Supplemental information

Co-agonist glycine controls the occurrence of bursts by activating extrasynaptic NMDARs in nigral dopamine neurons

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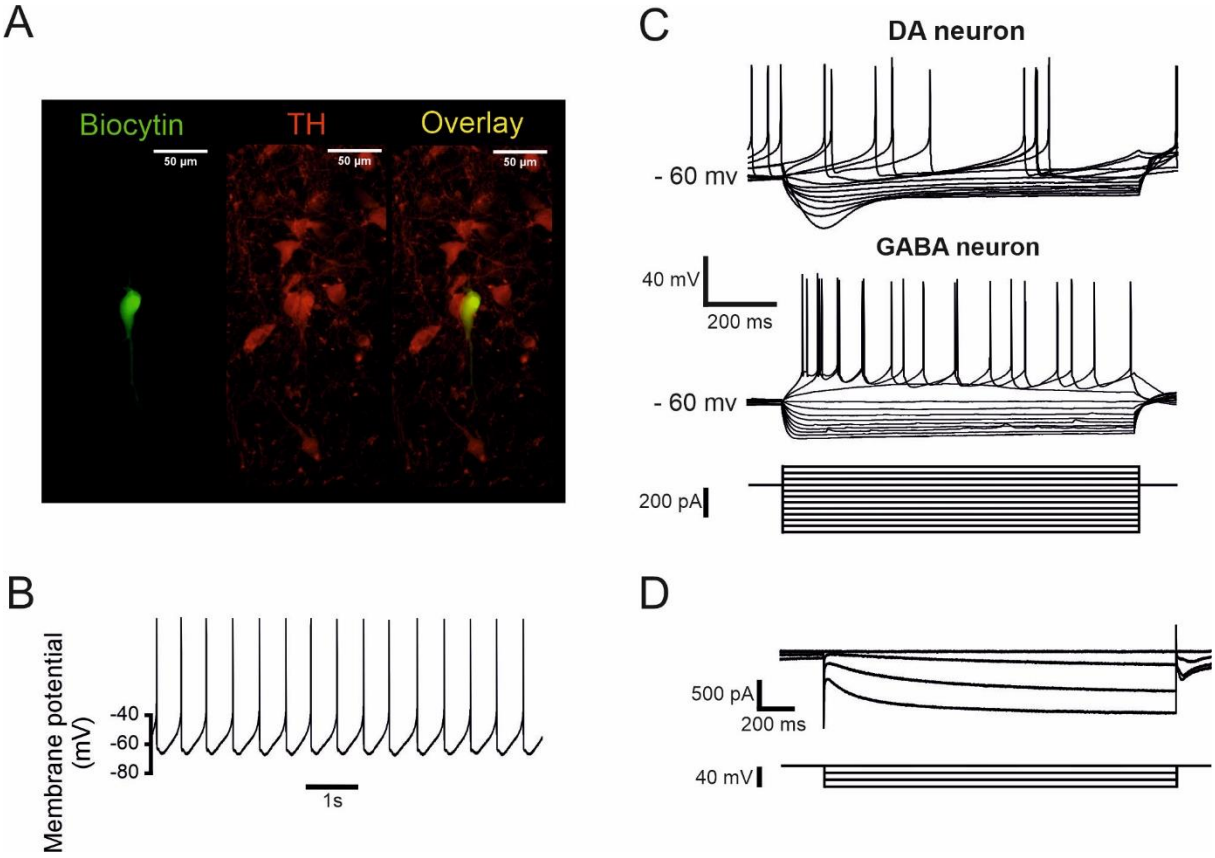


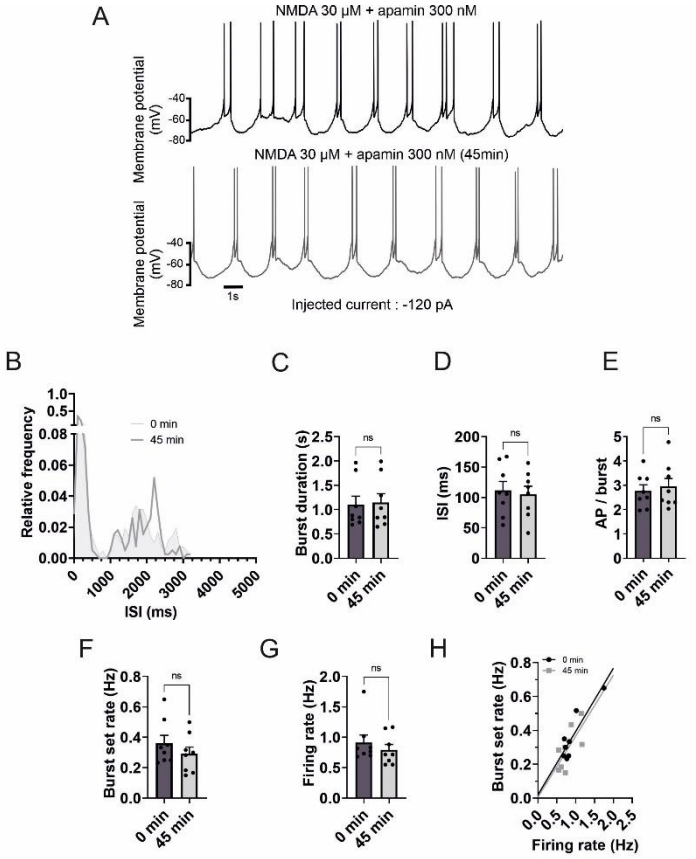
Supplementary Figure 1. Identification of SNc DA neurons

A, Epifluorescence photomicrographs of a DA neuron filled with biocytin and stained using fluorescein isothiocyanate (FITC)-conjugated avidin (left) and for tyrosine hydroxylase (TH, center). The overlay of FITC and TH is at the right.

B, Low frequency (typically ~1 – 4 Hz) spontaneous action potential firing recorded in a DA neuron in current-clamp with no current injection.

C, Voltage responses of a whole-cell recorded GABA neuron (top) and a DA neuron (middle) to 1-sec hyper- or depolarizing current pulses (−30 to 70 pA, 10 pA increment, bottom). Note the presence of a rectification in the membrane potential (sag) in the DA neuron in response to a hyperpolarizing current step which is absent in the GABA neuron.

D, Current traces in whole-cell voltage clamp in response to hyperpolarizing voltage steps showing slowly increasing currents typical for the activation of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels.



Supplementary Figure 2. Stability of bursting activity through time

A, Voltage traces showing spontaneous action potential bursts in a SNc DA neuron in the presence of NMDA (30 µM) and apamin (300 nM, top trace) and after a recording time of 45 minutes (bottom trace). The dashed square at the right side of the voltage trace is a single burst with a higher magnification.

B, ISI histogram demonstrating the distribution of burst activity in control (light grey), and after 45 min (dark grey).

C, Summary plot showing burst duration in control and after 45 min (control, 1.10 ± 0.18 s; after 45 min, 1.15 ± 0.18 s, p=0.8438). Bars are means ± SEM for n=8; Wilcoxon signed rank test.

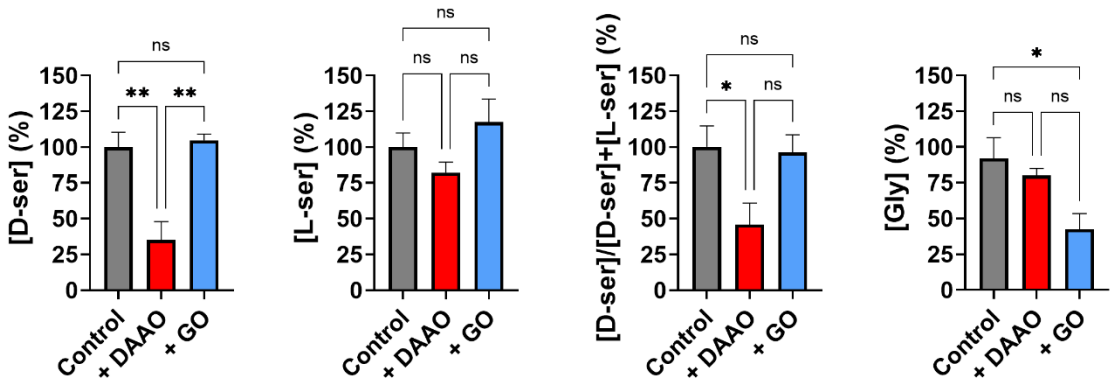
D, Summary plot showing ISI in control and after 45 min (control, 111.70 ± 14.68 ms; after 45 min, 105.20 ± 13.37 ms, p=0.6917). Bars are means ± SEM for n=8; Paired T test.

E, Summary plot showing action potential per burst in in control and after 45 min (control, 2.77 ± 0.25; after 45 min, 2.95 ± 0.33, p=0.4649). Bars are means ± SEM for n=8; Paired T-test.

F, Summary plot showing burst set rate in control and after 45 min (control, 0.36 ± 0.05 Hz; after 45 min, 0.29 ± 0.05 Hz, p=0.1752). Bars are means ± SEM for n=8; Paired T-test.

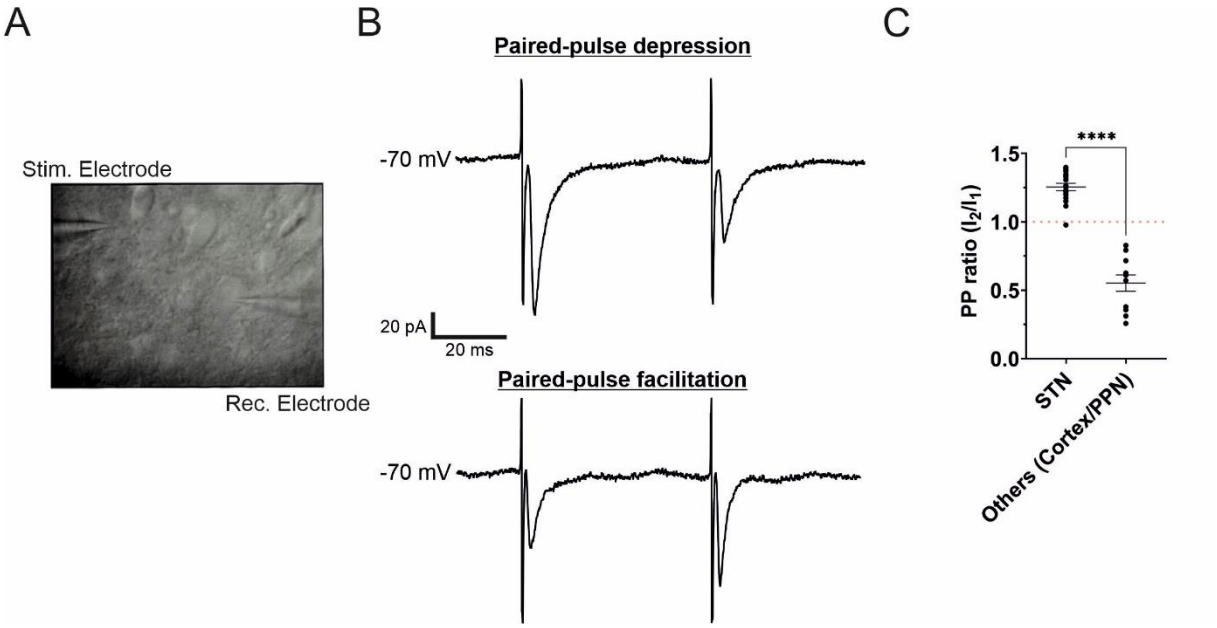
G, Summary plot showing firing rate in control and after 45 min (control, 0.91 ± 0.13 Hz; after 45 min, 0.79 ± 0.09 Hz, p=0.2656). Bars are means ± SEM for n=8; Wilcoxon signed rank test.

H, Linear regression graph between firing and burst set rate in control and after 45 min (control, R^2=0.80, slope 0.37 ± 0.08; after 45 min, R^2=0.51, slope 0.36 ± 0.15).



Supplementary Figure 3. Specificity of DAAO and GO effects in the substantia nigra.

Summary plots showing the average concentrations of D-serine, L-serine (and the D-serine/total Ser ratio) and glycine determined by HPLC analysis of aCSF medium from substantia nigra slices perfusion retrieved following experiments with control and samples treated with 0.2 U/mL of RgDAAO (+DAAO) or BsGO (+GO). Data are reported as percentage considering the control as 100% and as means ± SEM for n= 9 (control), n=6 (DAAO) and n=5 (GO); One-way anova (*P < 0.05; **P< 0.002). See text for details.

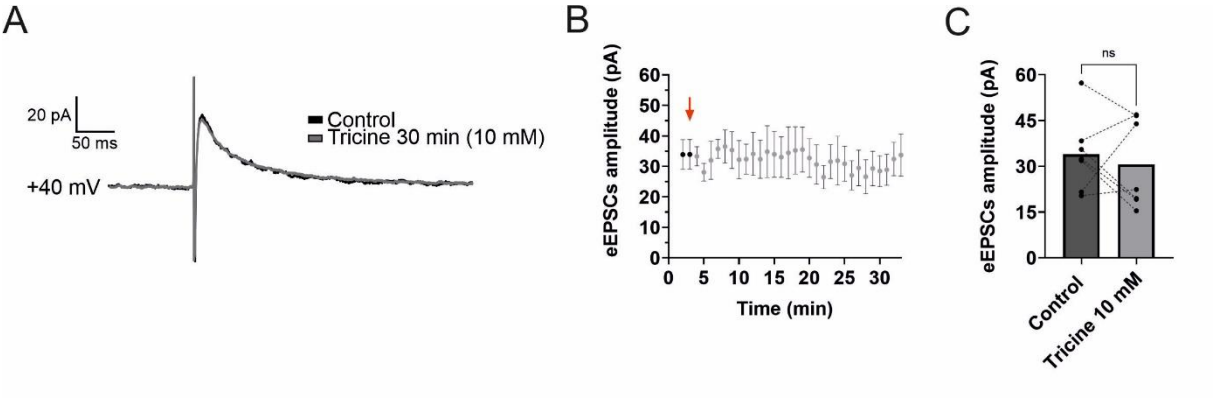


Supplementary Figure 4. Paired-pulse ratio of afferent fibers originating from the STN

***A*,** Infrared-Differential interference contrast photomicrograph of a DA neuron during whole-cell recording (right). The focal double-barreled synaptic stimulating theta electrode was placed rostrally from the recorded neuron at approximatively 50-100 µm from the soma.

B, Current traces in response to a paired-pulse protocol (50 Hz) showing a paired-pulse facilitation when stimulating STN fibers (top) and paired-pulse depression when stimulating PPN fibers (bottom). Low stimulation levels were used (typically 20–80 µA) to evoke eEPSCs.

C, Summary plot showing the PPR as a function of the origin of the fibers stimulated. For STN fibers (n=17), the PPR ≥ 1 and for PPN fibers (n=11), the PPR ≤ 1 (STN, 1.26 ± 0.03 ; PPN, 0.55 ± 0.06, $p<0.0001$) ; Unpaired T test. This distribution is in accordance with the distribution obtained using optogenetic techniques (Beaudoin et al., 2018).

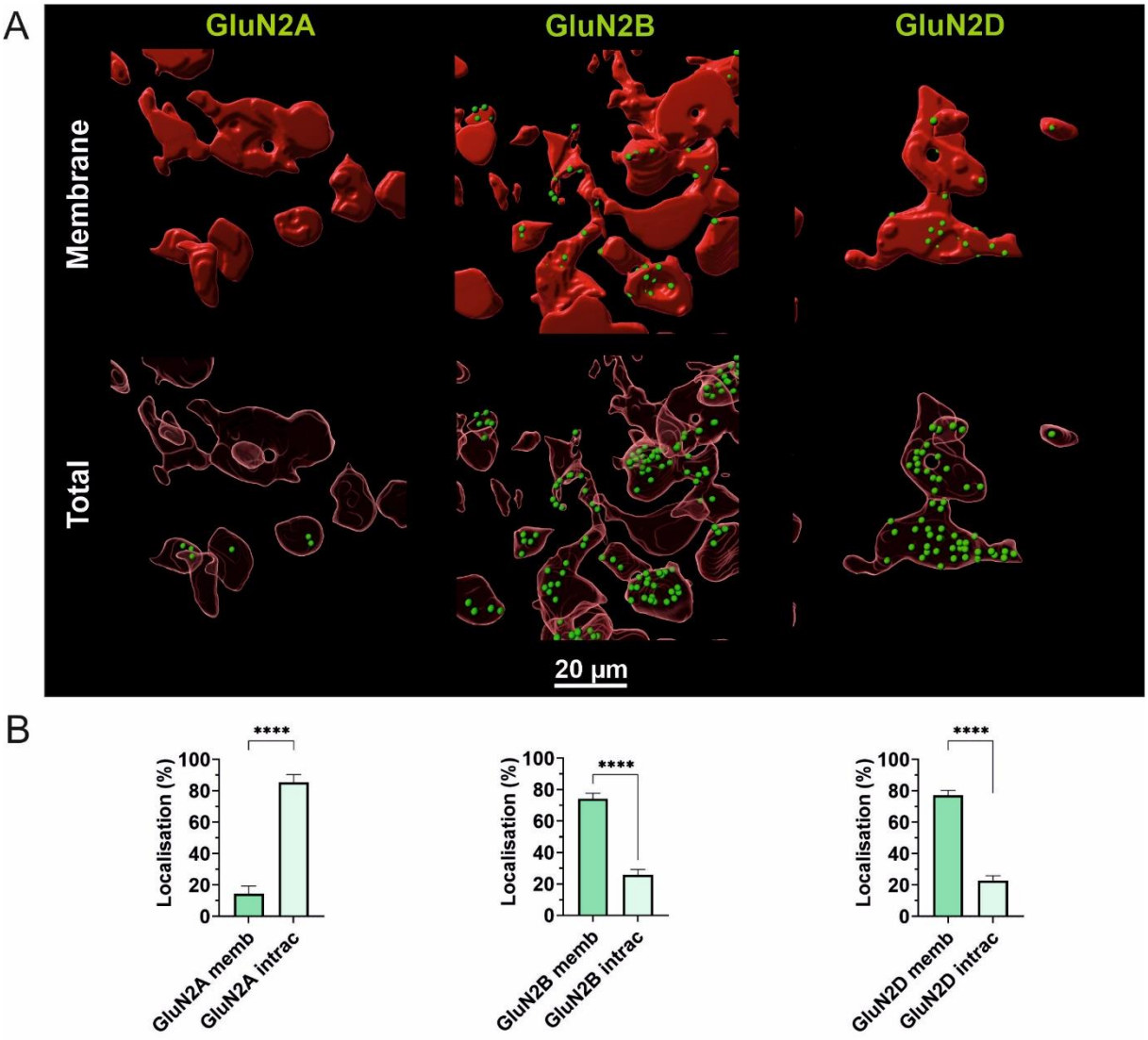


Supplementary Figure 5. Effects of tricine for longer applications

A, Averaged eEPSCs (~30 events) recorded from SNc DA neurons at +40 mV in control conditions (black traces) and after incubation with Tricine (gray trace) for > 30 min. Low stimulation levels were used (typically 20–80 µA) to evoke eEPSCs. For each condition traces were recorded in the presence of picrotoxin (50 µM), CGP 55485 (1 µM), CNQX (10 µM), and strychnine (10 µM).

***B*,** Averaged peak amplitude of NMDAR-eEPSCs immediately before (the two first points in black) and during the application of tricine for > 30 min. Note the constant amplitude of EPSCs during the application time.

C, Bar chart showing the amplitude of the NMDAR-eEPSCs in control conditions (dark grey bar) and after 30 min in Tricine (grey bar) (control, 33.92 ± 4.66 pA ; Tricine, 30.57 ± 5.46 pA, p= 0.5781) ; Bars are means ± SEM for n=7 ; Wilcoxon signed rank test.



Supplementary Figure 6. Spatial distribution of GluN2 NMDAR subunits in SNc DA neurons

A, 3D reconstruction of confocal images with DA neurons in red (TH+) and NMDAR subunits (green dots. Top panel represents the NMDAR subunits expressed only in the membrane of DA neurons and the bottom panel represents the expression of NMDAR subunits at the membrane, as well as, intracellularly.

***B*,** Bar chart showing the localization of NMDAR subunits for GluN2A (left; in membrane, 14.52 ± 4.88 % ; intracellular, 85.48 ± 4.88 %, p>0.0001) , GluN2B (middle; in membrane, 74.24 ± 3.55 % ; intracellular, 25.76 ± 3.55 %, p>0.0001) and GluN2D (right; in membrane, 77.16 ± 3.14 % ; intracellular, 22.84 ± 3.14 %, p>0.0001). Bars are means ± SEM for n=7, N=35 ; Mann-Whitney test.