

Supplementary information, Fig. 3 Cocaine conditioning increases frequency of Ca2+ transient events in both  $D1^{NAc-VM}$  and  $D1^{NAc-VP}$  projections.

**a,c,** Schematic of experimental design.  $AAV_9-EF1\alpha$ -DIO-hChR2-mCherry or  $AAV_9-EF1\alpha$ -DIO-mCherry was injected into the NAc of DI-Cre mice, and optical fibers were bilaterally implanted over the VM or VP. Laser stimulation was presented during saline conditioning. Cocaine and LiCl was conditioned in the other side. After three-day conditioning, the CPP/A test was performed. b,d, Bar graphs of CPP score. [Two-way RM ANOVA, VM: Control n = 8, ChR2 n = 9, F treatment-session (1, 33) = 11.258, p = 0.004; VP: Control n = 8, ChR2 n = 9, F treatment-session (1, 33) = 31.94, p < 0.001.] \*\*\*\*p < 0.001. e, Schematic of experimental design.  $AAV_9$ - $EF1\alpha$ -DIO-GCaMP6m was injected into the NAc of DI-Cre mice, with optical fiber implanted over the VM or VP. f,h, Representative photometry signal traces of D1-MSN axonal terminals in the VM (f) and VP (h) during saline and cocaine conditioning. Tick marks indicate events above threshold. g,i, Frequency of  $Ca^{2+}$  transient events in  $D1^{NAc-VM}$  (g) and  $D1^{NAc-VP}$  (i) terminals was calculated during 5-10 min after saline or cocaine treatment [Two-tailed Student's t-test, n = 11, t(20) = -2.336, p = 0.0282; Mann-Whitney U test, n = 13, Z = 2.052, p = 0.0387.] \*p < 0.05. Related to Figure 3.