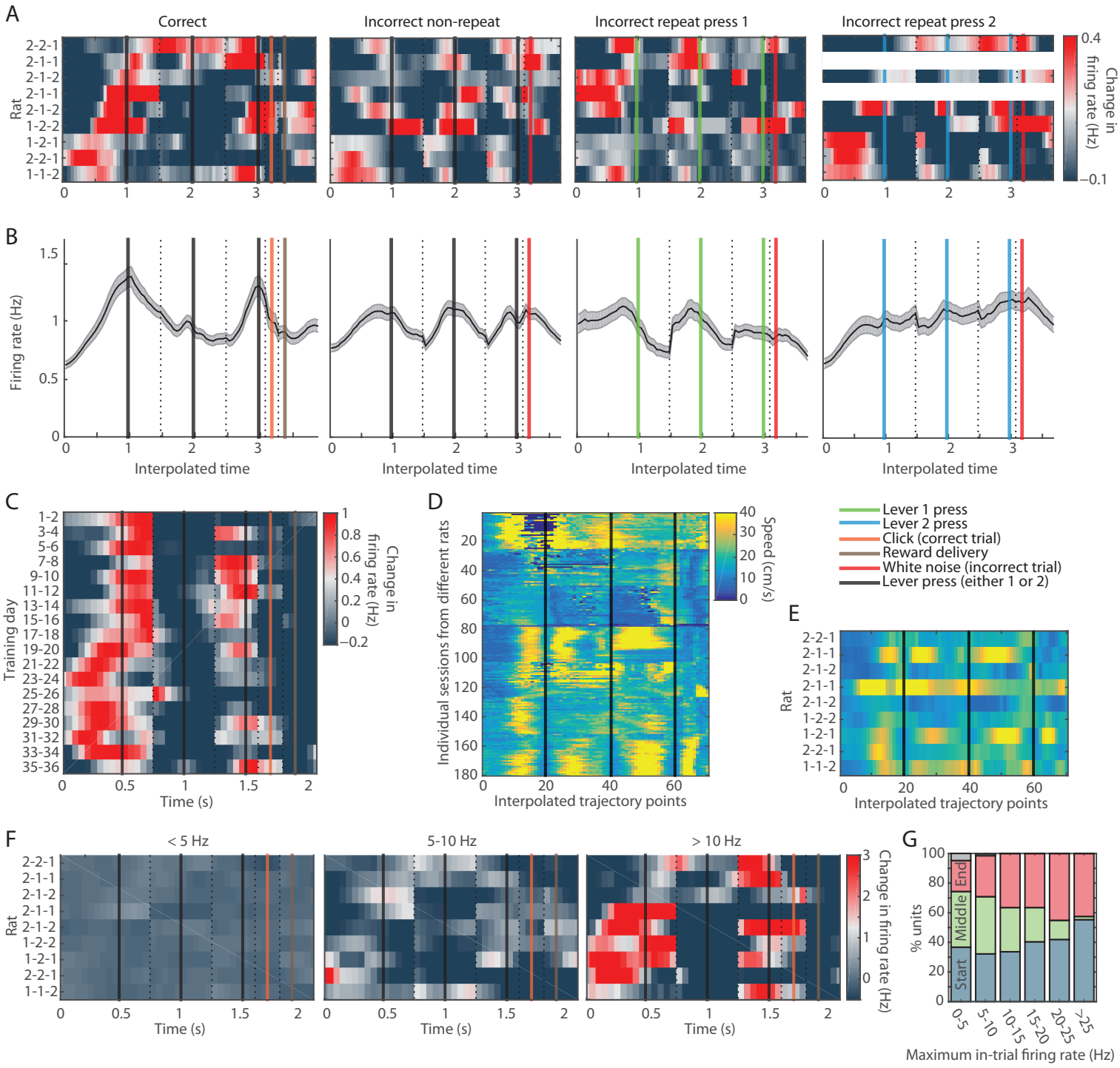

Figure S1. High Activity of SPNs in Dorsolateral Striatum at Learned Sequence Initiation and Termination Begins Early in Training, and This Activity Pattern Does Not Parallel Rats' Movement Speed. Related to Figure 3.

(A) Average peri-event spike activity of SPNs in the dorsolateral striatum during correct (first column), non-repeat incorrect (second column), lever 1 repeat (third column), and lever 2 repeat (fourth column) trials in 9 implanted rats that learned different lever-press sequences indicated on the left.

(B) Average peri-event activity for all SPNs during performance of correct sequences for the same trial types (n = 2501, 2501, 1143 and 1338 SPNs, respectively). Shading indicates SEM.

(C) Average firing rate of SPNs during performance of correct sequences across training days.

(D) Average speed of head movement in 180 sessions from 9 rats. Each trial's trajectory was interpolated to 70 points before being averaged and plotted. Lever-press events are indicated by black lines.

(E) Average speed of head movement for all 9 rats displayed in the same order as in A and Figure 3C.

(F) The spiking of SPNs that fired at less than 5 Hz (left, n = 1934), 5-10 Hz (middle, n = 329) and greater than 10 Hz (right, n = 237) during correct trial performance recorded in 9 implanted rats (rows), aligned to events in the learned sequence.

(G) Proportions of SPNs most active at the first (blue), second (green) or third (pink) lever presses, or at other trial times (gray) in the correct trial as a function of their peak in-task spiking rate.

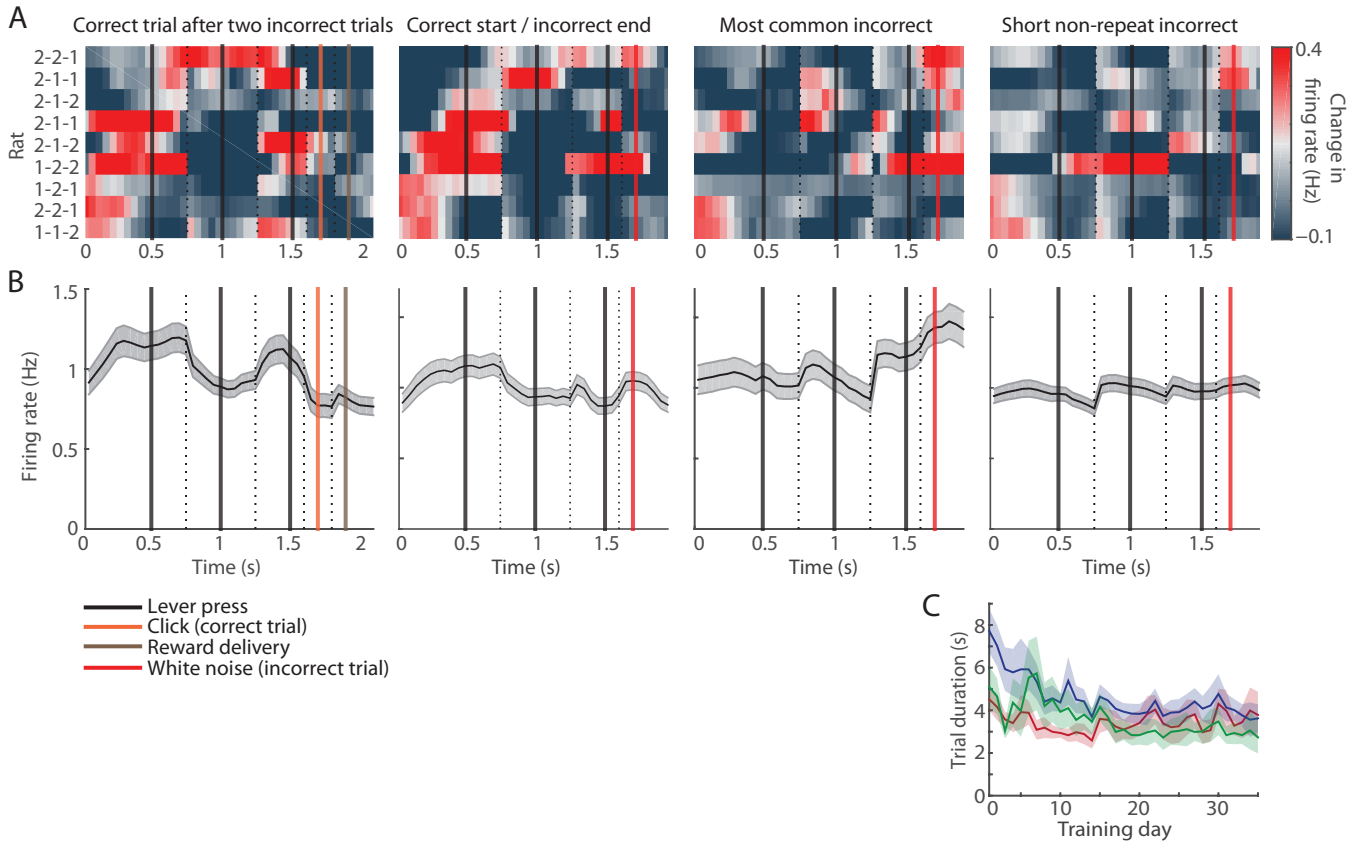

Figure S2. Striatal Beginning-and-End Activation Is Not Due to Reward in the Prior Trial, Number of Sequences Performed, or Short Trial Duration. Related to Figure 3.

(A) Mean peri-event SPN spiking in correct trials that occurred after two incorrect trials (first column), in trials in which the first two lever presses were correct but the last press was incorrect (second column), in the most common incorrect sequences in sessions in which that incorrect sequence was performed more frequently than the correct sequence (third column), and in short duration incorrect trials lasting < 3.5 s (fourth column) for 9 rats that learned the sequences indicated on the left.

(B) Mean peri-event spiking for all SPNs for the same trial types as in A (n = 1633, 1920, 753, and 2005, respectively). Shading indicates SEM.

(C) Mean trial duration (time from first press to third press) for correct (green), non-repeat incorrect (blue), and repeat incorrect (1-1-1 or 2-2-2, red) sequences as a function of training day in 9 rats.

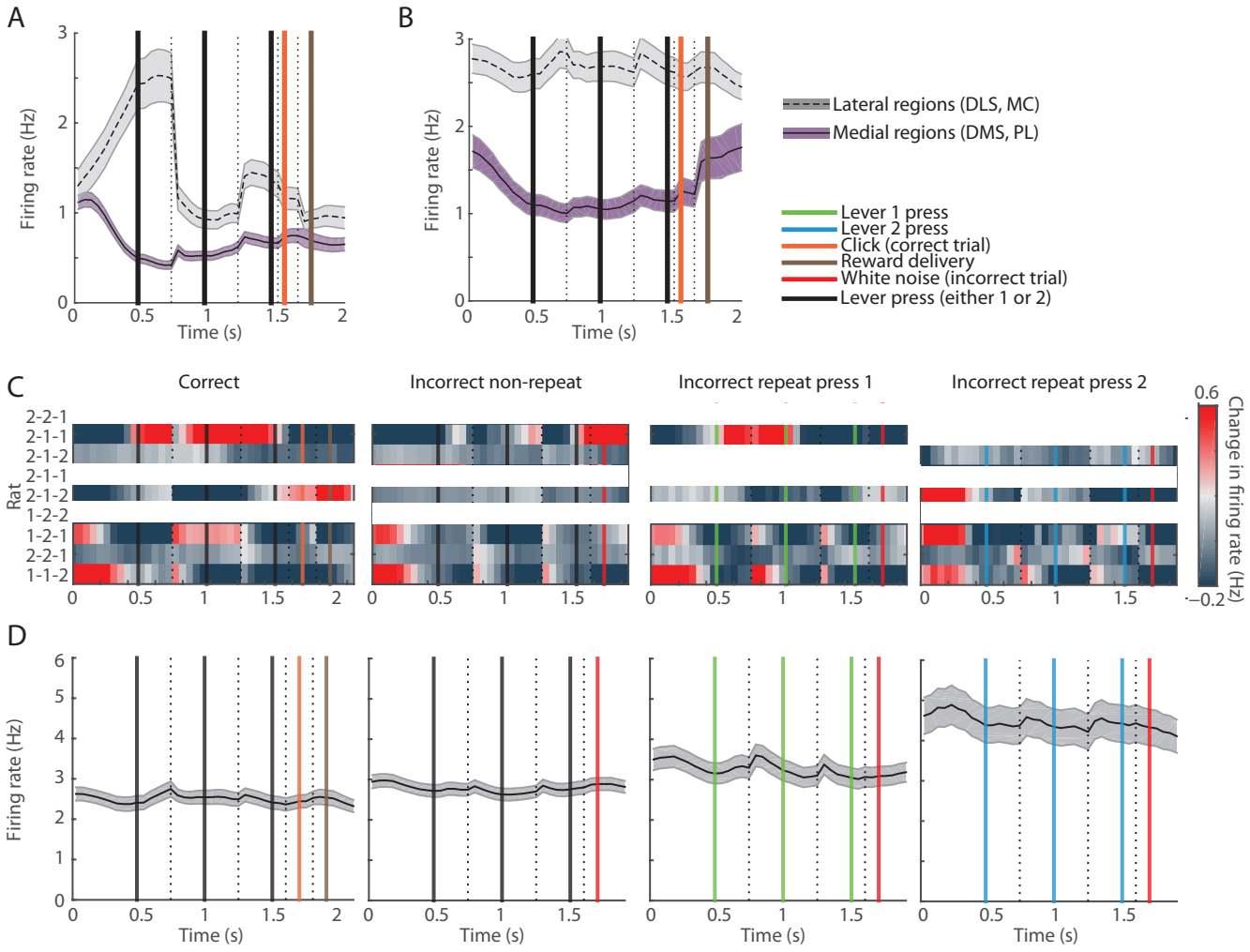

Figure S3. Dorsolateral, and Not Dorsomedial Corticostriatal Circuit Is Highly Activated during Lever-Press Sequence Task, but Motor Cortex Lacks the Task-Boundary Activity Seen in Dorsolateral Striatum. Related to Figure 3.

(A) Average peri-event firing rates of SPNs in dorsolateral (DLS, gray, n = 584) and dorsomedial (DMS, purple, n = 431) striatum during correct trials in three rats that had simultaneous recordings in the two striatal regions.

(B) Average peri-event firing rates of putative pyramidal neurons recorded in motor cortex of 6 rats (MC, gray, n = 817) and in prelimbic cortex of one rat (PL, purple, n = 187 units) during correct trials.

(C) Mean peri-event activity of putative pyramidal neurons in motor cortex during correct (first column), non-repeat incorrect (second column), lever 1 repeat (third column), and lever 2 repeat (fourth column) trials in 9 rats that learned different lever-press sequences indicated on the left. The 9 rats are the same as the rats shown in Figure 3C, but 3 of these rats did not have motor cortex recordings.

(D) Mean peri-event activity of all putative pyramidal neurons recorded in motor cortex for the same trial types as in C (n = 734, 807, 453 and 171, respectively).

All shading indicates ±SEM.

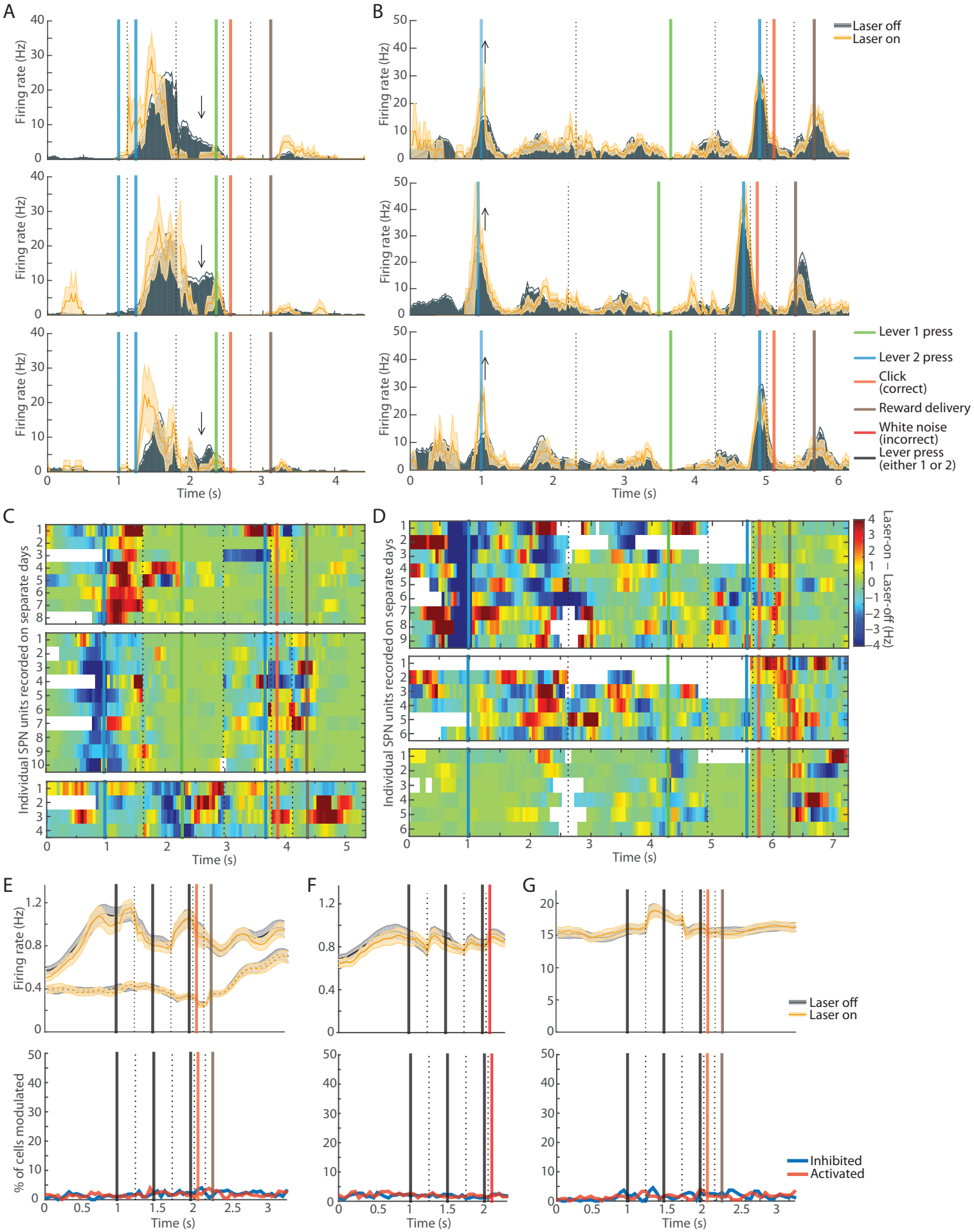
A
B
C
D
E
F
G
Firing rate (Hz)
Time (s)
Laser off
Laser on
Lever 1 press
Lever 2 press
Click (correct)
Reward delivery
White noise (incorrect)
Lever press (either 1 or 2)
Individual SPN units recorded on separate days
Laser-on – Laser-off (Hz)
% of cells modulated
Inhibited
Activated

Figure S4. Some Dorsolateral Striatal SPNs Were Reliably Modulated by Corticostriatal Terminal Inhibition, but the Population Task-Related SPN Activity Was Unaltered. Related to Figure 6.

(A and B) Examples of SPNs recorded across three days (rows) that were inhibited (A) or excited (B) at specific task-related time-points (arrows) by laser light directed at motor cortical terminals in the dorsolateral striatum. Gray, laser-off firing rate. Yellow, laser-on firing rate. Shading indicates SEM.

(C and D) Recurring task-time-specific effects of optogenetic terminal inhibition on activity of individual units recorded by 3 tetrodes (rows) in 2 separate rats (C and D). In each plot, rows indicate single units recorded on the tetrode on different recording days, and color indicates the difference in firing rate between laser-on and laser-off periods. In each tetrode, there are inhibitory (blue) or excitatory (red) effects in specific task-times. White indicates time bins that lacked sufficient laser trials to compare laser-on and laser-off periods.

(E) Top, population spiking of all SPNs (n = 1998 units) in correct trials during laser-off (gray) and laser-on (yellow) periods targeting corticostriatal terminals in the dorsolateral striatum. Dashed lines indicate spiking of low-task-responsive SPNs only, which never surpassed 5 Hz firing rate in correct trials (n = 1259). Bottom, proportions of all SPNs inhibited (blue) and activated (red) by laser.

(F and G) Same for SPNs in non-repeat incorrect trials (F, n = 1795 units) and FSIs in correct trials (G, n = 308 units).

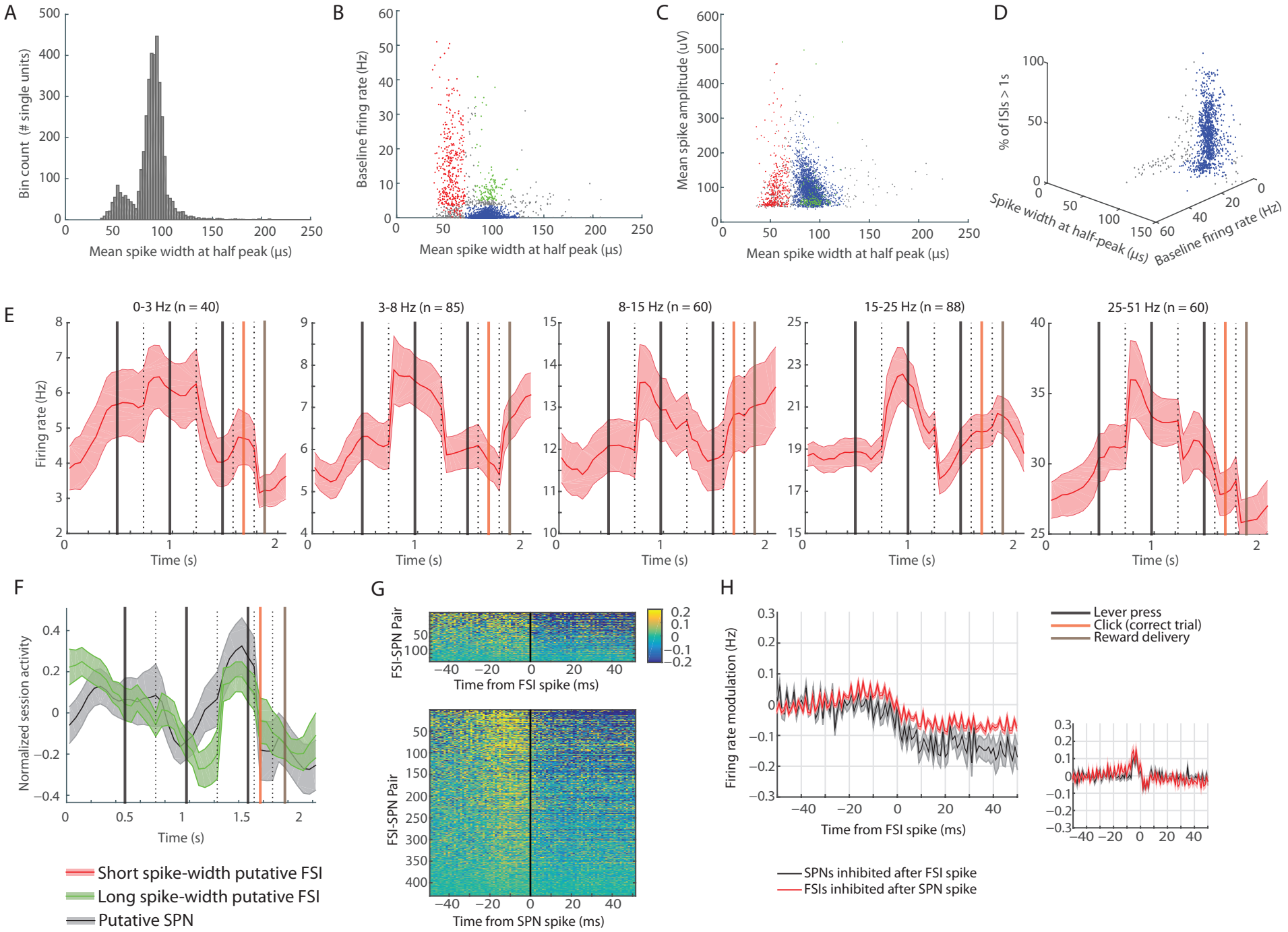
A
Bin count (# single units)
Mean spike width at half peak (µs)
B
Baseline firing rate (Hz)
Mean spike width at half peak (µs)
C
Mean spike amplitude (uV)
Mean spike width at half peak (µs)
D
% of ISIs > 1s
Spike width at half-peak (µs)
Baseline firing rate (Hz)
E
0-3 Hz (n = 40)
3-8 Hz (n = 85)
8-15 Hz (n = 60)
15-25 Hz (n = 88)
25-51 Hz (n = 60)
Firing rate (Hz)
Time (s)
F
Normalized session activity
Time (s)
Short spike-width putative FSI
Long spike-width putative FSI
Putative SPN
G
FSI-SPN Pair
Time from FSI spike (ms)
FSI-SPN Pair
Time from SPN spike (ms)
H
Firing rate modulation (Hz)
Time from FSI spike (ms)
Lever press
Click (correct trial)
Reward delivery
SPNs inhibited after FSI spike
FSIs inhibited after SPN spike

Figure S5. Bimodal Distribution of Spike Waveform Width in Striatal Single Units Delineates Groups of Units That Are Different in Electrophysiological Properties and in-Task Responses. **Related to Figure 7.**

(A) The distribution of mean spike width at half-peak for all units recorded in the dorsolateral striatum.

(B) Clusters of striatal units classified as SPNs (blue), FSIs (red), wide spike-width fast-spiking units (green), and unclassified (gray).

(C) The relationship of spike amplitude and spike width in the same units. The bimodal distribution of spike width in the dataset does not appear to be driven by spike amplitude differences.

(D) Properties of single units recorded in motor cortex. Units in blue were classified as putative pyramidal neurons, and units in gray were unclassified and were not used in analyses.

(E) Task-related spiking of narrow spike-width striatal units during learned sequence performance with different ranges of baseline firing rates. Shading indicates SEM.

(F) Comparison of normalized session activity of SPNs (gray) and wide spike-width fast-spiking units (green) recorded during correct sequence performance in 32 sessions.

(G) Top, SPN spiking aligned to the spikes of a simultaneously recorded FSI, in pairs of FSI-SPNs in which the SPN firing significantly decreased during the 20-ms period following the FSI spike. Bottom, FSI spiking aligned to the spikes of a simultaneously recorded SPN, in pairs of FSI-SPNs in which the FSI firing rate significantly decreased during the 20-ms period following the SPN spike.

(H) Activity modulation of the inhibited SPNs (black) and FSIs (red) shown in G (left), and SPNs and FSIs that were significantly inhibited in the first 5 ms after the reference spike (right).

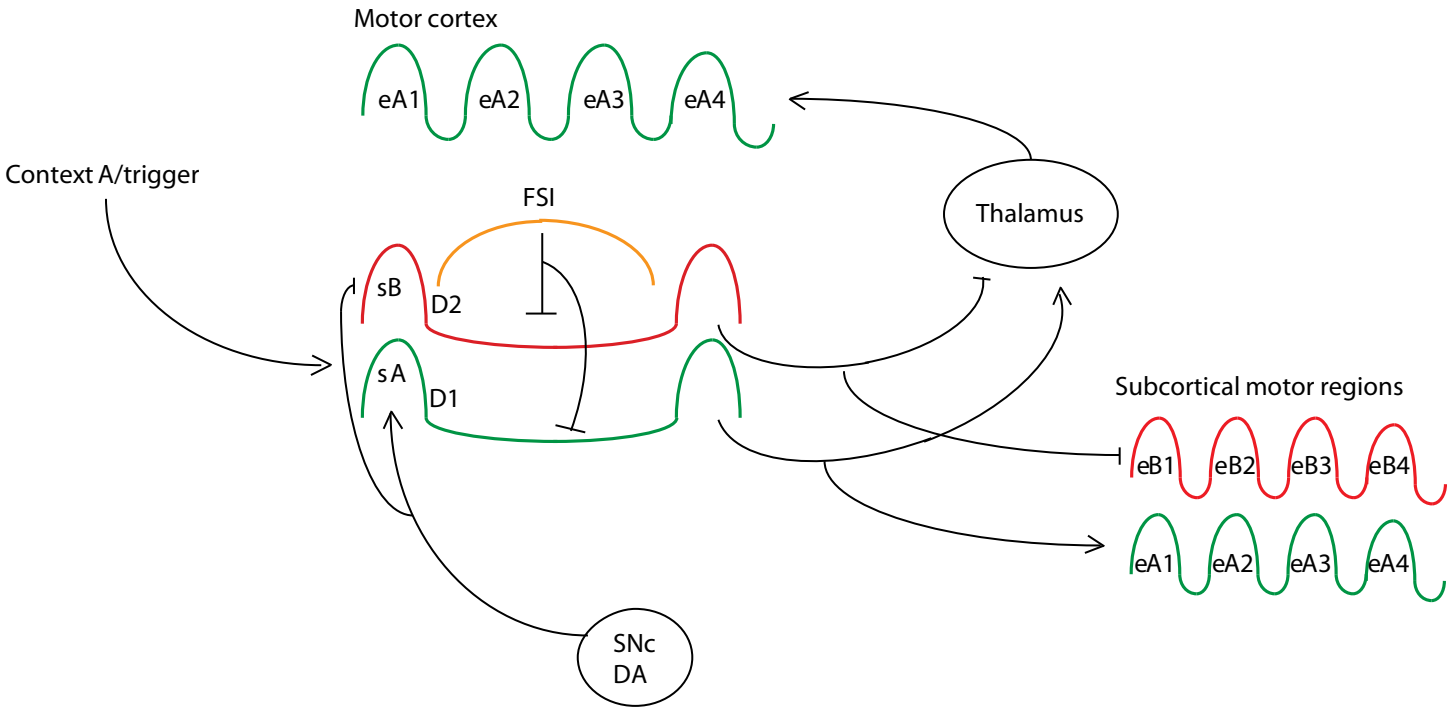

Figure S6. Modified Model for the Learning and Execution of Motor Programs. Related to Figures 3 and 7.
Sensory information about context and triggers for a particular behavior (in this case, behavioral program A) are delivered from cortical and thalamic regions (not shown) to the direct and indirect pathway SPNs in dorsolateral striatum. Within dorsolateral striatum, this input activates subsets of D1 SPNs that encode behavioral sequence A (sA) and also activates D2 SPNs that encode alternative behavioral sequences (in this case, sB). These D1 SPNs activate subsets of neurons that prompt the execution of individual actions (eA1-eA4) in behavioral program A, either in subcortical motor regions or in the motor cortex through the thalamus. Indirect pathway SPNs inhibit neurons that are involved producing alternative behaviors (in this case, behavioral program B). During the execution of the behavioral program, FSIs in dorsolateral striatum inhibit further activation of SPNs to allow the full program to become executed before another behavioral program can be triggered. In the case of a positive outcome, dopamine (DA) input from substantia nigra pars compacta (SNc) to dorsolateral striatum reinforces synaptic links between current sensory inputs and active D1 SPNs, further reinforcing the behavior.