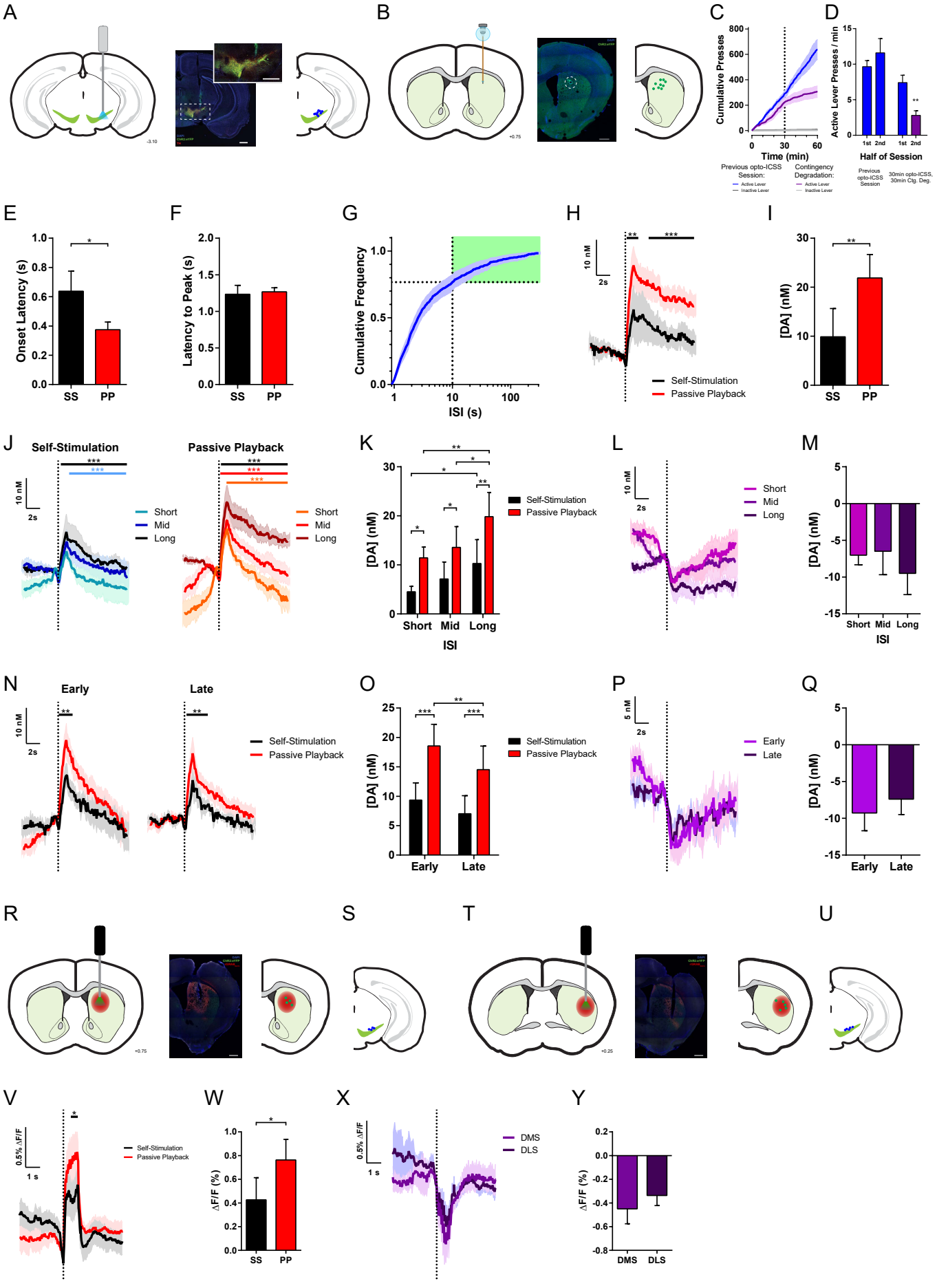
A
B
C
D
Cumulative Presses
Time (min)
Previous opto-ICSS Session:
Contingency Degradation:
Active Lever
Inactive Lever
Active Lever Presses / min
Half of Session
1st 2nd 1st 2nd
Previous opto-ICSS Session
30min opto-ICSS, 30min Ctg. Deg.
E
Onset Latency (s)
SS PP
F
Latency to Peak (s)
SS PP
G
Cumulative Frequency
ISI (s)
H
10 nM
2s
Self-Stimulation
Passive Playback
I
[DA] (nM)
SS PP
J
Self-Stimulation
Passive Playback
Short
Mid
Long
K
[DA] (nM)
Short Mid Long
ISI
L
M
N
Early
Late
O
Early Late
P
5 nM
Q
R
S
T
U
V
0.5% ΔF/F
1 s
W
ΔF/F (%)
X
DMS
DLS
Y

Figure S1. Opto-ICSS CRF cohort histology, contingency degradation, bout initiations, ISI, within-session comparisons, and DLS fiber photometry. Related to Figure 1.

(**A**) Left: Coronal schematic of fiber optic placement targeting the SNc (-3.10 mm posterior from bregma). Center: Representative image of fiber optic placement over the SNc in animal selectively expressing ChR2-eYFP in TH-positive dopamine neurons (scale bars = 500 μm). Right: Fiber optic placement for mice in the CRF cohort. (**B**) Left: Coronal schematic of FSCV carbon-fiber microelectrode placement in the dorsal striatum (+0.75 mm anterior to bregma). Center: Representative image of FSCV carbon-fiber microelectrode placement in the dorsal striatum (scale bar = 500 μm). The lesion was made by passing a current through the electrode just before perfusion after the conclusion of all experiments (see Methods). Right: FSCV electrode placement for mice in the CRF cohort. (**C**) Cumulative presses over time within the contingency degradation test session (30 min opto-ICSS followed by the 30 min contingency degradation test phase), overlaid with performance throughout the previous day's standard opto-ICSS session for comparison (n = 6 mice). (**D**) Summary of mean Active lever press rate during each phase of the contingency degradation test session (as in Fig. 1D), compared to the preceding day's standard opto-ICSS session (two-way repeated-measures ANOVA: main effect of Day, $F_{1,5}$ = 8.157, P = 0.0356; Day by Half of Session interaction, $F_{1,5}$ = 25.30, P = 0.0040; Sidak's multiple comparisons tests: Contingency Degradation 1st vs. 2nd Half, P = 0.0082; 2nd Half of Previous Day vs. Contingency Degradation test phase, P = 0.0004). (**E**) Latency for the onset of the stimulation-evoked dopamine response to exceed baseline: Time from stimulation onset until the dopamine response exceeded 2 standard deviations above the mean of baseline period (1 s preceding stimulation). Note that 1 animal's Self-Stimulation response did not cross this threshold within 2 s of stimulation onset, and was excluded from this analysis (n = 8 mice; paired t test: t_7 = 2.582, P = 0.0364). (**F**) Latency to the peak dopamine response, within 2 s following stimulation onset (n = 9 mice; paired t test: t_8 = 0.2722, P = 0.7924). (**G**) Cumulative frequency distribution of inter-stimulation intervals (ISIs) from the opto-ICSS FSCV recording session (n = 9 mice). Green shading indicates ISIs > 10 s, used to define bout initiation for the subset of stimulations analyzed in (H-I). (**H**) Mean dopamine concentration change to bout-initiating Self-Stimulation (ISI > 10s since previous stimulation) and corresponding Passive Playback stimulations. Black bars indicate time points where the Self-Stimulation response significantly differs from Passive Playback (permutation test, Ps = 0.007 and 0.0001 for first and second time clusters, respectively). (**I**) Mean change in dopamine concentration for the bout-initiating subset of stimulations in (H). (t_8 = 3.600, P = 0.0070). (**J**) Mean dopamine concentration changes to stimulations sorted by preceding ISI into short, medium (mid), and long ISI tertiles (permutation tests: Self-Stimulation Short vs. Long ISI, black bar, P = 0.0005; Short vs. Mid, blue bar, P = 0.0003; Playback Short vs. Long ISI, black bar, P = 0.0001; Mid vs. Long, red bar, P = 0.0001; Short vs. Mid, orange bar, P = 0.0009). (**K**) Mean change in dopamine concentration to stimulations sorted into ISI tertiles as in (J); (two-way repeated-measures ANOVA: main effect of Session Phase, $F_{1,8}$ = 16.07, P = 0.0039; main effect trend of ISI, $F_{2,16}$ = 3.295, P = 0.0633; Sidak's multiple comparisons tests: Self-Stimulation vs. Playback with Short ISIs, P = 0.0142; Mid, P = 0.0235; Long, P = 0.0011; Self-Stimulation Short vs. Long, P = 0.0439; Playback Short vs. Long, P = 0.0034; Playback Mid vs. Long, P = 0.0278). (**L**) Difference traces: Self minus Playback sorted by ISI from (J). (**M**) Mean Differences from (L); (one-way repeated-measures ANOVA is not significant, $F_{2,16}$ = 0.5891, P = 0.5664). (**N**) Mean dopamine concentration changes to the first 20 (Early) and final 20 (Late) stimulations within each phase of the recorded FSCV session (permutation tests: Early, P = 0.0038; Late, P = 0.0045). (**O**) Mean change in dopamine concentration to Early and Late stimulations as in (N); (two-way repeated-measures ANOVA: main effect of Session Phase, $F_{1,8}$ = 14.94, P = 0.0048; main effect of Early vs. Late, $F_{1,8}$ = 8.846, P = 0.0178; Sidak's multiple comparisons tests: Early Self vs. Playback, P < 0.0001; Late Self vs. Playback, P < 0.0001; Self-Stimulation Early vs. Late, P = 0.0788; Playback Early vs. Late, P = 0.0055). (**P**) Difference traces: Self minus Playback for Early and Late stimulations from (N). (**Q**) Mean Differences from (P) do not significantly differ (t_8 = 1.449, P = 0.1854). (**R**) Schematic (left), representative image (center), and fiber placement (right) for fiber photometry recordings of the red dopamine sensor rGRAB$_{DA1h}$ in the dorsomedial striatum (DMS) for data in Figure 1K-P. (**S**) Fiber placement for optogenetic stimulation of SNc dopamine neurons in this DMS rGRAB$_{DA1h}$ group. (**T-U**) Histology for dorsolateral striatum (DLS) rGRAB$_{DA1h}$ group, as in (R-S). (**V**) Mean ΔF/F rGRAB$_{DA1h}$ dopamine sensor response in DLS evoked by Self-Stimulation and Passive Playback stimulations (n = 5 mice; black bar, permutation test, P = 0.0162). (**W**) Mean ΔF/F rGRAB$_{DA1h}$ dopamine sensor response in DLS (paired t test, t_4 = 3.944, P = 0.0169). (**X**) Difference traces: Self minus Playback for DMS and DLS rGRAB$_{DA1h}$ groups. (**Y**) Mean Differences from (X) do not significantly differ (unpaired t test, t_7 = 0.7691, P = 0.467). Ctg. Deg., Contingency Degradation; ISI, Inter-stimulation interval; SS, Self-Stimulation; PP, Passive

Playback; DMS, dorsomedial striatum; DLS, dorsolateral striatum. Error bars are SEM here and for below figures.

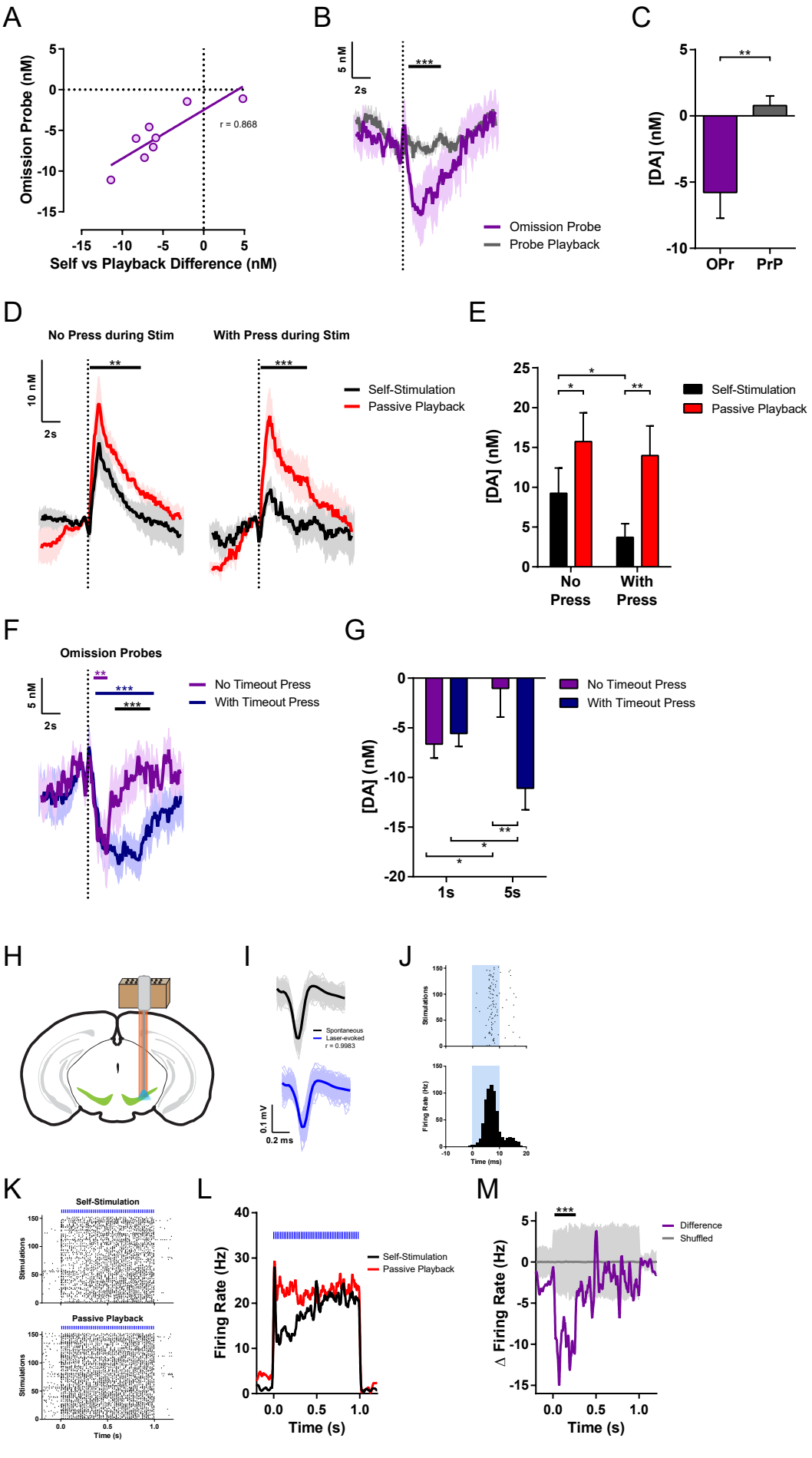
A
Omission Probe (nM)
Self vs Playback Difference (nM)
r = 0.868
B
5 nM
2s
***
Omission Probe
Probe Playback
C
[DA] (nM)
**
OPr
PrP
D
No Press during Stim
With Press during Stim
10 nM
2s
Self-Stimulation
Passive Playback
E
[DA] (nM)
No Press
With Press
Self-Stimulation
Passive Playback
F
Omission Probes
5 nM
2s
No Timeout Press
With Timeout Press
G
[DA] (nM)
1s
5s
No Timeout Press
With Timeout Press
H
I
Spontaneous
Laser-evoked
r = 0.9983
0.1 mV
0.2 ms
J
Stimulations
Firing Rate (Hz)
Time (ms)
K
Self-Stimulation
Passive Playback
Stimulations
Time (s)
L
Firing Rate (Hz)
Time (s)
Self-Stimulation
Passive Playback
M
Δ Firing Rate (Hz)
Time (s)
Difference
Shuffled

Figure S2. Inhibition of dopamine to isolated omissions, augmented suppression by additional presses, and *in vivo* electrophysiology. Related to Figure 2.

(**A**) Scatter plot depicting significant correlation between the amplitude of Omission Probe dopamine response and the stimulation response Difference (Self minus Playback; n = 8 mice, Pearson correlation r = 0.868, P = 0.0052). (**B**) Mean dopamine concentration change following temporally isolated Omission Probes (latency > 5 s since previous stimulation) and corresponding time points from the Passive Playback phase (n = 8 mice; permutation test, P = 0.0005). (**C**) Mean change in dopamine concentration following temporally isolated Omission Probes and equivalent time points from Playback phase (paired t test, t_7 = 3.511, P = 0.0098). (**D**) Mean dopamine concentration changes evoked by Self-Stimulations without (left) or with (right) an additional lever press during the ongoing stimulation, and the corresponding Passive Playback stimulations (n = 9 mice; permutation tests: Self-Stimulation with no press during stim vs. its Playback, P = 0.0011; Self-Stimulation with press during stim vs. its Playback, P = 0.0003; Self-Stimulation with vs. no press during stim, P = 0.0028). (**E**) Mean change in dopamine concentration for Self-Stimulations with or without additional presses during the stimulation, and their Playback (two-way repeated-measures ANOVA: main effect of Press, $F_{1,8}$ = 8.144, P = 0.0214; main effect of Session Phase, $F_{1,8}$ = 16.62, P = 0.0035; Sidak's multiple comparisons tests: Self-Stimulation with vs. no additional press, P = 0.0350; Self-Stimulation without additional press vs. Playback, P = 0.0163; Self-Stimulation with additional press vs. Playback, P = 0.0011). (**F**) Mean dopamine concentration changes during Omission Probes with or without additional presses during the probe timeout period (n = 8 mice; permutation tests: Omission Probe with no timeout press vs. 0, magenta bar, P = 0.002; Omission Probe with timeout press vs. 0, blue bar, P = 0.0001; Omission Probe with vs. without press, black bar, P = 0.0003). (**G**) Mean change in dopamine concentration at early (1 s) vs. late (5 s) time points during Omission Probes with or without additional presses during the timeout period (two-way repeated-measures ANOVA: main effect of Press, $F_{1,7}$ = 8.181, P = 0.0243; Press by Time interaction, $F_{1,7}$ = 16.70, P = 0.0047; Sidak's multiple comparisons tests: with vs. no press, late, P = 0.0025; no press, early vs. late, P = 0.0444; with press, early vs. late, P = 0.0479). (**H**) Schematic of experimental preparation for *in vivo* extracellular electrophysiology recordings with optogenetic identification of SNc dopamine neurons. (**I**) Waveforms of an optogenetically identified dopamine neuron for spontaneous (top) and laser-evoked (bottom) spikes (Pearson correlation, r = 0.9983, P < 0.0001). (**J**) Raster plot (top) and peri-event time histogram (bottom) of this dopamine neuron response to 10-ms optogenetic stimulation pulse. Each row in the raster represents one stimulation, and black ticks are spikes. (**K**) Raster plot of the same dopamine neuron responses aligned to Self-Stimulation (top) and Passive Playback stimulations (bottom). (**L**) Firing rate of the optogenetically identified dopamine neuron in (K) in response to Self-Stimulation versus Passive Playback stimulations. (**M**) Difference trace for the dopamine neuron in (K-L) depicting Self-Stimulation minus Playback difference in stimulation-evoked firing rate between session phases. Gray shaded region depicts the 95% confidence interval of the null distribution generated from shuffled data (permutation test, P = 0.0001). OPr, Omission Probe; PrP, Probe Playback.

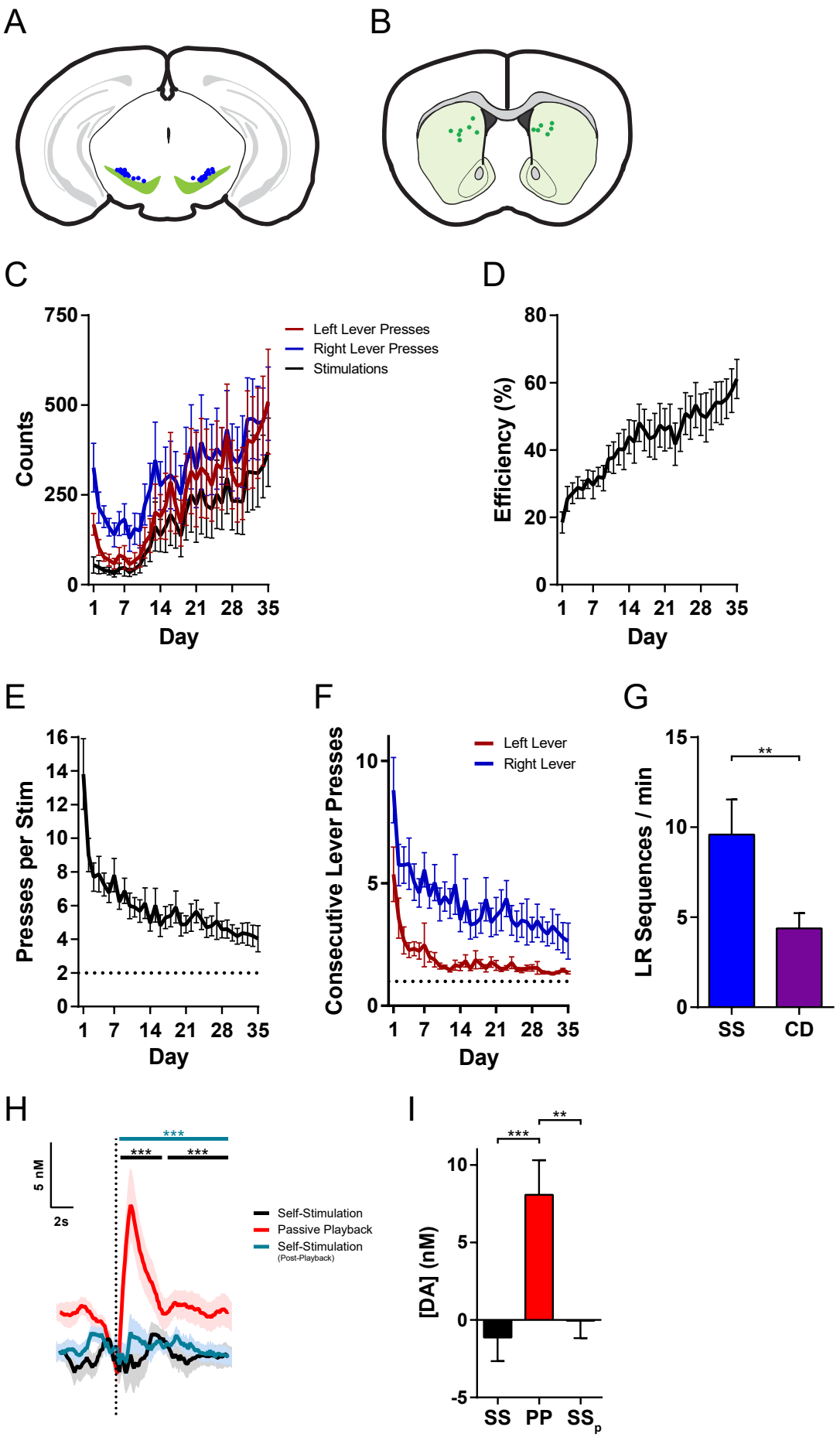
A
B
C
750
500
250
0
Counts
1 7 14 21 28 35
Day
Left Lever Presses
Right Lever Presses
Stimulations
D
80
60
40
20
0
Efficiency (%)
1 7 14 21 28 35
Day
E
16
14
12
10
8
6
4
2
0
Presses per Stim
1 7 14 21 28 35
Day
F
10
5
0
Consecutive Lever Presses
1 7 14 21 28 35
Day
Left Lever
Right Lever
G
15
10
5
0
LR Sequences / min
**
SS
CD
H
***
***
***
5 nM
2s
Self-Stimulation
Passive Playback
Self-Stimulation
(Post-Playback)
I
***
**
10
5
0
-5
[DA] (nM)
SS
PP
SS$_p$

Figure S3. Left-Right sequence cohort histology, additional behavior, and session phase order control. Related to Figure 3.

(**A**) Fiber optic placement for mice in the LR sequence cohort. (**B**) FSCV electrode placement for mice in the LR sequence cohort. (**C**) Presses on each lever and stimulations earned across days of training (n = 13 mice; statistics for stimulations presented in Fig. 3B; two-way repeated-measures ANOVA for Lever by Day: main effect of Day, $F_{34,408}$ = 3.189, P < 0.0001; main effect of Lever, $F_{1,12}$ = 16.67, P = 0.0015; Lever by Day interaction, $F_{34,408}$ = 1.535, P = 0.0307). (**D**) Efficiency across days of training, calculated as the number of stimulations per pair of lever presses (one-way repeated-measures ANOVA, $F_{34,408}$ = 6.936, P < 0.0001). (**E**) Total presses per stimulation (either lever) across days of training (one-way repeated-measures ANOVA, $F_{34,408}$ = 8.122, P < 0.0001). (**F**) Consecutive presses on each lever across days of training (two-way repeated-measures ANOVA: main effect of Day, $F_{34,408}$ = 8.430, P < 0.0001; main effect of Lever, $F_{1,12}$ = 23.07, P = 0.0004). (**G**) Contingency degradation test: 30 min of LR sequence opto-ICSS followed by 30 min contingency degradation test phase (n = 10 mice; paired t test, t_9 = 3.458, P = 0.0072). (**H**) Mean dopamine concentration change evoked by LR Self-Stimulation before or after the Passive Playback phase. The pre-playback Self-Stimulation (black) and Passive Playback responses are the same data as Fig. 3H, and the post-playback Self-Stimulation (teal) is an additional 30 min phase of LR sequence opto-ICSS following the Playback phase to control for potential order effects (n = 12 mice; permutation tests: Ps = 0.0001 for all time clusters, black bars for pre-playback Self-Stimulation vs. Playback, teal bar for post-playback Self-Stimulation vs. Playback). (**I**) Mean change in dopamine concentration for Self-Stimulation before or after Passive Playback (one-way repeated-measures ANOVA, $F_{2,22}$ = 26.39, P < 0.0001; Tukey's multiple comparisons tests: Self-Stimulation (pre) vs. Playback, P < 0.0001; Self-Stimulation (post) vs. Playback, P < 0.0001). SS, Self-Stimulation; CD, Contingency Degradation; PP, Passive Playback; SS_P, Self-Stimulation (post-Playback).

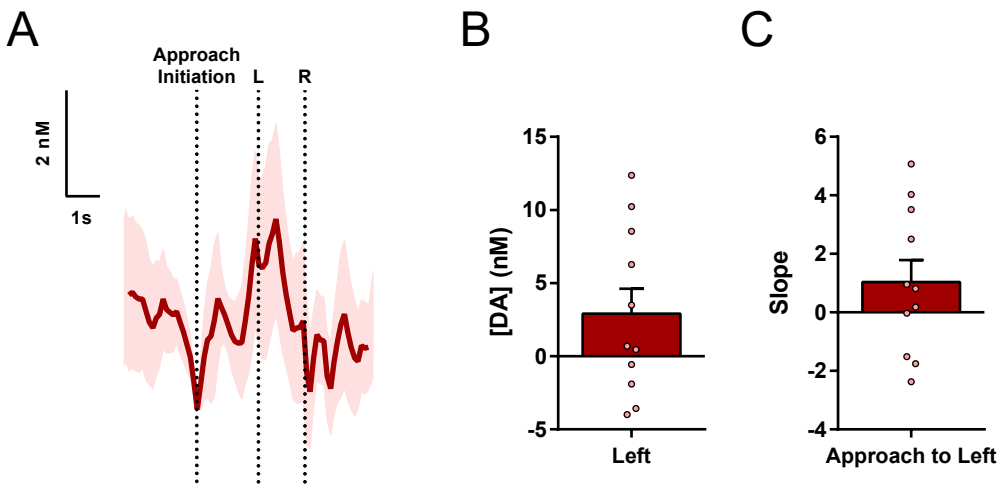

Figure S4. Left-Right sequences aligned to approach initiation. Related to Figure 4.
(**A**) Mean dopamine concentration change aligned to approach initiation (n = 11 mice). Intervals from approach initiation to Left lever press and from Left to Right press are scaled to each interval's median duration. (**B**) Mean change in dopamine concentration in the 0.5 s following the Left lever press, relative to the pre-approach baseline (one-sample t test vs 0, t_{10} = 1.700, P = 0.120). (**C**) Slope of linear regression fit to dopamine trace from approach initiation to Left press (one-sample t test vs 0, t_{10} = 1.388, P = 0.1952).