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Supplemental information

Activity-dependent somatodendritic dopamine release in the substantia nigra autoinhibits the releasing neuron

Takuya Hikima, Christian R. Lee, Paul Witkovsky, Julia Chesler, Konstantin Ichtchenko, and Margaret E. Rice

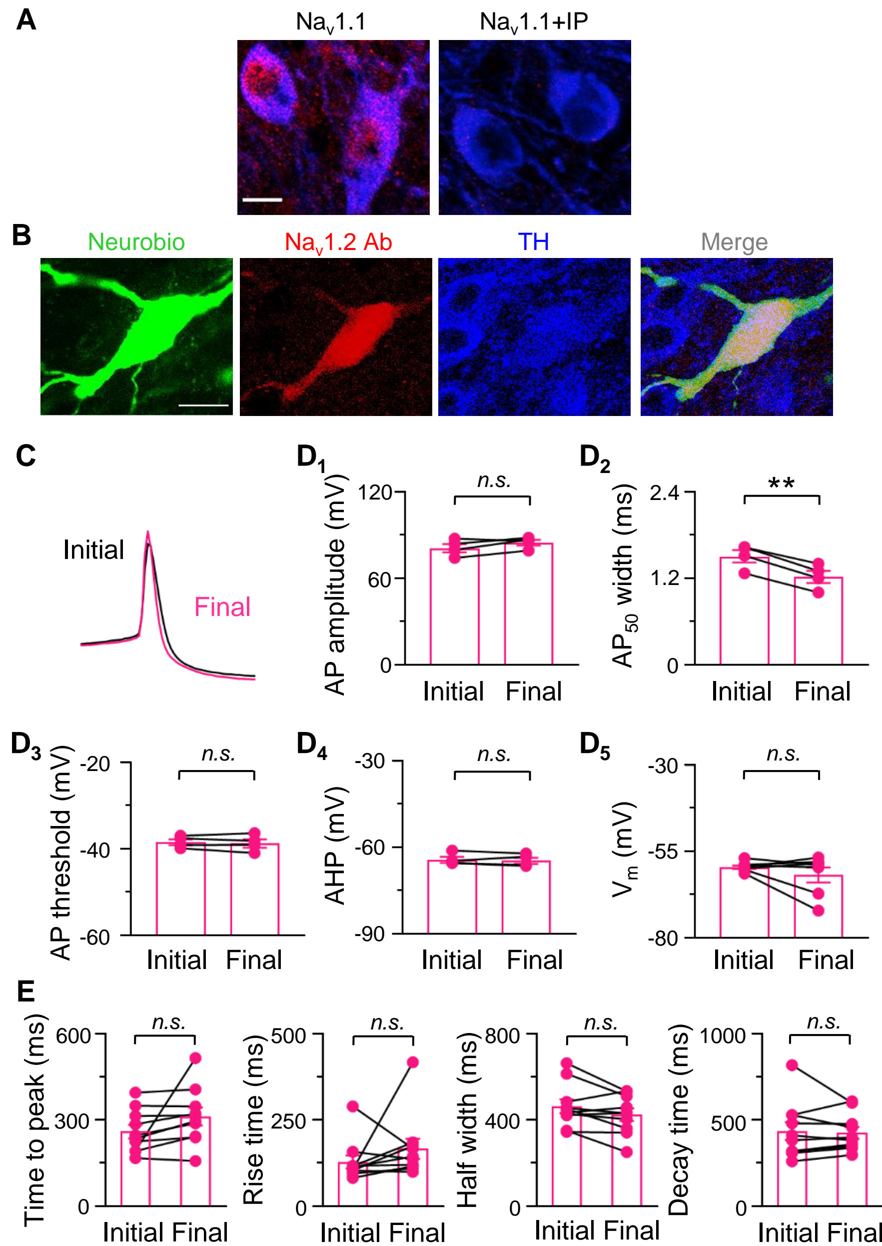

Figure S1 (Related to Main Figures 2 and 3). Effects of intracellular application of Na$_v$ Abs on action potential characteristics and lack of influence of Na$_v$ Abs on D2IC kinetics.

(A) Immunohistochemical staining of Na$_v$1.1 in SNc DA neurons**.** Left: Representative Na$_v$1.1-ir and TH-ir in the SNc, showing expression of Na$_v$1.1 in DA neurons (n = 3 mice), with the same Na$_v$1.1 Ab used in whole-cell recording studies. Right: Preadsorption of the Na$_v$1.1 Ab with its immunogenic peptide (IP) blocked immunostaining with Na$_v$1.1 Ab (from the same n = 3 mice). Scale bar, 10 µm.

(B) Confirmed infiltration of $Na_v1.2$ Ab-ir in a SNc DA neuron in a horizontal midbrain slice after whole-cell recording with a pipette containing $Na_v1.2$ Ab. The presence of $Na_v1.2$ Ab was shown by immunostaining with a secondary antibody only. The recorded cell was localized by Neurobiotin 488 that was introduced with $Na_v1.2$ Ab via the recording pipette, and identified as a DA neuron by TH-ir (n = 2 slices). Scale bar, 20 µm.

(C) Representative action potentials (APs) recorded using current clamp record in SNc DA neurons at the beginning (initial) and end of the 25 min recording period (final) in a cell in which spontaneous APs persisted with Na_v Abs in the pipette.

(D) Action potential (AP) characteristics of initial and final APs in SNc DA neurons in which spontaneous activity persisted with Na_v Abs in the pipette, and resting membrane potential (V_m) in all cells recorded with Na_v Abs. Parameters assessed were: (D_1) AP amplitude (initial, 81.3 ± 2.5 mV; final, 85.0 ± 1.7 mV, $p = 0.1$ for n = 4 neurons); (D_2) AP width at 50% peak amplitude (initial, 1.51 ± 0.07 ms; final, 1.23 ± 0.07 ms, $^{**}p < 0.01$ for n = 4 neurons); (D_3) AP threshold (initial, -38.4 ± 0.6 mV; final, -38.7 ± 0.8 mV, $p = 0.6$ for n = 4 neurons); (D_4) afterhyperpolarization (AHP) (initial, -62.2 ± 0.9 mV; final, -64.5 ± 0.9 mV, $p = 0.6$ for n = 4 neurons); and (D_5) resting membrane potential (V_m) (initial, -59.5 ± 0.5 mV; final, -61.6 ± 2.0 mV, $p = 0.2$ for n = 7 neurons). Bars are means ± S.E.M; paired *t*-test.

(E) Time to peak, rise time, half width and decay time of D2ICs after establishing whole-cell recording (initial) and at the end of the recording period (final) with Na_v Abs in the recording pipette. Parameters assessed were: time to peak (initial, 260 ± 22 ms; final, 214 ± 29 ms, n =10, $p = 0.1$); rise time (initial, 128 ± 18 ms; final, 167 ± 28 ms, $p = 0.3$); half-width (initial, 464 ± 31 ms; final, 425 ± 27 ms, $p = 0.08$); decay time (initial, 427 ± 50 ms; final, 428 ± 33 ms, $p = 0.7$; paired *t*-test).

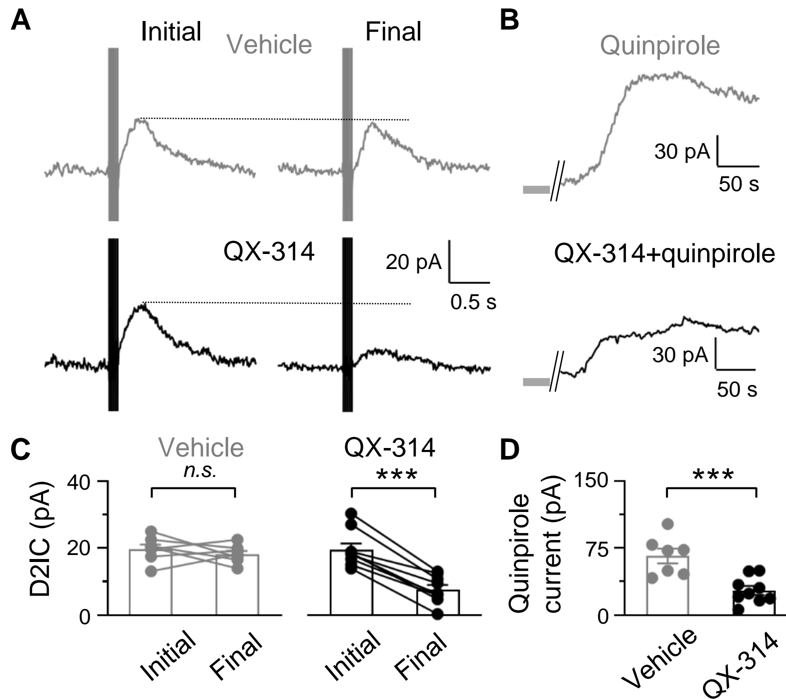

Figure S2 (Related to Main Figure 3). QX-314 in the pipette decreased D2IC amplitude, but also impaired D2 receptor activated GIRK currents.

(A) Representative evoked D2ICs recorded immediately after establishing whole-cell patch (initial) and after 16 min recording with vehicle or QX-314 (5 mM) in the pipette (final).

(B) Quinpirole-induced current (1 μM, 30 s) was also decreased by QX314 in the pipette compared to control conditions with vehicle, implying an effect on D2 receptors and/or GIRK channels. Gray bar indicates duration of quinpirole application.

(C) Quantification of D2IC amplitude at the beginning of recording (initial) and at the end (final) when recorded with vehicle alone or QX-314 (vehicle, 96 ± 9%, n = 7, $p = 0.4$; QX-314, 40 ± 6%, n = 9, ***$p < 0.001$; paired t-test).

(D) Quantification of final quinpirole-evoked D2 currents in the presence of vehicle or QX-314 (vehicle, 66.2 ±7.6 pA, n = 7; QX-314, 23.7 ± 4.2 pA, n = 9, ***$p < 0.001$; unpaired t-test).

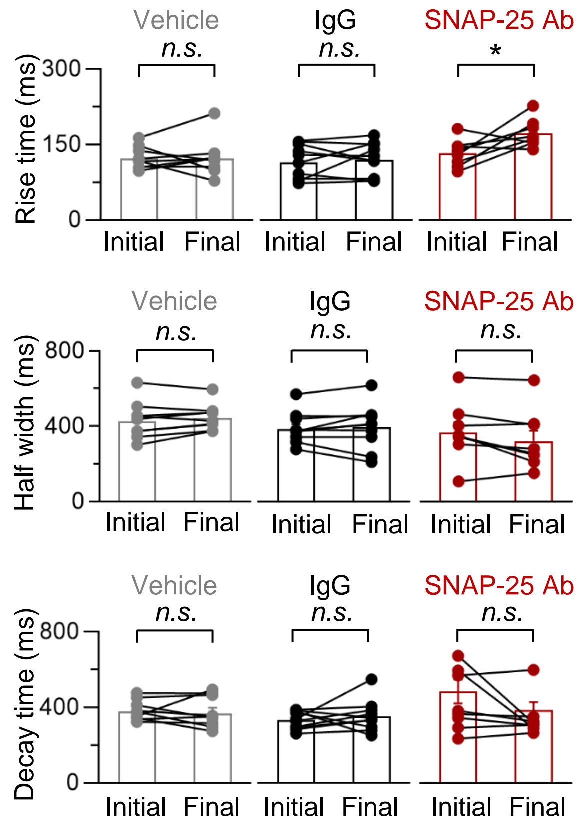

Figure S3 (Related to Main Figure 4). Effects of intracellular application of SNAP-25 Ab on the kinetics of evoked D2ICs in SNc DA neurons.

Single-cell application of SNAP25 Ab altered rise time, but not half width or decay time of D2ICs. Parameters were assessed after establishing whole-cell recording (initial) and at the end of the recording period (final) for vehicle (n = 9 neurons), IgG (n = 10 neurons), and SNAP-25 Ab (n = 8 neurons): rise time (vehicle initial 123 ± 6 ms, final 124 ± 11 ms, $p = 0.9$; IgG initial, 118 ± 10 ms, final 121 ± 10 ms, $p = 0.7$; SNAP-25 Ab initial, 133 ± 9 ms; final, 172 ± 9 ms, *$p < 0.05$); half-width (vehicle initial 431 ± 29 ms, final 447 ± 21 ms, $p = 0.2$; IgG initial 391 ± 25 ms, final 398 ± 35 ms, $p = 0.7$; SNAP-25 Ab initial 370 ± 51 ms, final 324 ± 52 ms, $p = 0.07$); decay time constant (vehicle initial 384 ± 16 ms; final 374 ± 24 ms, $p = 0.7$; IgG initial 374 ± 16 ms, final 398 ± 28 ms, $p = 0.5$; SNAP-25 Ab initial 486 ± 59 ms. final 388 ± 39 ms, $p = 0.2$). Bars are means ± S.E.M; paired *t*-test.

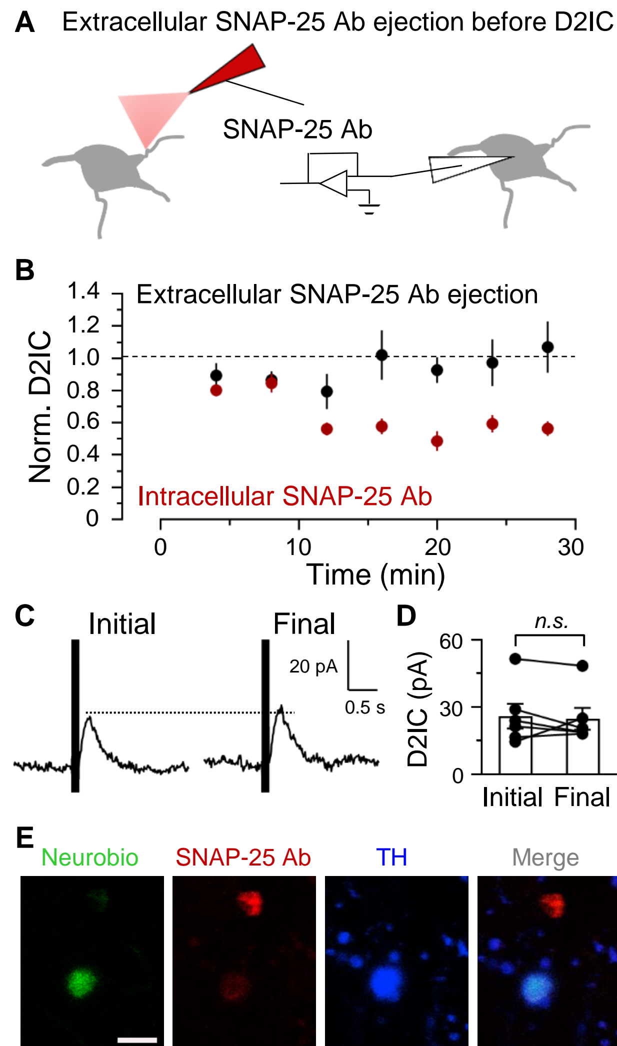

Figure S4 (Related to Main Figure 4). Extracellular SNAP-25 antibody (Ab) ejection did not alter D2IC amplitude.

(A) Schematic of experimental design used to obtain the data shown in (B-D). Left: a recording pipette containing SNAP-25 Ab was positioned near a DA neuron and SNAP-25 was ejected extracellularly from the pipette for 25 min. Right: the ejection pipette was then replaced with a patch pipette lacking SNAP-25, but containing Neurobiotin 488. Evoked D2ICs were recorded for 28 min, which is the time required for the usual effect of intracellular SNAP-25 Ab (*see* panel B).

(B) Average time course of changes in evoked D2IC amplitude recorded with extracellular or intracellular application of SNAP-25 Ab (extracellular SNAP-25 Ab, n = 6; intracellular SNAP-25 Ab, n = 10).

(C) Representative D2IC immediately after establishing whole-cell recording following extracellular SNAP-25 Ab application (initial) and at the end of the recording period (final, 28 min).

(D) Quantification of D2ICs in the presence of extracellular SNAP-25 Ab; mean final D2IC amplitude after extracellular SNAP-25 Ab application was 102 ± 13% of initial, $n = 6$, $p = 0.6$; paired *t*-test.

(E) Representative immunostaining for SNAP-25 Ab (secondary antibody only) and TH, with Neurobiotin 488 after whole-cell recording from the neuron in C; Neurobiotin 488 was used to identify the patch-clamped cell, and was ejected with SNAP-25 Ab via the extracellular pipette (n = 6 slices). Scale bar, 20 µm.

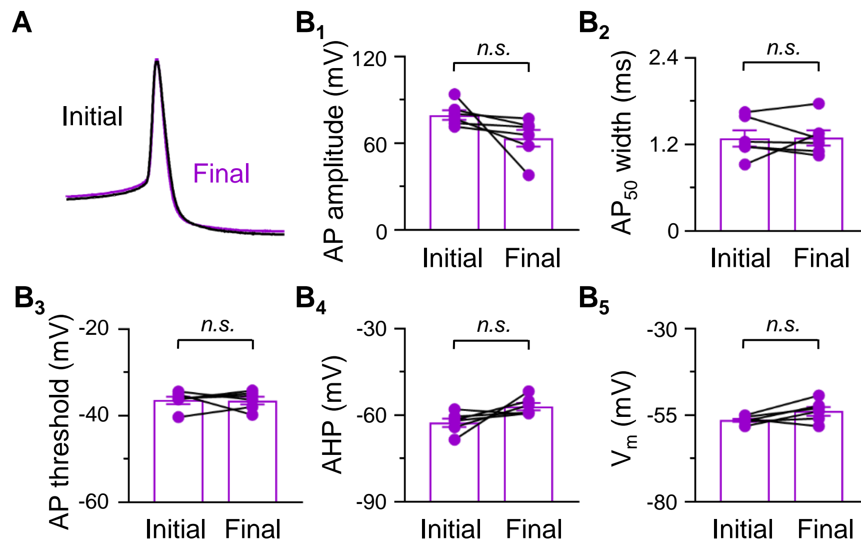

Figure S5 (Related to Main Figures 5). Intracellular application of BoNT/A LC (LC/A) did not alter action potential (AP) characteristics in SNc DA neurons.

(A) Representative current-clamp records from SNc DA neurons in SNc at the beginning (initial) and end of the 40-60 min recording period (final).

(B) Action potential (AP) characteristics of initial and final APs with LC/A in the pipette. Comparison of peak AP amplitude (B_1), AP width at 50% peak amplitude (B_2), AP threshold (B_3), afterhyperpolarization (AHP) (B_4), and resting potential (V_m) (B_5). Each point represents the average of 5 APs for a given SNc DA neuron. AP amplitude (initial, 79.9 ± 3.0 mV; final, 63.7 ± 5.3 mV, $p = 0.1$), AP width 50% (initial, 1.36 ± 0.09 ms; final, 1.28 ± 0.10 ms, $p = 0.9$), AP threshold (initial, -36.4 ± 0.8 mV; final, -36.4 ± 0.8 mV, $p = 0.9$), AHP (initial, -62.5 ± 1.3 mV; final, -56.9 ± 1.2 mV, $p = 0.09$), V_m (initial, -56.4 ± 0.4 mV; final, -53.8 ± 1.1 mV, $p = 0.08$). Bars are means ± S.E.M. for n = 6 neurons; paired *t*-test.

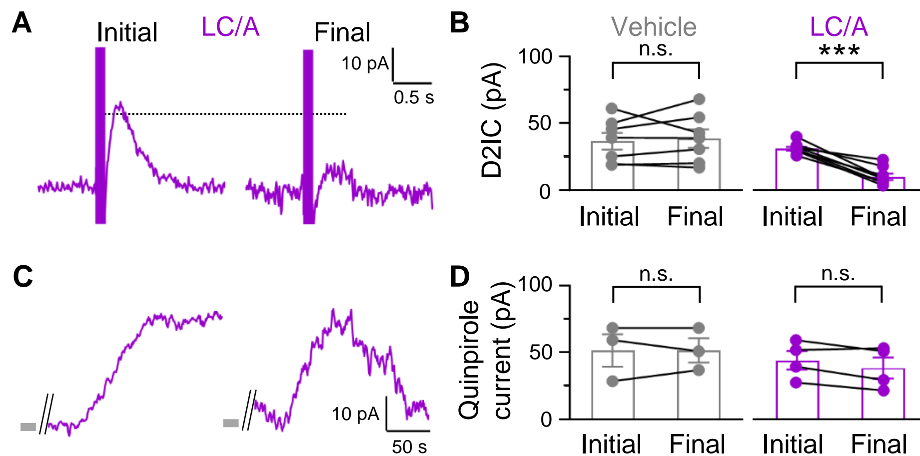

Figure S6 (Related to Main Figure 5). Single-cell application of BoNT/A LC (LC/A) decreased D2IC amplitude in SNc DA neurons from female mice.

(A) Representative evoked D2IC immediately after establishing whole-cell patch (initial) and at the end of the recording period (final).

(B) Mean changes in D2IC amplitude recorded with vehicle or LC/A in the pipette (vehicle, 109 ± 17% of initial, n=7, $p = 0.7$; LC/A, 28 ± 3%, n = 8, ***$p < 0.001$; paired *t*-test).

(C) Representative examples of quinpirole-evoked GIRK currents at the beginning and end of the experiment with BoNT/A LC in the recording pipette. Gray bar indicates duration of quinpirole superfusion.

(D) Quantification of quinpirole-evoked currents (vehicle, 105 ± 10% of initial, n = 3, $p = 0.9$; LC/A, 86 ± 5%, n = 4, $p = 0.11$; paired *t*-test).

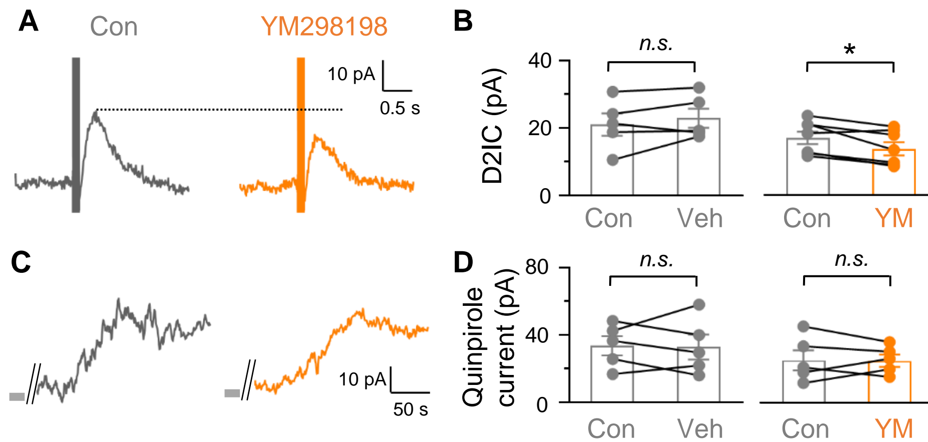

Figure S7 (Related to Main Figure 7). Antagonism of mGluR1 decreases the amplitude of evoked D2ICs in SNc DA neurons.

(A) Representative evoked D2IC immediately after establishing whole-cell patch (control, Con) and after application of a mGluR1 antagonist, YM298198 (YM, 50 μM, 12 min).

(B) D2IC amplitude recorded before (Con) and after application of vehicle (Veh) or YM298198 (YM) (vehicle, 115 ± 12% of control n=5, $p = 0.3$; YM298198, 81 ± 5%, n = 7, * $p < 0.05$; paired *t*-test).

(C) Representative examples of quinpirole-evoked GIRK currents at the beginning of the experiment and after YM298198 application. Gray bar indicates duration of quinpirole superfusion.

(D) Quantification of quinpirole-evoked currents (vehicle, 99 ± 13% of initial, n = 5, $p = 0.8$; YM298198, 112 ± 18%, n = 5, $p = 0.9$; paired *t*-test).