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Supplemental Information

Dopamine-Evoked Synaptic Regulation in the Nucleus Accumbens Requires Astrocyte Activity

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%Justin Lines 2019
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%This program takes photometry traces and analyzes
risetime/decaytime/onset

%Clear workspace
clear all
%Load Excel of Photometry Traces
[fname pathway]=uigetfile('*.xlsx');
data=xlsread([pathway fname]);
%Analyze traces
for i=1:size(data,2)
    thresh=mean(data(1:5000,i))+3*std(data(1:5000,i));
    digital(:,i)=data(:,i)>thresh;
    dummy=find(digital(:,i)>0);
    if ~isempty(dummy)
        onset(i)=dummy(1);
        backdown(i)=dummy(end);
        [amplitude(i),peak(i)]=max(data(5000:end,i));
        rise(i)=peak(i)-onset(i);
        decay(i)=backdown(i)-peak(i);
        width(i)=decay(i)+rise(i);
    else
        onset(i)=nan;
        backdown(i)=nan;
        [amplitude(i),peak(i)]=max(data(5000:end,i));
        rise(i)=nan;
        decay(i)=nan;
        width(i)=nan;
    end
end
%Setup outputs
output=[onset' backdown' rise' decay' width' peak' amplitude'];
%Export analysis
xlswrite([pathway '\' fname(1:end-5) 'data.xlsx'],output)
```

Data S1. MATLAB code. Related to STAR Methods. MATLAB code generated for fiber photometry analysis.

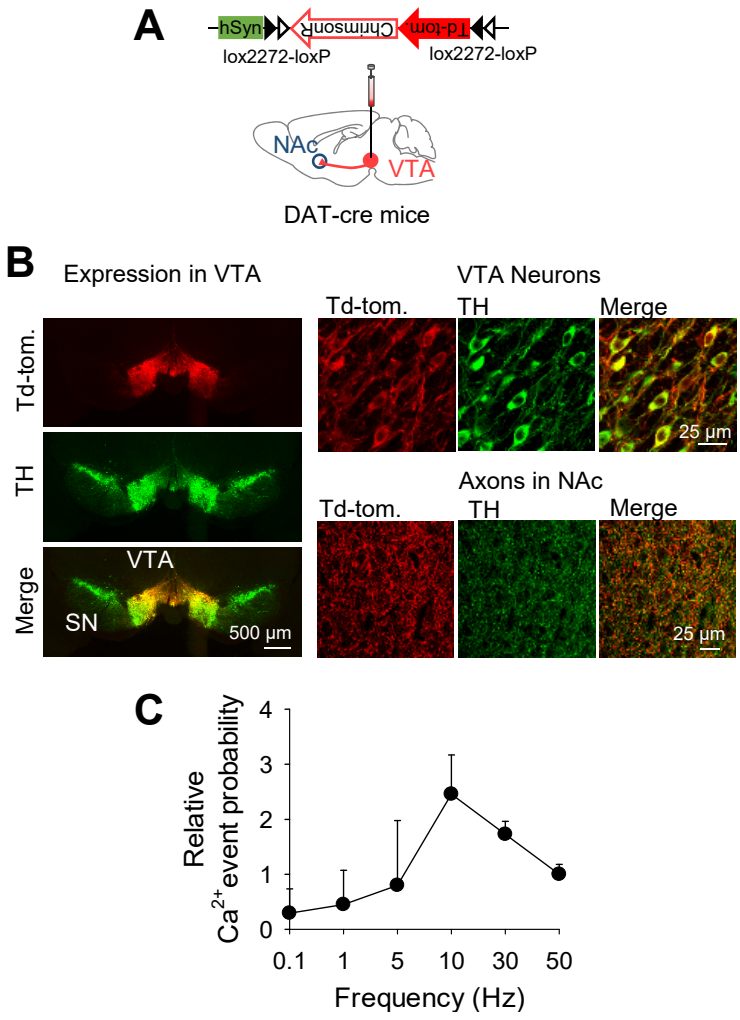

Figure S1. ChrimsonR expression in dopaminergic neurons. Related to Figure 2. **(A)** Viral vector injected into the VTA of DAT-cre mice. **(B)** Immunohistochemical images showing expression of AAV5-hSyn-ChrimsonR-TdTomato in neuronal somas in the VTA and axon terminals in the NAc. **(C)** Normalized mean astrocyte responses to different stimulation frequencies.

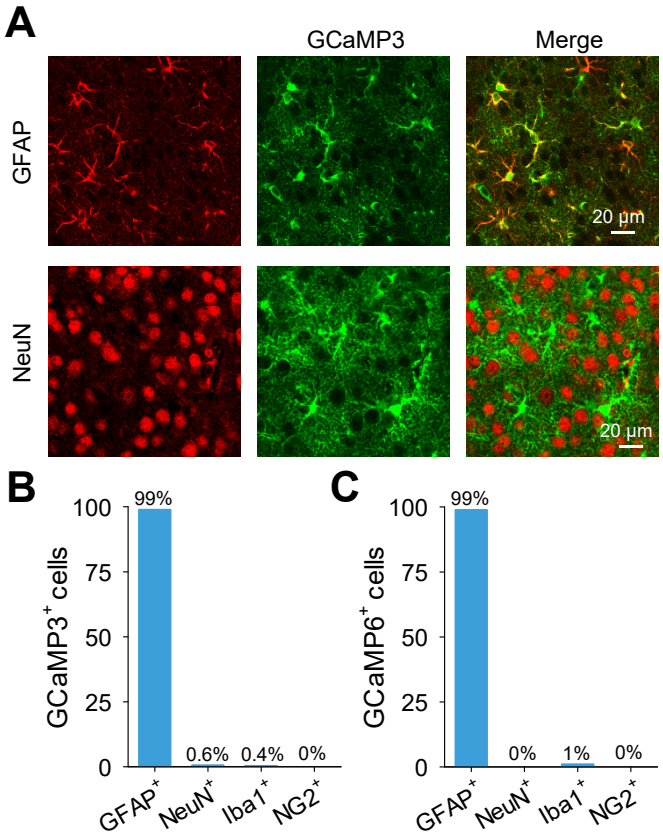

Figure S2. GCaMP expression in astrocytes. Related to STAR Methods. **(A)** Immunohistochemical images showing expression of GCaMP3 in NAc astrocytes and co-stained with GFAP or NeuN. From left to right: cell marker, GCaMP3 and merge. **(B)** Percent distribution of GCaMP3$^+$ cells (n = 511 cells). **(C)** Percent distribution of GCaMP6f$^+$ cells (n = 192 cells).

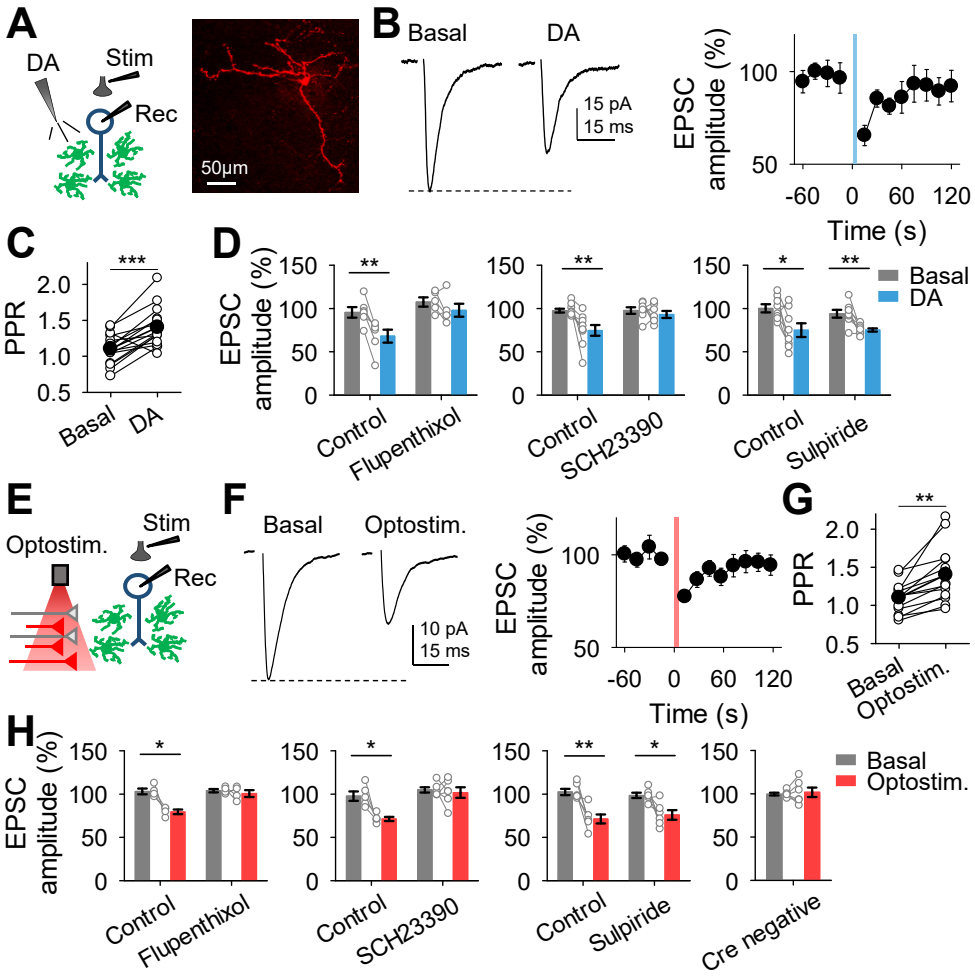

Figure S3. Dopamine depresses excitatory transmission. Related to Figure 3.
(A) Experimental scheme (right) and image of a nucleus accumbens medium spiny neuron filled with biocytin through a patch pipette (left). **(B)** Representative EPSC traces before (basal) and after DA application (right) and relative EPSC amplitude over time. **(C)** Paired pulse ratio (PPR) before (basal) and after dopamine application. Two-tailed student's paired t-test. **(D)** Relative EPSC amplitude. Two-tailed student's paired t-test. **(E-H)** as **a-d**, but for optical stimulation. Two-tailed student's paired t-test. Blue and red shadows indicate DA application and optical stimulation, respectively. Data are expressed as mean ± s.e.m., *p<0.05, **p<0.01, ***p<0.001.

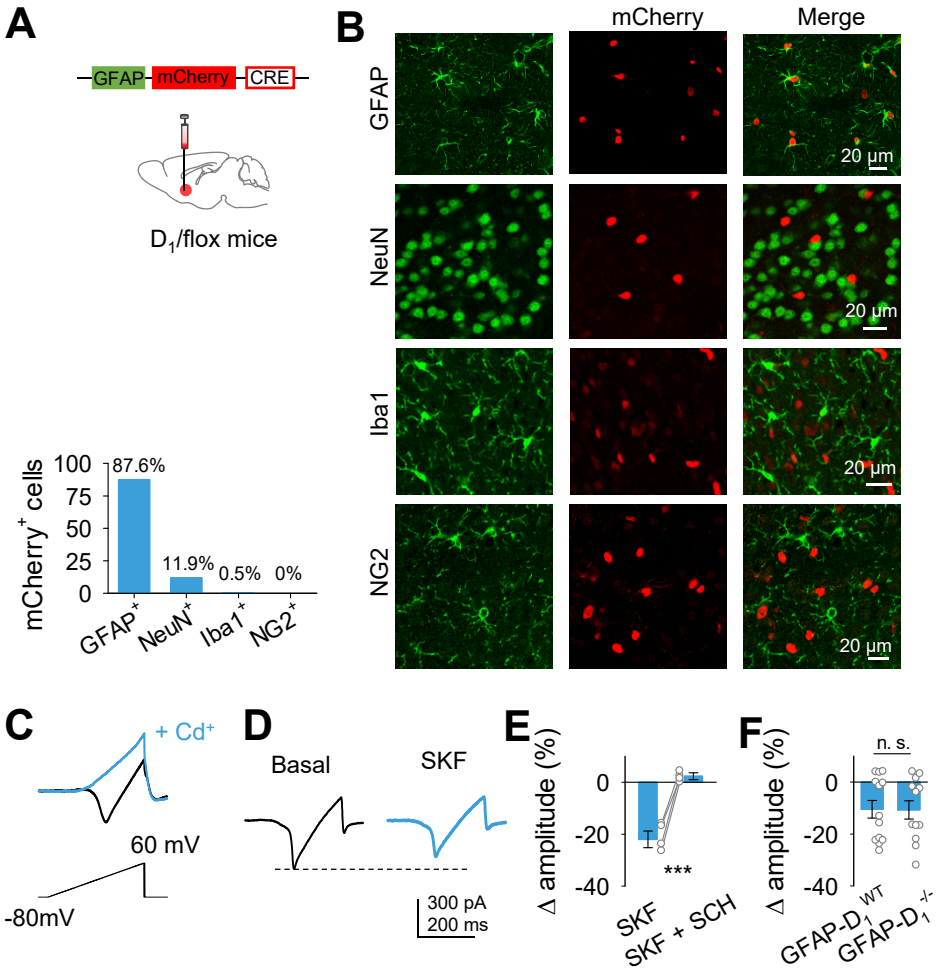

Figure S4. AAV8-GFAP-mCherry-Cre targets astrocytes. Related to Figure 4 and STAR Methods.

(A) Experimental scheme (top) and percent distribution of mCherry⁺ cells (bottom). **(B)** Immunohistochemistry images of mCherry⁺ cells co-stained with GFAP, NeuN, Iba1 or NG2 (n = 935 cells). From left to right: cell marker, mCherry, and merge. **(C)** Representative Cd+ sensitive currents. **(D)** Cd⁺ sensitive current before (basal) and after SKF 38393 application. **(E)** Relative change in amplitude of Cd⁺ sensitive current in SKF 38393 only conditions (SKF) or in SKF 38393 and SCH 23390 conditions (SKF + SCH). Twotailed student's unpaired t-test. **(F)** Relative change in amplitude of Cd⁺ sensitive current in response to SKF 38393. Data are expressed as mean ± s.e.m., ***p<0.001.

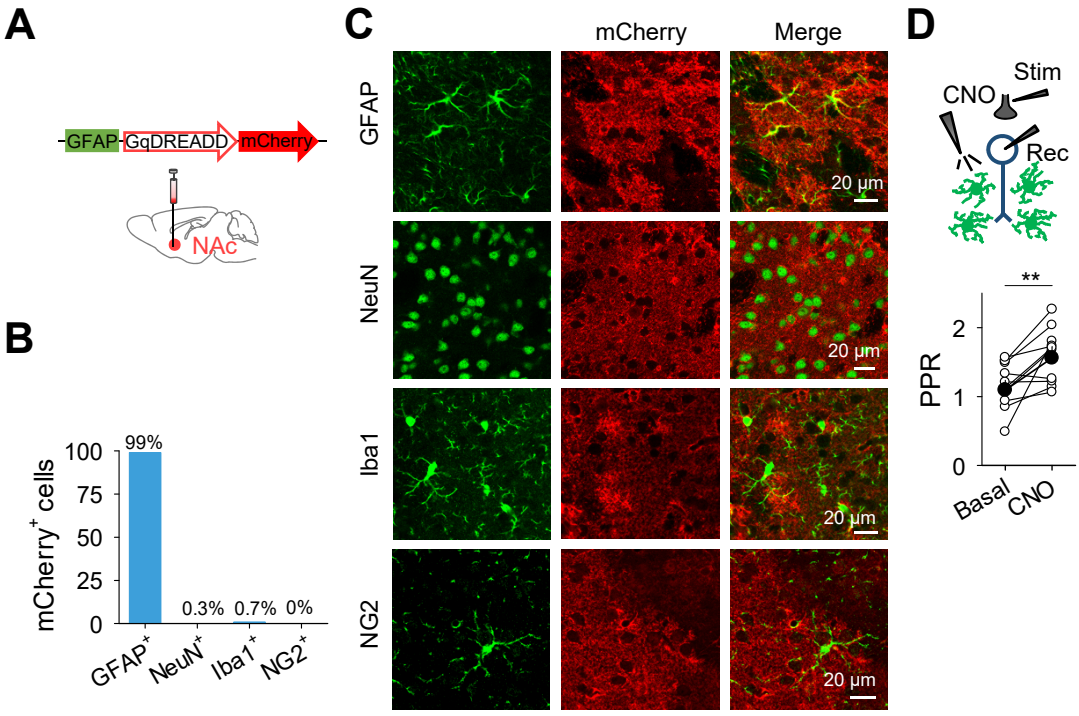

Figure S5: AAV8-GFAP-hM3D(Gq)-mCherry is specifically expressed in astrocytes. Related to Figure 6 and STAR Methods.

(A) Scheme of experimental approach. **(B)** Percent distribution of mCherry⁺ cells (n = 408 cells). **(C)** Immunohistochemistry images of mCherry⁺ cells co-stained with GFAP, NeuN, Iba1 or NG2. From left to right: cell marker, mCherry, and merge. **(D)** Experimental scheme (top) and paired pulse ratio (PPR) before (basal) and after CNO application (bottom) (n = 10). Two-tailed student's paired t-test. Data are expressed as mean ± s.e.m., **p<0.01.

Kruskal-Wallis One Way ANOVA values					
	H value	DF	P value	Post hoc	q and p values
Fig. 1D	38.085	3	<0.001	Dunn's Method	Control vs Flupenthixol: q=5. 428, p<0.001; Control vs GFP: q=3.502, p<0.001; Control vs Cre negative: q=4.949, p=0.003; Flupenthixol vs GFP: q=0.557, p=1; Flupenthixol vs Cre negative: q=1.114, p=1; GFP vs Cre negative: q=0.23, p=1

Table S1. Full report of Kruskal-Wallis One Way ANOVA values. Related to STAR Methods. Full report of H values, degrees of freedom (DF) and p value for the Kruskal-Wallis One Way ANOVA tests performed on non-parametric data and the post hoc analysis q and p values.

One way ANOVA values					
	F value	DF	p value	Post hoc	p values
Fig. 2B	4.539	4	0.004	Fisher LSD Method	Control vs Flupenthixol: p=0.008; Control vs SCH23390: p=0.03; Control vs Sulpiride: p=0.833; Control vs Cocktail: p=0.941
Fig. 2D	10.724	4	<0.001	Fisher LSD Method	Control vs Flupenthixol: p<0.001; Control vs SCH23390: p=0.003; Control vs Sulpiride: p=0.858; Control vs Cre Negative: p=0.005

Table S2. Full report of One way ANOVA values. Related to STAR Methods. Full report of F values, degrees of freedom (DF) and p value for the One way ANOVA tests performed and the post hoc analysis p values.

Transgenic mice generation using viral vectors				
			Mouse genotype	**Viral vector**
Mice expressing ChrimsonR in dopaminergic neurons	Fig. 1A-F; Fig. 2C-D; Fig. 3B, Fig. 3E-F; Fig. 5A-B; Fig. S1; Fig. S3E-H;	Control mice: "*Cre negative*"	DAT wild-type littermate mice lacking CRE	AAV5-hSyn-FLEX-ChrimsonR-tdTomato
		Transgenic mice	DAT-IRES-CRE	AAV5-hSyn-FLEX-ChrimsonR-tdTomato
GFAP-D_1 line	Fig. 4C-E; Fig. 6D, 6G-I; Fig. S4	Control mice: "*GFAP-D_1^{WT}*"	DRD1 wild-type littermate mice lacking flox	AAV8-GFAP-mCherry-CRE
		Transgenic mice: "GFAP-$D_1^{-/-}$"	DRD1 flox/flox	AAV8-GFAP-mCherry-CRE
Mice expressing DREADDs in astrocytes	Fig. 6A-C; Fig. S5	Control mice: "*mCherry*"	C57BL/6J	AAV8-GFAP -mCherry
		Transgenic mice	C57BL/6J	AAV8-GFAP-Gq-DREADD-mCherry

Table S4. Report of mice genotype and viral vectors used for generating transgenic mice. Related to STAR Methods. Report of the mice genotype and the viral vectors used to generate experimental transgenic mice and their respective control mice in main figures and supplementary figures.