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Regulation of dopamine release by tonic activity patterns in the striatal brain slice

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Abstract

Voluntary movement, motivation, and reinforcement learning depend on the activity of ventral midbrain neurons that extend axons to release dopamine (DA) in the striatum. These neurons exhibit two patterns of action potential activity: a low-frequency tonic activity that is intrinsically generated, and superimposed high-frequency phasic bursts that are driven by synaptic inputs. *Ex vivo* acute striatal brain preparations are widely employed to study the regulation of evoked DA release but exhibit very different DA release kinetics than *in vivo* recordings. To investigate the relationship between phasic and tonic neuronal activity, we stimulated the slice in patterns intended to mimic tonic activity, which were interrupted by a series of burst stimuli. Conditioning the striatal slice with low-frequency activity altered DA release triggered by high-frequency bursts, and produced kinetic parameters that resemble those *in vivo*. In the absence of applied tonic activity, nicotinic acetylcholine receptor and D2 dopamine receptor antagonists had no significant effect on neurotransmitter release driven by repeated burst activity in the striatal brain slice. In contrast, in tonically stimulated slices, D2 receptor blockade decreased the amount of DA released during a single burst and facilitated DA release in subsequent bursts. This experimental system provides a means to reconcile the difference in the kinetics of DA release *ex vivo* and *in vivo* and provides a novel approach to more accurately emulate pre- and post-synaptic mechanisms that control axonal DA release in the acute striatal brain slice.

Keywords: *dopamine, in vivo DA release, fast-scan cyclic voltammetry, striatal slice, D2 receptors, nicotinic acetylcholine receptor*

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Introduction

Basal ganglia dopaminergic neurons located in the substantia nigra pars compacta (SNpc) and ventral tegmental area of the midbrain send axonal projections to the striatum, where the release of dopamine (DA) plays pivotal roles in reinforcement learning, motivation, and motor control (Graybiel et al., 1994; Lanciego et al., 2012; Salamone, 2007). *In vivo*, these neurons predominantly exhibit two firing patterns: a tonic self-autonomous single-spike activity at 1-10 Hz (Grace & Bunney, 1984), and phasic burst activity at 13-20 Hz in anesthetized rodents and up to 80 Hz in awake animals or humans (Duda et al., 2016) that is driven by synaptic inputs. Tonic and phasic firing of DA neurons have been suggested to play distinct roles in animal behaviors: tonic neurotransmitter release is thought to be required for motivation and motor control, whereas phasic activity in response to environmental cues encodes reward prediction error and is important for learning (Eshel et al., 2024; Zweifel et al., 2009). Both tonic and phasic activity are regulated by pharmacological agents, including antipsychotic drugs, psychostimulants, and drugs of abuse including opiates, nicotine, and alcohol, as well as stress and pathological conditions. A dysregulation of firing patterns is associated with neurological disorders including Parkinson's disease, addiction, and schizophrenia (Juárez Olguín et al., 2016).

Many studies have analyzed the kinetics of DA exocytosis in acute striatal slices, providing fundamental insights into the regulation of evoked neurotransmitter release by drugs and local synaptic circuitry and demonstrating regional variations in DA release probability and short-term plasticity within the striatum (Cragg, 2003, 2006; Exley et al., 2008; Wu et al., 2002; H. Zhang & Sulzer, 2003). These *ex vivo* studies have shown that striatal DA release is modulated by many mechanisms, including changes in vesicular DA content, inhibition of the dopaminergic axons by D2 receptors (D2R) (Kehr et al., 1972; Lindgren et al., 2001; O'Hara et al., 1996; Schmitz et al., 2002), control by locally released acetylcholine (ACh) (Brimblecombe et al., 2018; Sulzer et al., 2016), and regulation of fusion pore kinetics (Staal et al., 2004). Additionally, the dynamic activity-dependent equilibrium between different pools of synaptic vesicles within the release sites – the readily releasable pool (RRP), the recycling pool and the reserve pool – is an important factor in the regulation of DA vesicle release probability and the refilling of recycling synaptic vesicles with neurotransmitter (Somayaji, Cataldi, Choi, et al., 2020; Somayaji, Cataldi, Edwards, et al., 2020; Yavich & MacDonald, 2000). Due to these mechanisms, phasic DA release has been suggested to be inversely dependent on the frequency of tonic activity, due to a larger readily releasable pool of synaptic vesicles and lower D2R-mediated autoinhibition in inactive dopaminergic axons (Eshel et al., 2024; Grace, 1991), although experimental evidence for this hypothesis has not been reported.

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While the acute striatal brain slice provides strong experimental advantages due to the ease of pharmacological or optogenetic manipulations of the local circuitry, limitations of this model system are highlighted by very different DA release kinetics *in vivo* (Shashaank et al., 2023; Somayaji, Cataldi, Choi, et al., 2020). Typically, kinetic studies of electrochemical recordings of evoked DA release *in vivo* are performed in anesthetized animals with the recording electrode implanted in the striatum and stimulation electrode in the ventral midbrain or the medium forebrain bundle. In contrast to slice recordings where single electric pulse evokes large release of DA (Schmitz et al., 2003; Stamford et al., 1991), a single stimulus pulse is typically insufficient to evoke detectable release of DA *in vivo* (Chergui et al., 1994; Somayaji, Cataldi, Choi, et al., 2020; Venton et al., 2003). Moreover, consecutive phasic bursts in striatal slices produce a profound release depression that contrasts with a facilitation of DA release observed *in vivo* (Somayaji, Cataldi, Choi, et al., 2020).

A notable difference between the *in vivo* and *ex vivo* recordings is that tonic activity is present *in vivo* but absent in coronal striatal slices that lack the cell bodies of dopaminergic neurons, where tonic activity is generated. To investigate whether tonic activity underlies the difference between *ex vivo* and *in vivo* kinetics of DA release and plasticity, we applied local electrical stimulation to the slice at tonic frequencies. The tonic conditioning in the striatal slice was followed by a series of high frequency bursts to determine changes in release facilitation or depression. Following characterization of these kinetics, we investigated the effect of nicotinic ACh receptor and D2 dopamine receptor antagonists on slice burst kinetics in the presence and absence of tonic activity. Our results indicate that superimposition of phasic firing patterns on tonic activity provides a means to use an *ex vivo* preparation for an improved analysis of the mechanisms that govern the release of monoamine neurotransmitters *in vivo*.

Results

To verify previously reported differences between *in vivo* and *ex vivo* striatal recordings, we compared evoked DA release in anesthetized mice and in acute striatal slices after a single stimulus followed by three high-frequency bursts of 30 pulses at 50 Hz (**Fig 1A**). *In vivo*, electrical stimuli were applied to the SNpc, while DA release was recorded in the dorsal striatum with fast scan cyclic voltammetry (FSCV). DA release in the striatal slice was stimulated locally in the dorsal striatum.

As expected, *in vivo* DA release was below the detection limit with a single pulse stimulation and was only detectable after a burst of stimuli (**Fig 1A**). In contrast, a single electrical stimulus

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produced a large and easily resolved FSCV signal in the slice, with burst stimulation further increasing the amount of released neurotransmitter. Importantly, facilitation of DA release during consecutive stimulation bursts was observed *in vivo* while a depression of release occurred *ex vivo* (**Fig 1B**).

To investigate whether lack of tonic activity in the slice is responsible for the differences in DA release kinetics *in vivo* and *ex vivo*, we stimulated slices at frequencies ranging from 0.5 to 5 Hz that were intended to emulate pacemaking activity of DA neurons (**Fig 2A**). We note that synaptic vesicle fusion in DA axons is expected to have a higher release probability with local electrical stimulation than with action potentials generated distally in the cell bodies, due to electric charge loss along lightly myelinated dopaminergic axons (Formisano et al., 2020; Liu et al., 2018). Introduction of artificial tonic activity in the striatal slice rapidly decreased DA release in individual pulses to undetectable levels, with more rapid depletion at higher stimulation frequencies (**Fig 2B-E**). No evoked DA peaks were observed at any tonic frequency stimulus by the end of a 5-minute train application.

Tonic stimulation was followed by three bursts, each consisting of 30 pulses at 50 Hz, to mimic phasic activity; and identical stimulus series was applied before the start of the tonic activity as an internal control for the effect of tonic stimuli (**Fig 2A**). An increase in tonic frequency from 0.5 to 2 Hz produced a progressive reduction of DA release following a single phasic burst (**Fig 2E-F**), while no release was detected at 5 Hz, likely due to the depletion of DA stored in synaptic vesicles (**Fig 2D**). Interestingly, neurotransmitter release during the 2nd and 3rd high-frequency bursts was much less affected by preceding tonic stimulation (**Fig 2E-F**). This resulted in a progressive relief of DA release depression during a series of burst stimuli that correlated with higher tonic activity (**Fig 2G**): at 2 Hz tonic stimulation, there was no significant reduction in the amplitude of DA evoked by sequential bursts, suggesting that the depression of release in *ex vivo* preparations is dependent on the lack of tonic activity.

Control of DA release by nAChR

Striatal cholinergic interneurons (ChI) constitute only 2%–5% of striatal neurons, but establish an extensive network of axons that provides a profound control over striatal DA neurotransmission via pre- and postsynaptic cholinergic receptors (Giorguieff et al., 1976; Rice & Cragg, 2004; H. Zhang & Sulzer, 2004; Zhou et al., 2001). Similar to previously published results (McGuirt et al., 2021; Threlfell & Cragg, 2011), application of the nAChR antagonist dihydro-β-erythroidine hydrobromide (DhβE, 1 μM) decreased DA release after a single stimulus but not after a burst stimulation, thus significantly increasing burst-to-single pulse ratio (**Fig 3A-C**). However, inhibition

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of nAChR in the presence of artificial tonic activity exerted no additional effect on a single burst release (**Fig 3D**) or on neurotransmitter release evoked by a series of bursts (**Fig 3E**), suggesting that nAChRs play a limited role in DA release modulation in the presence of artificial tonic activity.

Role of D2R

The modulation of dopamine release within the striatum originates from a dynamic interplay between dopamine receptors and a local striatal circuitry. D2 auto-receptors expressed on the axons of DA neurons provide feedback control of striatal DA release via activation of potassium GIRK channels that hyperpolarize the cells (Marcott et al., 2014), while D2 receptors on ChIs regulate ACh and DA release via postsynaptic mechanisms (Kharkwal et al., 2016).

Acute treatment of striatal slice with D2R antagonist sulpiride (10 μM) significantly increased DA released by a single pulse stimulus but did not change the amount of DA overflow following burst stimulation (**Fig 3A-C**). Tonic slice stimuli in the presence of sulpiride caused further decrease in DA released by a single burst (**Fig 3D**), consistent with a disinhibition of dopaminergic axons that produced a larger depletion of the RRP of dopaminergic vesicles. Furthermore, blockade of D2R combined with tonic stimulation caused more DA to be released in consecutive bursts, leading to a facilitation of neurotransmitter release (**Fig 3F, Table 1**).

Table 1. Summary of changes in DA and ACh levels during tonic slice stimulation and nAChR blockade

Treatment	Tonic activity	Single pulse *Figs 2C&3A*	Single burst *Figs 3B&D*	Multiple bursts *Figs 3E&F*
Ctrl	No			depression
DHβE	No	↓	↔	depression
Sulpiride	No	↑	↔	depression
Ctrl	Yes	n.d.	↓	no change
DHβE	Yes	n.d.	↓	no change
Sulpiride	Yes	n.d.	↓↓	Facilitation
In vivo	Yes	n.d.		Facilitation

n.d. is 'not detected'; ↔ stands for no difference relative to control.

Discussion

We introduce a simple experimental approach that can be employed to study the role of tonic activity in modulating phasic DA release in an acute striatal slice preparation. While the highest tested tonic frequency (5 Hz) caused complete cessation of DA neurotransmission (**Fig 2D**), likely

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due to the depletion of vesicular transmitter pools, at lower frequencies we uncovered several key characteristics of DA release that have not been previously reported in *ex vivo* preparations (**Table 1**).

First, tonic slice conditioning greatly diminished DA release following a single pulse stimulation, accompanied by a much smaller decrease in phasic neurotransmitter release, thus leading to an enhanced burst-to-single stimulus ratio. Such ‘high frequency filtering’ of DA neurotransmission, also observed *in vivo*, would lead to enhanced signal/noise during phasic DA release in response to environmental cues critical for learning (Bromberg-Martin et al., 2010). Interestingly, similar alteration in DA release kinetics has been reported in acute striatal slices treated with nAChR blockers (Brimblecombe et al., 2018; Rice & Cragg, 2004; Sulzer et al., 2016) (**Fig 3A,B**), suggesting that tonic DA and ACh tones have opposite effects on the signal/noise of DA neurotransmission. Nicotinic receptors are ligand-gated non-selective cation channels that potently modulate DA release in the striatum (Exley et al., 2008; T. Zhang et al., 2009; Zhou et al., 2001) and their activation on DA neurons drives depolarization and local action potentials (Liu et al., 2022). It is reported that tonic ACh tone facilitates DA release at lower firing frequencies but has no effect or caused depression of dopamine release at higher frequencies (>2 Hz) (McGuirt et al., 2021; Paladini & Roeper, 2014; Rice & Cragg, 2004; T. Zhang et al., 2009). The similarity between the effects of nAChR antagonists and the presence of tonic slice stimuli that both decrease single pulse-evoked DA release without affecting neurotransmitter release during bursts may be explained by the desensitization of nAChR (Rice & Cragg, 2004). During local tonic electrical stimulation of the slice, basal ACh concentration likely also increases, leading to nAChR desensitization. Since recovery from desensitization of nAChR may take up to a minute (Giniatullin et al., 2005), at the time of the burst stimulus (10 sec after the end of the tonic train) the effect of the tonic conditioning would be similar to nAChR pharmacological blockade (**Table 1**), although the contribution of this and other mechanisms requires further investigation.

Another mechanism that exerts control over striatal DA neurotransmission is via activation of D2R expressed on both dopaminergic axons and ChI cell bodies (Kharkwal et al., 2016; Kita et al., 2007; Schultz, 1998). In acute striatal slices, tonically released DA is expected to bind D2R and induce chronic depression of DA axons and ChI, thus decreasing the probability of DA release. Conversely, D2R blockade, not only allows more DA to be released from slices without tonic activity (**Fig 3A**), but also causes higher apparent synaptic vesicle depletion after tonic activity (**Fig 3D**).

As long reported, in naïve slices, sequential bursts of stimuli cause a profound depression of DA release (Phillips et al., 2002; Schmitz et al., 2002), but we now show that tonic stimulation of

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the slice leads to less dependence of released DA on the burst number, especially at higher tonic frequencies (**Fig 2G**). Furthermore, when tonic slice stimulation was combined with the pharmacological blockade of D2R, repeated stimulus bursts *facilitated* DA release, a response that has not been previously observed *ex vivo* (**Fig 3F**). A possible explanation for this phenomenon is that DA release from different vesicle pools possesses different calcium sensitivities. For example, if release from the RRP has higher Ca^{2+} affinity (i.e., can be triggered at lower cytosolic Ca^{2+} concentrations) than release from the recycling and the reserve pools, then depletion of the RRP during tonic activity would reveal synaptic facilitation due to Ca^{2+} buildup during high-frequency bursts. Consistently, we find that burst-to-burst depression of DA release was inversely proportional to tonic frequency, while release facilitation occurred in slices treated with a D2R antagonist, which as discussed above, drives a still stronger depletion of the RRP. These phenomena may further depend on muscarinic ACh receptors (Threlfell & Cragg, 2011), GABA receptors (Lopes et al., 2019) and possibly additional receptor-mediated mechanisms.

While the experimental system we introduce is relatively simple, it features many parameters that can be varied depending on the aims of the study, including the intensity and the frequency of tonic and phasic stimuli, the duration of tonic stimulation, and the delay before the burst stimulation and between the bursts. Furthermore, optogenetic stimulation of DA axons and/or ChI cell bodies, which should cause more targeted neuronal activation than electrical stimuli albeit with non-physiological activation mechanisms, as well as assessment of basal DA and ACh levels with electrochemical or optical techniques promise to further decipher the roles of tonic dopaminergic and cholinergic activities in modulating phasic DA neurotransmission.

In summary, we present a proof of principle study that demonstrates a critical role of tonic firing in shaping dopamine release dynamics and highlights the importance of studying neurotransmitter modulation in physiologically relevant settings. Bridging the gap between *ex vivo* and *in vivo* data will pave the way for understanding the mechanisms underlying dopamine signaling and its implications for neurological disorders and pharmacological interventions.

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Material and Methods

Experimental model and reagents

Male C57Bl6/J mice (3-6 months, The Jackson Laboratory, Bar Harbor, Maine) were used for both *in vivo* and *ex vivo* experiments. All animals were maintained on a 12:12 hour light/dark cycle with food and water *ad libitum*. All animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facilities. All experimental procedures were approved by the Columbia University Institutional Animal Care and Use Committee and followed guidelines established in the NIH Guide for the Care and Use of Laboratory Animals. Sulpiride (Cat#: 0894) and dihydro-β-erythroidine hydrobromide (Cat#: 2349) were from Tocris Bioscience.

Ex vivo striatal slices

Animals were euthanized by cervical dislocation, the brain was removed and placed in ice-cold sucrose cutting solution, containing (in mM): 10 NaCl, 2.5 KCl, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 180 sucrose and 10 glucose, oxygenated with 95% O_2/5% CO_2 to pH 7.4. Coronal slices (250 μm) that included the striatum were collected and allowed to rest at 34°C for 30 min in artificial cerebrospinal fluid (ACSF; in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.5 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4 and 10 glucose, oxygenated with 95% O_2/5% CO_2 to pH 7.4.

Fast-scan cyclic voltammetry (FSCV) ex vivo

FSCV recordings of evoked DA release by FSCV were performed as detailed previously (Lieberman et al., 2018; McGuirt et al., 2021). Striatal slices were incubated in oxygenated ACSF at room temperature for 30 min and transferred to a recording chamber with ACSF perfused at 2 mL/min and 34°C. A carbon fiber electrode was placed in the dorsolateral striatum ~50 μm into the slice. A triangular voltage ramps from a holding potential of - 450 mV to +800 mV over 8.5 ms (ramp of 294 V/s) were applied to the carbon fiber electrode every 100 ms. Current was recorded with an Axopatch 200B amplifier (Molecular Devices, Foster City, CA) filtered with a 10-kHz low-pass Bessel filter and digitized at 25 kHz (ITC-18 board, InstruTECH, Great Neck, NY). FSCV data acquisition and analysis were controlled by custom-built routines in Igor Pro (WaveMetrics Inc., Lake Oswego, OR). Slices were stimulated with a sharpened bipolar concentric electrode (400 μm max outer diameter; Pt/Ir; WPI) placed ~150 μm from the recording electrode, using an Iso-Flex stimulus isolator (AMPI) triggered by TTL pulses generated in the same custom-built Igor Pro routine and synchronized with FSCV pulses. Duration of each stimulus pulse was 0.2 ms. Carbon fiber electrodes were calibrated by quantifying background-subtracted voltammograms in

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standard solutions of DA in ACSF prepared fresh each recording day. Concentration traces were made in Igor Pro by collecting current over time at the peak DA oxidation potential normalized to the standard DA calibration factor.

FSCV recordings in vivo

We used previously published methods for *in vivo* FSCV recordings (Shashaank et al., 2023; Somayaji, Cataldi, Choi, et al., 2020). Briefly, male mice were anesthetized with iso-flurane (induction 4%, maintenance 0.8–1.4%), placed on a heating pad, and head-fixed on a stereotaxic frame. A craniotomy was performed to insert a 22G bipolar stimulating electrode (P1 Technologies, Roanoke, VA) in the ventral midbrain to trigger electrical pulse trains and a carbon-fiber microelectrode (5 μm diameter, ∼150 μm length) in the dorsal striatum to measure evoked DA release. The depth of the stimulating electrode was adjusted between 4 and 4.5 mm for maximal DA release. FSCV recordings and generation of the stimulation patterns were performed as described above. The electrical pulse trains were delivered to the stimulating electrode at a constant current of 400 μA using an Iso-Flex stimulus isolator; duration of each stimulus pulse was 4 ms. The carbon-fiber microelectrodes were calibrated in artificial cerebrospinal fluid using known concentrations of DA.

Statistical Analysis

Data was acquired and analyzed using the Igor Pro software and imported into Microsoft Excel (Microsoft Corp., Redmond, WA). All statistical analysis was performed in GraphPad Prism (version 10; La Jolla., San Diego, CA). All bar graphs show the mean ± SEM. Data comparing two variables were analyzed with a two-way ANOVA, followed by Bonferroni post-hoc test. Data comparing one variable among more than two groups were analyzed with one-way ANOVA and Tukey's post-hoc test.

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SAFETY

We have not found any unexpected, new, and/or significant hazards or risks associated with the reported work.

ABBREVIATIONS

DA: Dopamine

D2R: Dopamine D2 receptor

DHβE: Dihydro-β-erythroidine hydrobromide

FSCV: Fast-scan cyclic voltammetry

GABA: Gamma-aminobutyric acid

GIRK: G-protein-coupled inward rectifier potassium

MFB: Medium forebrain bundle

nAChR: Nicotinic acetylcholine receptors

RRP: Readily releasable pool

SNpc: Substantia nigra pars compacta

AUTHOR CONTRIBUTIONS

SB conducted all *ex vivo* FSCV experiments; EAM performed *in vivo* FSCV recordings in anesthetized mice. Data analysis and presentation was done by SB and EVM. All other authors contributed to experimental design, critical data analysis and writing of the manuscript.

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References

Brimblecombe, K. R., Threlfell, S., Dautan, D., Kosillo, P., Mena-Segovia, J., & Cragg, S. J. (2018). Targeted Activation of Cholinergic Interneurons Accounts for the Modulation of Dopamine by Striatal Nicotinic Receptors. eNeuro, 5(5), ENEURO.0397-17.2018. https://doi.org/10.1523/ENEURO.0397-17.2018

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: Rewarding, aversive, and alerting. *Neuron*, *68*(5), 815–834. https://doi.org/10.1016/j.neuron.2010.11.022

Chergui, K., Suaud-Chagny, M. F., & Gonon, F. (1994). Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. *Neuroscience*, *62*(3), 641–645. https://doi.org/10.1016/0306-4522(94)90465-0

Cragg, S. J. (2003). Variable Dopamine Release Probability and Short-Term Plasticity between Functional Domains of the Primate Striatum. *The Journal of Neuroscience*, *23*(10), 4378–4385. https://doi.org/10.1523/JNEUROSCI.23-10-04378.2003

Cragg, S. J. (2006). Meaningful silences: How dopamine listens to the ACh pause. *Trends in Neurosciences*, *29*(3), 125–131. https://doi.org/10.1016/j.tins.2006.01.003

Duda, J., Pötschke, C., & Liss, B. (2016). Converging roles of ion channels, calcium, metabolic stress, and activity pattern of Substantia nigra dopaminergic neurons in health and Parkinson's disease. *Journal of Neurochemistry*, *139*(Suppl Suppl 1), 156–178. https://doi.org/10.1111/jnc.13572

Eshel, N., Touponse, G. C., Wang, A. R., Osterman, A. K., Shank, A. N., Groome, A. M., Taniguchi, L., Cardozo Pinto, D. F., Tucciarone, J., Bentzley, B. S., & Malenka, R. C. (2024). Striatal dopamine integrates cost, benefit, and motivation. *Neuron*, *112*(3), 500-514.e5. https://doi.org/10.1016/j.neuron.2023.10.038

Exley, R., Clements, M. A., Hartung, H., McIntosh, J. M., & Cragg, S. J. (2008). Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *33*(9), 2158–2166. https://doi.org/10.1038/sj.npp.1301617

Formisano, R., Mersha, M. D., Caplan, J., Singh, A., Rankin, C. H., Tavernarakis, N., & Dhillon, H. S. (2020). Synaptic vesicle fusion is modulated through feedback inhibition by dopamine auto-receptors. *Synapse*, *74*(1), e22131. https://doi.org/10.1002/syn.22131

Giniatullin, R., Nistri, A., & Yakel, J. L. (2005). Desensitization of nicotinic ACh receptors: Shaping cholinergic signaling. *Trends in Neurosciences*, *28*(7), 371–378. https://doi.org/10.1016/j.tins.2005.04.009

Giorguieff, M. F., Le Floc'h, M. L., Westfall, T. C., Glowinski, J., & Besson, M. J. (1976). Nicotinic effect of acetylcholine on the release of newly synthesized (3H)dopamine in rat striatal slices and cat caudate nucleus. *Brain Research*, *106*(1), 117–131. https://doi.org/10.1016/0006-8993(76)90077-9

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Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience*, *41*(1), 1–24. https://doi.org/10.1016/0306-4522(91)90196-U

Grace, A. A., & Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: Burst firing. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *4*(11), 2877–2890. https://doi.org/10.1523/JNEUROSCI.04-11-02877.1984

Graybiel, A. M., Aosaki, T., Flaherty, A. W., & Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science (New York, N.Y.)*, *265*(5180), 1826–1831. https://doi.org/10.1126/science.8091209

Juárez Olguín, H., Calderón Guzmán, D., Hernández García, E., & Barragán Mejía, G. (2016). The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *2016*, 9730467. https://doi.org/10.1155/2016/9730467

Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T., & Atack, C. (1972). Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. *The Journal of Pharmacy and Pharmacology*, *24*(9), 744–747. https://doi.org/10.1111/j.2042-7158.1972.tb09104.x

Kharkwal, G., Brami-Cherrier, K., Lizardi-Ortiz, J. E., Nelson, A. B., Ramos, M., Del Barrio, D., Sulzer, D., Kreitzer, A. C., & Borrelli, E. (2016). Parkinsonism Driven by Antipsychotics Originates from Dopaminergic Control of Striatal Cholinergic Interneurons. *Neuron*, *91*(1), 67–78. https://doi.org/10.1016/j.neuron.2016.06.014

Kita, J. M., Parker, L. E., Phillips, P. E. M., Garris, P. A., & Wightman, R. M. (2007). Paradoxical modulation of short-term facilitation of dopamine release by dopamine autoreceptors. *Journal of Neurochemistry*, *102*(4), 1115–1124. https://doi.org/10.1111/j.1471-4159.2007.04621.x

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional Neuroanatomy of the Basal Ganglia. *Cold Spring Harbor Perspectives in Medicine*, *2*(12), a009621. https://doi.org/10.1101/cshperspect.a009621

Lieberman, O. J., McGuirt, A. F., Mosharov, E. V., Pigulevskiy, I., Hobson, B. D., Choi, S., Frier, M. D., Santini, E., Borgkvist, A., & Sulzer, D. (2018). Dopamine Triggers the Maturation of Striatal Spiny Projection Neuron Excitability during a Critical Period. *Neuron*, *99*(3), 540-554.e4. https://doi.org/10.1016/j.neuron.2018.06.044

Lindgren, N., Xu, Z. Q., Herrera-Marschitz, M., Haycock, J., Hökfelt, T., & Fisone, G. (2001). Dopamine D(2) receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. *The European Journal of Neuroscience*, *13*(4), 773–780. https://doi.org/10.1046/j.0953-816x.2000.01443.x

Liu, C., Cai, X., Ritzau-Jost, A., Kramer, P. F., Li, Y., Khaliq, Z. M., Hallermann, S., & Kaeser, P. S. (2022). An action potential initiation mechanism in distal axons for the control of dopamine release. *Science (New York, N.Y.)*, *375*(6587), 1378–1385. https://doi.org/10.1126/science.abn0532

Liu, C., Kershberg, L., Wang, J., Schneeberger, S., & Kaeser, P. S. (2018). Dopamine Secretion Is Mediated by Sparse Active Zone-like Release Sites. *Cell*, *172*(4), 706-718.e15. https://doi.org/10.1016/j.cell.2018.01.008

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Lopes, E. F., Roberts, B. M., Siddorn, R. E., Clements, M. A., & Cragg, S. J. (2019). Inhibition of Nigrostriatal Dopamine Release by Striatal GABAA and GABAB Receptors. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *39*(6), 1058–1065. https://doi.org/10.1523/JNEUROSCI.2028-18.2018

Marcott, P. F., Mamaligas, A. A., & Ford, C. P. (2014). Phasic dopamine release drives rapid activation of striatal D2-receptors. *Neuron*, *84*(1), 164–176. https://doi.org/10.1016/j.neuron.2014.08.058

McGuirt, A. F., Post, M. R., Pigulevskiy, I., Sulzer, D., & Lieberman, O. J. (2021). Coordinated Postnatal Maturation of Striatal Cholinergic Interneurons and Dopamine Release Dynamics in Mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *41*(16), 3597–3609. https://doi.org/10.1523/JNEUROSCI.0755-20.2021

O'Hara, C. M., Uhland-Smith, A., O'Malley, K. L., & Todd, R. D. (1996). Inhibition of dopamine synthesis by dopamine D2 and D3 but not D4 receptors. *The Journal of Pharmacology and Experimental Therapeutics*, *277*(1), 186–192.

Paladini, C. A., & Roeper, J. (2014). Generating bursts (and pauses) in the dopamine midbrain neurons. *Neuroscience*, *282*, 109–121. https://doi.org/10.1016/j.neuroscience.2014.07.032

Phillips, P. E. M., Hancock, P. J., & Stamford, J. A. (2002). Time window of autoreceptor-mediated inhibition of limbic and striatal dopamine release. *Synapse (New York, N.Y.)*, *44*(1), 15–22. https://doi.org/10.1002/syn.10049

Rice, M. E., & Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neuroscience*, *7*(6), 583–584. https://doi.org/10.1038/nn1244

Salamone, J. D. (2007). Functions of mesolimbic dopamine: Changing concepts and shifting paradigms. *Psychopharmacology*, *191*(3), 389–389. https://doi.org/10.1007/s00213-006-0623-9

Schmitz, Y., Benoit-Marand, M., Gonon, F., & Sulzer, D. (2003). Presynaptic regulation of dopaminergic neurotransmission. *Journal of Neurochemistry*, *87*(2), 273–289. https://doi.org/10.1046/j.1471-4159.2003.02050.x

Schmitz, Y., Schmauss, C., & Sulzer, D. (2002). Altered Dopamine Release and Uptake Kinetics in Mice Lacking D2 Receptors. *Journal of Neuroscience*, *22*(18), 8002–8009. https://doi.org/10.1523/JNEUROSCI.22-18-08002.2002

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology*, *80*(1), 1–27. https://doi.org/10.1152/jn.1998.80.1.1

Shashaank, N., Somayaji, M., Miotto, M., Mosharov, E. V., Makowicz, E. A., Knowles, D. A., Ruocco, G., & Sulzer, D. L. (2023). Computational models of dopamine release measured by fast scan cyclic voltammetry in vivo. *PNAS Nexus*, *2*(3), pgad044. https://doi.org/10.1093/pnasnexus/pgad044

Somayaji, M., Cataldi, S., Choi, S. J., Edwards, R. H., Mosharov, E. V., & Sulzer, D. (2020). A dual role for α-synuclein in facilitation and depression of dopamine release from substantia nigra neurons in vivo. *Proceedings of the National Academy of Sciences*, *117*(51), 32701–32710. https://doi.org/10.1073/pnas.2013652117

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Somayaji, M., Cataldi, S., Edwards, R. H., & Sulzer, D. (2020). *α-Synuclein facilitates dopamine release during burst firing of substantia nigra neurons in vivo* (p. 2020.06.10.145110). bioRxiv. https://doi.org/10.1101/2020.06.10.145110

Staal, R. G. W., Mosharov, E. V., & Sulzer, D. (2004). Dopamine neurons release transmitter via a flickering fusion pore. *Nature Neuroscience*, *7*(4), 341–346. https://doi.org/10.1038/nn1205

Stamford, J. A., Kruk, Z. L., & Millar, J. (1991). Differential effects of dopamine agonists upon stimulated limbic and striatal dopamine release: In vivo voltammetric data. *British Journal of Pharmacology*, *102*(1), 45–50. https://doi.org/10.1111/j.1476-5381.1991.tb12130.x

Sulzer, D., Cragg, S. J., & Rice, M. E. (2016). Striatal dopamine neurotransmission: Regulation of release and uptake. *Basal Ganglia*, *6*(3), 123–148. https://doi.org/10.1016/j.baga.2016.02.001

Threlfell, S., & Cragg, S. J. (2011). Dopamine signaling in dorsal versus ventral striatum: The dynamic role of cholinergic interneurons. *Frontiers in Systems Neuroscience*, *5*, 11. https://doi.org/10.3389/fnsys.2011.00011

Venton, B. J., Zhang, H., Garris, P. A., Phillips, P. E. M., Sulzer, D., & Wightman, R. M. (2003). Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing: Decoding dopamine neurotransmission. *Journal of Neurochemistry*, *87*(5), 1284–1295. https://doi.org/10.1046/j.1471-4159.2003.02109.x

Wu, Q., Reith, M. E. A., Walker, Q. D., Kuhn, C. M., Carroll, F. I., & Garris, P. A. (2002). Concurrent Autoreceptor-Mediated Control of Dopamine Release and Uptake during Neurotransmission: An In Vivo Voltammetric Study. *The Journal of Neuroscience*, *22*(14), 6272–6281. https://doi.org/10.1523/JNEUROSCI.22-14-06272.2002

Yavich, L., & MacDonald, E. (2000). Dopamine release from pharmacologically distinct storage pools in rat striatum following stimulation at frequency of neuronal bursting. *Brain Research*, *870*(1–2), 73–79. https://doi.org/10.1016/s0006-8993(00)02403-3

Zhang, H., & Sulzer, D. (2003). Glutamate Spillover in the Striatum Depresses Dopaminergic Transmission by Activating Group I Metabotropic Glutamate Receptors. *Journal of Neuroscience*, *23*(33), 10585–10592. https://doi.org/10.1523/JNEUROSCI.23-33-10585.2003

Zhang, H., & Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nature Neuroscience*, *7*(6), 581–582. https://doi.org/10.1038/nn1243

Zhang, T., Zhang, L., Liang, Y., Siapas, A. G., Zhou, F.-M., & Dani, J. A. (2009). Dopamine Signaling Differences in the Nucleus Accumbens and Dorsal Striatum Exploited by Nicotine. *The Journal of Neuroscience*, *29*(13), 4035–4043. https://doi.org/10.1523/JNEUROSCI.0261-09.2009

Zhou, F. M., Liang, Y., & Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nature Neuroscience*, *4*(12), 1224–1229. https://doi.org/10.1038/nn769

Zweifel, L. S., Parker, J. G., Lobb, C. J., Rainwater, A., Wall, V. Z., Fadok, J. P., Darvas, M., Kim, M. J., Mizumori, S. J. Y., Paladini, C. A., Phillips, P. E. M., & Palmiter, R. D. (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-

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dependent behavior. *Proceedings of the National Academy of Sciences*, *106*(18), 7281–7288. https://doi.org/10.1073/pnas.0813415106

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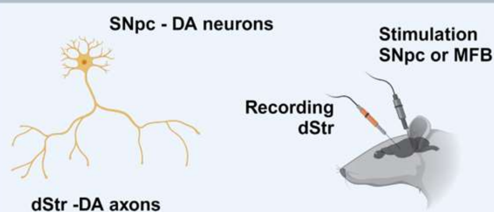
In Vivo



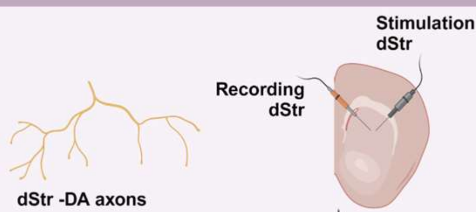
Tonic activity from the cell bodies

3 bursts

Single Stimulus: no detectable release

Consecutive Burst Stimulation: facilitation

Ex Vivo



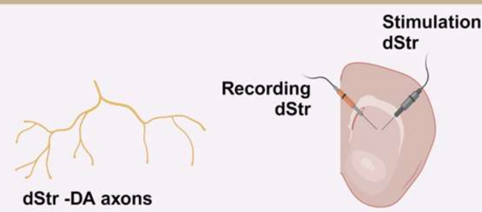
No tonic activity

3 bursts

Single Stimulus: release scales with stimulation intensity

Consecutive Burst Stimulation: depression

Our Model



Local tonic activity

3 bursts

Single Stimulus: no detectable release

Consecutive Burst Stimulation: no change or facilitation with D2R antagonist

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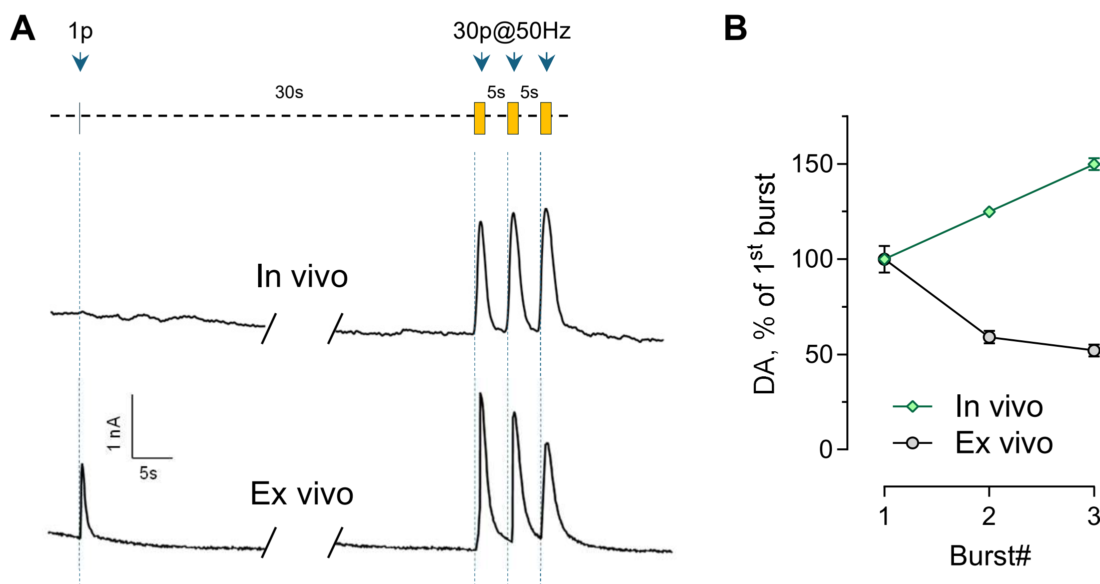

Figure 1: Comparison of DA release kinetics following single pulse and burst stimuli *in vivo* and *ex vivo*. Recordings *in vivo* were performed with FSCV electrode placed in the dorsal striatum and electrical stimulation in the SNpc. *Ex vivo* recordings were done in acute coronal dorsal striatum slices with local electrical stimulation. **(A)** Stimulation paradigm (top) and representative FSCV traces from *in vivo* (middle) and *ex vivo* (bottom) recordings. **(B)** Synaptic plasticity during a series of burst stimuli *in vivo* and in a slice. Curves are significantly different with $p<0.0001$ by 2-way ANOVA.

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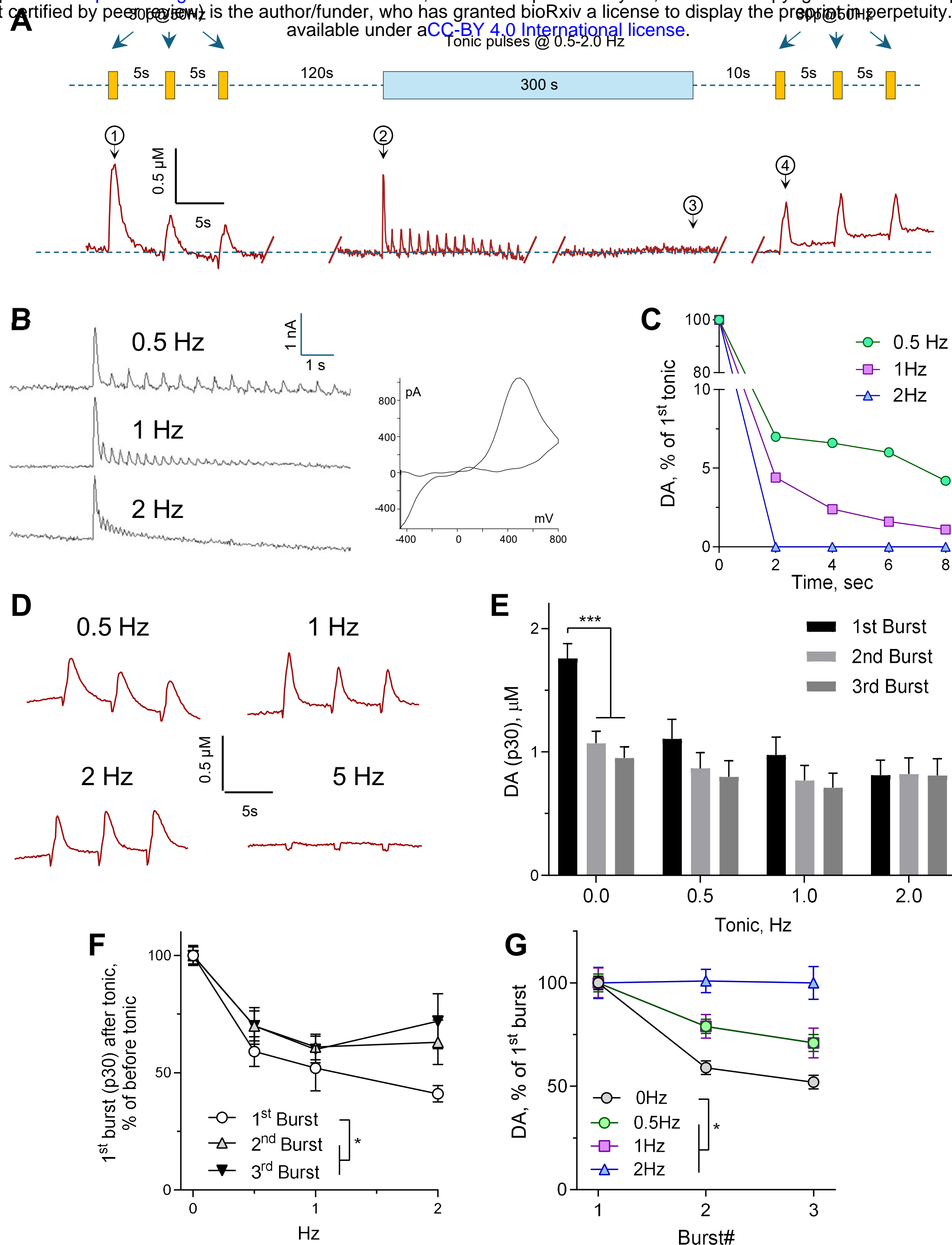

Figure 2: Effect of tonic frequency on the amplitude of evoked striatal dopamine release. (**A**) Stimulation paradigm used in each recording consisted of three phasic bursts (30 pulses at 50 Hz), followed by tonic stimulation at 0.5-5 Hz for 5 min, then three more phasic bursts. Representative FSCV traces are shown below. Numbers denote DA release following (1) 30p burst without tonic activity, (2) 1p stimulus without tonic activity, (3) 1p stimulus after tonic activity, and (4) 30p burst after tonic activity. **(B)** Representative FSCV traces of DA release peaks during the first 10 sec of tonic activity show frequency-dependent depression of DA release, followed by a complete cessation of neurotransmission. No DA release was detectable by the end of the 5 min tonic stimulation. Representative voltammogram of DA oxidation/reduction after the 1st stimulus is shown on the right.. **(C)** Average amplitudes of DA release peaks during the 1st 10 sec of tonic activity. **(D)** Representative FSCV traces of burst stimulation-dependent DA release after 5 min of tonic slice conditioning. No burst DA release was detected after 5 Hz tonic stimulation. **(E)** Average amplitudes of DA release peaks following a series of stimuli bursts (***- $p < 0.001$ by one-way ANOVA; n= 46 0Hz, 12 0.5Hz, 12 1Hz and 7 2Hz). **(F)** Effect of preceding tonic slice stimulation on DA release evoked by phasic bursts 1-3. * - $p<0.05$ by two-way ANOVA. **(G)** Increasing tonic frequency correlates with progressive relief from depression during a series of phasic bursts. * - $p<0.05$ between curves by two-way ANOVA.

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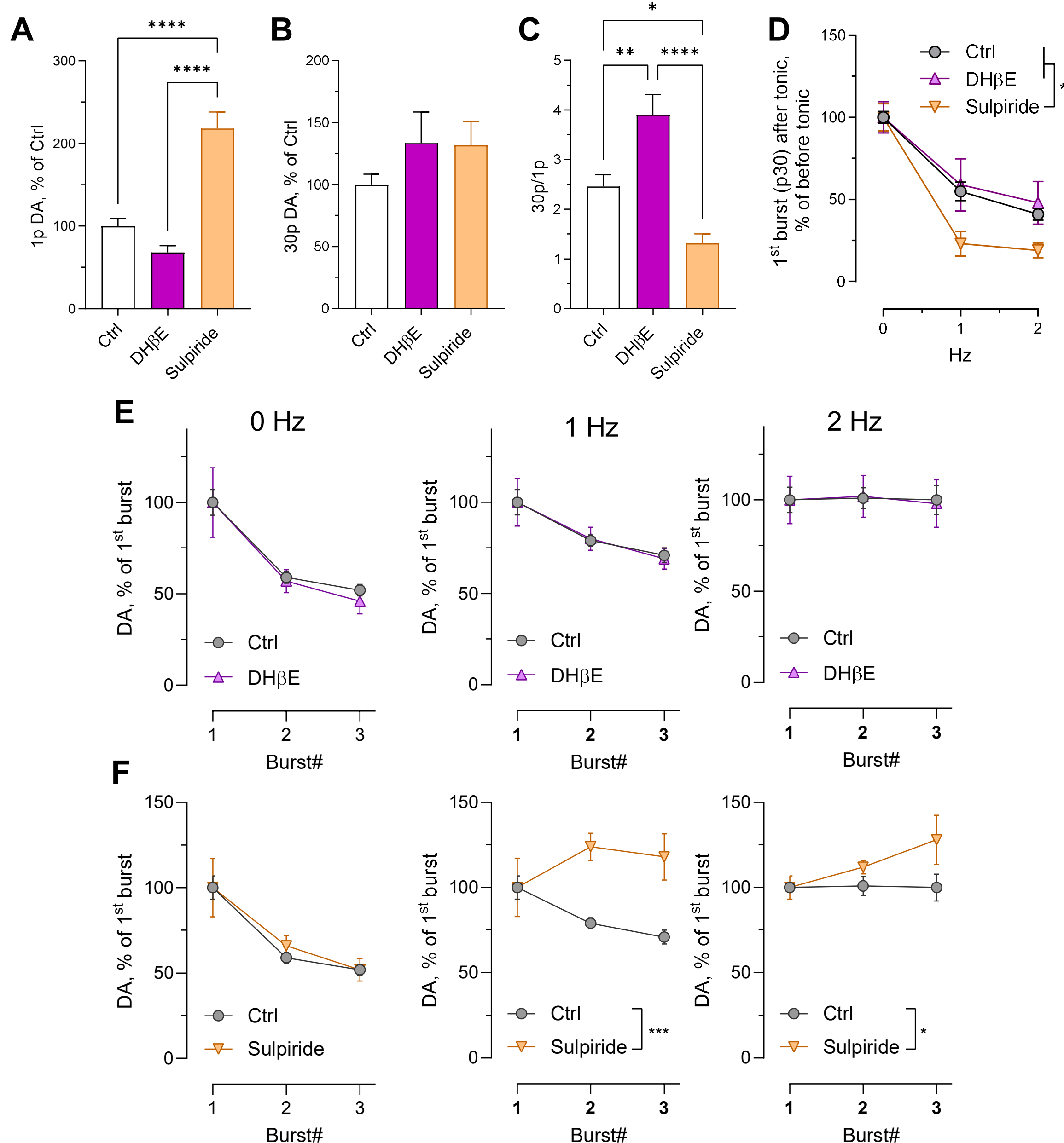

Figure 3: Modulation of evoked striatal DA release by nAChR and D2 antagonists. (A-C) Effect of nAChR antagonist DHβE (1 µM) and D2R antagonist Sulpiride (10 µM) on single pulse (A), single burst stimulation (B) and their ratio (C) in slices without tonic activity (*,**,**** - $p < 0.05$, 0.005 and 0.0001 by one-way ANOVA; n = 33 Ctrl, 13 Sulpiride and 10 DHβE). **(D)** Effect of artificial tonic activity on DA release evoked by a single burst (* - $p<0.05$ by two-way ANOVA; n = 46 (0Hz), 25 (1Hz) and 8 (2Hz) for Ctrl; 10, 6 and 4 for DHβE; 13, 7 and 4 for Sulpiride). **(E-F)** Changes in the amplitude of DA release evoked by three sequential burst stimuli in the presence of DHβE (E) and Sulpiride (F; *,*** - $p<0.05$ or 0.001 by two-way ANOVA).