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Supplemental Information

Dopamine Autoreceptor Regulation of a Hypothalamic Dopaminergic Network

Stefanos Stagkourakis, Hoseok Kim, David J. Lyons, and Christian Broberger

SUPPLEMENTAL INFORMATION

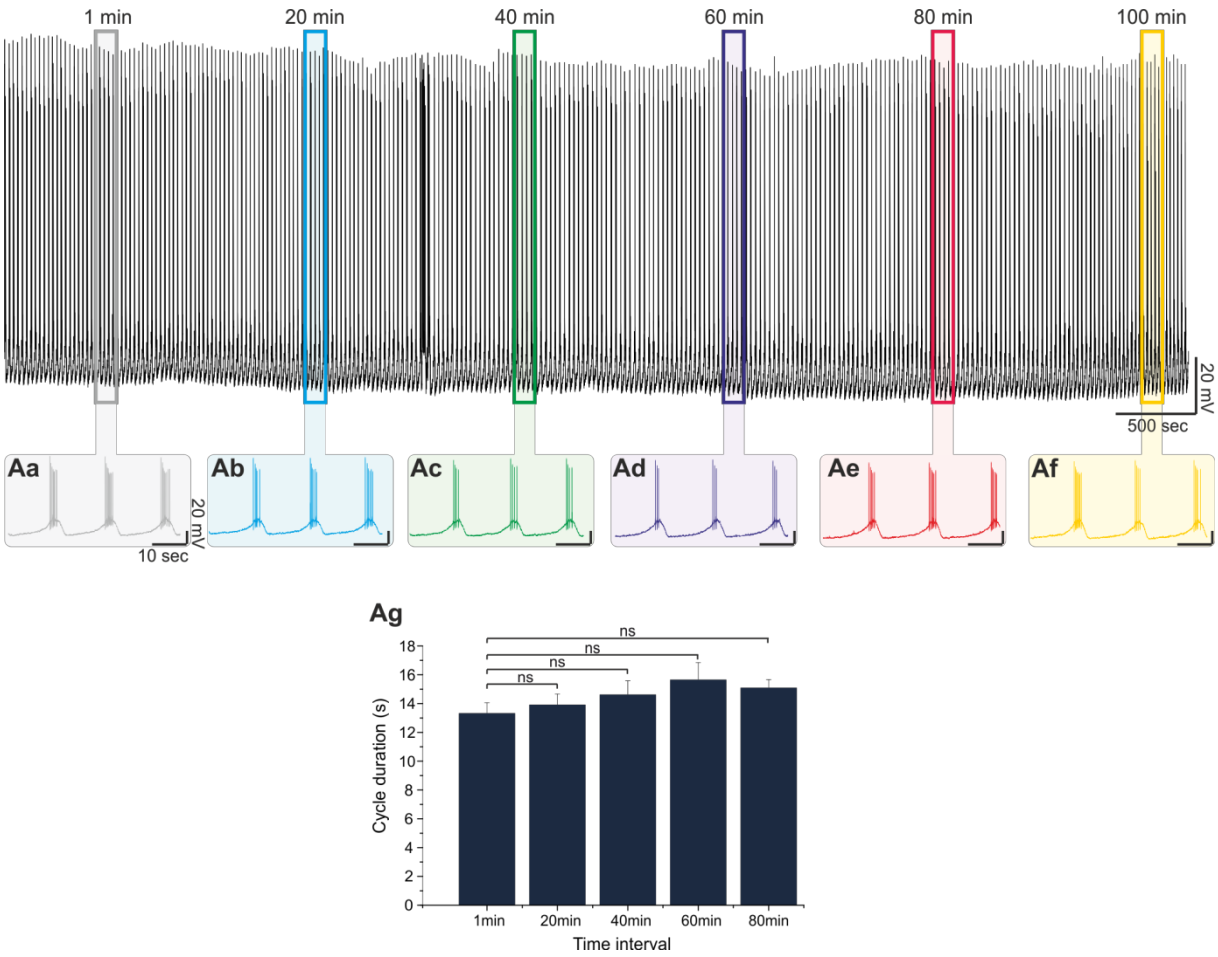


Figure S1. Robustness of the TIDA oscillation and maintenance of rhythmicity in long whole-cell recordings, see Experimental Procedures.

(Aa-g) Cycle duration does not change in control long-term recordings (+0.71±0.55 s; p>0.05 at t=40 min vs t=1 min; n=6). **(Ag)** Cycle duration does not change in control long-term recordings in an 80 min period (+0.97±1.03 s; p>0.05 at t=80 min vs t=1 min; n=3).

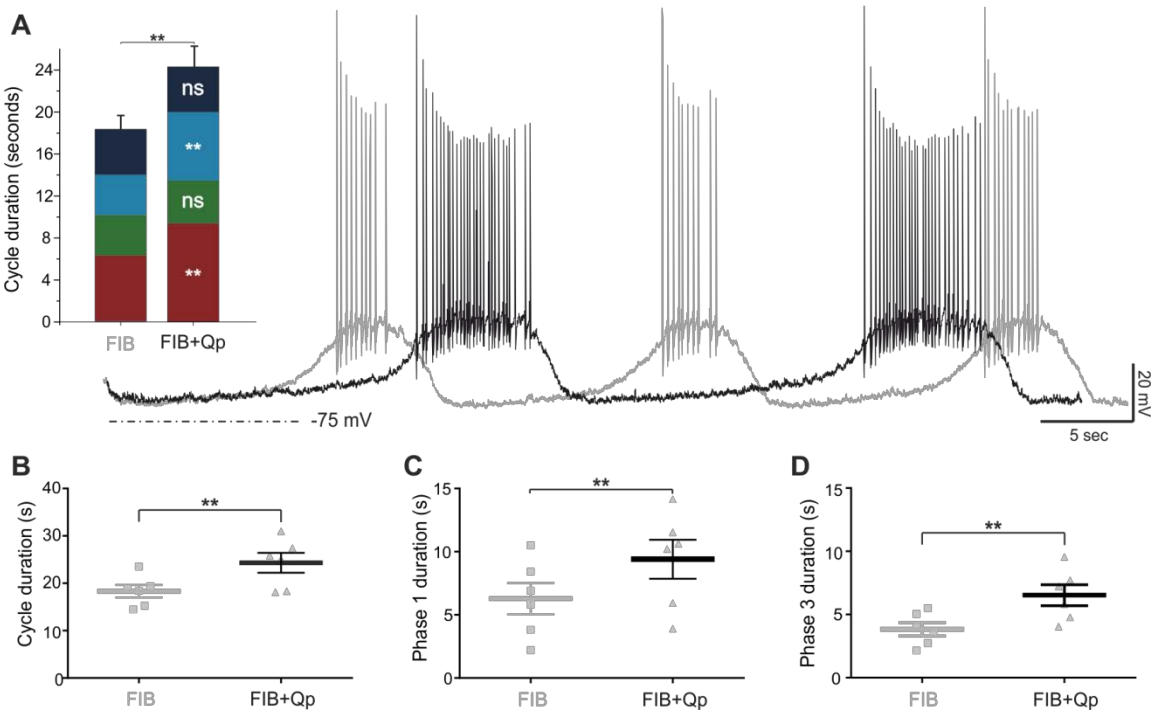
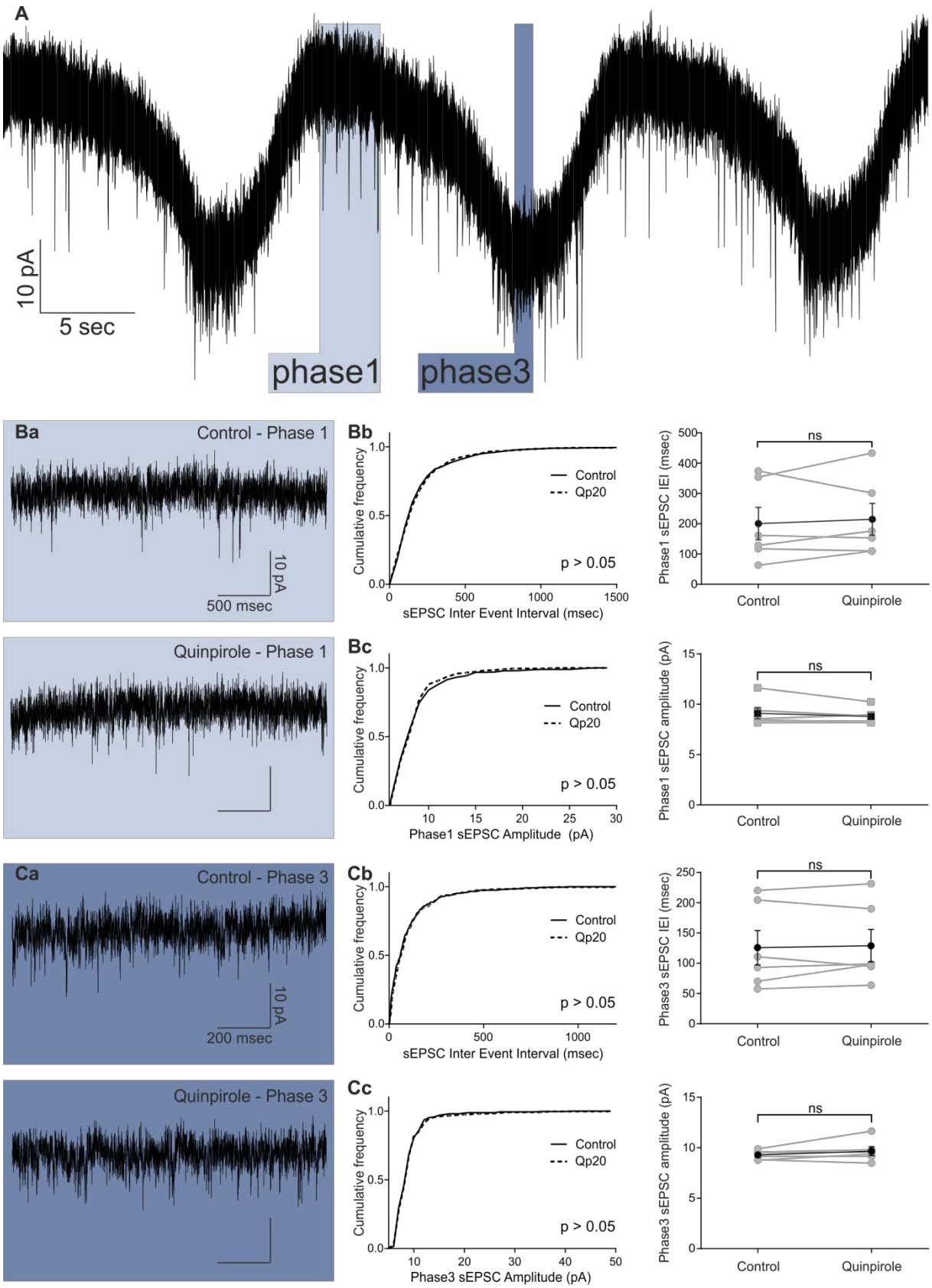


Figure S2. Related to Figure 1; D2R activation effect on cycle and phase duration persists in fast ionotropic blockade.

(A) Quinpirole application leads to an increase in cycle duration in the presence of fast ionotropic blockade (FIB; Picrotoxin 100 μM, CNQX 10 μM and AP-5 25 μM) via phase 1 and phase 3 prolongation. **(B)** Cycle duration increase (+5.99±0.91 s; $p<0.01$ *vs* FIB; n=6). **(C)** Phase 1 duration increase (+3.12±0.52 s; $p<0.01$ *vs* FIB; n=6). **(D)** Phase 3 duration increase (+2.69±0.47 s; $p<0.01$ *vs* FIB; n=6).


A
10 pA
5 sec
phase1
phase3
Ba
Control - Phase 1
10 pA
500 msec
Bb
Cumulative frequency
Control
Qp20
p > 0.05
sEPSC Inter Event Interval (msec)
Phase1 sEPSC IEI (msec)
ns
Control
Quinpirole
Quinpirole - Phase 1
Bc
Phase1 sEPSC Amplitude (pA)
Phase1 sEPSC amplitude (pA)
Ca
Control - Phase 3
200 msec
Cb
Phase3 sEPSC IEI (msec)
Quinpirole - Phase 3
Cc
Phase3 sEPSC Amplitude (pA)
Phase3 sEPSC amplitude (pA)

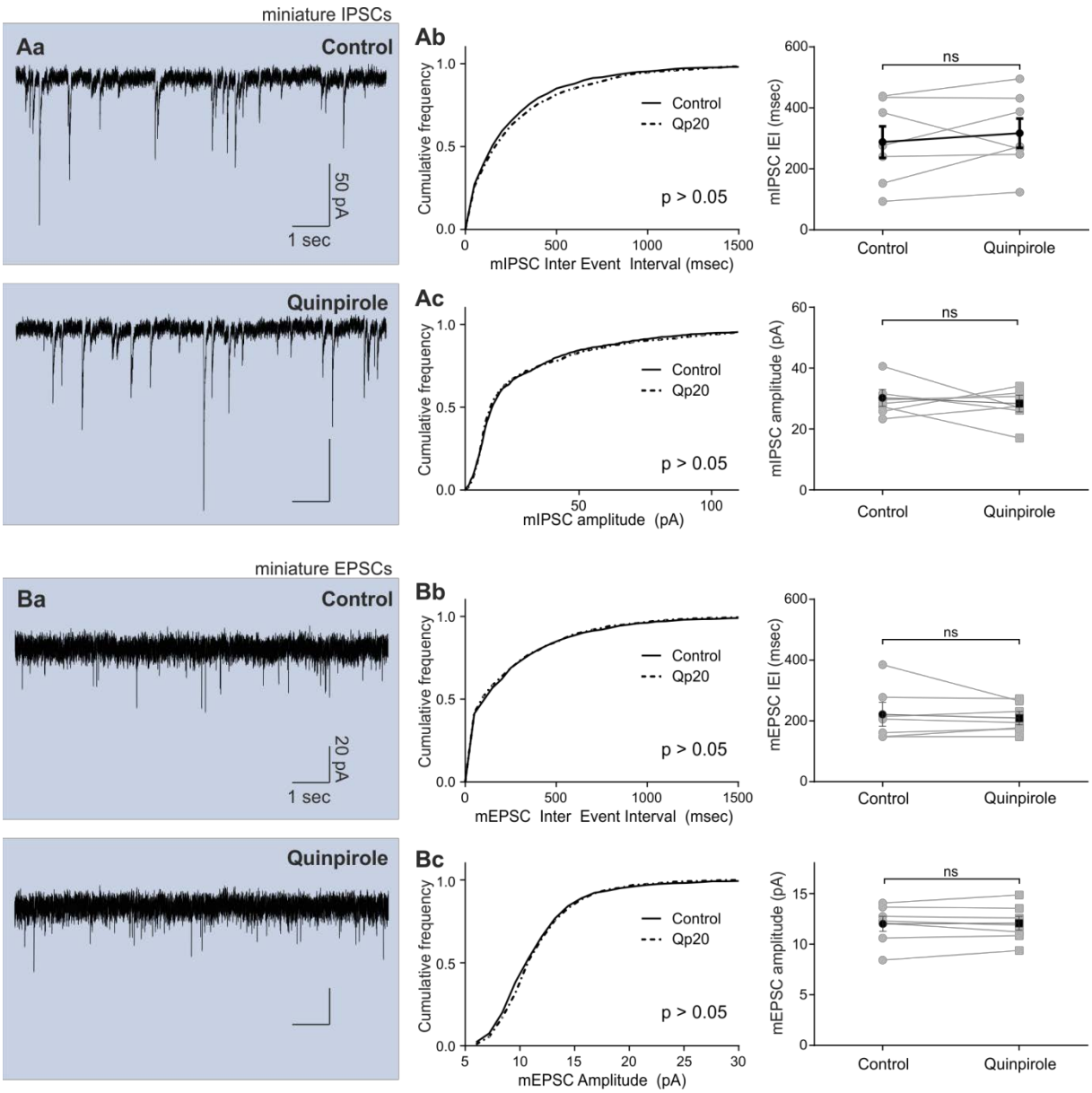
20

21

22

Figure S3. Related to Figure 5; Spontaneous EPSCs are not affected by D2R activation.

(Aa) Raw voltage clamp trace demonstrating the time intervals from which sEPSCs were sampled during phase 1 (light blue) and phase 3 (dark blue). (Ba) Raw voltage clamp traces in control *vs* quinpirole illustrating no change in sEPSC frequency or amplitude during phase 1. (Bb) Cumulative frequency distribution of sEPSC inter-event interval demonstrating no difference in frequency (+14.18±22.30 ms, KS-2 $p<0.05$; *t*-test $p<0.05$; n=5) or amplitude (Bc) (-0.29±0.25 pA, KS-2 $p>0.05$; *t*-test $p>0.05$; n=5) during phase 1. (Ca) Raw voltage clamp trace illustrating no difference in frequency or amplitude of sEPSCs during phase 3 after quinpirole application. (Cb) Cumulative frequency distribution of sEPSC inter event interval demonstrating no difference in frequency (+3.28±6.73 ms, KS-2 $p<0.05$; *t*-test $p<0.05$; n=5) or amplitude (Cc) (+0.38±0.30 pA, KS-2 $p>0.05$; *t*-test $p>0.05$; n=5) during phase 3.



FigureS4. Related to Figure 5; Miniature synaptic transmission is unaffected by D2R activation in TIDA neurons.

(Aa) Raw voltage clamp traces in control *vs* quinpirole illustrating no change in miniature IPSC frequency or amplitude. (Ab) Cumulative frequency distribution of mIPSC inter-event interval demonstrating no difference in frequency (+29.07±30.56 ms, KS-2 $p<0.05$; *t*-test $p<0.05$; n=7) or amplitude (Ac) (-1.78±3.07 pA, KS-2 $p>0.05$; *t*-test $p>0.05$; n=7). (Ba) Raw voltage clamp trace illustrating no difference in frequency or amplitude of mEPSCs as a result of quinpirole application. (Bb) Cumulative frequency distribution of mEPSC inter-event interval demonstrating no difference in frequency (-10.75±18.73 ms, KS-2 $p<0.05$; *t*-test $p<0.05$; n=7) or (Bc) amplitude (+0.06±0.24 pA, KS-2 $p>0.05$; *t*-test $p>0.05$; n=7).

Table S2. Related to Figure 2 Ac-Ad and Bc-Bd; D2R antagonists decrease the amplitude and broaden the TIDA action potentials.

		Control	Eticlopride 1 μM
Amplitude (mV)	AP1	97.41 ± 1.12	85.83 ± 2.05 **
	AP2	85.87 ± 1.79	71.77 ± 2.19 **
	AP3	79.74 ± 1.43	61.55 ± 3.86 **
	AP4	75.35 ± 1.65	57.79 ± 3.36 **
	AP5	72.35 ± 1.54	54.34 ± 3.01 **
Half-width (ms)	AP1	1.49 ± 0.10	1.56 ± 0.09
	AP2	1.74 ± 0.14	1.93 ± 0.14 **
	AP3	1.91 ± 0.16	2.36 ± 0.26 *
	AP4	2.08 ± 0.18	2.54 ± 0.25 **
	AP5	2.17 ± 0.18	2.79 ± 0.39 *

		Control	Haloperidol 1 μM
Amplitude (mV)	AP1	95.09 ± 1.91	89.11 ± 2.26 **
	AP2	85.00 ± 2.28	76.82 ± 2.76 **
	AP3	79.73 ± 2.93	68.85 ± 2.94 ***
	AP4	74.38 ± 2.25	63.95 ± 2.77 **
	AP5	74.16 ± 2.44	59.28 ± 2.13 **
Half-width (ms)	AP1	1.37 ± 0.02	1.41 ± 0.04
	AP2	1.53 ± 0.04	1.63 ± 0.04 *
	AP3	1.67 ± 0.06	1.82 ± 0.05 **
	AP4	1.79 ± 0.06	1.97 ± 0.07 **
	AP5	1.82 ± 0.07	2.14 ± 0.07 **

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Ca^{2+} current studies. To examine modulation of Ca^{2+} channels, bath application of TTX (0.5μM) was used to block the voltage-gated Na^{+} conductance (Cohen et al., 1981) and Ba^{2+} (1mM) was added to the extracellular solution to augment the Ca^{2+} channel currents (Kostiuk et al., 1985, Smith et al., 1986). The Cs^{+}-based intracellular solution described for recordings of EPSCs (see above) was used to block K^{+} currents (Adelman and Senft, 1966, Clay and Shlesinger, 1984). At the end of every experiment $CdCl_2$ (400 μM) was applied to abolish the Ca^{2+} conductances (Thevenod and Jones, 1992, Swandulla and Armstrong, 1989); that recording was used as the negative control. Due to incomplete blockade of the K^{+} leak conductance by Cs^{+}, the Cd^{2+} trace was subtracted digitally from Ca^{2+} recordings of the same neuron. Run-down of Ca^{2+} currents was minimized by using the Cs^{+}-based intracellular solution (Zhang et al., 1994), by applying brief (150 ms) ramp protocols adapted from (Chen and Kirchgessner, 2002), and by waiting for 10 mins between activating currents, a time interval that was used for pharmacological reagents to reach a peak of effect. In these conditions, run-down of the HVA Ca^{2+} current was calculated to be 3.3±0.69% per 10 min (n=3). The values of all Ca^{2+} peak currents were corrected based on the run-down estimate.

Oscillation phase analysis. We developed a Matlab script in order to isolate the contribution of each phase to the oscillation cycle duration. The stereotyped traits of the oscillation were used to develop an algorithm, to allow analysis of duration and membrane voltage of the four phases of the duty cycle. Four phases were defined on the basis of slope (dV/dt) changes (Figure 1A): Phase 1 was defined as the slow depolarizing phase beginning at the nadir of the membrane potential (the lowest mV value of an oscillation cycle) and ending within a time window of 0.5-4sec prior to the first action potential of the oscillation cycle, where the point of maximal change in slope defines the phase 2 initiation point. Phase 2 was thus defined as the fast depolarizing phase beginning at the phase 2 initiation point and ending at the firing threshold (defined as dV/dt>5 mV/ms) of the first action potential of the oscillation cycle. Phase 3 was defined as the action potential firing plateau phase, extending between the firing threshold of the first action potential of the oscillation cycle until the end of firing where the maximal change in slope defines the end of phase 3 and beginning of phase 4. Phase 4 constitutes the fast hyperpolarizing phase between end of phase 3 and the ensuing nadir point.

Recording of EPSCs and IPSCs. In recordings of sIPSCs and mIPSCs whole-cell voltage-clamp recordings were performed with micropipettes filled with intracellular solution containing (in mM), 150 KCl, 10 HEPES, 10 EGTA, and 2 Na_2ATP, pH 7.3 (with KOH), in the presence of fast excitatory neurotransmission blockade by CNQX (10 μM) and AP-5 (25 μM). The final part of every s/mIPSCs recording was application of picrotoxin (100 μM) and the observation of complete loss of all synaptic events. In recordings of sEPSCs and mEPSCs whole-cell voltage-clamp recordings were performed with micropipettes filled with intracellular solution containing (in mM), 140 K-gluconate, 10 KCl, 10 HEPES, 10 EGTA, and 2 Na_2ATP, pH 7.3 (with KOH), in the presence of fast inhibitory neurotransmission blockade by picrotoxin (100 μM). The final part of every s/mEPSCs recording was application of picrotoxin (100 μM) and the observation of complete loss of all synaptic events. sIPSCs and sEPSCs along phases were compared via manual selection. Beginning of phase 1 in voltage-clamp was defined as the most positive point (in pA), of a new oscillation cycle and beginning of phase 3 was selected as the most negative point (in pA) of the oscillation cycle.

*Reagents***.** Dopamine hydrochloride, apomorphine hydrochloride hydrate, picrotoxin, cadmium chloride, haloperidol, $BaCl_2$ and (TEA)-Cl_2 were purchased from Sigma. TTX and charybdotoxin were purchased from Alomone Labs. (-)-quinpirole hydrochloride, SKF-81297 hydrobromide, (-)-Eticlopride hydrochloride, CNQX, AP-5, nimodipine and CGP 55845 hydrochloride were purchased from Tocris Bioscience. GVIA ω-conotoxin was purchased from Abcam biochemical.