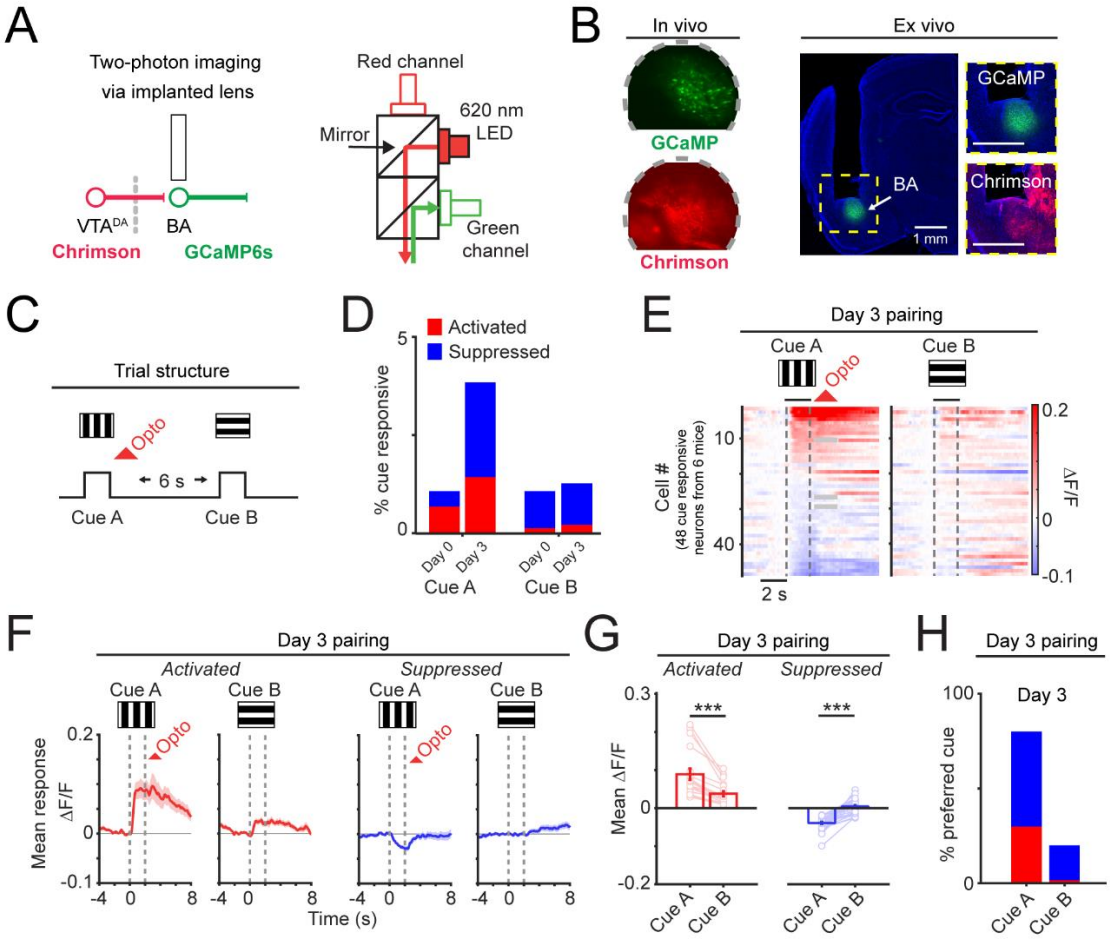

Figure S1. Two-photon calcium imaging during pairing of a visual stimulus with photostimulation of VTA$^{DA\rightarrow BA}$ axons. Related to Figures 1 to 4.

A) *Left*: schematic of *in vivo* two-photon calcium imaging of GCaMP6s fluorescence and photostimulation of dopamine release. VTA$^{DA\rightarrow BA}$ dopamine axons expressing Chrimson were stimulated using red-light illumination via the same implanted lens used to record GCaMP6s signals from BA glutamatergic neurons. *Right*: schematic of two-photon microscope optical path for red-light illumination and concurrent collection of green GCaMP6s fluorescence emission. **B)** *Top left*: example *in vivo* image of GCaMP6s expression in BA glutamatergic neurons. *Bottom left*: *in vivo* image of VTA$^{DA\rightarrow BA}$ axons expressing Chrimson-tdTomato in the same field of view. *Right*: *ex vivo* post-hoc histology from the same animal as *left*, showing precise targeting of cADDis expression to BA and as well as Chrimson-expressing VTA$^{DA\rightarrow BA}$ dopamine axons. **C)** Diagram of trial structure design for pairing one visual stimulus (vertically oriented drifting bars, "Cue A"; 2 s duration) followed 200 ms later by photostimulation (5 mW, 2 s duration; 15.5 Hz). A second visual stimulus (horizontally oriented drifting bars, "Cue B"; 2 s duration) was not paired

with photostimulation. **D)** Percentage of all neurons with significant cue responses on "Day 0" before photostimulation (Cue A: 14/1283 neurons; Cue B: 14/1283 neurons from 7 mice) and following three days of photostimulation pairing (Cue A: 48/1199 neurons; Cue B: 12/1199 neurons from 6 mice). **E)** Heatmap with rows depicting mean response of BA neurons (n = 48 neurons, 14 fields of view from 6 mice) during presentation of each cue on the third day of photostimulation. **F)** Mean time course of responses to visual stimuli and photostimulation across all BA neurons that were significantly activated (red; n = 18 neurons) or suppressed (blue; n = 30 neurons) by "Cue A". Cue-evoked suppression is likely a consequence of activation of local inhibitory interneurons within the BA. **G)** Mean response to "Cue A" or "Cue B" for activated (*left*, n = 18 neurons from 6 mice, ***, $p < 0.001$) and suppressed neurons (*right*, n = 30 neurons from 6 mice, ***, $p < 0.001$). Error bars: s.e.m. across neurons. Two-sided Wilcoxon sign-rank. **H)** Percentage of cue-responsive neurons preferring (i.e., maximally responsive to) a given cue following three days of photostimulation (Cue A: 48/60 neurons; Cue B: 12/60 neurons).

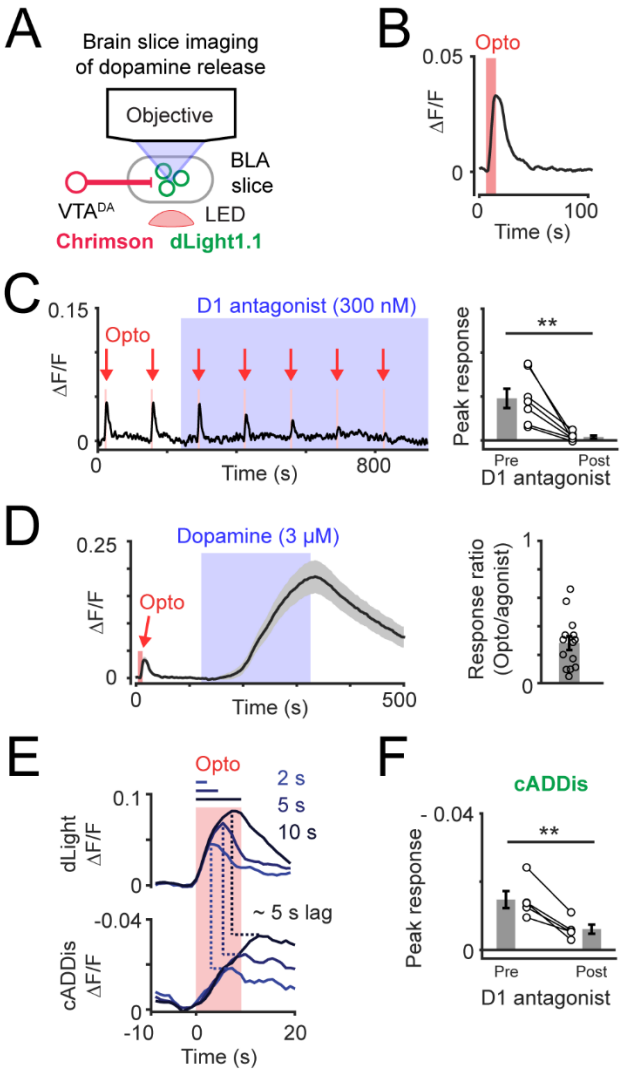

Figure S2. Photostimulation of VTA$^{DA→BA}$ axons transiently elevates dopamine in BA. Related to Figure 1 and 2.

A) Schematic of brain slice imaging of photostimulation-evoked dopamine release. VTA$^{DA→BA}$ dopamine axons expressing Chrimson were stimulated using red-light illumination from below the slice. Dopamine levels were determined by two-photon imaging of dLight1.1 expressed in BLA neurons. **B)** Mean dLight1.1 fluorescence during photostimulation of dopamine release (5 s duration; 15.5 Hz). n = 14 slices from 5 mice. **C)** Application of dopamine D1 receptor antagonist (SCH23390; 300 nM) blocks photostimulation-evoked dLight1.1 signal. *Right*: peak photostimulation-evoked dLight1.1 response before and after antagonist. ** p = 0.006, n = 7 slices from 2 mice. Two-tailed paired t-test. **D)** *Left*: mean time course in response to photostimulation (5 s duration; 15.5 Hz) followed by application of dopamine (3 μM). Mean ± s.e.m. *Right*: response ratio of exogenous dopamine vs.

photostimulation-evoked dopamine release. n = 14 slices from 5 mice. **E)** *Top*: mean time course of dLight1.1 fluorescence change in response to photostimulation of $VTA^{DA \rightarrow BA}$ axons for 2, 5, or 10 s. *Bottom*: mean time course of cADDis fluorescence changes in response to photostimulation of $VTA^{DA \rightarrow BA}$ axons for 2, 5, or 10 s. **F)** Peak photostimulation-evoked cADDis response before and after D1 antagonist (SCH23390; 300 nM). **, p = 0.005, n = 5 slices from 3 mice. Two-tailed paired t-test. All panels show mean ± s.e.m.

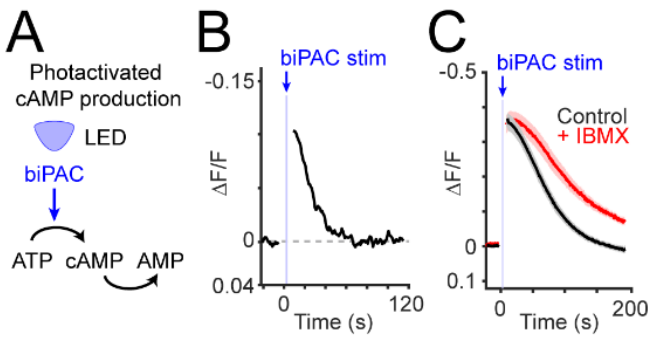

Figure S3. Photostimulation of direct cAMP production drives persistently elevated cAMP. Related to Figure 1.

A) Schematic of cAMP production using biPAC (blue-light stimulation of adenylate cyclase). **B)** Example recording of cADDis fluorescence during biPAC photostimulation (2 s duration; continuous illumination). Recording performed at 32°C. **C)** Stimulation of biPAC (2 s duration) in control slices (black; n = 13 slices from 4 mice) or in the presence of a phosphodiesterase inhibitor (IBMX, 100 µM; red; n = 4 slices from 4 mice). Recording performed at room temperature. Mean ± s.e.m.

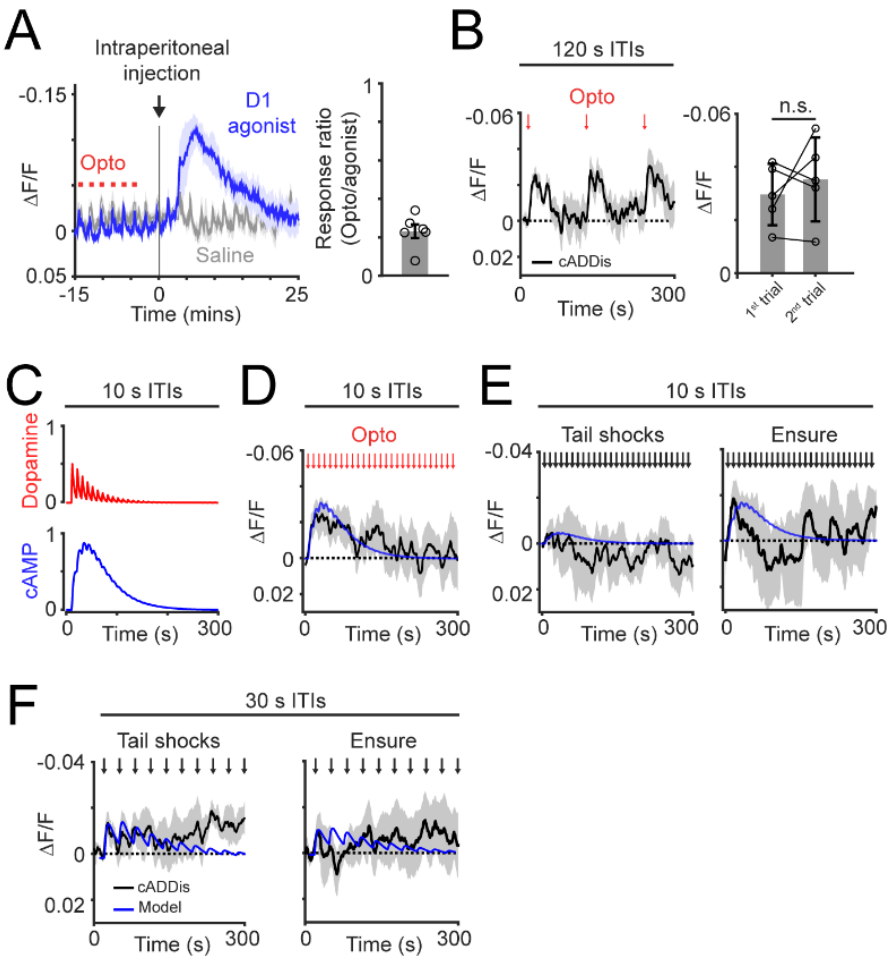

Figure S4. Additional fiber photometry of cADDis. Related to Figure 3.

A) *Left*: mean time course of cADDis fluorescence in response to intraperitoneal injection of a Type 1 dopamine receptor (D1) agonist (blue; SKF81297, 20 mg/kg, 150 µL; n = 6 mice) or saline (gray, 150 µL; n = 5 mice). Right: ratio of responses to photostimulated dopamine release vs. application of a D1 agonist. n = 6 mice. **B)** *Left:* mean time course of cADDis fluorescence in response to photostimulation of $VTA^{DA \rightarrow BA}$ axons (2 s duration; 20 Hz) every 120 s. n = 5 mice. *Right:* comparison of peak response following first or second trial of photostimulation (n.s., $p > 0.05$, n = 5 mice). Two-sampled paired t-test. **C)** Simulation of repeated 2 s-duration stimulations of dopamine release occurring with 10 s inter-trial intervals (ITI). **D)** Mean time course of cADDis fluorescence (black line) in response to photostimulation of $VTA^{DA \rightarrow BA}$ axons (2 s duration; 20 Hz) every 10 s. Simulated cAMP dynamics (blue line) scaled to peak photometry amplitude. n = 5 mice. **E)** *Left:* mean time course of cADDis fluorescence in response to uncued tail shock delivery (0.3 mA; 50 ms duration) delivered every 10 s. Mean ± s.e.m. n = 4 mice. Simulated cAMP dynamics (blue line) scaled to peak photometry amplitude. *Right:* mean time course of cADDis

fluorescence in response to uncued Ensure delivery (single 5 µL droplet) delivered every 10 s. Simulated cAMP dynamics (blue line) scaled to peak photometry amplitude. n = 3 mice. **F)** *Left:* mean time course of cADDis fluorescence in response to uncued tail shock delivery (0.3 mA; 50 ms duration) every 30 s. *Right:* mean time course of cADDis fluorescence in response to uncued Ensure delivery (single 5 µL droplet) every 30 s. Recording normalized to baseline period before first trial. n = 5 mice. Simulated cAMP dynamics (blue line; based on model shown in Figure 3E) scaled to peak photometry amplitude. All panels show mean ± s.e.m.