

**Supplemental materials for:**

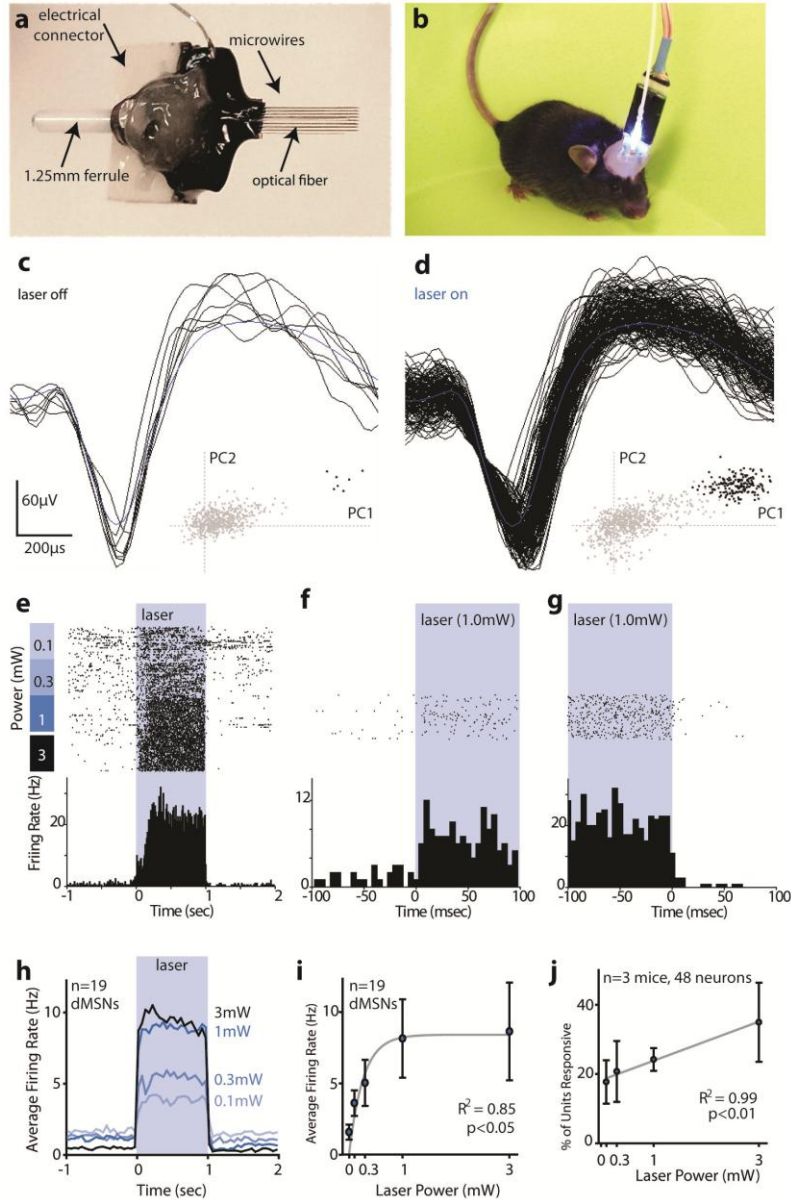
**Distinct roles for direct and indirect pathway striatal neurons in reinforcement**

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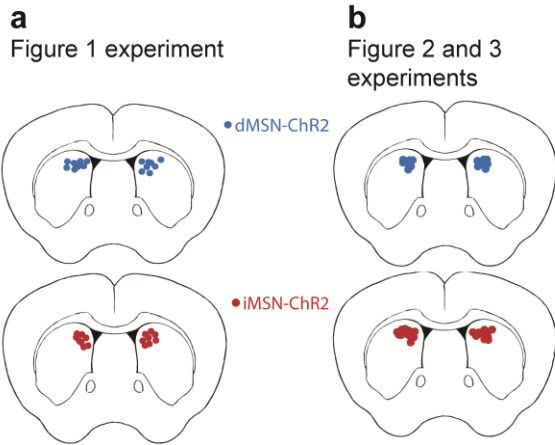
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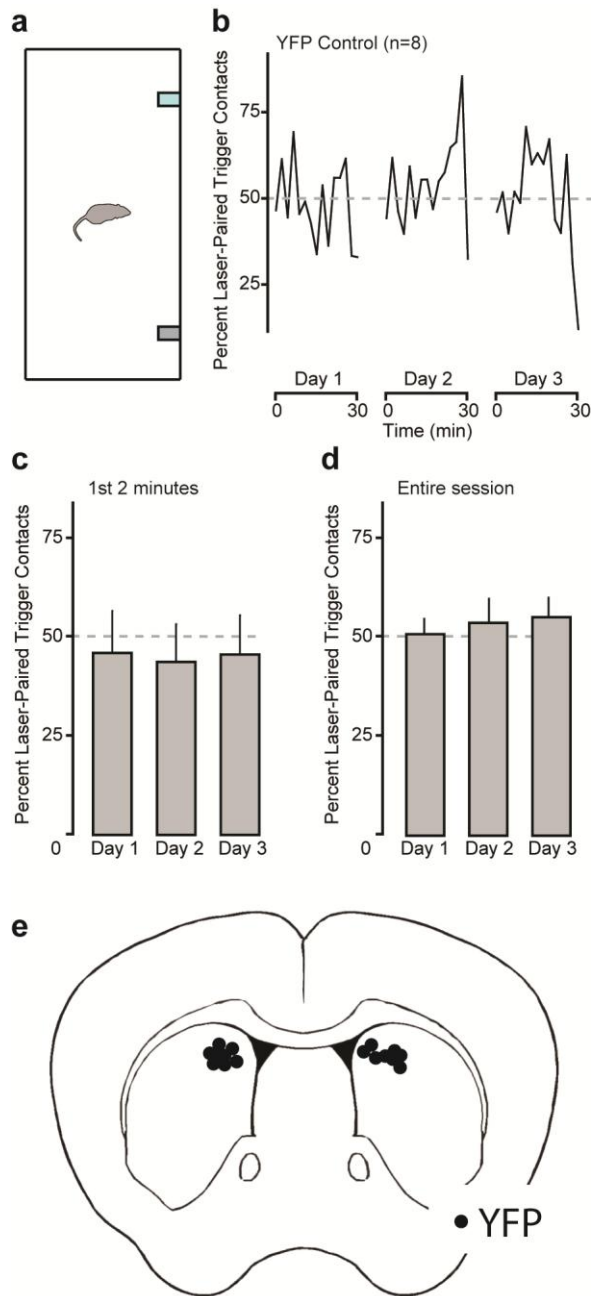
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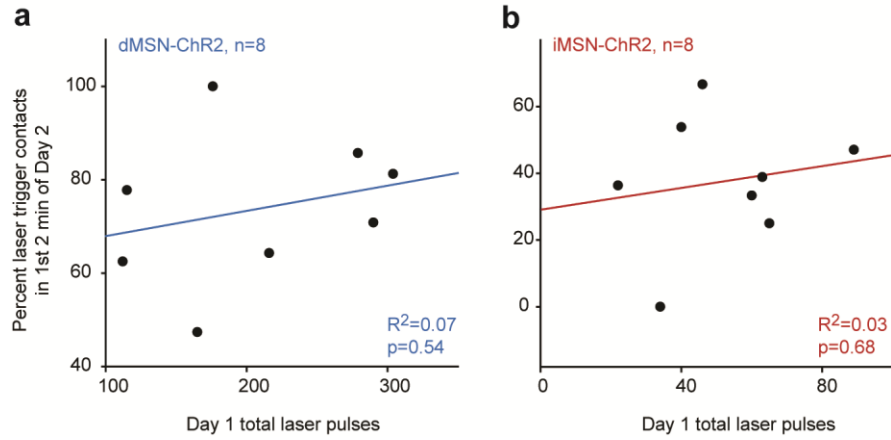
**Supplemental Figure S1. Optogenetic identification of dMSNs in awake *in vivo* recordings.** (a) photograph of an optrode array consisting of 16 tungsten microwires and one optical fiber. (b) photograph of a mouse with an optrode array hooked up to the recording and illumination systems. (c) example spike waveforms from 3 minutes of recording of a ChR2-expressing dMSN with the laser off. inset: principal component analysis showing cluster separation. (d) waveforms of the same dMSN with the laser on at 1mW. (e) example rasterplot and peri-laser histogram of a dMSN recorded from an awake mouse, showing light-evoked spiking at 0.1mW, 0.3mW, 1mW and 3mW. (f) rasterplot and peri-laser histogram of neuron in (e) zoomed in on 100msec before and after the start of the 1mW laser pulse. (g) rasterplot and peri-laser histogram of neuron in (e) zoomed in on 100 msec before and after the end of a 1mW laser pulse. (h) average firing of all identified dMSNs at each laser intensity. (i) scatterplot of average firing rate at different laser intensities, fit with an exponential rise to max regression. (j) scatterplot of number of responsive neurons at different laser intensities, fit with a linear regression.



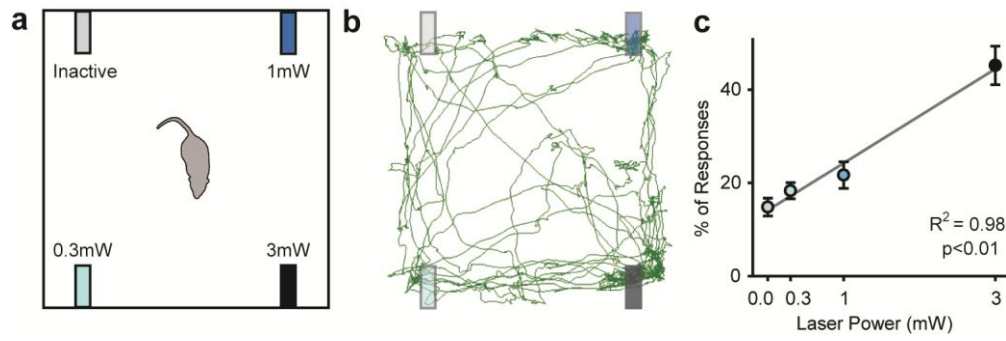
**Supplemental Figure S2. Fiber placements for experiments in the main text. (a)** Fiber placements for the operant experiment in Figure 1. **(b)** Fiber placements for the dopamine antagonist experiment and place preference experiment in Figures 2 and 3.



**Supplemental Figure S3. YFP control data.** (a) schematic of operant chamber with laser-paired and inactive triggers. (b-d) percent of laser-paired trigger contacts for YFP control mice (2-minute bins). (e) fiber placements for YFP control mice.



**Supplemental Figure S4. Laser experience on Day 1 does not predict behavior at the start of Day 2.** (a) Correlation between number of contacts with laser-paired trigger on Day 1 and initial trigger preference on Day 2 dMSN-ChR2 mice. (b) same as (a) for iMSN-ChR2 mice.



**Supplemental Figure S5. Characterization of reinforcing properties of dMSN stimulation at different laser intensities.** (a) schematic of operant chamber with four operant triggers. (b) 3-minute path plot of a dMSN-ChR2 mouse interacting with the four triggers. (c) scatterplot of the percent of responses on each trigger, fit with a linear regression.

Waveform Statistics	n	Waveform Amplitude	Trough-Peak Duration
dMSNs	19	169.3 ± 16.9	442.1 ± 46.4
Unidentified	29	188.1 ± 12.9	434.5 ± 39.0
p-value		0.377	0.901

Channel Statistics	n	Clustering MANCOVA p-value	J3 Statistic	Davies-Bouldin Statistic
with dMSNs	18	0.002 ± 001	2.3 ± 0.4	0.17 ± 0.03
Unidentified	21	0.015 ± 0.013	2.6 ± 0.6	0.17 ± 0.04
p value		0.327	0.701	0.899

**Supplemental Table S1. Waveform and channel clustering statistics for identified dMSN vs. other cells and channels. Top,** Amplitude and duration of recorded waveforms of identified dMSNs and unidentified units in these same recordings. **Bottom,** Statistics comparing clustering quality of channels that contained identified dMSNs vs. channels that did not.

Group	Session	Inactive responses	Laser responses	p-value
dMSN-ChR2	Day 1	86.3 ± 8.9	207.1 ± 27.3	0.000615*
dMSN-ChR2	Day 2	96.6 ± 10.8	249.9 ± 31.3	0.003151*
dMSN-ChR2	Day 3	77.8 ± 11.0	273.0 ± 14.7	0.000030*
iMSN-ChR2	Day 1	121.9 ± 13.9	52.4 ± 7.5	0.000026*
iMSN-ChR2	Day 2	108.3 ± 13.6	54.6 ± 8.9	0.000099*
iMSN-ChR2	Day 3	123.9 ± 24.9	62.3 ± 6.2	0.037403*
Control	Day 1	67.8 ± 11.0	74.9 ± 12.3	0.552512
Control	Day 2	67.3 ± 12.1	90.8 ± 22.2	0.400039
Control	Day 3	79.5 ± 20.9	92.0 ± 20.2	0.619431

Group	Session	Inactive responses	Laser responses	p-value
dMSN-ChR2	Retraining	78.9 ± 6.5	220.5 ± 23.6	0.000035*
dMSN-ChR2	Extinction	55.9 ± 6.5	110.1 ± 19.1	0.005453*
iMSN-ChR2	Retraining	104.5 ± 14.5	43.3 ± 7.7	0.001810*
iMSN-ChR2	Extinction	72.3 ± 16.5	71.0 ± 18.8	0.478446

**Supplemental Table S2. Trigger counts for operant experiment. Top,** Number of responses on the laser and inactive triggers for the three daily sessions in Figure 1. **Bottom,** Number of responses on the laser and inactive triggers for retraining and extinction sessions. \* in the p-value column marks sessions with significant differences between laser and inactive responses ( $p < 0.05$ ).



Group	Session	Inactive responses	Laser responses	p-value
dMSN-ChR2 Saline-Treated	Day 1	87.6 ± 19.0	226.4 ± 35.9	0.028376*
dMSN-ChR2 Saline-Treated	Day 2	78.2 ± 5.2	267.8 ± 35.9	0.005026*
dMSN-ChR2 Saline-Treated	Day 3	52.0 ± 5.1	235.4 ± 10.0	0.000018*
dMSN-ChR2 Antagonist-Treated	Day 1	61.5 ± 13.6	164.2 ± 49.6	0.031785*
dMSN-ChR2 Antagonist-Treated	Day 2	58.7 ± 12.2	208.7 ± 21.7	0.000997*
dMSN-ChR2 Antagonist-Treated	Day 3	48.2 ± 8.6	191.7 ± 16.5	0.000489*
iMSN-ChR2 Saline-Treated	Day 1	124.0 ± 13.5	51.9 ± 4.4	0.001220*
iMSN-ChR2 Saline-Treated	Day 2	83.6 ± 11.0	53.7 ± 7.5	0.020731*
iMSN-ChR2 Saline-Treated	Day 3	81.3 ± 20.1	52.6 ± 6.4	0.132385
iMSN-ChR2 Antagonist-Treated	Day 1	71.4 ± 14.0	44.6 ± 8.9	0.031517*
iMSN-ChR2 Antagonist-Treated	Day 2	102.4 ± 17.8	54.0 ± 10.3	0.011596*
iMSN-ChR2 Antagonist-Treated	Day 3	116.8 ± 26.0	76.1 ± 15.2	0.046400*

Group	Session	Inactive responses	Laser responses	p-value
dMSN-ChR2 Antagonist-Treated	Day 4	74.6 ± 12.2	250.4 ± 50.2	0.023472*
iMSN-ChR2 Antagonist-Treated	Day 4	76.1 ± 12.6	26.7 ± 6.9	0.002097*

**Supplemental Table S3. Trigger counts for operant experiment with dopamine antagonists. Top,** Number of responses on the laser and inactive triggers for the three daily sessions in Figure 3, with dopamine antagonists or saline pre-treatment. **Bottom,** Number of responses on the laser and inactive triggers for 4<sup>th</sup> day of training for previously-saline treated mice. \* in the p-value column marks sessions with significant differences between laser and inactive responses ( $p < 0.05$ ).

### **Supplemental video captions**

**Video S1:** Reinforcement of laser-paired trigger contact in a naïve dMSN-ChR2 mouse. Mouse has never received laser stimulation at start of the video, and gains a strong preference within ~10 minutes of training. Video shows first 15 minutes of the first day of training, sped up 10x.

**Video S2:** Punishment of laser-paired trigger contact in a naïve iMSN-ChR2 mouse. Mouse has never received laser stimulation at start of the video, and starts avoiding the laser-paired trigger, as well as exhibiting an “escape response” within ~10 minutes of training. Video shows first 15 minutes of the first day of training, sped up 10x.