Supplementary Figure 1

**Confirmation that optogenetic inhibition of dopaminergic neurons affects choice**

(a) Sample behavioral trace as in Figure 1d, but with NpHR stimulation trials depicted as green blocks (stimulation on a randomly selected 10% of all trials; 200 example trials from an NpHR-YFP animal). (b) Whole cell recording in voltage clamp of the photocurrent in an example NpHR-YFP expressing neuron from a TH::Cre mouse injected with Cre-dependent NpHR virus (green bar, 560 nm light). Inset shows the average and SEM across the population (n=14 neurons; peak current: 689+/−19 pA, steady state current: 455+/−13 pA). (c) Light-induced inhibition of spikes generated by current injections (150pA injections). During 10-seconds of photostimulation (green bar), action potentials. Trace is a single trial of a single neuron. (d) Population summary for c. Normalized spike rate before (left bar), during (middle bar) and after (right bar) 10s of photostimulation, averaged across the population. (n=14 neurons, paired two-tailed t-
test, $p=1.8\times10^{-8}$, $t(11)=8.59$, comparison of baseline and stim period firing rate; $p=1.6\times10^{-10}$, $t(11)=11.15$, comparison of stim and recovery). (e) Surgical schematic. Cre-dependent NpHR is injected into the VTA/SN of DAT::Cre mice and the optical fiber is implanted above the structure. (f) Coefficients from a logistic regression model demonstrating the influence of VTA/SN cell body inhibition on lever choice in subsequent trials in NpHR-YFP and YFP-control DAT::Cre mice. A negative coefficient indicates a reduction in the return probability to the lever chosen on the previous trial. Conversely, a positive coefficient indicates that the animal is more likely to return to the previously chosen lever. Rewarded choices with stimulation decreased the probability of returning to the chosen lever in comparison to rewarded choices without stimulation in NpHR-YFP mice (left panel; $p = 0.007$, $t(5)=4.46$ for 1 trial back; two-tailed t-test comparing coefficients of “rewarded choice” in blue with “rewarded choice+ rewarded choice x stim” in purple; $n=6$ mice). Likewise, unrewarded choice with stimulation significantly decreased the probability of returning to the chosen lever compared to unrewarded choice alone (left panel; $p=0.03$, $t(5)=3.04$ for 1 trial back, two-tailed t-test comparing “unrewarded choice” in red with “unrewarded choice+unrewarded choice x stim” in orange). (g) Same as f but stimulation was limited to only part of the trial. Left: inhibition from the time of the initial nose poke to the time of the lever press. Right: inhibition from the time of the lever press until the end of reward consumption. In either case, no significant effect of light stimulation on either rewarded or unrewarded choice; $p>0.1$. 

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Supplementary Figure 2

Largely non-overlapping populations of TH⁺ neurons project to DMS and NAc

(a) The retrograde tracer CTB 488 was injected into the NAc and the retrograde tracer CTB 555 was injected in the DMS of an example mouse. Scale bar: 1mm. (b) Retrogradely labeled neurons were evident in the lateral VTA / medial SNc. Scale bar: 100 µm. (c) Of TH⁺ neurons labeled with either CTB, 52±7% were labeled with the CTB injected in the NAc (green bar), while 56±5% were labeled with the CTB injected in the DMS (red bar). Notably, only 8±4% of TH⁺ neurons that were labeled with either CTB were labeled with both CTBs (gray bar), signifying that DAergic inputs to NAc and DMS are largely independent. For these data, 3 coronal midbrain slices were considered from each of n=6 mice (total of 487 neurons counted). Error bars are SEM.
Supplementary Figure 3

Individual sites show similar responses to reward consumption and reward-predictive cues in VTA/SN::DMS and VTA/SN::NAc terminal recordings

(a) gCaMP6f responses time-locked to either CS+ (left panel), CS− (middle panel) or Reward Consumption (right panel) in VTA/SN::DMS (blue) and VTA/SN::NAc terminals (n=11). Each line is the average response of between 500 and 1500 trials of an individual animal. The average of these traces is represented in Figure 4d. (b) Same as a except each trace is the response kernel derived from the regression model outlined in Figure 4a instead of the gCaMP6f response.
Supplementary Figure 4

**Difference in ipsilateral and contralateral responses in individual recording sites in VTA/SN::DMS and VTA/SN::NAc terminals**

(a) The difference in gCaMP6f time-locked responses between contralateral and ipsilateral choice trials in VTA/SN::DMS. (b) Same as a only and VTA/SN::NAc terminals. The time locked response to the nose poke is shown in the left panel, lever presentation on the right. A positive number indicates a larger contralateral response while a negative number indicates a larger ipsilateral response. Each trace is the average of an individual animal. (c,d) Same as in a, b except traces are the difference between the contralateral and ipsilateral response kernels derived from the regression model outlined in Figure 4a. (e) VTA/SN::NAc terminal time-locked gCaMP6f responses from individual animals separated into ipsilateral (grey) and contralateral (orange) trials for nose poke and lever presentation (left and right panels, respectively). (f) Same as in e except for VTA/SN::DMS terminal recordings (ipsilateral trials in grey, contralateral in blue). (g,h) Same as in e,f only traces are response kernels derived from the regression model outlined in Figure 4a.
Control striatal recordings in mice expressing GFP (not gCaMP6f) in VTA DA terminals does not reveal contralateral preference.

(a) Control recordings in DMS and NAc of TH::Cre mice injected with GFP virus in the VTA (n=4 recording sites). Each row represents GFP Z-score data from a different trial, time locked to either the nose poke (left) or the lever presentation (right). No obvious modulation in the GFP fluorescence signal at the time of these events is evident. (b) Kernels were calculated exactly as in Fig. 6b,d, and, no significant ipsilateral/contralateral modulation is evident in the GFP signal, indicating that the modulation with upcoming movement is not a movement artifact.
Supplementary Figure 6

*Upcoming lever choice is a better predictor of VTA/SN::DMS terminal responses than previous lever choice*

A linear regression model of the VTA/SN::DMS terminal responses that includes all behavioral events (trial start, nose poke, lever presentation, lever press, CS+, CS- and reward consumption), as well as interaction kernels between the choice on the upcoming trial and the event kernels, and finally interaction with choice on the previous kernel and the event kernel. This reveals non-zero interaction kernels for the upcoming but not previous trial interaction kernels. In other words, upcoming choice is a better predictor of neural activity than previous choice. Error bars are SEM across recording sites.
Supplementary Figure 7

**Difference in contralateral and ipsilateral responses in individual recording sites in VTA/SN::DMS cell bodies**

(a) The difference in gCaMP6f time-locked responses between contralateral and ipsilateral choice trials in VTA/SN::DMS cell body recordings (n=7, surgery schematic outlined in Fig 7a). The time locked response to the nose poke is shown in the left panel, lever presentation on the right. A positive number indicates a larger contralateral response while a negative number indicates a larger ipsilateral response. Each trace is the average of an individual animal. (b) Same as in a except traces are the difference between the contralateral and ipsilateral response kernels derived from the regression model outlined in Figure 4a. (c) VTA/SN::DMS cell body time-locked gCaMP6f responses from individual animals separated into ipsilateral (grey) and contralateral (blue) trials for nose poke and lever presentation (left and right panels, respectively). (d) Same as in c only traces are again response kernels derived from the regression model outlined in Figure 4a.