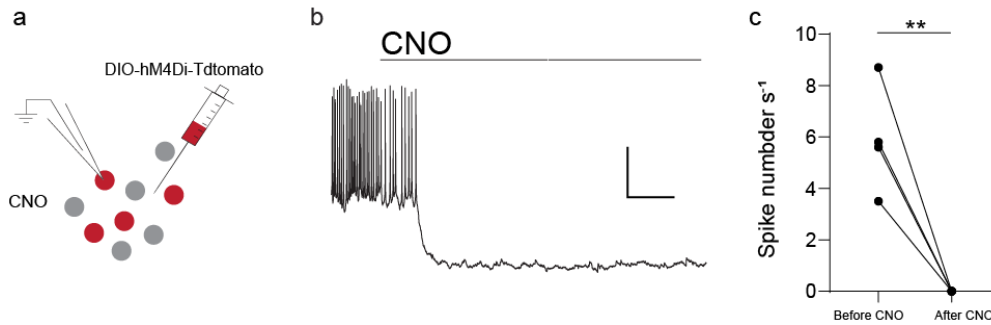**Supplementary Figure 3 | Control experiments for silencing PBN, NST and MS.**

a, Optrode recording from MS GABAergic neurons while infusing PBN with saline vehicle for muscimol. **b**, Heatmap plot of Z-score for sucrose responses of MS GABAergic neurons (n = 16) before and after infusion of saline in PBN. **c**, Population average. All shades indicate s.e.m. **d**, Mean Z-score before and after infusing saline in PBN. Data points for the same neuron are connected with a line. "ns", not significant, p = 0.6147, paired t test, two-sided. **e-h**, Infusing saline in NST. "ns", not significant, p = 0.5867, paired t test, two-sided. **i-l**, Infusing saline in MS and recording from LHb neurons. "ns", not significant, p = 0.3455, paired t test, two-sided. Source data are provided as a Source Data file.
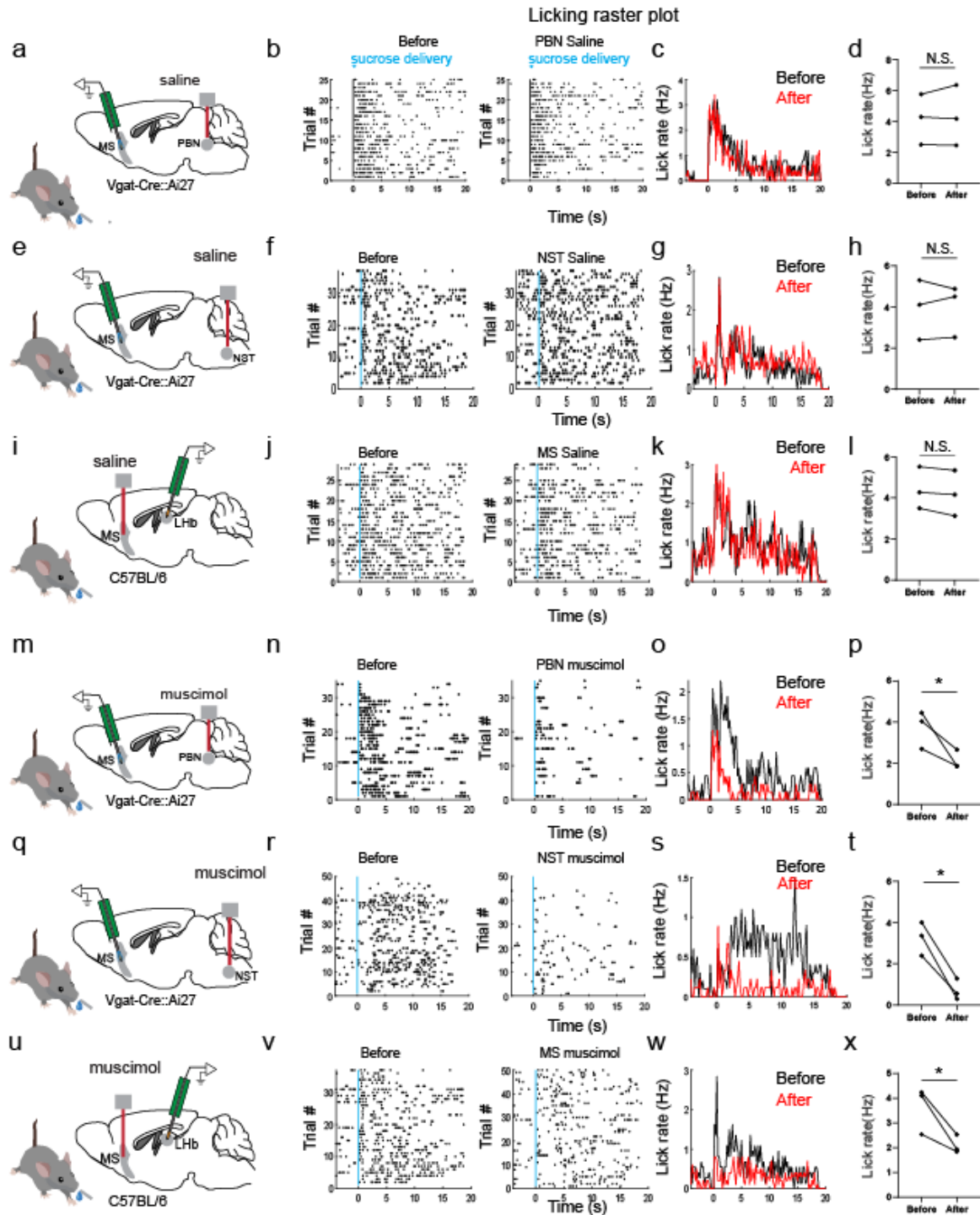
**Supplementary Figure 4 | Sucrose licking behavior in control and silencing experiments.**

a, Illustration of experimental scheme. Sucrose licking behavior was monitored when optrode recording was performed from MS GABAergic neurons. Saline was infused into PBN. **b**, Raster plot of licks to sucrose delivery before and after infusion of saline in PBN. **c**, Plot of lick rate before (in black) and after (in red) saline infusion. **d**, Mean lick rate before and after infusing saline in PBN. Data points for the same animal are connected with a line. N.S., p = 0.2209, not significant, paired t test, two-sided. **e-h**, Infusing

saline into NST, p=0.9204, paired t test, two-sided. **i-l**, Infusing saline into MS, p=0.103, paired t test, two-sided. **m-p**, Lick rate before and after infusing muscimol into PBN. *, p = 0.0479, paired t test, two-sided. **q-t**, Lick rate before and after infusing muscimol into NST. *, p = 0.0199, paired t test, two-sided. **u-x**, Lick rate before and after infusing muscimol into MS. *, p = 0.0476, paired t test, two-sided. Source data are provided as a Source Data file.

Supplementary Figure 5 | Efficiency of chemogenetic silencing of hM4Di-expressing neurons in MS.

a, Illustration of experimental scheme. Slice whole-cell recording was performed from neurons that expressed hM4Di (red). **b**, Example recorded trace showing that CNO application effectively suppressed spiking activity of an hM4Di-expressing MS neurons. Scale: 20mV, 5s. **c**, Mean spike rate (Hz) before and after CNO application. **, p= 0.0053, paired t test, one-sided. Source data are provided as a Source Data file.
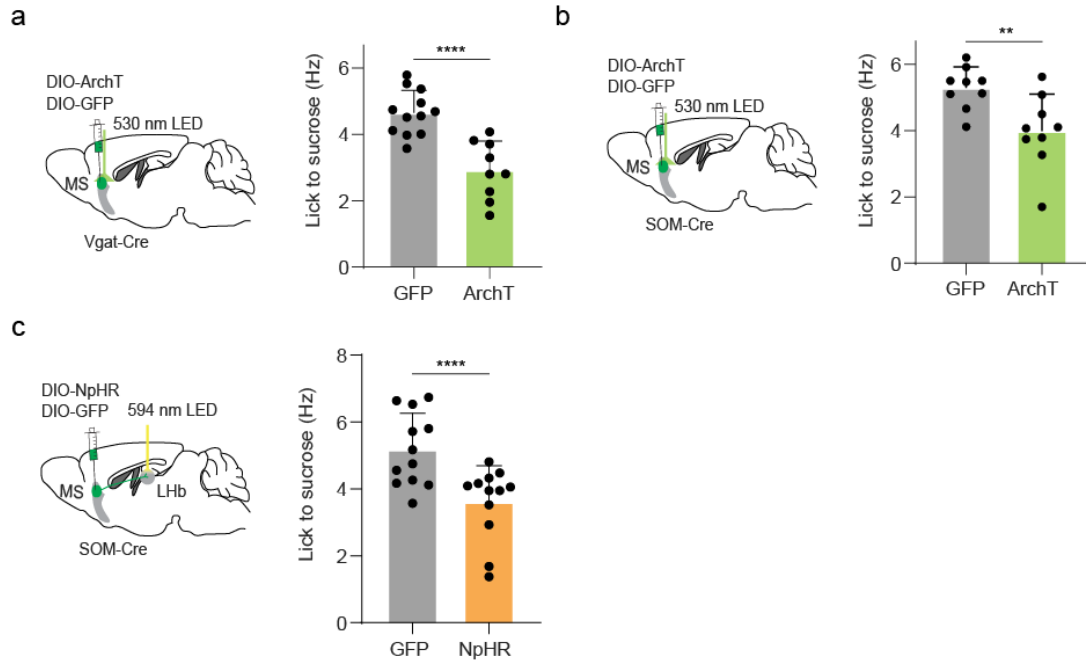
Supplementary Figure 6 | Lick rates to sucrose in optogenetic silencing experiments.

a, Left, illustration of experimental scheme. Either ArchT or GFP was expressed in MS GABAergic neurons in Vgat-Cre mice, with the optic fiber been implanted to target MS to deliver 530nm LED light. Right, lick rates to sucrose delivery in GFP or ArchT expressing mice. ****p<0.0001, t test, two-sided. **b**, Either ArchT or GFP was expressed in MS of SOM-Cre mice. Right, lick rates to sucrose in GFP or ArchT expressing mice. **p=0.0077, t test, two-sided. **c**, Either NpHR or GFP was expressed in MS of SOM-Cre mice, two-sided. Right, lick rates to sucrose in GFP or NpHR expressing mice. ****p=0.002, t test, two-sided. Source data are provided as a Source Data file.