Figures and figure supplements

Motor thalamus supports striatum-driven reinforcement

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Figure 1. dMSN self-stimulation elicits rapid and persistent NMDAR-independent reinforcement. (a) Schematic of a sagittal brain section showing optogenetic stimulation of striatal dMSNs (left), and slice showing fluorescent dMSNs after striatal infusion of a Cre-dependent virus encoding eYFP in a D1-Cre mouse (right). (b) Schematic of the behavioral apparatus showing active (laser-paired) and inactive nosepokes. (c) Example cumulative plot of active nosepokes from a D1-ChR2 (blue) and D1-eYFP (black) mouse. (d) Average nosepokes during a single self-stimulation session for D1-ChR2 and D1-eYFP mice (n = 11 and 8, two-way ANOVA, interaction poke x group, F(1,17)=31.03, p<0.001, posthoc Sidak's multiple comparisons test, ChR2 active vs eYFP active p<0.001, ChR2 active vs inactive p<0.001, eYFP active vs inactive p=0.921). (e) Average nosepokes during four consecutive daily sessions (n = 8, one-way ANOVA, F(1.908,13.36) = 1.237, p=0.319). (f) Distribution of (left) and average (right) inter-poke intervals for days 1 and 4 of dMSN self-stimulation (n = 8, paired t-test, p=0.0575). (g) Average nosepokes during an extinction session for D1-ChR2 and D1-eYFP mice (n = 8 and 8, two-way ANOVA, interaction poke x group, F(1,14)=47.86, p<0.0001, posthoc Sidak’s multiple comparisons test, ChR2 active vs eYFP active p<0.0001, ChR2 active vs inactive p<0.0001, eYFP active vs inactive p=0.5703). (h) Example cumulative plot of active nosepokes from D1-ChR2 mice expressing (D1-Cre, blue) or lacking (D1-Cre x NR1f/f, red) NMDA receptors in dMSNs. (i) Figure 1 continued on next page.
Figure 1 continued

Average nosepokes during a single self-stimulation session for D1-ChR2 mice with (‘D1’) or without (‘D1-NR1\(^{KO}\)’) NMDA receptors in dMSNs (n = 9 and 7, two-way ANOVA, interaction poke x group, F(1,14)=0.638, p=0.438, posthoc Sidak’s multiple comparisons test, D1 active vs D1-NR1 active p=0.418). (j) Average distance travelled by D1-ChR2 mice with (‘D1’) or without (‘KO’) NMDA receptors in dMSNs (Mann Whitney U test, p=0.023).

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Figure 1—figure supplement 1. Fiber placement and infusion sites. (a) Fiber tip placement in the striatum for D1-ChR2 (blue), D1-eYFP (white) and D1-NR1 (red) cohorts shown in Figure 1 (left), and intracellular slice recording from a dMSN expressing ChR2 from a D1-Cre x NR1f/f mouse injected with Lalive et al. eLife 2018;7:e34032. DOI: https://doi.org/10.7554/eLife.34032.
DIO-ChR2, showing absence of NMDA receptor-dependent current at +40 mV. (b) Fiber tip placement in the SNr for Arch3 (green) and eYFP (white) cohorts shown in Figure 3. (c) Fiber tip placement in VM for ChR2 (blue) and eYFP (white) cohorts shown in Figure 3. (d) Fiber tip placement in the DRN for ChR2 (blue) and eYFP (white) cohorts shown in Figure 3. (e) Fiber tip placement for axonal dMSN stimulation over the cerebral peduncle/ anterior SNr (left), and coronal slices from mice infused with saline or FLEx-Caspase three in the DRN showing intact or absent 5HT expression in DRN, respectively (right), from mice used in Figure 4. (f) Fiber tip placement in the striatum (left) and infusion site (right) for D1-ChR2 mice infused saline (blue) or muscimol (red) used for self-stimulation or locomotion (grey) assays shown in Figure 5. (g) Left: Fiber tip placement for dMSN axonal stimulation over the cerebral peduncle (cp) in vGAT-cre mice infused with DIO-ChR2 in the striatum and DIO-ChR2 (blue) or DIO-eYFP (white) in the SNr, and coronal slice showing eYFP + dMSN fibers and fiber tip. Right: In the same mice, fiber tip placement for SNr terminal stimulation in VM, and coronal slice from a vGAT-Cre mouse infused with DIO-eYFP in the striatum and DIO-mCherry in the SNr, showing segregation of dMSN axons (green) en route to the SNr, and SNr terminals (red) in VM. From mice used in Figure 5.

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Figure 1—figure supplement 2. Characterization of dMSN self-stimulation. (a) Relationship between laser duration and poke rate. Dotted box highlights chosen parameter for further experiments. (b) Relationship between stimulation pattern and poke rate (all stimuli delivered for 1 s). Dotted box highlights chosen parameter for further experiments. (c) Relationship between the number of nosepokes required to obtain laser (Fixed Ratio), poke rate, and laser exposure. (d) Average nosepoke rate before, during, and after a contingency degradation session (Deg), during which mice received an average of 5 x 1 s of laser per minute non-contingent on nosepokes (n = 8, one-way ANOVA, treatment F(1.405,9.832) = 10.11, p=0.007, posthoc Tukey’s multiple comparisons test, preTrain vs Deg p=0.016, Deg vs postTrain p=0.001). (f) Relationship between stimulation pattern and poke rate (all stimuli delivered for 1 s) for dMSN axon self-stimulation over the cerebral peduncle.

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Figure 1—figure supplement 3. dMSN self-stimulation pattern is stable over days. (a) Average number of poking bursts for days 1 and 4 of dMSN self-stimulation with criterion of inter poke interval (ipi) <2 s or <6 s (paired t-test, <2 s p=0.364; <6 s p=0.396). (b) Average number of nosepokes/bursts for days 1 and 4 of dMSN self-stimulation with criterion of inter poke interval (ipi) <2 s or <6 s (Wilcoxon signed rank test, <2 s p=0.383; <6 s p=0.383). DOI: https://doi.org/10.7554/eLife.34032.006
**Figure 2.** No measurable changes in excitatory synaptic transmission or excitability in dMSNs after self-stimulation. (a) Coronal section of the striatum showing ChR2-eYFP expression in DMS, optic fiber track and recording area (dotted box), corresponding approximately to the portion of striatum illuminated during behavior. (b) Whole-cell recordings of dMSNs (tmt+) from trained D1-ChR2 mice (including ChR2 +and ChR2- neurons, identified by the presence or absence of a blue light-evoked photocurrent, inset) and naive mice (tmt+), showing average (left) and example traces (right) for mEPSC frequency and amplitude (frequency: Kruskal-Wallis test, p=0.505; amplitude, one-way ANOVA, F(2,32)=0.6141, p=0.547). (c,d) Whole-cell recordings of dMSNs (tmt+) from trained D1-ChR2 mice (including ChR2 +and ChR2- neurons), trained D1-eYFP mice (eYFP +neurons), or naive mice (tmt+), showing averages (left) and example traces (right) for AMPA/NMDA ratio and paired-pulse ratio (c) and excitability (d) [AMPA/NMDA ratio: n = 8,7,6,7; one-way ANOVA, F(3,24)=0.062, p=0.9793; paired-pulse ratio: n = 8,11,11,7; one-way ANOVA, F(3,32)=1.358, p=0.273; excitability: n = 6,8,14; two-way ANOVA, interaction current x group F(10,125)=0.3801, p=0.9533].

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Figure 2—figure supplement 1. No measurable changes in excitatory synaptic transmission or excitability in iMSNs after dMSN self-stimulation. (a,b) Whole-cell recordings of putative iMSNs (tmt-) from trained D1-ChR2 (ChR2), D1-eYFP (eYFP) or naive mice (naive), showing average and example traces for AMPA/NMDA and paired-pulse ratios (a) and excitability (b) [AMPA/NMDA ratio: n = 7,6,5; one-way ANOVA, treatment F(2,15)=0.3426, p=0.715; Paired-pulse ratio: n = 10,6,5; Kruskal Wallis test, p=0.753; excitability: n = 11,6; two-way ANOVA, interaction current x group F(5,75)=0.438, p=0.820].

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Figure 3. Optogenetic control of basal ganglia output neurons or their projection targets is reinforcing. (a) Schematic of a sagittal brain section showing optogenetic inhibition of SNr (left), and coronal slice showing Arch3-eYFP and TH expression after SNr infusion of a Cre-dependent construct in a vGAT-Cre mouse and fiber track (right). (b) Example cumulative plot of active nosepokes from a SNr-Arch3 (green) and SNr-eYFP (black) mouse. (c) Average nosepokes during a single self-inhibition session for SNr-Arch3 and SNr-eYFP mice (n = 7 and 10, two-way ANOVA, interaction poke x group, F(1,15)=13.01, p=0.003, posthoc Sidak’s multiple comparisons test, Arch3 active vs eYFP active p=0.007). (d) Schematic of a sagittal brain section showing optogenetic excitation of VM (left), and coronal slice showing ChR2-eYFP expression after VM infusion of CaMKIIα-ChR2 in a WT mouse (right). (e) Example cumulative plot of active nosepokes from a VM-ChR2 (blue) and VM-eYFP (black) mouse. (f) Average nosepokes during a single self-stimulation session for VM-ChR2 and VM-eYFP mice (n = 8 and 8, two-way ANOVA, interaction poke x group, F(1,14)=22.17, p=0.003, posthoc Sidak’s multiple comparisons test, ChR2 active vs eYFP active p<0.001). (g) Schematic of a sagittal brain section showing optogenetic excitation of DRN (left), and coronal slice showing ChR2-eYFP overlapping with 5HT expression after infusion of DIO-ChR2 in a SERT-Cre mouse and fiber track (right). (h) Example cumulative plot of active nosepokes from a DRN-ChR2 (blue) and DRN-eYFP (black) mouse. (i) Average nosepokes during a single self-stimulation session for DRN-ChR2 and DRN-eYFP mice (n = 4 and 6, two-way ANOVA, interaction poke x group, F(1,8)=38.09, p<0.001, posthoc Sidak’s multiple comparisons test, ChR2 active vs eYFP active p<0.001). (j) Schematic of a sagittal brain section showing optogenetic excitation of the MLR (left), and coronal slice showing ChR2-eYFP expression after MLR infusion (centered around the PPTg) of DIO-ChR2 in a vGLUT2-Cre mouse and fiber tracks (right). (k) Example cumulative plot of active nosepokes from an MLR-ChR2 responsive (resp, blue) and MLR-ChR2 non-responsive (non-resp, black) mouse. (l) Average nosepokes during a single self-stimulation session for MLR-ChR2 responsive and non-responsive mice (n = 2 and 3).

Figure 3 continued on next page
Abbreviations: 5HT, 5-hydroxytryptamine; DRN, dorsal raphe nucleus; MLR, mesencephalic locomotor region, PPTg, pedunculopontine tegmentum; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; TH, tyrosine hydroxylase; VM, ventromedial thalamus; VTA, ventral tegmental area.

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Figure 4. Silencing DRN serotonergic neurons or MLR glutamatergic neurons does not disrupt dMSN self-stimulation. (a) Sagittal schematic showing injection of a hSyn-ChR2 construct in the striatum and DIO-Casp3 in the DRN of a SERT-Cre mouse, and optic fiber placement above dMSN axons. (b) Example cumulative plot of active nosepokes for dMSN axon stimulation in mice infused with saline (sal, blue) or DIO-Casp3 (casp, orange). (c) Average nosepokes during a single self-stimulation session for dMSN axon self-stimulation in mice with intact (sal) or lesioned (casp) serotonergic neurons in DRN (sal versus casp, two-way ANOVA, interaction pokes x stim F(1,8)=0.45, p=0.521, posthoc Sidak’s multiple comparisons test, sal active vs casp active p=0.76). (d) Sagittal schematic showing optogenetic stimulation of dMSNs and inhibition of MLR glutamatergic neurons (CaMKIIα-eNpHR3.0). (e) Example cumulative plot of active nosepokes from D1-ChR2 mice for dMSN stimulation alone (blue) or paired with MLR inhibition (green). (f) Average nosepokes during a single self-stimulation session for dMSN stimulation alone or paired with MLR inhibition, or MLR inhibition alone (D1+ versus D1+ and MLR-, two-way ANOVA, interaction pokes x stim F(1,10)=1.618, p=0.232, posthoc Sidak’s multiple comparisons test, D1+ active vs D1+ and MLR- active p=0.142; MLR-, Wilcoxon signed rank test, active vs inactive p=0.625).

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Figure 4—figure supplement 1. MLR glutamatergic neurons are necessary for dMSN-driven locomotion. (a) Sagittal schematic showing optogenetic stimulation of dMSNs and inhibition of MLR glutamatergic neurons (CaMKIIα-eNpHR3.0). (b) Average distance travelled before (pre), during (laser) and after (post) dMSN stimulation with (green) or without (blue) MLR inhibition, and example tracks (right) (n = 6 mice, two-way ANOVA, interaction epoch x stim F(2,10)=10.15, p=0.004, posthoc Sidak’s multiple comparisons test, laser D1 vs D1 +MLR p<0.001).

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Figure 5. VM thalamus inhibition disrupts dMSN self-stimulation. (a) Schematic of a sagittal brain section showing optogenetic excitation of striatal dMSNs combined with muscimol infusion in VM, and coronal slice from a D1-Cre mouse showing ChR2-eYFP-expressing fibers from dMSNs en route to SNr (green) and infusion site in VM (DiI, orange). (b) Example cumulative plot of active nosepokes from D1-ChR2 mice infused with saline (sal, blue) or muscimol (mus, red). (c) Average nosepokes during a single self-stimulation session for D1-ChR2 mice infused with saline (sal) or muscimol (mus, n = 8 and 7, two-way ANOVA, interaction poke x group, F(1,13)=6.33, p=0.026, posthoc Sidak’s multiple comparisons test, sal active vs mus active p=0.0009, sal active vs inactive p<0.001, musactive vs inactive p=0.018). (d) Average spontaneous locomotion in D1-ChR2 mice infused in VM with muscimol or saline (left), and example tracks (right) (n = 3, Wilcoxon matched-pairs signed rank test, p=0.5). (e) Average distance travelled before (pre), during (laser) and after (post) dMSN stimulation in the same mice as in g (left), and example tracks (right) (two-way ANOVA, interaction laser x drug F(2,4)=0.873, p=0.485, main effect of laser F(2,4)=14.16, p=0.015, posthoc Tukey’s multiple comparisons test, sal pre vs laser p=0.002, mus pre vs laser p=0.001, sal laser vs mus laser p=0.994). (f) Schematic of a sagittal brain section showing injection of Cre-dependent ChR2 in the stratum and SNr of a vGAT-Cre mouse (left) and fiber placement above dMSN axons and in VM for optogenetic excitation of dMSN combined with excitation of SNr terminals in VM, respectively (right). (g) Example cumulative plot of active nosepokes for dMSN axon stimulation paired with SNr-eYFP (D1+, blue) or SNr-ChR2 terminal stimulation in VM (D1+ and SNr+, red). (h) Average nosepokes during a single self-stimulation session from the same mice as in e (n = 6 and 6, two-way ANOVA, interaction poke x group, F(1,10)=12.95, p=0.0049, posthoc Sidak’s multiple comparisons test, D1+active vs D1+ and SNr+active p<0.001, D1+active vs inactive p<0.001, D1+ and SNr+active vs inactive p=0.018, SNr+, Wilcoxon signed rank test, active vs inactive p=0.623). DOI: https://doi.org/10.7554/eLife.34032.019
Figure 6. dMSN stimulation increases firing in VM thalamus. (a) Sagittal schematic depicting dMSN optogenetic stimulation with simultaneous in vivo single-unit recording from VM in awake, head-fixed mouse. (b) Peri-event time histogram of all 163 cells recorded in VM, aligned to dMSN stimulation (blue bar, 1 s duration). Units are sorted by response type (increase [excit], no significant change [ns], decrease [inhib]) and modulation index (see below). (c) Average z-scored response of all VM neurons to dMSN stimulation (blue shading, 1 s duration, n = 163, baseline vs stim, Wilcoxon signed rank test, p = 0.0006). (d–f) Detailed analysis of responses for the first 20 ms (d), first 100 ms (e) or full 1 s (f) of dMSN stimulation (blue shading). Average firing rate (top) and modulation index (bottom) for excited (red), inhibited (blue) or unmodulated (grey) units. Latency to significant change in firing rate is shown in d, top inset. Bottom insets, pie charts showing fraction of excited (red), inhibited (blue) and unmodulated (grey) VM neurons during dMSN stimulation for each time window. See materials and methods for modulation index calculation.

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Figure 6—figure supplement 1. Stimulation and recording sites for VM in vivo recordings. (a) Left, coronal section showing axonal eYFP expression in VM from a vGAT-Cre mouse expressing ChR2-eYFP in the SNr. Right, whole-cell recording at +40 mV in VM showing a GABA_{A}R-mediated IPSC elicited by optical stimulation (blue tick) of ChR2-expressing terminals of SNr neurons, and absence of current at −70 mV (E_{Cl}). IPSC at +40 mV is blocked by picrotoxin (red trace). (b) Optic fiber tip placement in DMS for direct pathway stimulation during in vivo recordings in VM. (c) Coronal section of VM thalamus showing recording sites and modulation index.

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