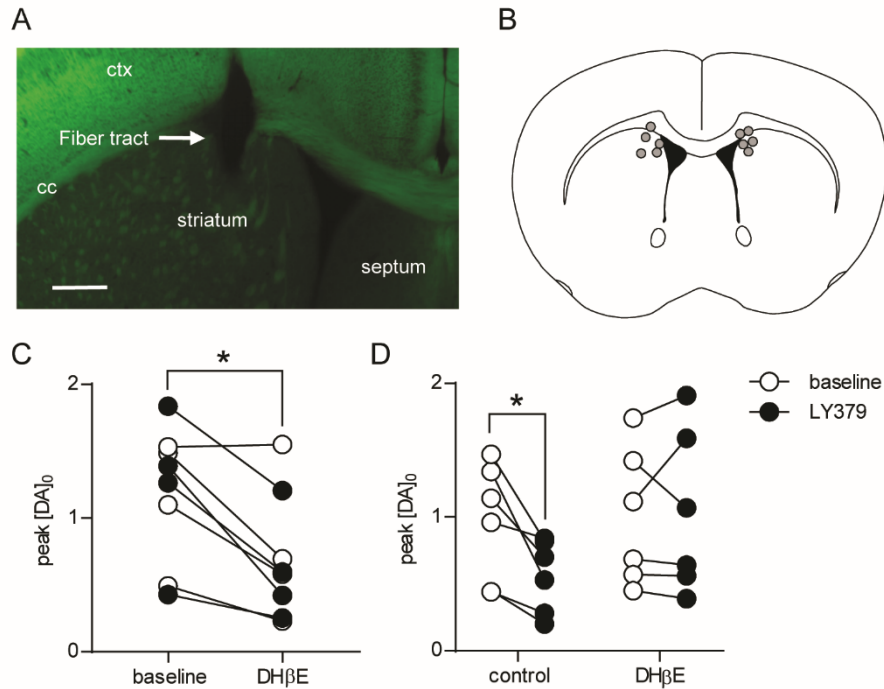
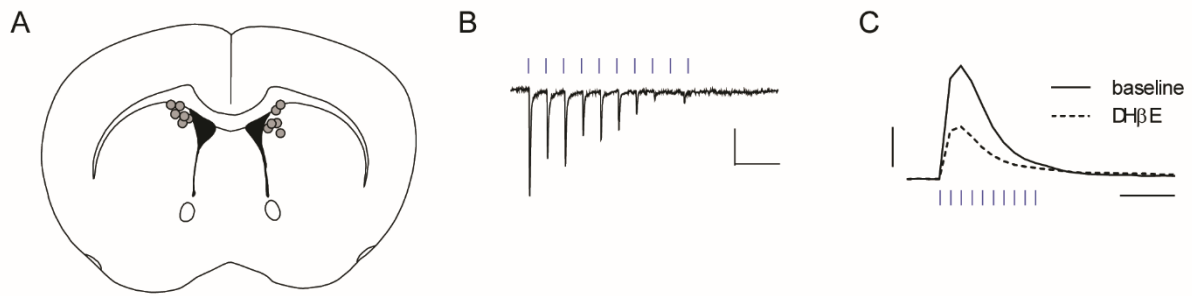


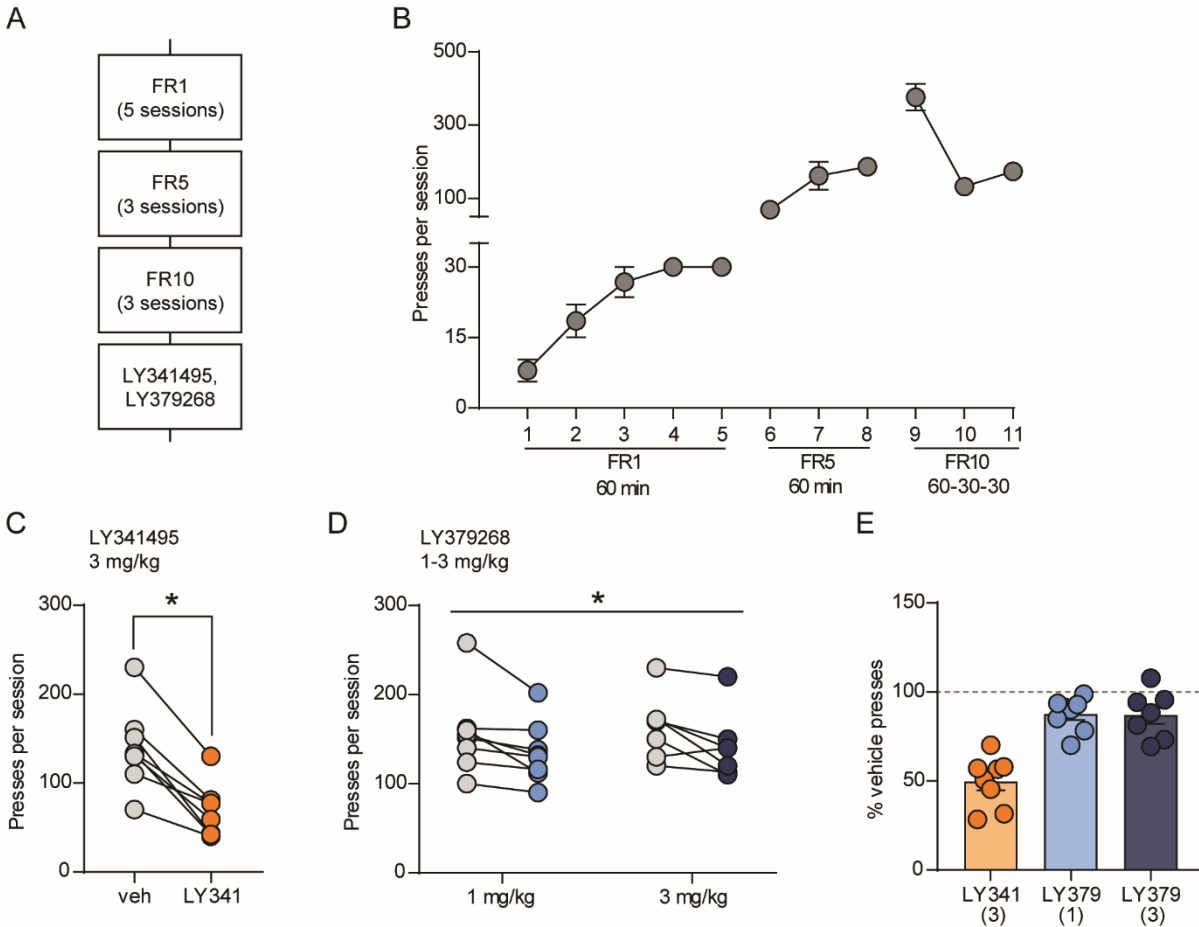
## Supplemental Figures



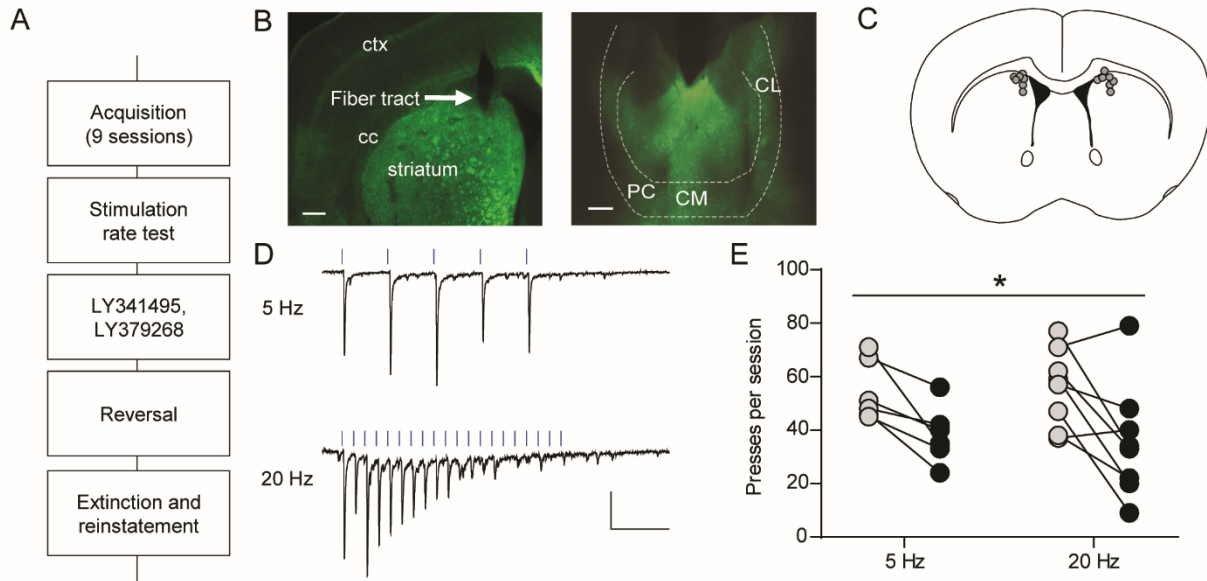
**Figure S1. Optical fiber placement and optically-evoked dopamine release in the DMS of *Vglut2-Cre<sup>+/+</sup>;Ai32<sup>+</sup>* mice.** (a) Representative image of EYFP expression in a coronal section from a *Vglut2-Cre<sup>+/+</sup>;Ai32<sup>+</sup>* mouse. Arrow indicates the optical fiber tract terminating in the DMS. Scale bar: 500  $\mu\text{m}$ . (b) Schematic of optical fiber placement in mice represented in Figs. 1 and 2 ( $n = 5$ ). (c) Peak dopamine concentrations before and after bath application of DH $\beta$ E (1  $\mu\text{M}$ ) measured in response to 20 pulses of 20-Hz optical stimulation ( $n = 4$ , closed circles) or 10 pulses of 10-Hz stimulation ( $n = 4$ , open circles) in the DMS of *Vglut2-Cre<sup>+/+</sup>;Ai32<sup>+</sup>* using FSCV. DH $\beta$ E reduced peak dopamine responses to  $56.2 \pm 7.4\%$  of baseline.  $*t_{(7)} = 4.22$ ,  $p = 0.0039$ , paired t-test. (d) Peak dopamine concentrations before (open circles) and after (closed circles) bath application of LY379268 (100 nM) in the absence or presence of DH $\beta$ E (1  $\mu\text{M}$ ). Dopamine release was evoked by 10 pulses of 10-Hz stimulation. Two-way RM ANOVA revealed a main effect of LY379268 ( $F_{(1,10)} = 5.518$ ,  $p = 0.0407$ ) and a significant LY379268 x DH $\beta$ E interaction ( $F_{(1,10)} = 7.363$ ,  $p = 0.0218$ ). *Post hoc* comparisons demonstrated that LY379268 reduced peak dopamine release in control slices, but not in the presence of DH $\beta$ E ( $*p = 0.01$  without DH $\beta$ E;  $p = 0.96$  with DH $\beta$ E; Sidak's multiple comparisons test). Abbreviations: ctx, cortex; cc, corpus callosum.



**Figure S2. Optical fiber placement and optically-evoked EPSCs and dopamine release in the DMS of *Vglut2-Cre<sup>+/+</sup>;Ai32<sup>+</sup>* mice.** (a) Schematic of optical fiber placement in mice represented in Fig. 3 (n = 6). (b) Representative whole-cell recording of EPSCs in a DMS medium spiny neuron in response to 10 pulses of 10-Hz optical stimulation. Scale bars: 100 pA, 0.25 s. (c) Representative FSCV traces of dopamine release measured in the DMS in response to 10 pulses of 10-Hz optical stimulation, before and after bath application of DHβE (1 μM). Scale bars: 0.5 μM dopamine, 0.5 s.



**Figure S3. Modulation of operant responding for food reinforcement by pharmacological manipulation of mGlu<sub>2/3</sub>.** (a) Timeline of experimental procedures. (b) Acquisition of operant responding for food reinforcement over 11 training sessions ( $n = 8$ ). (c-d) Within-subject comparisons of lever presses per session after injection of vehicle or 3 mg/kg LY341495 (c), or 1 mg/kg and 3 mg/kg LY379268 (d) ( $n = 7-8$ ). For (c), paired t-test:  $t_{(7)} = 6.846$ ,  $*p = 0.0002$ . For (d), two-way RM ANOVA: main effect of LY379268 treatment ( $F_{(1,13)} = 16.07$ ;  $*p = 0.0015$ ). There was not a significant LY379268 x dose interaction ( $F_{(1,13)} = 0.002$ ;  $p = 0.96$ ). (e) Average lever presses per session (normalized to vehicle presses for each mouse) for each drug treatment. Bars represent mean  $\pm$  SEM, and individual data points are overlaid. Doses of each drug (mg/kg) are indicated in parentheses.



**Figure S4. ChR2 expression, optical fiber placement, and stimulation rate modulation of active lever pressing in C57BL/6J mice expressing ChR2 in thalamostriatal terminals.** (a) Timeline of experimental procedures. (b) Representative images of EYFP expression in the anterior intralaminar nuclei of the thalamus (right; scale bar: 250  $\mu\text{m}$ ) and optical fiber placement in the DMS (left; scale bar: 500  $\mu\text{m}$ ). ChR2 expression also extended posterior to the parafascicular nucleus in most animals. Arrow indicates the optical fiber tract terminating in the DMS. (c) Schematic of optical fiber placement in mice represented in Figs. 4 and 5 ( $n = 7$ ). (d) Representative whole-cell recording of EPSCs in a DMS medium spiny neuron in response to 5 pulses of 5-Hz optical stimulation or 20 pulses of 20-Hz optical stimulation. Scale bars: 100 pA, 0.25 s. (e) Within-subject comparisons of active lever presses for 10-Hz vs. 5-Hz or 20-Hz stimulation ( $n = 6-8$ ). Two-way ANOVA revealed a main effect of altered stimulation rate ( $F_{(1,12)} = 16.97$ ,  $*p = 0.002$ ), but no rate-specific differences ( $F_{(1,12)} = 0.009$ ,  $p = 0.93$ ). Abbreviations: ctx, cortex; cc, corpus callosum; CL, centrolateral nucleus of the thalamus; CM, centromedial nucleus of the thalamus; PC, paracentral nucleus of the thalamus.

## **Supplemental Results**

### **Emergence of movements associated with optical stimulation**

In both Vglut2-Cre<sup>+/-</sup>;Ai32<sup>+/-</sup> mice and C57BL/6J mice injected with ChR2-encoding virus in the thalamus, we observed movements associated with optical stimulation in the DMS. Movements varied in type and magnitude both within and between animals, and included head turns, rearing, and partial or full rotations. Movements were not observed during passive delivery of stimulation trains during shaping or during the first session in which the mouse began lever pressing, but emerged by the third session in which the mouse pressed the lever, suggesting that the movements represent conditioned responses (**Supplemental video 1**). Interestingly, electrical stimulus-driven ICSS of dorsal striatum sites has been reported to produce similar movements [1].

### **Detailed results associated with Fig. 4b,c**

ChR2-injected mice on average engaged in 37.17 (95% CI: [19.08, 55.25]) more active lever presses per session compared to EGFP-injected mice during later sessions ( $t_{(10)} = 4.03$ ,  $p = 0.0024$ ). On average, ChR2-injected mice performed 45.47 (95% CI: [37.53, 53.40]) more active lever presses per session on later sessions compared to the first session ( $t_{(193)} = 5.60$ ,  $p < 0.0001$ ). Similarly, ChR2-injected mice engaged in an average of 47.22 (95% CI: [30.71, 63.74]) fewer inactive lever presses per session than active lever presses on later sessions, ( $t_{(193)} = 11.23$ ,  $2 p < 0.0001$ ). Finally, among ChR2-injected mice, there was a significant interaction between lever-type and session ( $t_{(193)} = 3.64$ ,  $p = 0.0003$ ), indicating that the difference between active and inactive lever presses was on average 43.38 (95% CI: [20.03, 66.74]) greater on later sessions compared to the first session among ChR2-injected mice.

### **References associated with Supplemental Results**

1. Phillips AG, Carter DA, Fibiger HC. Dopaminergic substrates of intracranial self-stimulation in the caudate-putamen. Brain Res. 1976;104:221-232.