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Supplemental Information

Gain Modulation by Corticostriatal and Thalamostriatal Input Signals during Reward-Conditioned Behavior

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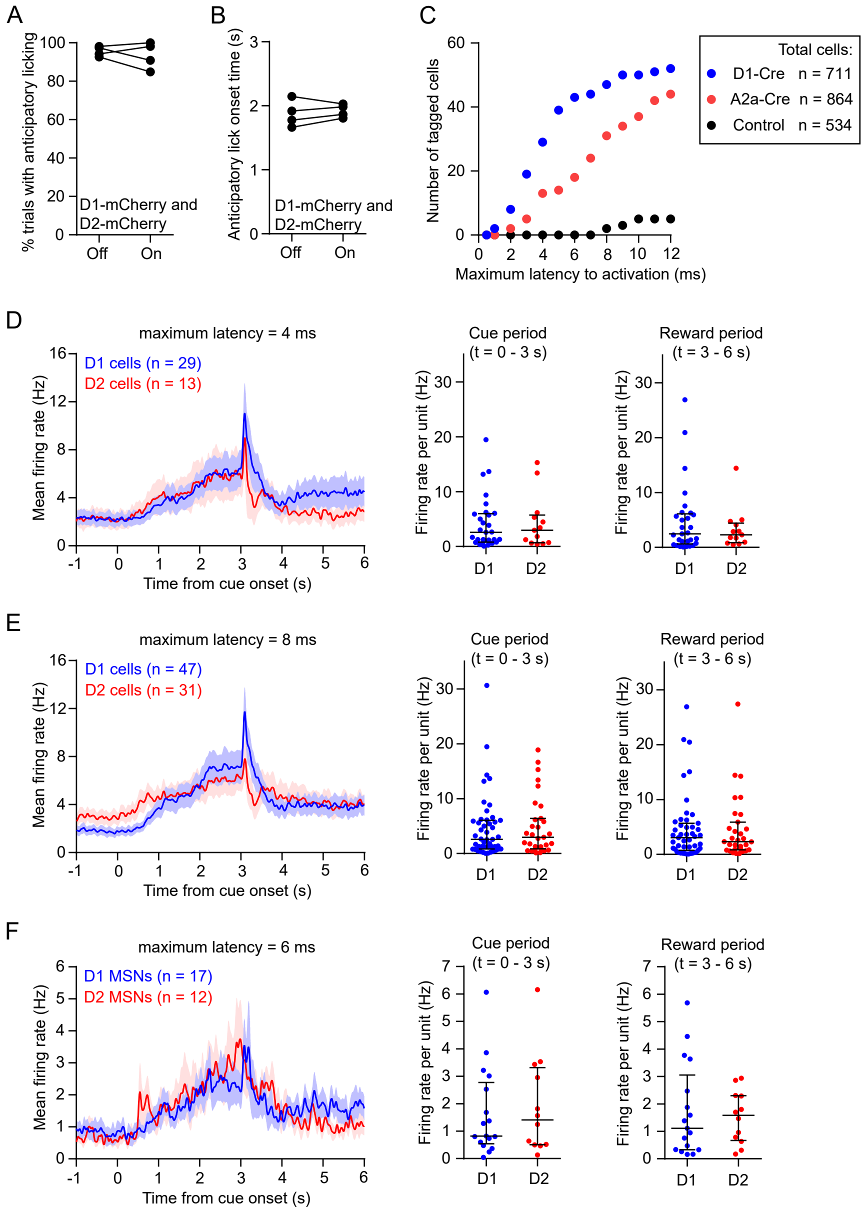

Figure S1. Related to Figure 1.
Optogenetic Manipulations on D1 and D2 MSNs.

A. Optical stimulation did not significantly alter the probability of anticipatory licking in mCherry$^+$ control mice (n = 4; 2 D1-Cre and 2 A2a-Cre mice, Wilcoxon signed rank test, p = 0.62).
B. Optical stimulation did not significantly alter the anticipatory licking onset time in mCherry$^+$ control mice (n = 4; 2 D1-Cre and 2 A2a-Cre mice, Wilcoxon signed rank test, p = 0.62).
C. Cumulative number of optogenetically tagged cells as a function of maximum latency to activation. Black circles denote the number of false positive tagged cells from control recordings which had no opsin expression. Total cells represent the number of all cells (tagged + untagged) from each group.
D. Optogenetic tagging analysis repeated with the maximum latency to activation set to 4 ms. There were 29 D1 cells and 13 D2 cells. Left: mean firing rate versus time. Data represent mean ± SEM. Middle and right: The median firing rate of individual tagged D1 and D2 cells was not significantly different either in the cue period (Mann Whitney test, p = 0.98) or reward period (p = 0.91). Lines and error bars represent median and IQR.
E. Optogenetic tagging analysis repeated with the maximum latency to activation set to 8 ms. There were 47 D1 cells and 31 D2 cells. Left: mean firing rate versus time. Middle and right: The median firing rate of individual tagged D1 and D2 cells was not significantly different either in the cue period (Mann Whitney test, p = 0.74) or reward period (p = 0.94).
F. Optogenetic tagging analysis repeated with cells that were both positively tagged and identified electrophysiologically as MSNs. The maximum latency to firing was 6 ms. There were 17 D1 MSNs and 12 D2 MSNs. Left: mean firing rate versus time. Middle and right: The median firing rate of individual tagged D1 and D2 MSNs was not significantly different either in the cue period (Mann Whitney test, p = 0.78) or reward period (p = 0.81).

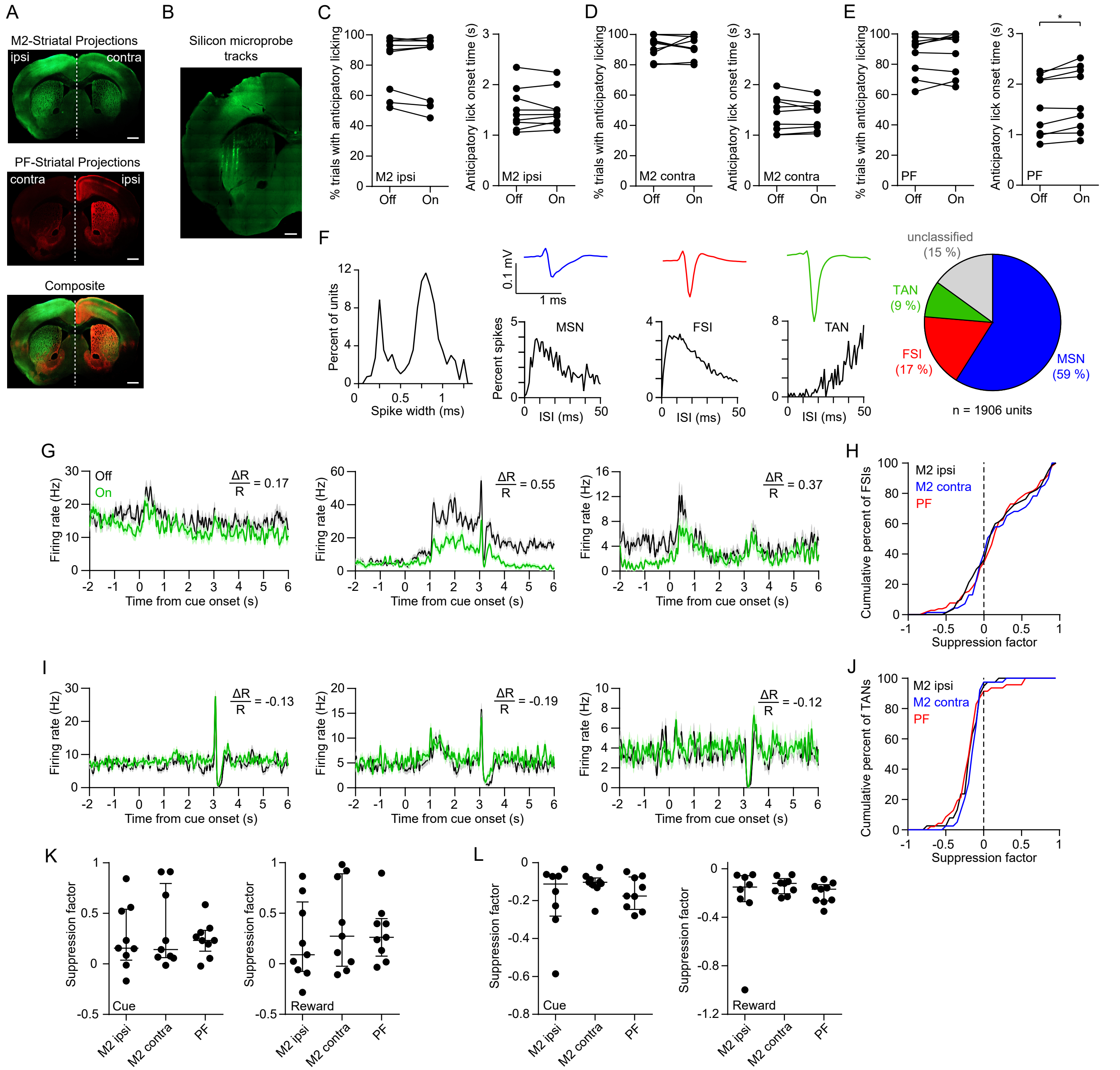

Figure S2. Related to Figures 2 and 3.
Effects of Suppressing Corticostriatal and Thalamostriatal Projections.

A. Overlapping anterograde projections from M2 and PF in the lateral striatum. Images correspond to the same animal. Scale bar = 1 mm.
B. Track of the four-pronged silicon microprobe in the lateral striatum. Scale bar = 0.5 mm.
C. Suppressing ipsilateral M2 projections in the striatum did not significantly alter the probability of anticipatory licking (left, n = 9, Wilcoxon signed rank test, p = 0.5), or the licking onset time (right, p = 0.82).
D. Suppressing contralateral M2 projections in the striatum did not significantly alter the probability of anticipatory licking (left, n = 9, Wilcoxon signed rank test, p = 0.98), or the licking onset time (right, p = 0.82).
E. Suppressing PF projections in the striatum did not significantly alter the probability of anticipatory licking (left, n = 9, Wilcoxon signed rank test, p = 0.38), but significantly increased the licking onset time (right, median of differences = 0.1 s, p = 0.012).

Figure S2 (continued).

F. Left: Histogram of spike waveform trough to peak width of all units pertaining to recordings in Figure 2. The bimodal distribution is used to putatively distinguish FSIs (narrow spikes) from other cell types (predominantly MSNs). Middle: Spike waveform and ISI distribution for a representative MSN (blue), FSI (red), and TAN (green). Right: Proportion of each cell type in the recordings.

G. Mean firing rate and corresponding suppression factor of three FSIs with laser on (green) and off (black). Left: cell from the ipsilateral M2 group. Middle: cell from the contralateral M2 group. Right: cell from the PF group. Data represent mean ± SEM.

H. Cumulative distribution of the FSI suppression factor for the ipsilateral M2 (black), contralateral M2 (blue), and PF (red) input groups. The median value was significantly higher than zero in each group (Wilcoxon signed rank test, $p < 0.001$). The median suppression factor was 0.09 for ipsilateral M2 ($n = 80$ FSIs from 9 mice), 0.07 for contralateral M2 ($n = 69$ FSIs from 9 mice), 0.18 for PF ($n = 104$ FSIs from 9 mice).

I. Same as G but for three TANs.

J. Same as H but for TANs. The median value was significantly lower than zero in each group (Wilcoxon signed rank test, $p < 0.0001$). The median suppression factor was -0.15 for ipsilateral M2 ($n = 38$ TANs from 9 mice), -0.11 for contralateral M2 ($n = 39$ TANs from 9 mice), -0.15 for PF ($n = 47$ TANs from 9 mice). Suppression factors in G-J were calculated in the time period from -2 to 6 s relative to cue onset corresponding to the laser presentation period.

K. There was no significant difference in FSI suppression factor between the three input groups during either the cue period ($n = 9$ per group, Kruskal-Wallis test, $H = 0.11$, $p = 0.95$), or reward period ($H = 0.86$, $p = 0.65$).

L. There was no significant difference in TAN suppression factor between the three input groups during either the cue period ($n = 8$ M2 ipsi, 9 M2 contra, 9 PF, Kruskal-Wallis test, $H = 0.91$, $p = 0.63$), or reward period ($H = 1.9$, $p = 0.39$). Note that $n = 8$ for the M2 ipsi group because one subject contained no TANs.

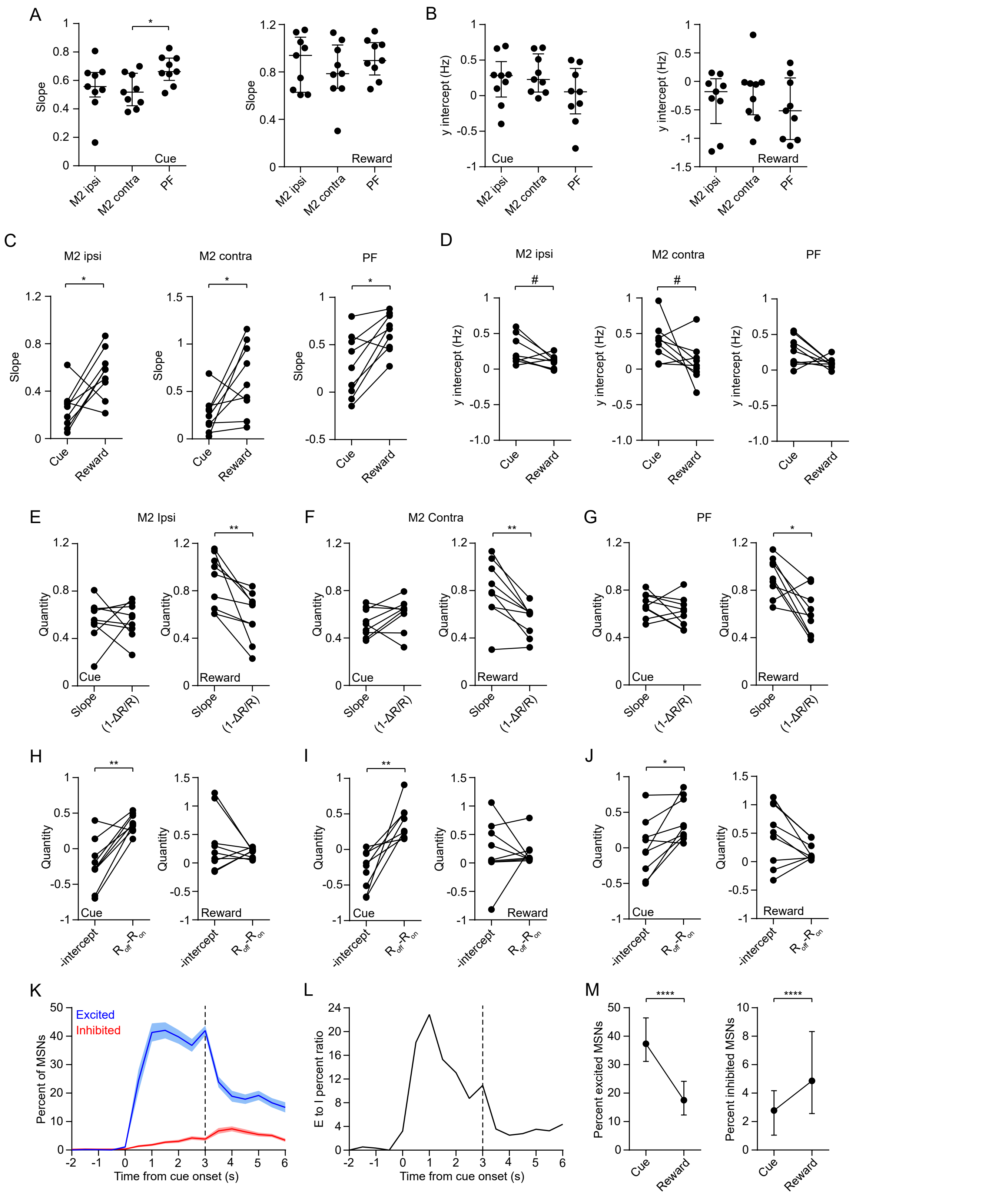

Figure S3. Related to Figure 4.

Differential MSN Gain and Excitation-to-Inhibition Ratio in the Cue and Reward Periods.

A. Comparison of slope between the three input groups (Kruskal-Wallis test). Left, cue period: H = 6.21, p = 0.045. *Post hoc* Dunn's multiple comparisons test showed that M2 ipsi *vs* M2 contra p > 0.99, M2 ipsi *vs* PF p = 0.25, M2 contra *vs* PF p = 0.046. Right, reward period: H = 0.74, p = 0.69.

B. Comparison of y intercept between the three input groups (Kruskal-Wallis test). Left, cue period: H = 2.65, p = 0.27. Right, reward period: H = 0.6, p = 0.74.

Figure S3 (continued).

C. After removing units with R_{off} greater than 4 Hz, the slope of the R_{on} vs R_{off} line was still significantly lower in the cue compared to the reward period for the ipsilateral M2 group (n = 9, Wilcoxon signed rank test, $p = 0.027$), contralateral M2 group (n = 9, $p = 0.027$), and PF group (n = 9, $p = 0.012$).

D. After removing units with R_{off} greater than 4 Hz, the y intercept of the R_{on} vs R_{off} line showed a trend for being significantly lower in the reward period compared to the cue period for the ipsilateral M2 group ($p = 0.055$) and contralateral M2 group ($p = 0.055$), and no significant difference for the PF group ($p = 0.1$).

E. Comparison of slope to (1 – suppression factor) for the ipsilateral M2 group in the cue (left) and reward (right) period (n = 9). Wilcoxon signed rank test is used for C-J and M. Cue period: $p > 0.99$. Reward period: $p = 0.004$.

F. Same as E but for the contralateral M2 group (n = 9). Cue period: $p = 0.3$. Reward period: $p = 0.008$.

G. Same as E but for the PF group (n = 9). Cue period: $p = 0.25$. Reward period: $p = 0.02$.

H. Comparison of the negative of the y intercept to the median ($R_{off} - R_{on}$), for the ipsilateral M2 group in the cue (left) and reward (right) period (n = 9). Cue period: $p = 0.008$. Reward period: $p = 0.65$).

I. Same as H but for the contralateral M2 group (n = 9). Cue period: $p = 0.004$. Reward period: $p = 0.91$.

J. Same as H but for the PF group (n = 9). Cue period: $p = 0.012$. Reward period: $p = 0.1$.

K. Mean percentage of significantly excited and inhibited MSNs per subject as a function of time. Data represent mean ± SEM of recordings from 35 mice pertaining to Figure 2 (n = 27 NpHR3, and 8 YFP mice). Only laser off trials were used in K-M. The dashed vertical line indicates the reward time.

L. Ratio of the mean percentage of excitatory to inhibited units from I.

M. Left: Comparison of the mean percentage of significantly excited MSNs in the cue and reward period (n = 35, $p < 0.0001$). Right: Comparison of the mean percentage of significantly inhibited MSNs in the cue and reward period ($p < 0.0001$). Data represent median and IQR.

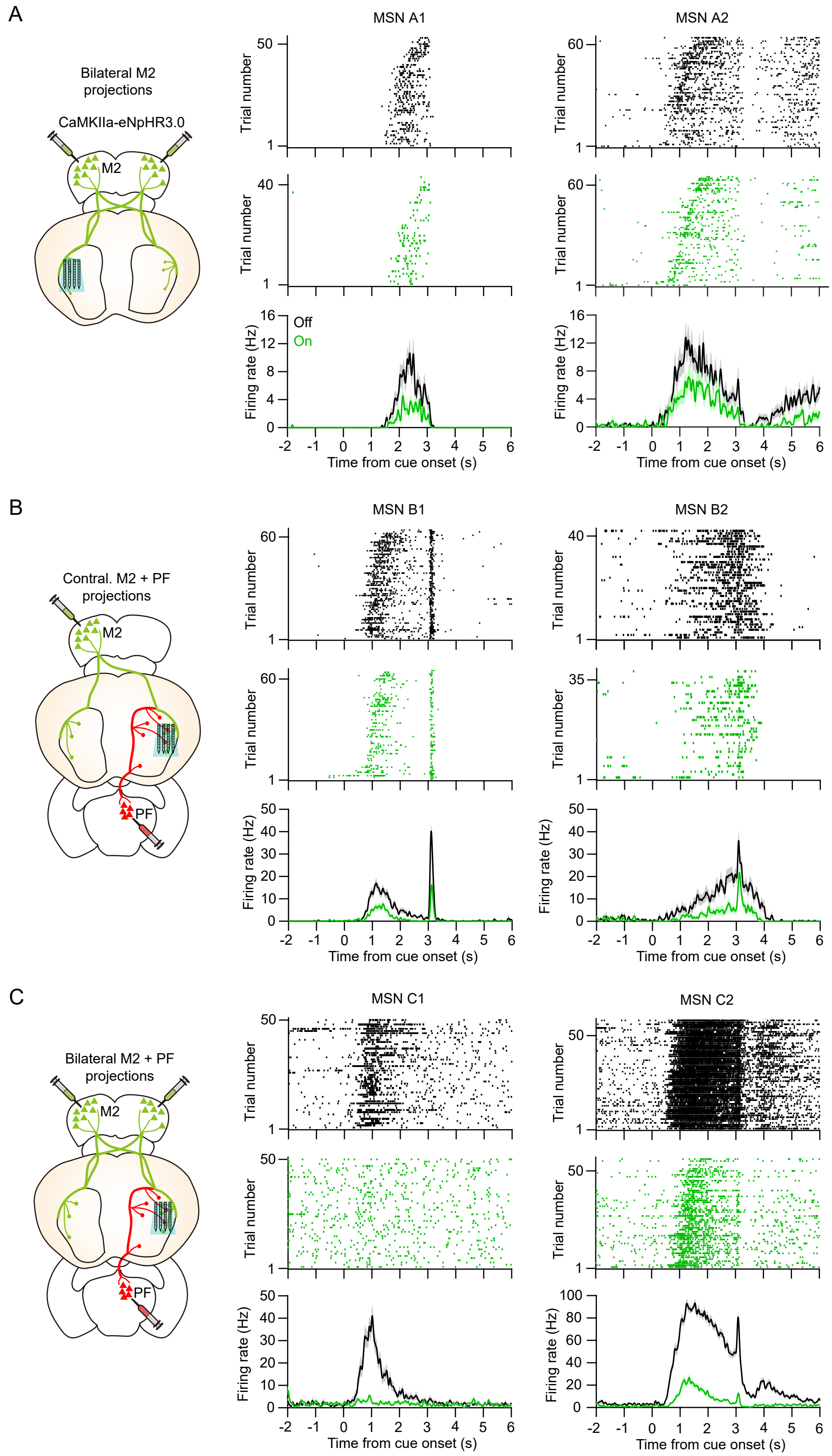

Figure S4. Related to Figure 5.
Representative MSN Spike Rasters during Suppression of Multiple Inputs.

A. Left: Approach used to determine the combined contribution of two inputs corresponding to bilateral M2, on striatal activity. Middle and right: spike rasters and mean firing rate of two corresponding MSNs with laser on (green) and off (black). The suppression factor is 0.63 (cue period) and 0.61 (reward period) for MSN A1; 0.45 (cue) and 0.74 (reward) for MSN A2.

Figure S4 (continued).

B. Left: Approach used to determine the combined contribution of two inputs corresponding to contralateral M2 plus PF, on striatal activity. Middle and right: spike rasters and mean firing rate of two corresponding MSNs. The suppression factor is 0.65 (cue) and 0.67 (reward) for MSN B1; 0.7 (cue) and 0.62 (reward) for MSN B2.

C. Left: Approach used to determine the combined contribution of three inputs corresponding to bilateral M2 plus PF, on striatal activity. Middle and right: spike rasters and mean firing rate of two corresponding MSNs. The suppression factor is 0.82 (cue) and 0.18 (reward) for MSN C1; 0.82 (cue) and 0.85 (reward) for MSN C2.

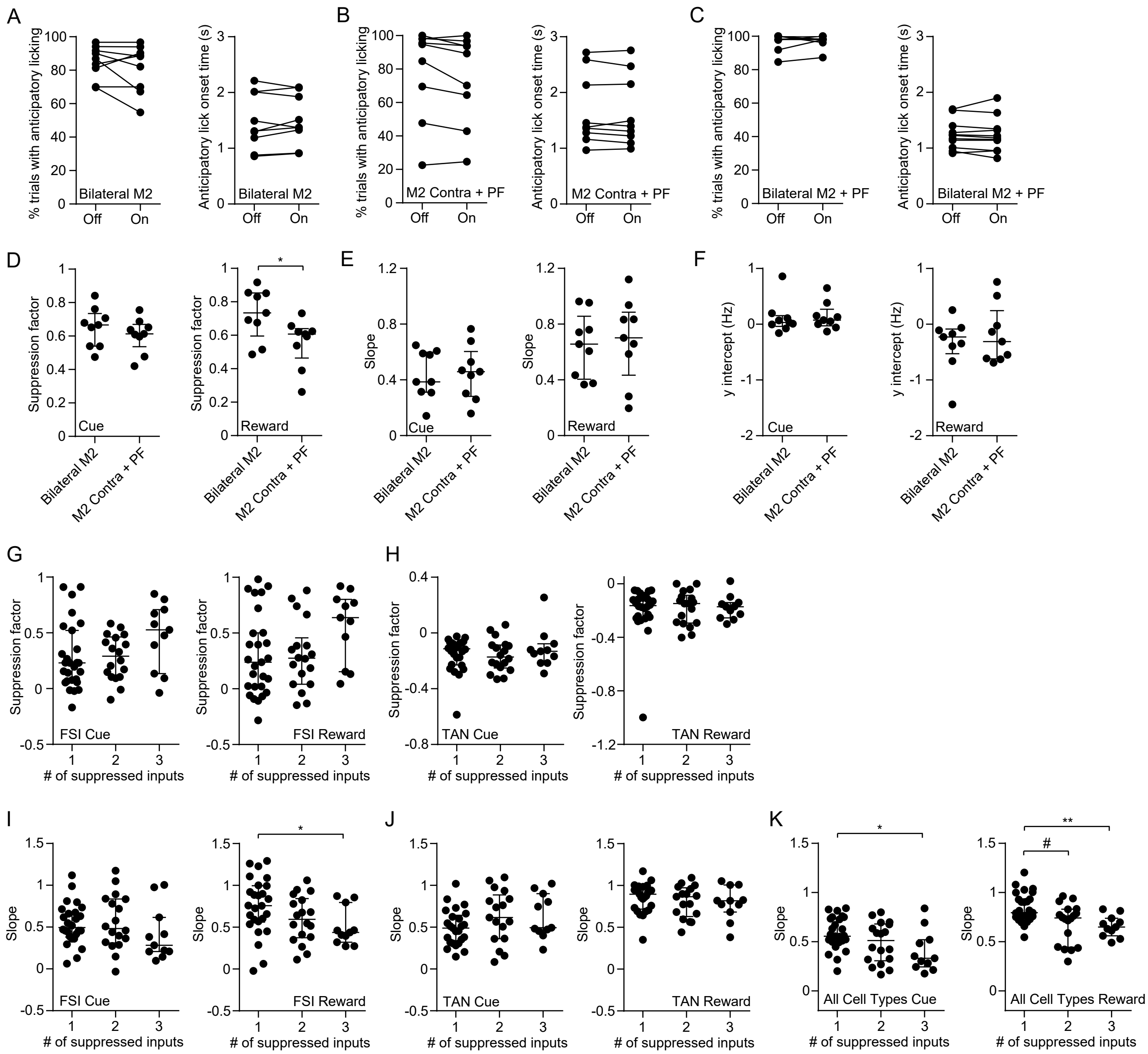

Figure S5. Related to Figures 5 and 6.
Results from Simultaneously Suppressing Multiple Inputs.

A. Suppressing bilateral M2 projections in the striatum did not significantly alter the probability of anticipatory licking (left, n = 9, Wilcoxon signed rank test, p = 0.38), or the licking onset time (right, p = 0.65).
B. Same as A but for contralateral M2 plus PF input suppression (n = 9, left: p = 0.07, right: p = 0.43).
C. Same as A but for bilateral M2 plus PF input suppression (n = 11, left: p = 0.91, right: p = 0.33).
D. Left: The suppression factor of the bilateral M2 group was not significantly different from the contralateral M2 plus PF group in the cue period (n = 9, Mann Whitney test, p = 0.34). Right: In the reward period the suppression factor was significantly greater in the bilateral M2 group (p = 0.032).
E. Left: The slope in the cue period is not significantly different between the two groups (p = 0.99). Right: The slope in the reward period is not significantly different between the two groups (p = 0.86).
F. Left: The y intercept in the cue period is not significantly different between the two groups (p = 0.6). Right: The y intercept in the reward period is not significantly different between the two groups (p = 0.86).
G. The cue (left) and reward (right) period FSI suppression factor did not vary significantly as a function of the number of simultaneously suppressed inputs (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, cue period: H = 3.53, p = 0.17; reward period: H = 4.56, p = 0.1).
H. The cue (left) and reward (right) period TAN suppression factor did not vary significantly as a function of the number of simultaneously suppressed inputs (n = 26 single, 18 double, 11 triple inputs, Kruskal-Wallis test, cue period: H = 0.71, p = 0.7; reward period: H = 0.22, p = 0.89).

Figure S5 (continued).

I. Left: The cue period FSI slope did not vary significantly as a function of the number of simultaneously suppressed inputs (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, H = 3.2, p = 0.2). Right: The reward period FSI slope varied significantly as a function of the number of simultaneously suppressed inputs (Kruskal-Wallis test, H = 6.59, p = 0.04). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs p = 0.31, 1 *vs* 3 inputs p = 0.046, 2 *vs* 3 inputs p = 0.99).

J. The cue (left) and reward (right) period TAN slope did not vary significantly as a function of the number of simultaneously suppressed inputs (n = 26 single, 18 double, 11 triple inputs, Kruskal-Wallis test, cue period: H = 3.24, p = 0.2; reward period: H = 0.99, p = 0.61). Note that in H and J, n = 26 for the single input group because one subject contained no TANs.

K. Left: The cue period slope calculated from all cell types varied significantly as a function of the number of simultaneously suppressed inputs (Kruskal-Wallis test, H = 6.44, p = 0.04). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs p = 0.54, 1 *vs* 3 inputs p = 0.04, 2 *vs* 3 inputs p = 0.63). Right: The reward period slope calculated from all cell types varied significantly as a function of the number of simultaneously suppressed inputs (Kruskal-Wallis test, H = 11.86, p = 0.003). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs p = 0.055, 1 *vs* 3 inputs p = 0.005, 2 *vs* 3 inputs p = 0.85). Lines and error bars in D-K represent median and IQR.

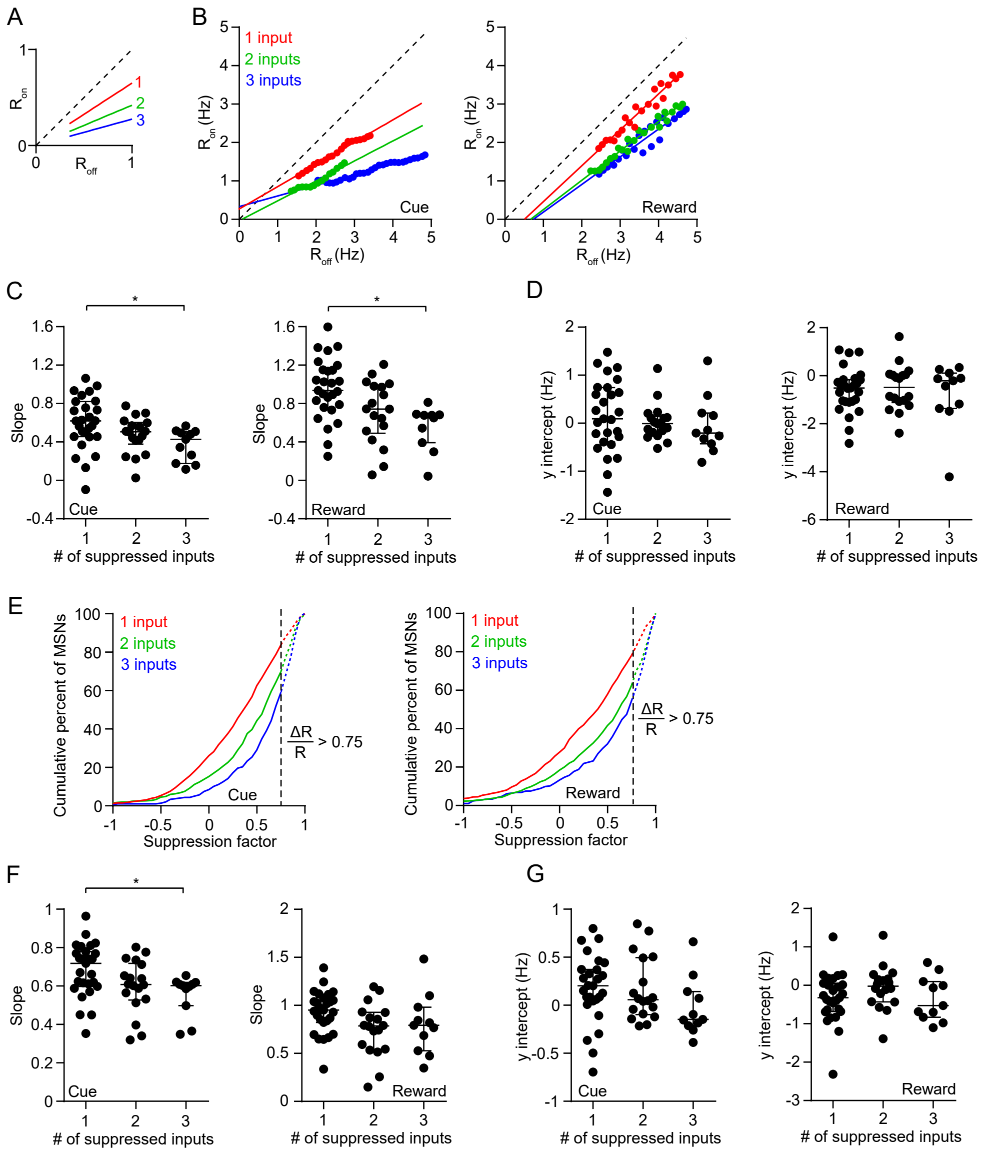

Figure S6. Related to Figure 6.

Controlling for Floor Effects in Analysis of Gain Modulation.

A. Illustration of removal of lower tercile of points from the R_{on} versus R_{off} plot, as a first control for possible floor effects on the estimation of slope from the linear fit.

B. R_{on} versus R_{off} calculated from the pooled population of MSNs from single (red), double (green), and triple (blue) input group data. The lower tercile of points have been removed. The solid lines represent linear fits to the data. Left and right plots represent data from the cue and reward period, respectively.

C. Even after removing the lower tercile of points, the slope still showed a significant reduction as a function of the number of simultaneously suppressed inputs. Left: cue period (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, H = 8.69, $p = 0.01$). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs $p = 0.28$, 1 *vs* 3 inputs $p = 0.01$, 2 *vs* 3 inputs $p = 0.55$). Right: reward period (Kruskal-Wallis test, H = 9.35, $p = 0.009$). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs $p = 0.09$, 1 *vs* 3 inputs $p = 0.02$, 2 *vs* 3 inputs $p > 0.99$).

D. Even after removing the lower tercile of points, the y intercept still showed no significant reduction as a function of the number of simultaneously suppressed inputs. Left: cue period (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, H = 1.38, $p = 0.5$). Right: reward period (Kruskal-Wallis test, H = 0.36, $p = 0.84$).

E. Cumulative distribution of the MSN suppression factor from the single (red), double (green), and triple (blue) input group data. As a second control for possible floor effects, all MSNs with suppression factor above a value of 0.75 (cutoff indicated by the dashed vertical line) were excluded from the calculation of R_{on} versus R_{off} used to obtain the slope. Left and right plots represent data from the cue and reward period, respectively.

Figure S6 (continued).

F. Even after removing MSNs exceeding the cutoff suppression factor, the slope still showed a significant reduction as a function of the number of simultaneously suppressed inputs. Left: cue period (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, H = 8.47, p = 0.01). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs p = 0.13, 1 *vs* 3 inputs p = 0.02, 2 *vs* 3 inputs p > 0.99). Right: reward period (Kruskal-Wallis test, H = 6.04, p = 0.049). *Post hoc* Dunn's multiple comparisons test showed that 1 *vs* 2 inputs p = 0.076, 1 *vs* 3 inputs p = 0.26, 2 *vs* 3 inputs p > 0.99).

G. Even after removing MSNs exceeding the cutoff suppression factor, the y intercept still showed no significant reduction as a function of the number of simultaneously suppressed inputs. Left: cue period (n = 27 single, 18 double, 11 triple inputs, Kruskal-Wallis test, H = 4.38, p = 0.11). Right: reward period (Kruskal-Wallis test, H = 3.1, p = 0.21). Lines and error bars in C, D, F, G represent median and IQR.