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Supplemental Information

High-Frequency Activation of Nucleus Accumbens D1-MSNs Drives Excitatory Potentiation on D2-MSNs

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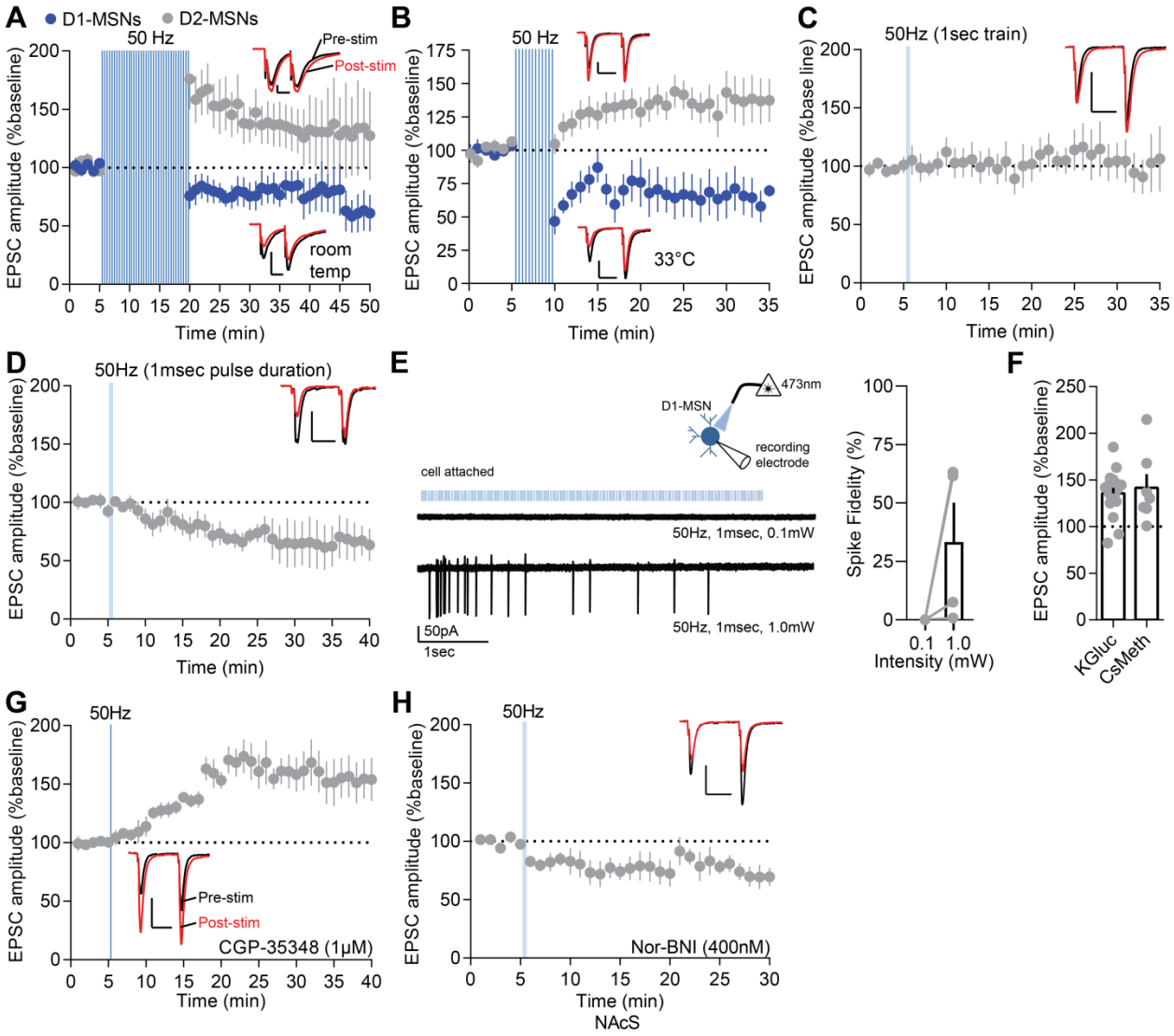

Figure S1 (Related to Figure 1). Characterization of stimulation factors or receptors required for D2-MSN LLP in the NAcS and NAcC. (A) 15 min of 50 Hz stimulation (5 sec light on, 5 sec light off) promotes excitatory potentiation on D2-MSNs (One-way RM ANOVA $F_{11,55}$=4.414, P<0.0001, n=6) and no observable effect on D1-MSNs (One-way RM ANOVA $F_{11,33}$=1.122, P=0.3758, n=4). **(B)** 5 min of 50 Hz stimulation (5 sec light on, 5 sec light off) promotes excitatory potentiation on D2-MSNs (One-way RM ANOVA $F_{16,112}$=15.57, P=0.0016, n=8) and depression on D1-MSNs (One-way RM ANOVA $F_{16,64}$=37.11, P<0.0001, n=5). **(C)** A 50 Hz train (10 msec pulse length) of one second was not capable of producing D2-MSN LLP (One-way RM ANOVA with Geisser-Greenhouse (GG) correction (GG): $F_{2.123,12.74}$=7.226, P=0.5103, n=6). **(D)** Lowering 50 Hz light pulses (5 sec train) to 1 msec produced excitatory depression on D2-MSNs (One-way RM ANOVA with GG $F_{3.020,18.12}$=3.246, P=0.0459, n=7). **(E)** 50 Hz light stimulation at 1 msec pulses is not capable of maintaining high fidelity firing. **(F)** No difference in stimulation-induced potentiation of EPSC amplitude is observed between cells recorded with K-gluconate internal (KGluc) or Cesium methanosulfonate (CsMeth) internal (Two-tailed t_6=0.4759, P=0.6510). **(G)** $GABA_A$ receptor (picrotoxin, 100 μM) and $GABA_B$ receptor (CGP-35348, 1μM) has no effect on stimulation-induced potentiation (One-way RM ANOVA with GG: $F_{1.734,12.14}$=7.228, P=0.0103, n=8). **(H)** Kappa receptor antagonism with nor-BNI did not block excitatory depression on D2-MSNs caused by 50 Hz D1-MSN stimulation in the NAcS (One-way RM ANOVA with GG $F_{3.374,23.62}$=2.688, P=0.0638, n=7). *p<0.05.

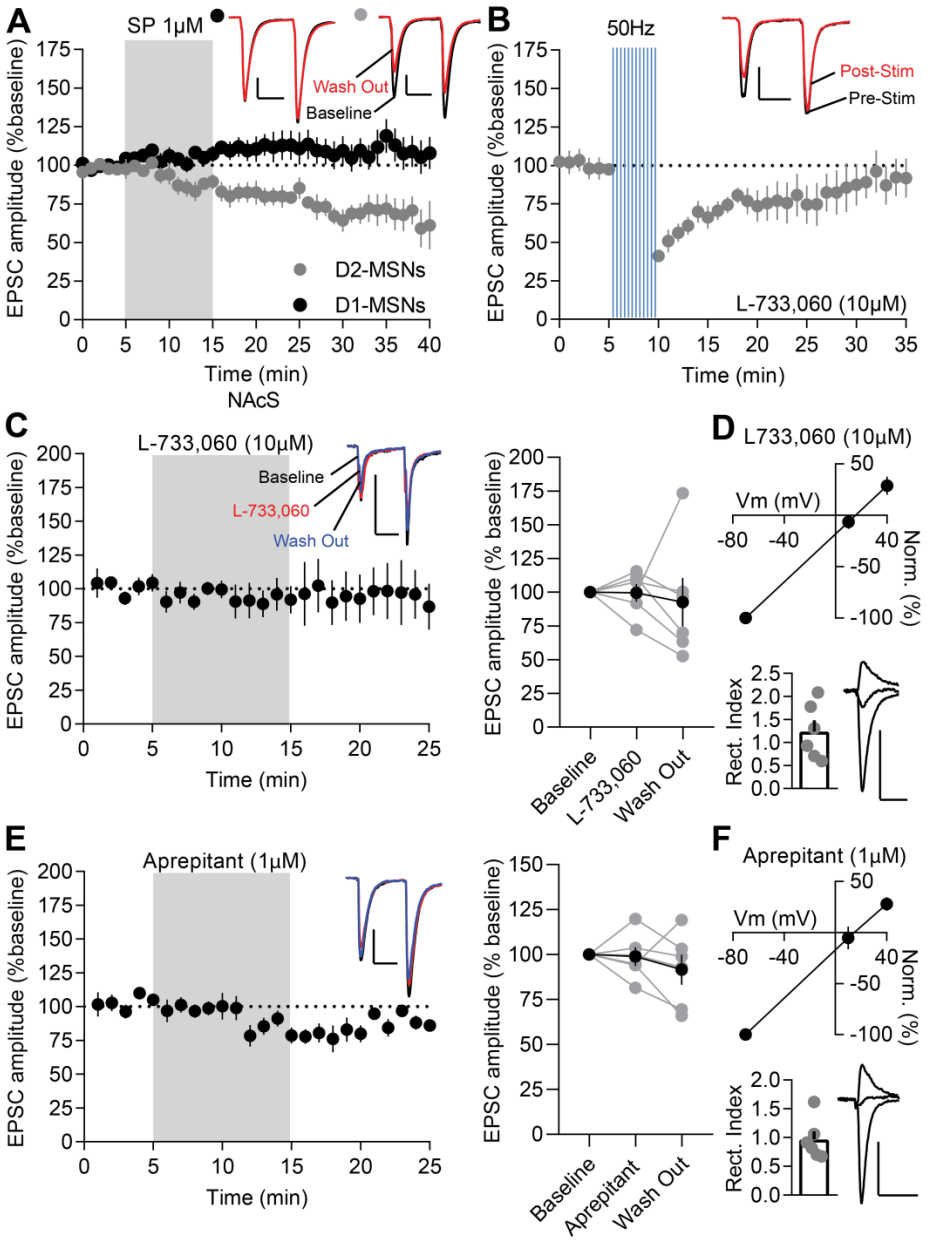

Figure S2 (Related to Figure 2). Substance P promotes excitatory depression on D2-MSNs in the NAcS and NK1 receptor antagonists block stimulation induced D2-MSN LLP without affecting baseline synaptic properties. (A) NAcS D2-MSNs EPSCs display excitatory depression (One-way RM ANOVA $F_{20,140}$=3.487, P<0.0001, n=8) and NAcS D1-MSNs show no change (One-way RM ANOVA $F_{20,80}$=1.270, P=0.2043, n=10) following bath application of substance P (1 μM). Scale bar for synaptic recordings: 100 pA, 25 msec. **(B)** EPSCs are transiently depressed following 50 Hz stimulation in the presence of the NK1 receptor antagonist L-733,060 (10 μM) (One-way RM ANOVA $F_{11,55}$=12.92, P<0.0001, n=6). **(C)** L-733,060 does not affect EPSC amplitude in D2-MSNs (One-way RM ANOVA $F_{2,10}$=1.379, P=0.8567, n=6). **(D)** L-733,060 does not affect AMPA receptor inward rectification at +40 mV (One-sample two-tailed t-test: theoretical mean 1.0, actual mean 1.234; t_5=0.9531, P=0.3843, n=6). **(E)** Aprepitant does not affect EPSC amplitude in D2-MSNs (One-way RM ANOVA $F_{2,10}$=0.8344, P=0.4623, n=6). **(F)** Aprepitant does not affect AMPA receptor inward rectification at +40 mV (One-sample t-test: theoretical mean 1.0, actual mean 0.9645; t_5=0.2476, P=0.8143, n=6).

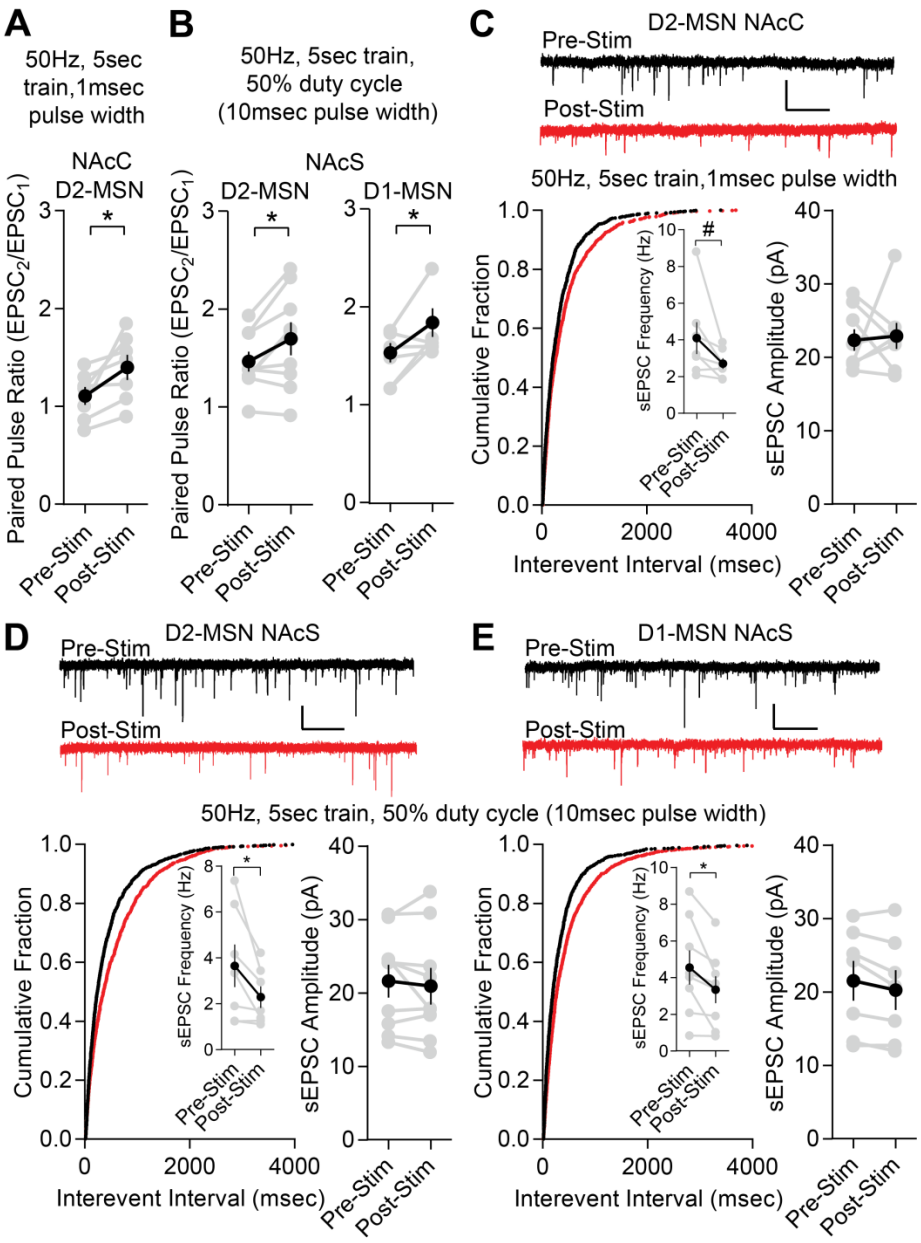

Figure S3 (Related to Figure 3). Stimulation-induced excitatory depression in the NAcS or the NAcC (1 msec pulses) is governed by a pre-synaptic mechanism. (A) Paired pulse ratios are significantly increased for NAcC D2-MSNs following 50 Hz D1-MSN stimulation (1 msec pulses) (Paired two-tailed, t_6=2.711, P=0.0350, n=7). **(B)** Paired pulse ratios are significantly increased for NAcS D2-MSNs (Paired two-tailed t_6=2.092, P=0.0407, n=7) and D1-MSNs (Paired two-tailed t_5=2.042, P=0.0483, n=6). **(C)** NAcC D2-MSN display a trend toward a decrease in sEPSC frequency (Paired two-tailed t_6=2.114, P=0.0789, n=7), but no change in amplitude (Paired two-tailed t_6=0.2319, P=0.8223, n=7) following 50 Hz stimulation with 1 msec pulses. **(D)** NAcS D2-MSN sEPSC frequency (Paired two-tailed t_7=2.526, P=0.0449, n=8), but not amplitude (Paired two-tailed t_7=0.7378, P= 0.4817, n=8) was significantly decreased following 50 Hz stimulation. **(E)** NAcS D1-MSN sEPSC frequency (Paired two-tailed t_6=2.995, P=0.0201, n=7), but no change in amplitude was observed (Paired two-tailed t_6=2.295, P=0.0615, n=7) was significantly decreased following 50Hz stimulation. Scale bars: 25pA, 1sec. *p<0.05, #p=0.08.

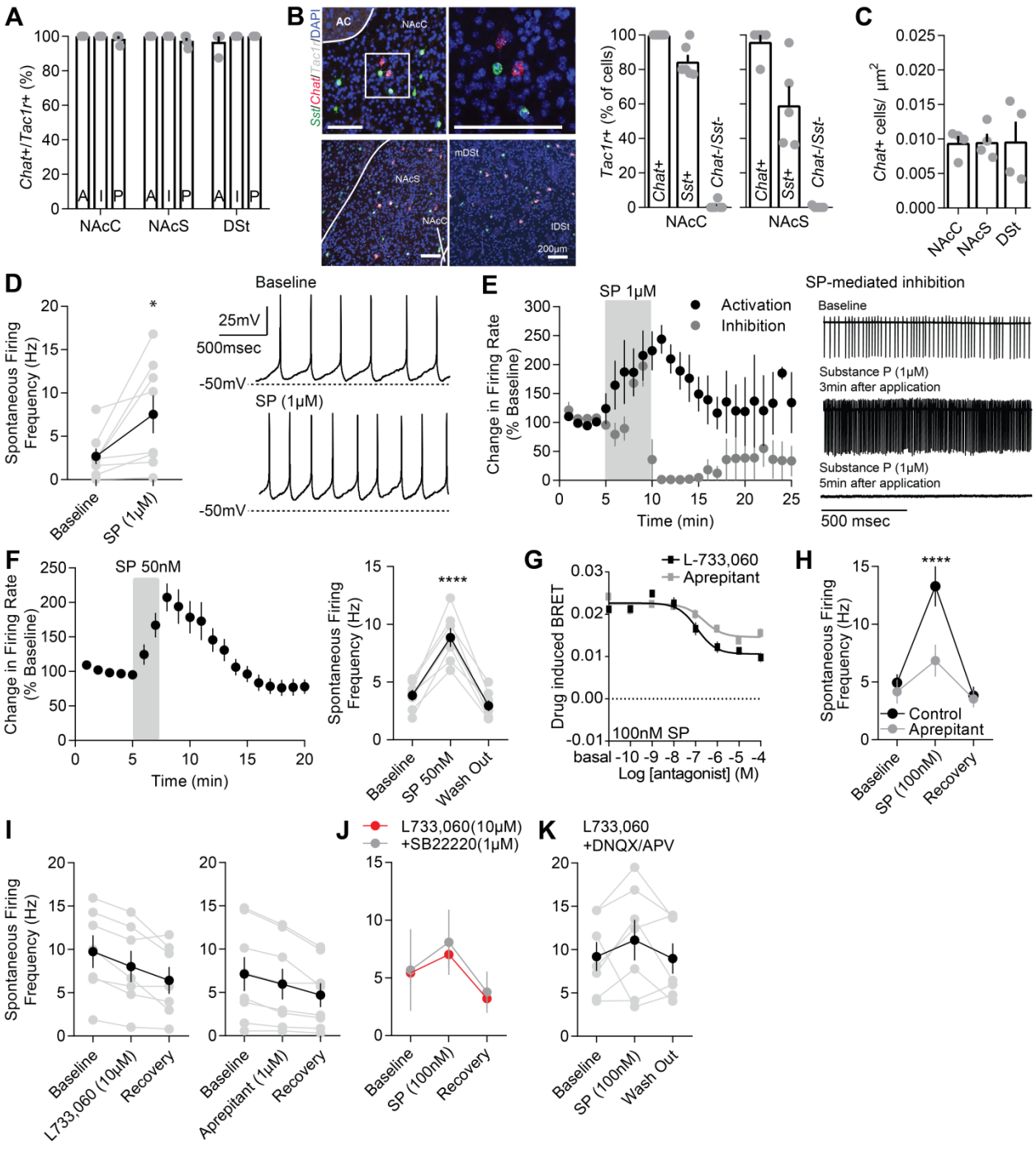

Figure S4 (Related to Figure 4). Substance P enhances ChAT firing rate at varying concentrations via activation of the NK1 receptor. (A) Coexpression of choline acetyltransferase (*Chat*) RNA and neurokinin 1 receptor (*Tac1r*) does not differ across Bregma coordinates from anterior (A), intermediate (I), and posterior (P) slices. **(B)** Nearly all *Chat* positive cells coexpress *Tac1r* RNA across all regions (NAcC, NAcS, and DSt). The remaining coexpression is found in somatostatin (*Sst*) RNA positive cells. **(C)** The density of *Chat* positive cells does not differ across all striatal regions assessed. **(D)** Whole cell spontaneous firing is significantly enhanced by bath application of substance P (1 μM) (Paired t_7=2.631, P=0.0339, n=8). **(E)** Spontaneous activity in a subset of ChAT neurons was rapidly enhanced then suppressed by substance P (1 μM) via a depolarization block mechanism.

(F) Substance P (50 nM) significantly enhanced spontaneous firing rate of ChAT neurons (One-way RM ANOVA $F_{12,84}$=8.886, P<0.0001, n=8). Spontaneous firing frequency is enhanced by substance P (50nM) (One-way RM ANOVA $F_{2,12}$=54.50, P<0.0001, n=8). **(G)** Suppression of Gq-NK1 receptor substance P-induced BRET by increasing concentrations of NK1 receptor antagonists L-733,060 and aprepitant. **(H)** Aprepitant suppresses enhanced spontaneous firing of ChAT neurons caused by bath application of substance P (100 nM) (Two-way RM ANOVA Interaction $F_{2,28}$=9.537, P<0.0001, n=8 cells per group). **(I)** Despite some rundown effects, spontaneous firing frequency is not altered by neurokinin antagonist bath application (L-733,060: One-way RM ANOVA $F_{2,14}$=5.868 P=0.0141, n=8, Dunnett Baseline vs. L-733,060 q_{14}=1.909, p>0.05; Aprepitant: One-way RM ANOVA $F_{2,16}$=4.066 P=0.0373, n=9, Dunnett Baseline vs. Aprepitant q_{16}=1.740, p>0.05). **(J)** The addition of the NK3 receptor antagonist SB22220 does not further suppress the substance P antagonism by L-733,060 (Two-way RM ANOVA No Interaction $F_{2,18}$=0.1790, P=0.8376; Main effect of time $F_{2,18}$=17.42, P<0.0001, n=3-8 cells per group). **(K)** Glutamate receptor antagonists DNQX and APV are not capable of further suppressing the substance P antagonism by L-733,060 (One-way RM ANOVA $F_{2,12}$=1.112, P=0.3607, n=7). *p<0.05, ****p<0.0001.

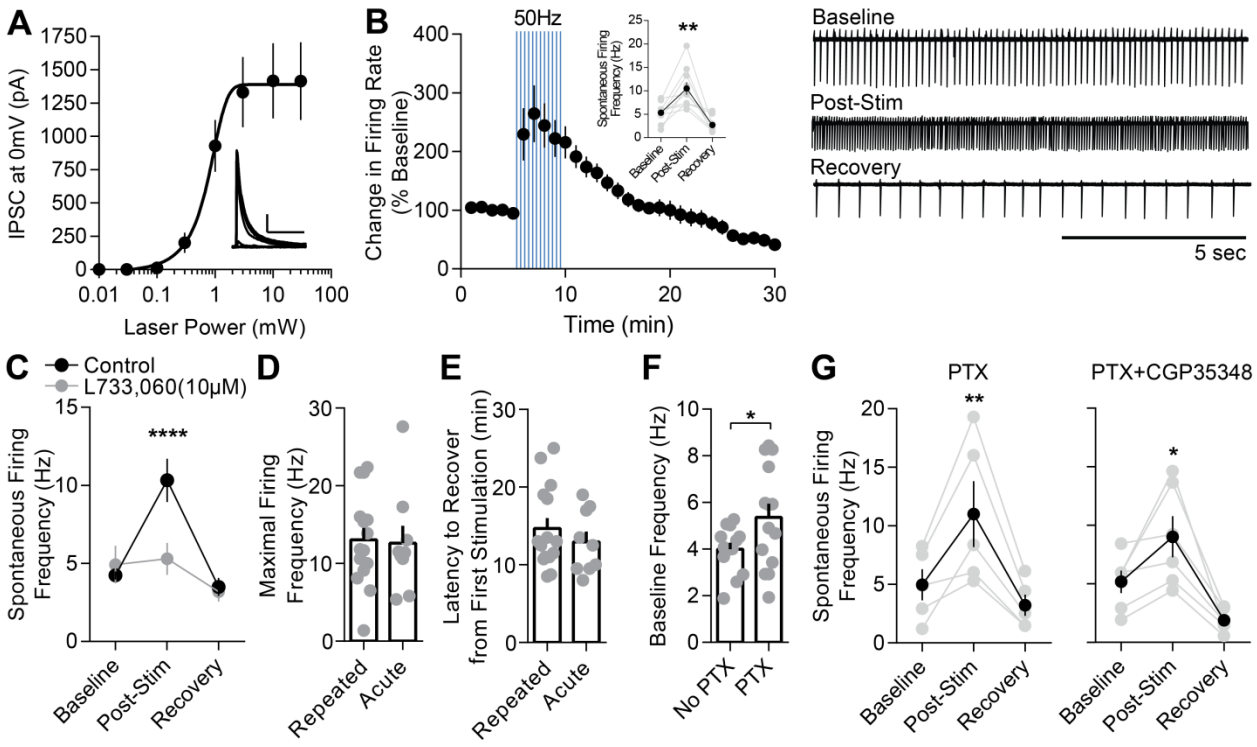

Figure S5 (Related to Figure 5). Acute or repeated stimulation enhances ChAT activity and is not dependent on GABA receptor disinhibition. (A) Increasing inhibitory post-synaptic currents in response to varying intensities of 473 nM blue light stimulation. **(B)** 5 min of repeated 50 Hz stimulation significantly enhances spontaneous firing rate of ChAT neurons (Frequency: One-way RM ANOVA $F_{2,20}$=17.87, P<0.0001, n=11, Baseline vs. Post-Stim q_{20}=3.909, p<0.01). **(C)** L-733,060 NK1 receptor antagonism blocks stimulation-induced enhancement in spontaneous ChAT firing frequency (Two-way RM ANOVA Interaction $F_{2,22}$=8.705, P=0.0016; Sidak's multiple comparisons (baseline vs. post-stim): L-733,060 t_{22}=0.3409, P<0.05; control t_{22}=6.115, P<0.0001, n=6-7). **(D)** No difference in maximal spontaneous firing frequency of ChAT neurons after repeated stimulation or acute stimulation (Unpaired two-tailed t_{22}=0.1626, P=0.8723, n=15,9). **(E)** No difference in latency of spontaneous firing frequency to recover to baseline levels is observed after repeated stimulation or acute stimulation (Unpaired two-tailed t_{22}=0.8385, P=0.4108, n=15,9 cells). **(F)** A small difference in baseline spontaneous firing frequency of ChAT in the presence of picrotoxin (PTX) neurons is observed (Unpaired two-tailed t_{25}=2.066, P=0.0493, n=13,14 cells). **(G)** GABA receptor antagonists have no effect on D1-MSN 50 Hz stimulation-induced enhancement in firing frequency ($GABA_A$: One-way RM ANOVA $F_{2,8}$=12.73, P=0.0033, n=5, baseline vs. Post-Stim q_8=3.727, p<0.05; $GABA_A$ and $GABA_B$: One-way RM ANOVA $F_{2,10}$=, P=0.0009, n=6, baseline vs. Post-Stim q_{10}=2.982, p<0.05). *p<0.05, **p<0.01, ****p<0.0001.

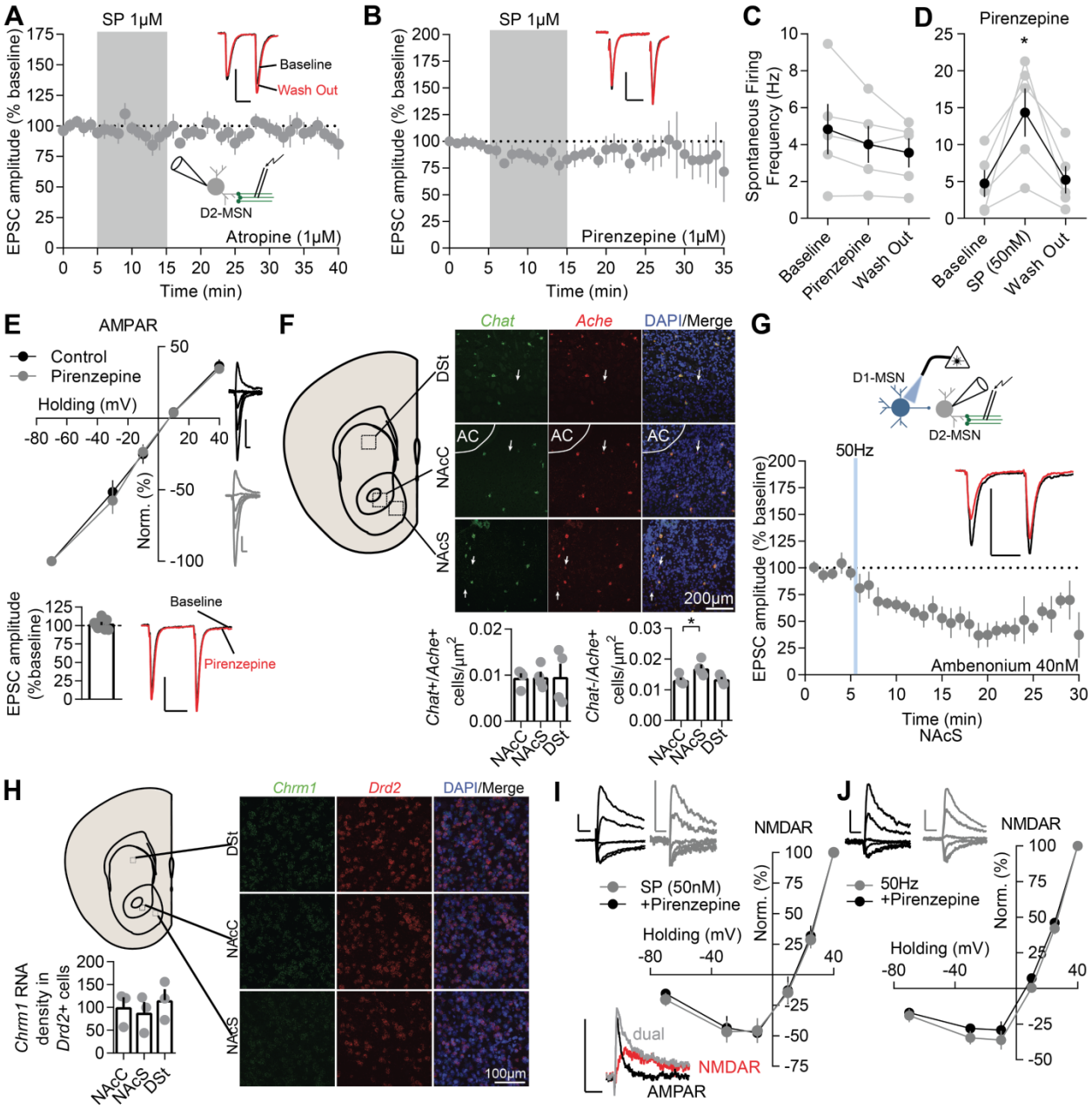

Figure S6 (Related to Figure 6). Muscarinic 1 receptor blockade does not affect baseline firing rates and expression is not different across striatal compartments. (A) Atropine (1 μM) blocks LLP on D2-MSNs caused by substance P (1 μM) (One-way RM ANOVA GG correction $F_{2.885,23.08}$=0.6758, P=0.5702, n=9). Scale bar for synaptic recordings at -70 mV: 100 pA, 25 msec. **(B)** Pirenzepine (10 μM) blocks LLP on D2-MSNs caused by substance P (1 μM) (One-way RM ANOVA GG correction $F_{2.541,15.25}$=0.7473, P=0.5200, n=7). **(C)** Pirenzepine alone does not affect spontaneous firing rate of ChAT neurons (One-way RM ANOVA $F_{2,8}$=2.712, P=0.1261, n=5). **(D)** Pirenzepine does not affect substance P-induced enhancements in ChAT firing rate (One-way RM ANOVA $F_{2,8}$=10.15, P=0.0064, n=5). **(E)** Pirenzepine does not affect the D2-MSN current-voltage relationship (Two-way RM ANOVA No interaction $F_{5,40}$=0.3566, P=0.8749, n=5 cells per group), Pirenzepine does not affect EPSC amplitude in the NAcC at a 1 μM concentration (One-sample t_8=1.187, P=0.2694, n=9). **(F)** Expression of acetylcholinesterase (*Ache*) RNA is found in all *Chat*+ cells and some *Chat*- cells (arrows showing examples) across all regions of the striatum. No difference in density is observed in *Chat*+ cells that coexpress *Ache* RNA (One-way

ANOVA: $F_{2,9}$=0.0028, P=0.9972, n=4). Density of cells displaying coexpression of *Ache* RNA in *Chat*- is higher in the NAcS (One-way ANOVA: $F_{2,9}$=5.064, P=0.0336, n=4; NAcC vs. NAcS: q_9=4.029, p<0.05). **(G)** Inhibition of acetylcholinesterase (AchE) with Ambenonium (40 nM) was not capable of blocking 50 Hz D1-MSN stimulation-mediated excitatory depression in the NAcS (One-way RM ANOVA with GG: $F_{3.374,23.62}$=4.300, P=0.0587, n=4). **(H)** Muscarinic 1 receptor (*Chrm1*) RNA expression does not differ across striatal compartments (One-way RM ANOVA GG: $F_{1.010,2.109}$=8.368, P=0.1006, n=3). **(I)** NMDA receptor currents on D2-MSNs do not differ between substance P and substance P+pirenzepine treatment (Two-way RM ANOVA No interaction $F_{5,75}$=0.9926, P=0.0955, n=8,9 cells). Scale bar for NMDA receptor currents 50 pA, 25 msec. **(J)** NMDA receptor currents on D2-MSNs do not differ between D1-MSN 50 Hz stimulation and stimulation+pirenzepine (Two-way RM ANOVA No interaction $F_{5,70}$=0.4128, P=0.8383, n=9,7 cells). *p<0.05.

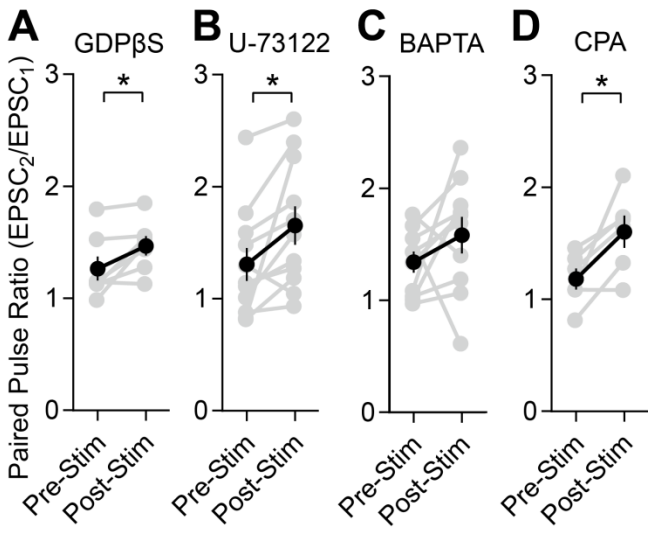

Figure S7 (Related to Figure 7). Presynaptic excitatory depression on NAcC D2-MSNs is observed in response to 50 Hz D1-MSN stimulation in most conditions where muscarinic 1 receptor signaling is blocked. (A) Blocking G-protein signaling with GDPβS enhances PPR (Paired two-tailed t_6=2.686, P=0.0362, n=7) **(B)** Antagonism of PLC with U-73122 enhances PPR (Paired two-tailed t_{10}=2.654, P=0.0242, n=11) **(C)** Calcium chelation with BAPTA does not change PPR (Paired two-tailed t_9=1.255, P=0.2410, n=10). **(D)** Internal calcium store depletion with CPA enhances PPR (Paired two-tailed t_5=3.066, P=0.0279, n=6). *p<0.05

Table S1. Exact Statistics for All Main Text Figures, Related to STAR Methods

Fig	Panel	Test	Subcategory	Test Statistic	*P*-value	*n*
1	B	Mixed-effects model (some 50 Hz measurements unpaired)		Frequency vs. Light Intensity $F_{2,12}$=4.928	0.0266	5,9
		Sidak's Multiple Comparison	50 Hz: 0.1 mW vs. 1.0 mW	t_{24}=4.569	0.0004	5,9
	C	RM One-Way ANOVA, with Geisser-Greenhouse's correction (GG)	D1-MSNs	$F_{3.732,29.86}$= 1.187	0.3356	9
			D2-MSNs	$F_{3.025,39.33}$= 8.927	0.0001	14
	D	RM One-Way ANOVA, GG	D1-MSNs	$F_{3.339,16.70}$= 2.883	0.0346	9
			D2-MSNs	$F_{4.242,33.94}$= 3.139	0.0491	8
	F	Dunnett's Multiple Comparisons from Panel C and D, Baseline vs. 20min time point, 15min after stimulation	D1-MSNs NAcC	q_8= 1.031	n.s., not significant	9
			D2-MSNs NAcC	q_{13}= 6.623	<0.001	14
			D1-MSNs NAcS	q_7= 4.323	<0.05	8
			D2-MSNs NAcS	q_8= 5.369	<0.01	9
	G	RM One-Way ANOVA, GG	10 Hz	$F_{1.940,7.758}$= 1.725	0.2397	5
			20 Hz	$F_{2.728,10.91}$= 0.9608	0.4383	5
2	A	RM One-Way ANOVA, GG	D1-MSNs	$F_{2.829,11.32}$= 0.7073	0.5594	5
			D2-MSNs	$F_{2.096,14.67}$= 6.930	0.0071	8
	B	RM One-Way ANOVA, GG		$F_{4.448,35.58}$ = 0.8104	0.5380	9
	D	RM One-Way ANOVA, GG	D1-MSNs	$F_{2.969,14.84}$= 2.134	0.1396	6
			D2-MSNs	$F_{2.189,28.45}$= 3.400	0.0436	14
		Dunnett's Multiple Comparisons from Panel D, Baseline vs. 20 min time point	D1-MSNs NAcC	q_5= 1.004	n.s.	5
			D2-MSNs NAcC	q_{13}= 3.759	<0.05	14
	F	One-sample two-tailed *t*-test, at least 10 min after SP, Theoretical mean 100	D1-MSNs NAcC	t_7= 1.908, actual mean 84.53	0.0980	8
			D2-MSNs NAcC	t_{23}= 3.181, actual mean 133.4	0.0042	24
	G	RM One-Way ANOVA, GG		$F_{3.280,22.96}$= 0.6235	0.6206	8
	H	RM One-Way ANOVA, GG	1 to 2	$F_{3.032,18.19}$= 3.836	0.0271	7
			2 to 3	$F_{2.616,13.08}$= 1.951	0.1749	7
3	A	Paired *t*-test, one-tailed	D2-MSNs	t_{14}= 0.9873	0.1701	15
			D1-MSNs	t_{10}= 0.4445	0.3331	11
	B	Paired *t*-test, one-tailed	D2-MSNs	t_{21}= 1.839	0.0800	22
			D1-MSNs	t_7= 1.045	0.3309	8
	C	Paired *t*-test, two-tailed	sEPSC Freq	t_{11}= 0.7521	0.4665	12
			sEPSC Amp	t_{11}= 4.500	0.0009	12
	D	Paired *t*-test, two-tailed	sEPSC Freq	t_{10}= 1.759	0.1090	11

			sEPSC Amp	t_{10}= 1.145	0.2791	11
	E	Paired *t*-test, two-tailed	sEPSC Freq	t_{16}= 0.8701	0.3971	17
			sEPSC Amp	t_{16}= 4.206	0.0007	17
	F	Paired *t*-test, two-tailed	sEPSC Freq	t_{7}= 2.919	0.0224	8
			sEPSC Amp	t_{7}= 0.5790	0.3971	8
4	B	One-Way ANOVA		$F_{2,16}$=1.062	0.3690	6,6,7
	C	RM One-Way ANOVA, GG, selected from set of neurons not undergoing depolarization block		$F_{1.457,7.284}$= 70.91	<0.0001	6
		Dunnett's Multiple Comparisons, Baseline vs. SP	Baseline vs. SP	q_{5}= 3.759	<0.01	6
	F	RM One-Way ANOVA, GG	SP	$F_{2.452,31.87}$= 14.11	<0.0001	14
			+L-733,060	$F_{2.824,16.94}$= 13.44	=0.0001	7
		Dunnett's Multiple Comparisons, Baseline vs. 10min	SP	q_{13}= 3.759	<0.05	14
			+L-733,060	q_{6}= 2.435	n.s.	7
	G	RM Two-Way ANOVA	Control, L-733,060	Interaction $F_{2,26}$= 13.25	0.0001	7,8
		Sidak's multiple comparison	Baseline vs. SP (Control)	t_{26}=8.269	<0.0001	7,7
			Baseline vs. SP (L-733,060)	t_{26}=1.864	n.s.	7,7
5	B	RM One-Way ANOVA, GG, time course	50 Hz	$F_{1.632,14.69}$= 19.75	0.0001	10
			+Aprepitant	$F_{2.586,18.10}$= 6.150	0.0059	8
		Dunnett's Multiple Comparisons, Baseline vs. 10min	50 Hz	q_{9}= 4.100	<0.05	10
			+Aprepitant	q_{7}= 0.1722	n.s.	8
		RM Two-Way ANOVA, Frequency (insert)	Frequency: 50Hz, Aprepitant	Interaction $F_{2,34}$= 14.08	<0.0001	6,13
		Bonferroni multiple comparisons	Baseline vs. Post-Stim (Control)	t_{34}=7.184	<0.0001	6,6
			Baseline vs. Post Stim (Aprepitant)	t_{34}=0.8769	n.s.	13,13
	C	One-Way ANOVA	0.5, 1, 5, 5 repeated (sec)	$F_{3,29}$= 4.837	0.0075	4,8,10, 11
		Dunnett's Multiple Comparisons	0.5 vs. 1	q_{29}=0.988	0.5746	4,8
			0.5 vs. 5	q_{29}=2.728	0.0250	4,10
			0.5 vs. 5 repeated	q_{29}=3.122	0.0098	4,11
	D	One-Way ANOVA	10,20,50 (Hz)	$F_{2,24}$= 6.401	0.0059	8,9,10
		Dunnett's Multiple Comparisons	10 vs. 50	q_{24}=4.465	0.0114	8,10

			20 vs. 50	q_{24}=4.164	0.0187	9,10
	E	One-Way ANOVA	0, 10, 50 (Hz)	$F_{3,32}$= 11.42	<0.0001	16, 7, 7, 6
		Dunnett's Multiple Comparisons	0 vs. 50	q_{32}= 7.579	<0.0001	10,6
			10 vs. 50	q_{32}= 5.262	0.0040	7,6
			20 vs. 50	q_{32}= 7.339	<0.0001	7,6
6	A	RM One-Way ANOVA, GG		$F_{2.917,23.34}$= 0.5407	0.6544	9
	B	RM One-Way ANOVA, GG		$F_{2.714,21.71}$= 1.528	0.2371	9
	C	RM Two-Way ANOVA	SP, +Pirenzepine	Interaction $F_{4,60}$=0.1183, Main effect for treatment (Control vs. Antagonist) $F_{1,15}$=0.2630	Interaction 0.1183, Main effect for treatment =0.0631	7,10
		Sidak's Multiple Comparison	-70 vs. +40 mV	t_{75}= 3.107	<0.05	7,10
		Unpaired two-tailed *t*-test	Rectification Index	t_{14}= 2.296	0.0377	7,10
	D	RM Two-Way ANOVA	50 Hz, +Pirenzepine	Interaction $F_{5,100}$=4.275; Main effect for treatment $F_{1,20}$=3.599	Interaction 0.0014; Main effect for treatment =0.0114	10, 12
		Sidak's Multiple Comparison	-70 vs. +40 mV	t_{120}= 4.744	<0.0001	10, 12
		Unpaired two-tailed *t*-test	Rectification Index	t_{22}= 2.452	0.0226	10, 12
	E	RM Two-Way ANOVA		Interaction $F_{2,14}$=4.694; Main effect for treatment $F_{2,14}$=3.204	Interaction 0.0275; Treatment 0.0207	5,4
	F	RM Two-Way ANOVA		Interaction $F_{2,24}$=7.508; Main effect for treatment $F_{1,12}$=9.586	Interaction 0.0149; Treatment 0.0093	7,7
7	A	RM One-Way ANOVA GG		$F_{3.672,25.71}$= 2.902	0.0452	8
	B	RM Two-Way ANOVA		Interaction $F_{2,10}$=11.87; Main effect for condition $F_{1,5}$=5.073;	Interaction <0.0001; Condition 0.0741	6,6
		Bonferroni multiple comparison	+40 mV	t_{10}=5.152	<0.01	6,6
		Paired two-tailed *t*-test	Rectification Index	t_5=3.742	0.0134	6,6
	C	RM One-Way ANOVA GG		$F_{2.919,23.35}$= 3.088	0.0479	9
	D	One-sample *t*-test	Rectification Index	Theoretical mean 1.0; Actual mean 0.9379; t_7=0.471	0.6500	8

	E	RM One-Way ANOVA GG		$F_{2.268,18.15}$= 0.373	0.7192	9
	F	RM Two-Way ANOVA, post-hoc	+40 mV	Interaction $F_{2,12}$=20.20; Main effect for condition $F_{1,6}$=33.40;	Interaction <0.0001; Condition 0.0012	7,7
		Bonferroni multiple comparison	+40 mV	t_{12}=8.806	<0.0001	7,7
		Paired two-tailed *t*-test	Rectification Index	t_6=3.095	0.0213	7,7
	G	RM One-Way ANOVA GG		$F_{2.707,18.95}$= 2.45	0.0995	8
	H	One-sample *t*-test	Rectification Index	Theoretical mean 1.0; Actual mean 1.021; t_7=0.173	0.8670	8