## Title page

# Tonic dendritic GABA release by substantia nigra dopaminergic neurons


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## Abstract

Recent studies have demonstrated the importance of extrastriatal dopamine release in the emergence of the network dysfunction underlying motor deficits in Parkinson's disease (PD). To better characterize the actions of dopamine on substantia nigra pars reticulata (SNr) GABAergic neurons, optogenetic and electrophysiological tools were used in *ex vivo* mouse brain slices to monitor synaptic transmission arising from globus pallidus externa (GPe) neurons. As predicted by previous work, activation of D2 dopamine receptors (D2Rs) suppressed GABA release evoked by stimulation of GPe axons. However, D2R activation also suppressed a tonic, $GABA_A$ receptor mediated inhibition of SNr spiking. D2R-mediated inhibition of tonic GABA release led to a roughly 30% increase in SNr spiking rate. Chemogenetic inhibition of GPe terminals or excitation of astrocytes had no effect on tonic GABA release in the SNr. In contrast, chemogenetic inhibition of dopaminergic neurons or knocking down the expression of aldehyde dehydrogenase 1A1 (ALDH1A1) blunted tonic GABAergic signaling. Lastly, in a progressive mouse model of PD targeting dopaminergic neurons, the tonic inhibition of SNr neurons by GABA release also was lost. Taken together, these observations suggest that dopamine and GABA are co-released by the dendrites of ALDH1A1-expressing dopaminergic neurons that course through the SNr. The co-release of these transmitters could serve to promote movement by making SNr neurons less responsive to phasic activity arising from the indirect pathway circuitry and by lowering basal spiking rates.


## Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease [1, 2]. The cardinal motor symptoms of the disease – bradykinesia and rigidity – are attributable to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) that innervate the basal ganglia. Although the classical model of the network dysfunction underlying PD motor symptoms emphasizes the importance of the dopaminergic innervation of the striatum (the largest of the basal ganglia nuclei),

recent work has demonstrated that other basal ganglia nuclei also are important in the transition to the parkinsonian state, including the substantia nigra pars reticulata (SNr) [3, 4].

From the network standpoint, the SNr is particularly interesting. The principal SNr neurons are autonomously active GABAergic neurons that tonically inhibit motor control circuits in the brainstem and diencephalon [5]. The basal activity of SNr neurons is bidirectionally regulated by synaptic connections with other basal ganglia neurons, including GABAergic neurons in the GPe and striatum (direct pathway spiny projection neurons) and glutamatergic neurons in the subthalamic nucleus (STN) [6, 7]. These synaptic connections are generally thought to be modulated by dopamine through presynaptic G-protein coupled receptors (GPCRs). The dopamine that acts on these receptors is released locally by the dendrites of SNc neurons that course through the SNr [8, 9]. Previous studies have suggested that dopamine released into the SNr suppresses synaptic transmission arising from GPe and STN neurons, while enhancing transmission from direct pathway spiny projection neurons (dSPNs). However, the neuropil in this region is complex, making a definitive assessment of how dopamine modulates these pathways problematic with conventional electrical stimulation methodologies. Optogenetic methods offer a way of surmounting this difficulty, but this approach has not been used to determine how dopamine modulates either GPe or STN input to SNr neurons. Sorting this out in a rigorous way is critical to understanding not only how dopamine modulates SNr activity in the healthy brain, but how this activity goes awry in PD. The potential impact of this local dopaminergic modulation has been driven home by the observation that in parkinsonian MCI-Park mice, restoration of dopaminergic signaling within the SNr alone alleviates open field deficits [10].

Recent work points to another way in which nominally dopaminergic neurons in the SNc might modulate SNr activity. Optogenetic stimulation of genetically defined dopaminergic neurons has revealed that some of these neurons co-release glutamate and others co-release GABA from their axon terminals [11-13]. It is unclear whether transmitter co-release is a phenomenon limited to axons or whether it extends to the dendrites of SNc dopaminergic neurons.

The goal of the study presented here was to define how dopamine modulates the activity of SNr neurons. The initial experiments utilized optogenetic approaches to characterize how dopamine modulated the release of GABA from GPe synaptic terminals on SNr neurons. These studies were consistent with inferences drawn from previous work that presynaptic D2 dopamine receptors (D2Rs) inhibit phasic release of GABA from GPe terminals. However, these experiments also revealed that D2Rs suppressed a tonic GABAergic inhibition of SNr spiking. This tonic GABAergic signal was traced back to SNc dopaminergic neurons, suggesting that dendritic co-release of dopamine and GABA was serving to promote movement by making SNr neurons less responsive to phasic activity arising from the indirect pathway circuitry and by lowering basal spiking rates. Consistent with this conjecture, the

tonic release of GABA was lost in a progressive mouse model of PD that specifically targeted dopaminergic neurons.

## Results

### D2R agonists inhibited phasic GABA release from GPe terminals

To characterize how dopamine modulated GABA release from GPe terminals, GPe neurons were induced to express the opsin Chronos by injecting an adenoassociated virus (AAV) carrying a synapsin promoter driven expression plasmid with a tdTomato fluorescent protein reporter (see Methods) into the GPe of mice (Fig. 1A). Three to four weeks later, mice were sacrificed, and mesencephalic slices prepared for study. Optogenetic stimulation of GPe axons evoked robust post-synaptic currents in SNr neurons voltage clamped at -60 mV with a $Cs^+$ containing whole-cell electrode (Fig. 1B). These optically evoked currents were blocked by bath application of the $GABA_A$ receptor ($GABA_AR$) antagonist gabazine (10 µM). To assess dopaminergic modulation of synaptic transmission, the D2R agonist quinpirole (2 µM) was bath applied while optogenetically stimulating GPe axons. Quinpirole reduced the amplitude of the GPe evoked inhibitory postsynaptic currents (IPSCs) by roughly 30% ($p = 0.02$, $n = 5$) (Fig. 1 B, C). Consistent with a presynaptic locus of the modulation, the paired-pulse ratio of GPe evoked IPSCs rose significantly in the presence of quinpirole ($p = 0.02$, $n = 6$) (Fig. 1 D, E).

To assess the impact of this presynaptic modulation on the ability of GPe to modulate the basal spiking of SNr neurons, they were recording in cell-attached mode (to preserve the intracellular environment) and then GPe axons stimulated at 30 Hz for 2 seconds. Initially, this stimulation effectively suppressed SNr activity, but the efficacy of this inhibition waned with time (Fig. 2A-C). The waning strength of the GPe inhibition is likely attributable to both biologically relevant factors, like depression of GABA release, but also to the limitations posed by the optogenetic methods [14]. As expected, quinpirole attenuated the ability of GPe stimulation to slow the spiking of SNr neurons ($p = 0.02$, $n = 8$) (Fig. 2A-C). Taken together, these results are consistent with the inferences drawn from previous work using electrical stimulation [15].

### D2R agonists lowered tonic GABA release

One of the unexpected observations in these experiments was the elevation in the basal spiking rate of SNr neurons with bath application of quinpirole, in the absence of GPe axon stimulation ($p = 0.01$, $n = 8$) (Fig. 3A). The quinpirole-induced elevation in discharge rate was about 40% when monitored in cell-attached mode. Bath application of gabazine (10 µM) increased the basal spiking rate of SNr neurons recorded in cell-attached mode by roughly 50%, suggesting that they were tonically inhibited by activation of $GABA_ARs$ ($p < 0.01$, $n = 10$) (Fig. 3B). These results suggest that quinpirole and gabazine might be acting through a common mechanism – inhibition of tonic GABA release. If this was the case, bath application of gabazine should occlude the modulation of SNr spiking by quinpirole. Indeed, in the

presence of gabazine, quinpirole did not significantly modulate SNr spiking rate ($p = 0.13$, $n = 7$) (Fig. 3C).

These results demonstrate that there is a sustained source of GABA that significantly modulates the spiking rate of SNr neurons and this tonic GABA release is negatively modulated by D2-class receptors. Within the SNr, there are several possible sources of tonic GABA release that might be shaped by D2R signaling (Fig. 3D): GPe terminals, astrocytes, and SNc dopaminergic neurons. As SNr neurons are not known to express D2-class dopamine receptors, they were excluded as possible sources of tonic GABAergic signaling.

### D2R-regulated tonic GABA release was not derived GPe terminals or astrocytes

One potential source of tonic GABA release that might be negatively modulated by D2Rs is the GPe terminal. To test this hypothesis, an AAV carrying a synapsin-driven hM4D (Gi) designer receptor exclusively activated by designer drugs (Gi-DREADD)-tdTomato expression construct was injected into the GPe. Three-four weeks later, mice were sacrificed and ex vivo experiments performed. Bath application of the DREADD agonist CNO reduced the amplitude of electrically evoked IPSCs in voltage-clamped SNr neurons ($p = 0.02$, $n = 4$) (Fig. 4A). This modulation was similar in magnitude to that seen in optogenetically evoked IPSCs following D2R stimulation. However, CNO application had no effect on the basal spiking rate of SNr neurons recorded in cell-attached mode, arguing that GPe terminals were not contributing to tonic GABA release ($p = 0.19$, $n = 5$) (Fig. 4B).

Many astrocytes express D2Rs [16-18]. Moreover, in some regions of the brain, astrocytes are known to release GABA [19]. Astrocytic GABA release is thought to occur through bestrophin1 channels in response to an elevation in intracellular $Ca^{2+}$ concentration [20]. To test if astrocytes in the SNr mediate tonic GABA, an AAV carrying a plasmid in which a glial fibrillary acidic protein (GFAP) promoter fragment controlled expression of an excitatory hM3 (Gq) DREADD construct was stereotaxically injected into the SNr of mice. These vectors have been used to chemogenetically elevate intracellular $Ca^{2+}$ in astrocytes [21] (Fig. 5A). Three-four weeks after the AAV injection, mice were sacrificed and brain slices prepared. In these slices, bath application of the DREADD agonist CNO elevated the basal spiking rate of SNr neurons recorded in cell-attached mode ($p = 0.03$, $n = 8$) (Fig. 5B). In line with the notion that astrocytes were exciting (not inhibiting) SNr neurons, bath application of the bestrophin 1 inhibitor NPPB decreased SNr spiking rate ($p = 0.03$, $n = 6$) (Fig. 5C).

### Tonic GABA release originated from SNc dopaminergic neurons

Another possible source of tonic GABA release is SNc dopaminergic neuron. As mentioned above, SNc dopaminergic neurons co-release dopamine and GABA, at least from their striatal axons [13]. Moreover, the somatodendritic region of these neurons is richly invested with D2Rs that are coupled to Kir2 $K^+$ channels that suppress spiking and dopamine release [16, 22]. One of the enzymes

thought to be important in the production of releasable GABA is aldehyde dehydrogenase 1α1 (ALDH1A1) [23]. To assess the extent to which ALDH1A1 dendrites innervate the SNr, mice expressing Cre recombinase in ALDH1A1+ neurons were crossed with mice expressing Flippase under the control of the dopamine transporter promoter (DAT-Flp). These mice were then injected with an AAV Cre-On/Flp-On EYFP reporter construct, and an AAV Cre-Off/Flp-On mCherry reporter construct. 3 weeks after the injection, mice were sacrificed for histological analysis. In tissue from these mice, ALDH1A1+ dopaminergic neurons were marked by EYFP expression, whereas ALDH1A1- dopaminergic neurons were marked with mCherry. In both rostral and caudal regions, the dendrites of ALDH1A1+ dopaminergic neurons coursed through much of dorsoventral extent of SNr, providing an anatomical substrate for the distributed release of GABA (Fig. 6A, B) [9, 24].

As a first test of the role of ALDH1A1 in generating the tonic GABA signal, a viral knockdown strategy was used. The SN of mice expressing Cre recombinase under control of the DAT promoter (DAT-Cre), were stereotaxically injected with an AAV carrying a FLEX ALDH1A1 shRNA expression construct with an mCherry reporter (Fig. 6C) [23]. Two weeks later, mice were sacrificed and brain slices prepared. In ex vivo, cell-attached recordings from SNr neurons, the basal spiking rate was significantly higher than in controls ($p < 0.001$, $n = 18$ control / 15 shRNA) (Fig. 6D). Moreover, bath application of gabazine did not change the basal spiking rate of SNr neurons from ALDH1A1 shRNA treated mice ($p = 0.10$, $n = 15$) (Fig. 6E). To provide an orthogonal test of the hypothesis that ALDH1A1 was driving GABA synthesis, a pharmacological approach was used. First, the ALDH1A1 inhibitor disulfiram (10 µM) was bath applied to control slices and then the effect of gabazine on basal spiking rate monitored. Disulfiram blocked the effect of gabazine ($p = 0.80$, $n = 5$) (Fig. 6G). Second, another inhibitor of ALDH1A1 N,N-diethylaminobenzaldehyde (DEAB, 10 µM) was bath applied and the effect of gabazine on basal spiking monitored. As with disulfiram, gabazine had no effect on DEAB-exposed SNr neurons ($p = 0.19$, $n = 5$) (Fig. 6H).

As a final test of the hypothesis that SNc dopaminergic neurons were the source of tonic GABA release in the SNr, a chemogenetic approach was used. The SN of DAT-Cre mice was stereotaxically injected with an AAV carrying a FLEX construct coding for the glycine pharmacologically selective actuator module (PSAM-L141-Y115F-GlyR). Three weeks later, mice were sacrificed and mesencephalic slices prepared. Histological analysis of the mesencephalon of these mice showed good overlap of tyrosine hydroxylase immunoreactivity and the PSAM reporter (Fig. 7A, B). In principle, once activated, the glycine PSAM should modestly hyperpolarize SNc dopaminergic neurons and decrease $Ca^{2+}$-dependent GABA release (Fig. 7C). In cell attached patch recordings from PSAM-expressing neurons, bath application of the pharmacologically selective effector molecule (PSEM) uPSEM817 tartate (0.5 µM) modestly reduced the tonic spiking of SNc dopaminergic neurons ($p = 0.03$,

n = 10) (Fig. 7D). Furthermore, as predicted, bath application of the PSEM also significantly increased SNr spiking rate, consistent with the hypothesis that autonomously active SNc dopaminergic neurons were the source of tonic GABAergic inhibition (p = 0.02, n = 8) (Fig. 7E).

### Tonic GABA release was lost in a mouse model of PD

In the MC1-Park model of PD, the emergence of parkinsonism parallels the down-regulation of the dopaminergic phenotype in the somatodendritic region of SNc dopaminergic neurons [ref]. This phenotypic down-regulation is accompanied by a loss of dopamine release in the SNr. If dopamine and GABA were co-released by dendrites, tonic GABA release also should be down-regulated in parkinsonian MC1-Park mice. Indeed, in contrast to littermate controls (Fig. 8A), bath application of gabazine failed to increase the spiking rate of SNr neurons recorded in *ex vivo* slices from P60-70 MC1-Park mice (p = 0.69, n = 7) (Fig. 8B).

## Discussion

### Conclusions/Summary

### Evidence for GABA release from SNc dopaminergic neurons

### The source of GABA being released

### ALDH1A1-dependent synthesis

### Mitochondrial sources?

### Functional implications of co-release in SN

The model we use to describe and explain the connectivity and function of the basal ganglia network influences our capability to discover or design novel therapies for disease pathology. Regenerative therapies targeting the putamen with viruses promoting AADC expression or grafting of dopamine neuron progenitor cells are designed and tested in clinical trials based on reversing the well documented deterioration of the nigrostriatal pathway during the progression of Parkinson's disease [25, 26]. The canonical models that we currently use in translational research reflect our deep understanding of dopamine signaling in the striatum, but under represent the current understanding of how dopamine neuron activity modulates the function of the ventral midbrain. It is already understood that the dopamine signal from SNc neurons facilitates GABA release from spiny neuron inputs to SNr and depresses glutamate release from STN inputs to SNr, both effects suppressing SNr output and facilitating movement. We can now add to this the understanding that SNc neurons also directly inhibit SNr and facilitate movement by releasing GABA neurotransmitter from their dendrites alongside the canonical dopamine signal. The remaining contradiction is the D2R inhibition of GPe input, which appears to facilitate SNr firing and suppress movement. Either the reduction of GABA release from GPe inputs is an exception to the other prokinetic effects of dopamine neuron signaling, or there are network dynamics at play in vivo that are not readily resolved in an ex vivo experiment. The results of this study give us a more complete understanding of how dopamine neuron signaling in the midbrain modulates

basal ganglia output, and how therapeutic replacement of dopamine neurotransmitter alone to this structure may be insufficient for full restoration of normal function.

It is well understood that the presentation of certain Parkinson's disease symptoms arises from pathological changes in multiple neurotransmitter signals within the basal ganglia and associated structures [27], so finding dopamine neurons releasing an additional neurotransmitter to modulate the output of the basal ganglia is surprising but not unreasonable. The demonstration that SNc dopamine neurons had the capacity to corelease GABA in the striatum was documented previously [13], and while alternative explanations for the mechanism through which dopamine neurons acquire the GABA they corelease were presented [28, 29], a physiological salience to this GABA neurotransmitter release remained elusive. The current study delivers on the promise of these previous works by proving the significance of GABA release from dopamine neurons upon basal ganglia output, and this was achieved by highlighting and distinguishing the midbrain region specific signaling of dopamine neurons.

Our data shows that the tonic GABA observed from control mice is absent from p60 MCI-Park mice that are fully symptomatic. From the disinhibition of spontaneous SNr firing rate, we interpret that GABA depletion has occurred alongside dopamine depletion. What remains untested is the therapeutic potential of replacing this lost tonic GABA. A conservative prediction of the result of GABA replacement would be reduction or reversal of locomotor deficits in open field tests. A more speculative prediction would be that replacement of tonic GABA would reduce the therapeutic dosage of levodopa required to overcome locomotor deficits, leading to a widening of the therapeutic window of treatment that avoids dyskinesia symptoms. The challenge remains for exploring these predictions in that one must devise a means of modulating the GABA synthesis activity of ALDH1a1 without interfering with its other essential physiological functions in dopamine neurons [30].

## Methods

### Animals

All housing, breeding and procedures were performed according to the Northwestern University Animal Studies committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were group housed with food and water provided ad libitum under a 12 h-12 h light-dark cycle and temperatures of 65-75 °F with 40-60% humidity. Male and female mice were used for experiments. No significant differences were observed between both groups. We obtained *DAT-cre* mice (B6.SJL-Slc6a3$^{tm1.1(cre)Bkmn}$/J) from the Jackson Laboratories. We obtained C57BL/6J and PV-cre mice (B6.129P2-Pvalb$^{tm1(cre)Arbr}$/J) from the Jackson Laboratories. MC1-Park mice (DAT-cre x Ndufs2 $^{-/-}$ ) were bred in house [10].

**Stereotaxic injections**

Mice were anaesthetized using an isoflurane precision vapourizer (SomnoSuite - Kent Scientific) and placed in a stereotaxic frame (David Kopf Instruments) with a Cunningham adaptor (Harvard Apparatus) to maintain anesthesia delivery throughout surgery. After exposing the skull, a small hole was bored with a micro drill bit and 200 nl of viral vector was delivered through a glass micropipette (Drummond Scientific Company) pulled on a Sutter P-97 puller. The SNr was targeted at the following coordinates (mm): anterioposterior (AP) = -3.40, mediolateral (ML) = 1.41 and dorsoventral (DV) = -4.45 (all (AP, ML, DV) relative to the bregma). The SNc was targeted at the following coordinates: AP = -3.0, ML = 1.2, DV = 4.2. The GP was targeted at the following coordinates: AP = -0.34, ML = 1.81, DV = 3.61, and AP = -0.82, ML = 2.33, DV = 3.72.

Source of coordinates and diagrams: Allen Mouse Brain Atlas

**Recombinant AAV vectors**

The following AAV viruses were used in this study: AAV9-hSyn-hM4Di-mCherry (Addgene; RRID:Addgene_50475), AAV9-hSyn-DIO-hM4Di-mCherry (Addgene; RRID:Addgene_44362), AAV9-Ef1a-DIO-PSAM-L141F-Y115F-GlyR-2A-FusionRed (Virovek), AAV2/5-GfAABC$_1$D-hM3Dg-mCherry (Addgene; RRID:Addgene_50478), AAV-CMV-Aldh1a1-shRNA (Stanford, [23])

**Ex vivo brain slice preparation**

Mice were anaesthetized with a mixture of ketamine (50 mg kg$^{-1}$) and xylazine (4.5 mg kg-1) then transcardially perfused with ice-cold, oxygenated modified artificial cerebrospinal fluid (aCSF) containing 210 mM sucrose, 2.5 mM KCl, 1.25 mM $NaH_2PO_4$, 25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 10 mM $MgSO_4$ and 10 mM glucose, pH 7.3 (315–320 mOsm l$^{-1)}$. Once perfused, the brain was rapidly removed and either sagittal or coronal slices containing the SNr (thickness, 240 µm) were sectioned using a vibratome (VT1200S Leica Microsystems). Brain slices were incubated in oxygenated modified aCSF containing 124 mM NaCl, 4.5 mM KCl, 25 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4$, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$ and 10 mM glucose [..] at 34 °C for 30 min, then at room temperature for another 30 min before the experiments. All solutions were pH 7.4, 310-320 mOsm l$^{-1}$ and were continually bubbled with 95%$O_2$/5% $CO_2$. Experiments were performed at 32-33 °C.

**Immunohistochemistry**

After recording, brain slices were fixed in 4% PFA for 15 min to preserve the epitope for TH antibody staining. Rabbit-anti TH (Millipore sigma AB152) was used as primary to label dopamine neurons in fixed tissue.

**Analysis and statistics**

Electrophysiology data were generally analyzed using Clampfit 10.3 software (Molecular Devices; https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite. Data are summarized using box plots showing median values, first and third quartiles, and range, unless otherwise specified. Statistical analysis was performed with Mathematica 13 (Wolfram; https://www.wolfram.com/mathematica/?source=nav) and GraphPad Prism 8 (GraphPad Software; https://www.graphpad.com/). Probability threshold for statistical significance was $P < 0.05$.

**Table for Materials & Methods**

| Reagent or Resource | Source | Identifier |
|---|---|---|
| *Experimental Models: Organisms/Strains* | | |
| C57BL/6J | Jackson Laboratories | RRID:IMSR JAX:000664 |
| DAT-cre x Ndufs2 $^{-/-}$ | Available at Jackson Laboratories, strain bred in house for these experiments | RRID:IMSR_JAX:036313 |
| DAT-Cre (B6.SJL-Slc6a3$^{tm1.1(cre)Bkmn}$/J) | Jackson Laboratories | RRID:IMSR_JAX:006660 |
| PV-cre mice (B6.129P2-Pvalb$^{tm1(cre)Arbr}$/J) | Jackson Laboratories | RRID:MGI:5504648 |
| Materials | | |
| AAV9-hSyn-hM4Di-mCherry | Addgene | RRID:Addgene_50475 |
| AAV9-hSyn-DIO-hM4Di-mCherry | Addgene | RRID:Addgene_44362 |
| AAV9-Ef1a-DIO-PSAM-L141F-Y115F-GlyR-2A-FusionRed | Virovek | |
| AAV2/5-GfAABC$_1$D-hM3Dg-mCherry | Addgene | RRID:Addgene_50478 |
| Rabbit-anti TH antibody | Millipore-Sigma | Millipore Cat# AB152, RRID:AB_390204 |
| AAV-CMV- | Stanford: Virus generated by Jun Ding Lab; | |

| | | |
|---|---|---|
| Aldh1a1-shRNA | Plasmid provided by Lu Chen Lab | |
| *Software and Algorithms* | | |
| Mathematica 13 | Wolfram; https://www.wolfram.com/mathematica/?source=nav | RRID:SCR_014448 |
| Clampfit v 10.3 | Molecular Devices; https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite | RRID:SCR_011323 |
| GraphPad Prism v.8 | URL: http://www.graphpad.com/ | RRID:SCR_002798 |
| Allen Mouse Brain Common Coordinate Framework | URL: https://atlas.brain-map.org/ | RRID:SCR_020999 |
| Methods | | |
| Protocol Title | Section in Methods | DOI (Protocols.io) |
| Mouse Stereotaxic Surgeries for Intracranial Viral Injection V.1 | Stereotaxic viral injections | **dx.doi.org/10.17504/protocols.io.81wgby191vpk/v1** |
| Preparation of ex-vivo brain slices for physiology experiments V.2 | Ex vivo brain slice preparation | dx.doi.org/10.17504/protocols.io.dm6gp328jvzp/v2 |
| Ex vivo mouse brain patch clamp recordings combined with optogenetic stimulation | Slice recording and optogenetic stimulation | dx.doi.org/10.17504/protocols.io.5qpvokxp9l4o/v1 |
| Immunofluorescence for confocal imaging after slice recording | Immunohistochemistry | dx.doi.org/10.17504/protocols.io.6qpvr8xmolmk/v1 |
| Datasets and Code | | |
| Zenodo Name | Description | DOI |
| Data and Images for Tonic dendritic GABA release by substantia nigra dopaminergic neurons | Data sets and Images for Figures | 10.5281/zenodo.10850897 |
| Mathematica code for Tonic | Mathematica code used to generate datasets | 10.5281/zenodo.10855007 |

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## References

1. Beitz, J.M., *Parkinson s disease a review.* Frontiers in Bioscience, 2014. **S6**(1): p. 65-74.
2. McGregor, M.M. and A.B. Nelson, *Circuit Mechanisms of Parkinson's Disease.* Neuron, 2019. **101**(6): p. 1042-1056.
3. Smith, Y. and J.Z. Kieval, *Anatomy of the dopamine system in the basal ganglia.* Trends Neurosci, 2000. **23**(10 Suppl): p. S28-33.
4. Mallet, et al., *Cellular and Synaptic Dysfunctions in Parkinson's Disease: Stepping out of the Striatum.* Cells, 2019. **8**(9): p. 1005.
5. McElvain, L.E., et al., *Specific populations of basal ganglia output neurons target distinct brain stem areas while collateralizing throughout the diencephalon.* Neuron, 2021. **109**(10): p. 1721-1738.e4.
6. Albin, R.L., A.B. Young, and J.B. Penney, *The functional anatomy of basal ganglia disorders.* Trends in Neurosciences, 1989. **12**(10): p. 366-375.
7. Cui, G., et al., *Concurrent activation of striatal direct and indirect pathways during action initiation.* Nature, 2013. **494**(7436): p. 238-42.
8. Damier, P., et al., *The substantia nigra of the human brainI. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D28K immunohistochemistry.* Brain, 1999. **122**(8): p. 1421-1436.
9. Rice, M.E. and J.C. Patel, *Somatodendritic dopamine release: recent mechanistic insights.* Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 2015. **370**(1672): p. 20140185.
10. González-Rodríguez, P., et al., *Disruption of mitochondrial complex I induces progressive parkinsonism.* Nature, 2021. **599**(7886): p. 650-656.
11. Chuhma, N., et al., *Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses.* J Neurosci, 2004. **24**(4): p. 972-81.
12. Stuber, G.D., et al., *Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate.* J Neurosci, 2010. **30**(24): p. 8229-33.

13. Tritsch, N.X., J.B. Ding, and B.L. Sabatini, *Dopaminergic neurons inhibit striatal output via non-canonical release of GABA.* Nature, 2012. **490**(7419): p. 262-266.
14. Lin, J.Y., *A user's guide to channelrhodopsin variants: features, limitations and future developments.* Exp Physiol, 2011. **96**(1): p. 19-25.
15. Aceves, J.d.J., et al., *Dopaminergic Presynaptic Modulation of Nigral Afferents: Its Role in the Generation of Recurrent Bursting in Substantia Nigra Pars Reticulata Neurons.* Frontiers in System Neuroscience, 2011. **5**: p. 6.
16. Beckstead, M.J., et al., *Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons.* Neuron, 2004. **42**(6): p. 939-46.
17. Bal, A., et al., *Evidence for dopamine D2 receptor mRNA expression by striatal astrocytes in culture: in situ hybridization and polymerase chain reaction studies.* Brain Res Mol Brain Res, 1994. **23**(3): p. 204-12.
18. Qiu, J., et al., *Sinomenine activates astrocytic dopamine D2 receptors and alleviates neuroinflammatory injury via the CRYAB/STAT3 pathway after ischemic stroke in mice.* J Neuroinflammation, 2016. **13**(1): p. 263.
19. Liu, J., et al., *Astrocytes: GABAceptive and GABAergic Cells in the Brain.* Frontiers in Cellular Neuroscience, 2022. **16**: p. 892497.
20. Park, H., et al., *Bestrophin-1 Encodes for the Ca2+-Activated Anion Channel in Hippocampal Astrocytes.* The Journal of Neuroscience, 2009. **29**(41): p. 13063-13073.
21. Khakh, B.S. and B. Deneen, *The Emerging Nature of Astrocyte Diversity.* Annual Review of Neuroscience, 2019. **42**(1): p. 187-207.
22. Lacey, M.G., N.B. Mercuri, and R.A. North, *Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta.* J Physiol, 1987. **392**: p. 397-416.
23. Kim, J.-I., et al., *Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons.* Science, 2015. **350**(6256): p. 102-106.
24. Damier, P., et al., *The substantia nigra of the human brainII. Patterns of loss of dopamine-containing neurons in Parkinson's disease.* Brain, 1999. **122**(8): p. 1437-1448.
25. Christine, C.W., et al., *Magnetic resonance imaging–guided phase 1 trial of putaminal AADC gene therapy for Parkinson's disease.* Annals of Neurology, 2019. **85**(5): p. 704-714.
26. Barker, R.A., et al., *Human Trials of Stem Cell-Derived Dopamine Neurons for Parkinson's Disease: Dawn of a New Era.* Cell Stem Cell, 2017. **21**(5): p. 569-573.
27. Snijders, A.H., et al., *Physiology of freezing of gait.* Annals of Neurology, 2016. **80**(5): p. 644-659.
28. Tritsch, N.X., et al., *Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis.* eLife, 2014. **3**: p. e01936.
29. Melani, R. and N.X. Tritsch, *Inhibitory co-transmission from midbrain dopamine neurons relies on presynaptic GABA uptake.* Cell Reports, 2022. **39**(3): p. 110716.
30. Carmichael, K., et al., *Function and Regulation of ALDH1A1-Positive Nigrostriatal Dopaminergic Neurons in Motor Control and Parkinson's Disease.* Frontiers in Neural Circuits, 2021. **15**: p. 644776.

## Figure legends

**Figure 1. Optically Evoked GPe mediated IPSCs are depressed by D2 Receptor activation.** (A) Coronal slice, injection site of AAV-Chronos/tdTomato into GPe. (B) Photo-evoked GPe mediated IPSCs recorded in whole cell configuration from SNr neurons. (C) Paired T-test of photo-evoked IPSC amplitudes before and after bath application of 2 µM quinpirole; n = 5, p = 0.02. (D) Paired Pulses of GPe-mediated IPSCs at 50 ms interval before and after quinpirole bath application. (E) Paired T-test of paired pulse ratio of GPe-mediated IPSCs before and after quinpirole application; n = 6, p = 0.02

**Figure 2**. **Train stimulation of GPe input inhibits SNr spiking.** (A) A 6 second cell attached recording of SNr spiking inhibited by a 2 sec 20 Hz train stimulation of GPe-mediated IPSCs, before and after bath application by quinpirole. (B) A 6 second PSTH of cell attached spiking inhibited by a 2 sec 20 Hz train stimulation of GPe-mediated IPSCs, before and after bath application of quinpirole. (C) Wilcoxon matched-pairs signed rank test of percent inhibition (change in discharge rate / basal discharge rate) before and after quinpirole, n = 8, p = 0.02

**Figure 3. GABA-A receptor blockade occludes disinhibition by quinpirole.** (A) Example trace and summary data of cell attached spiking from SNr neurons before and after bath application of quinpirole. Paired T-test, n = 8, p = 0.01. (B) Example trace and summary data of cell attached spiking from SNr neurons before and bath application of gabazine. Paired T-test, n = 10, p < 0.01. (C) Example trace and summary data of cell attached spiking from SNr neurons pretreated with gabazine, before and after bath application of quinpirole. Paired T-test, n = 7, p = 0.13. (D) Schematic of cells in SNr and SNc, showing the expression of D2 receptors that could be modulating GABA release upon SNr neurons.

**Figure 4. Inhibition of GPe input does not alter tonic spiking in SNr.** GPe injected with inhibitory Gi-DREADDs. (A) Example trace and summary data of electrically evoked GPe-mediated IPSCs before and after bath application of DREADD agonist CNO. Paired T-test, n = 4, p = 0.02. (B) Example trace and summary data of cell attached spiking from SNr neurons before and after bath application of CNO. Paired T-test, n = 5, p = 0.19

**Figure 5. Astrocyte modulation of SNr spiking is unrelated to Tonic GABA** (A) Schematic of Gq-DREADD expression in Astrocytes. CNO application triggers intracellular calcium release and modulates conductance of bestrophin 1 channels. (B) Example trace and summary data of cell attached spiking from SNr neurons before and after bath application of DREADD agonist CNO. Paired T-test, n = 8, p = 0.03. (C) Example trace and summary data of cell attached spiking from SNr neurons before and after bath application of BST1 antagonist NPPB. Paired T-test, n = 6, p = 0.03.

**Figure 6. Blockade of ALDH1a1 disrupts tonic GABA in SNr.** (A) Coronal image of Dat-Cre+ / ALDH1A1+ (green) and Dat-Cre+ / ALDH1A1- (red) neurons in rostral substantia nigra. (B) Coronal image of Dat-Cre+ / ALDH1A1+ (green) and Dat-Cre+ / ALDH1A1- (red) neurons in caudal substantia nigra. (C) Coronal image of ALDHA1-shRNA-mCherry expression in SNc. (D) Example trace and summary data of cell attached spiking from control SNr neurons, and SNr neurons from animals injected with ALDH-shRNA. Welch's T-test, n = 18 control/15 shRNA, p < 0.001. (E) Example trace and summary data of cell attached SNr neuron spiking from mice injected with ALDH-shRNA before and

after bath application with gabazine. Paired T-test, n = 15, p = 0.10. (F) Example trace and summary data of SNr spiking from control neurons and neurons pretreated with ALDH1A1 antagonists DEAB or disulfiram. Welch's T-test, n = 18 control/18 blocked, p = 0.01. (G) Example trace and summary data of SNr spiking pretreated with DEAB, before and after bath application of gabazine. Paired T-test, n = 5, p = 0.19. (H) Example trace and summary data of SNr spiking pretreated with disulfiram, before and after bath application of gabazine. Paired T-test, n = 5, p = 0.80.

**Figure 7. Chemogenetic inhibition of SNc disinhibits SNr**. (A) 10X image of substantia nigra of a Dat-Cre mouse. TH labelled with Rb-anti TH 488. Dat-Cre+ dopamine neurons infected with AAV9-Ef1a-DIO-PSAM-L141F-Y115F-GlyR-2A-FusionRed. (B) 60X image of substantia nigra, TH is green, PSAM-GlyR is red. (C) Schematic of PSEM agonist activating chloride influx into SNc neurons; hyperpolarization reduces GABA efflux from SNc neurons. (D) Example trace and summary data of SNc neurons infected with PSAM-GlyR before and after bath application of PSEM agonist uPSEM817 tartate. Summary data shows change in rate from SNc neurons after bath application of PSEM agonist, n = 10. (E) Example trace and summary data of SNr neuron spiking from PSAM-GlyR infected mice before and after bath application of PSEM agonist. Paired T-test, n = 8, p = 0.02.

**Figure 8. Tonic GABA is lost in dopamine depleted MC1-Park mice.** (A) Example trace and summary data of SNr neurons from MCI-Park- mice before and after bath application of gabazine. (B) Example trace and summary data of SNr neurons from P60 – P70 MC1-Park+ mice before and after bath application of gabazine. Paired T-test, n = 7, p = 0.69.

**Figure 1. Optically Evoked GPe mediated IPSCs are depressed by D2 Receptor activation.**

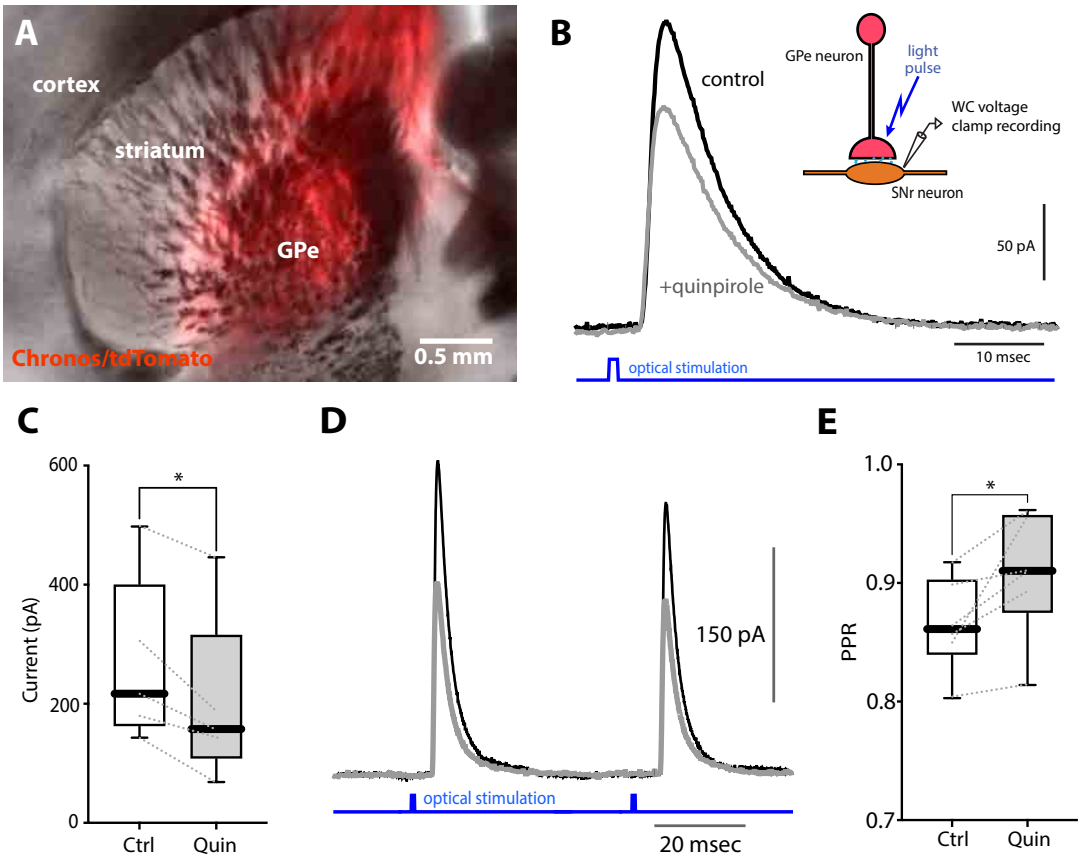


**Figure 2. Train stimulation of GPe input inhibits SNr spiking.**

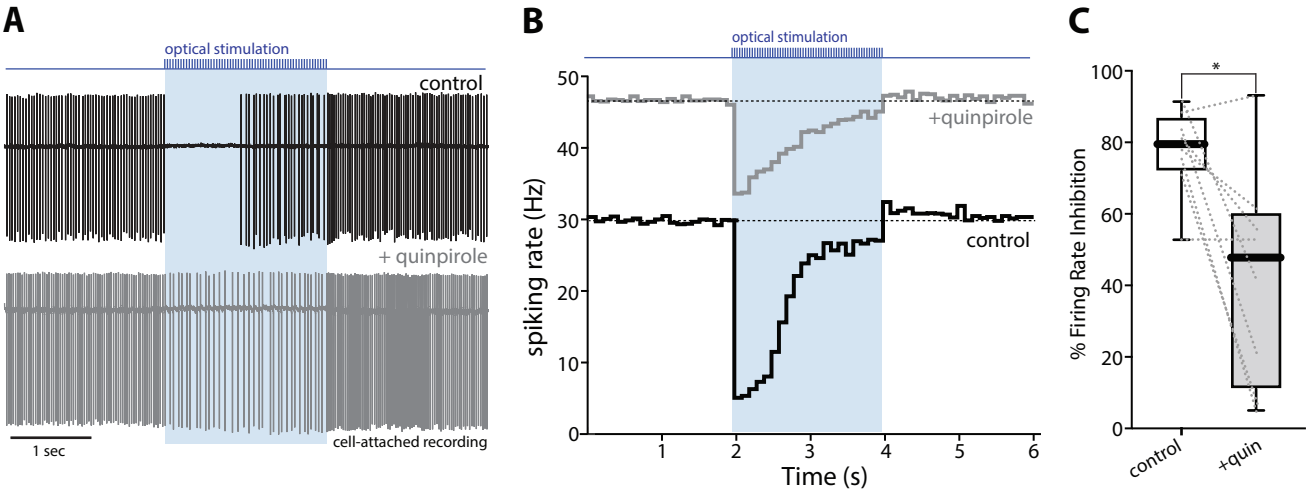


**Figure 3. GABA-A receptor blockade occludes disinhibition by quinpirole.**

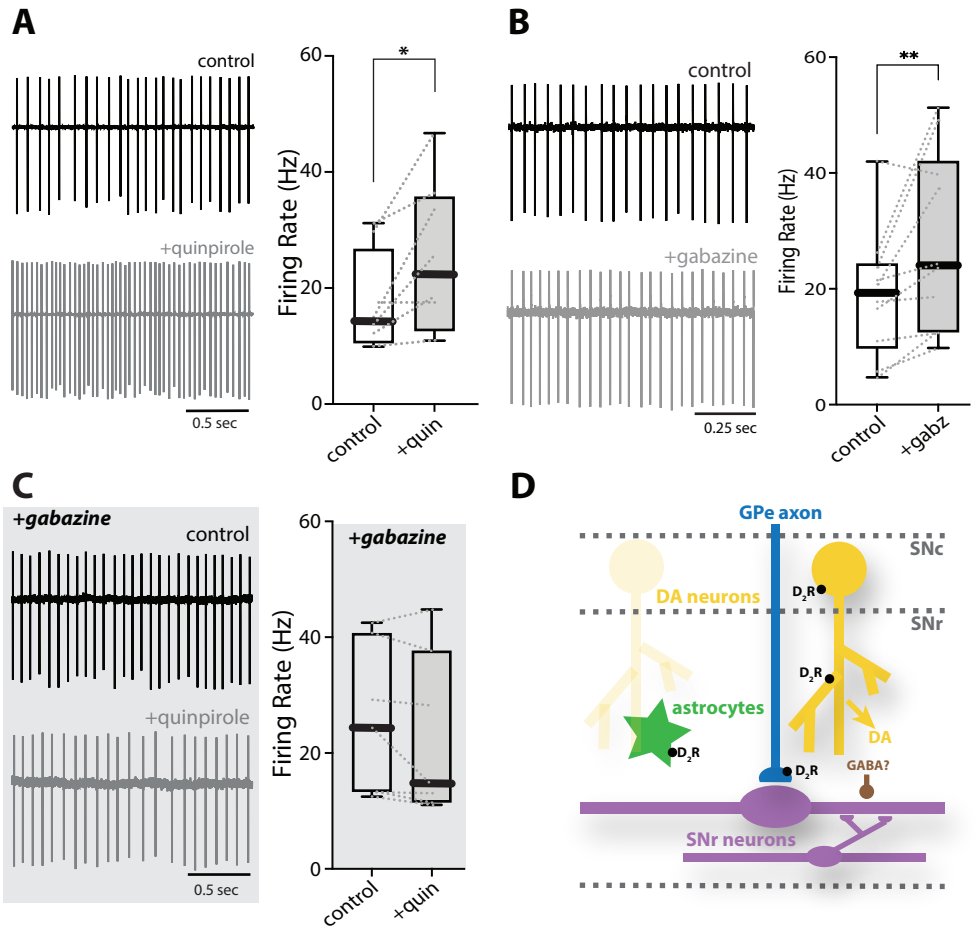
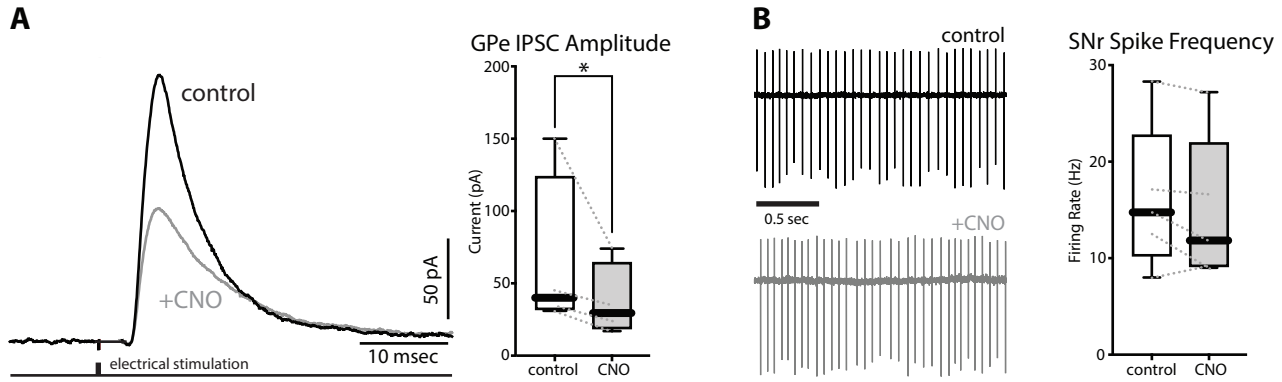


**Figure 4. Inhibition of GPe input does not alter tonic spiking in SNr.**



## Figure 5. Astrocyte modulation of SNr spiking is unrelated to Tonic GABA

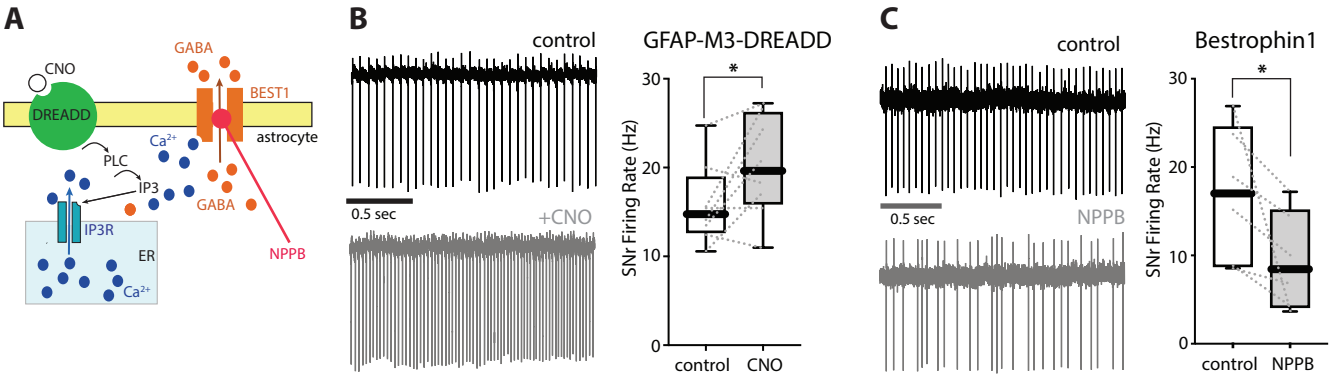


**Figure 6. Blockade of ALDH1a1 disrupts tonic GABA in SNr.**

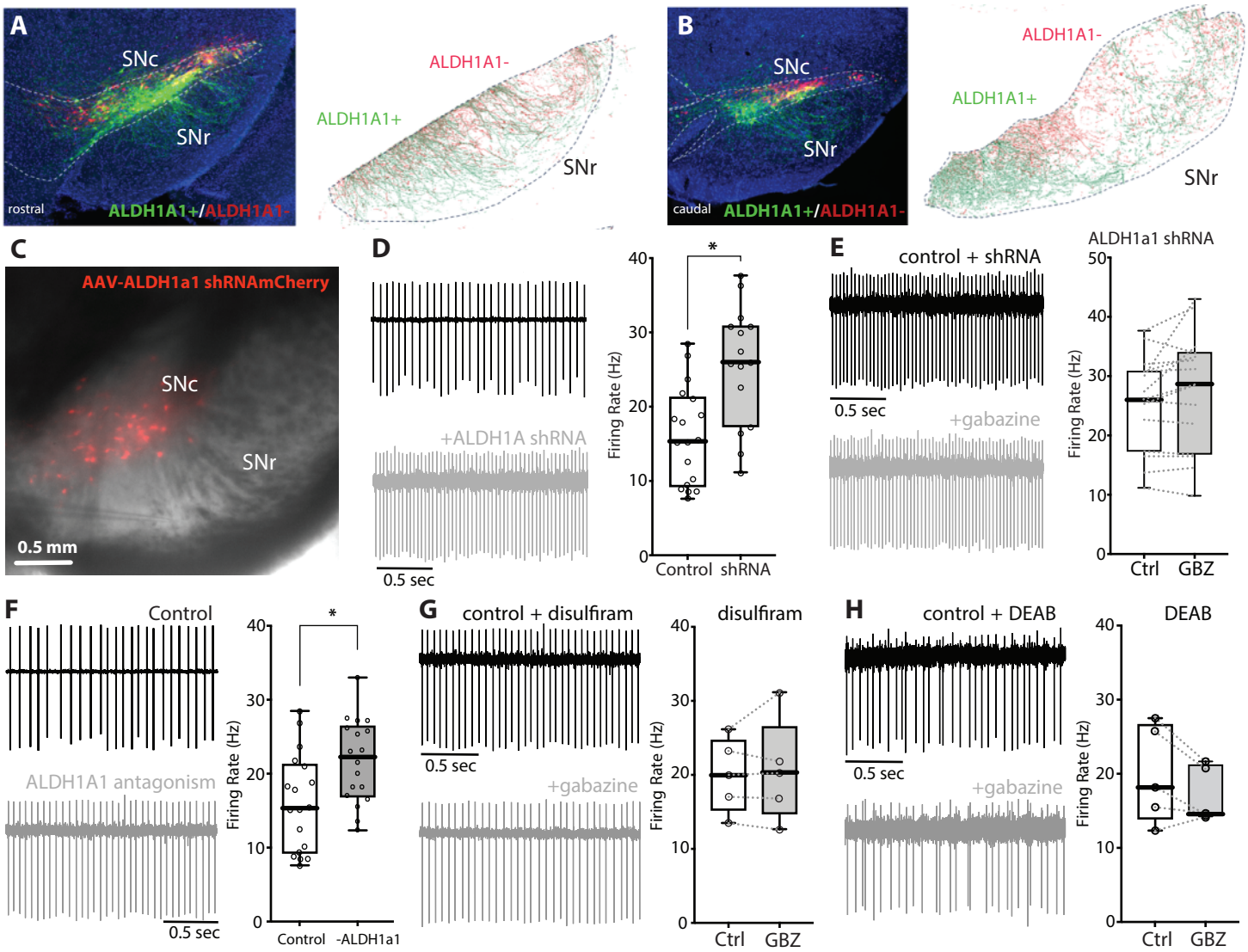
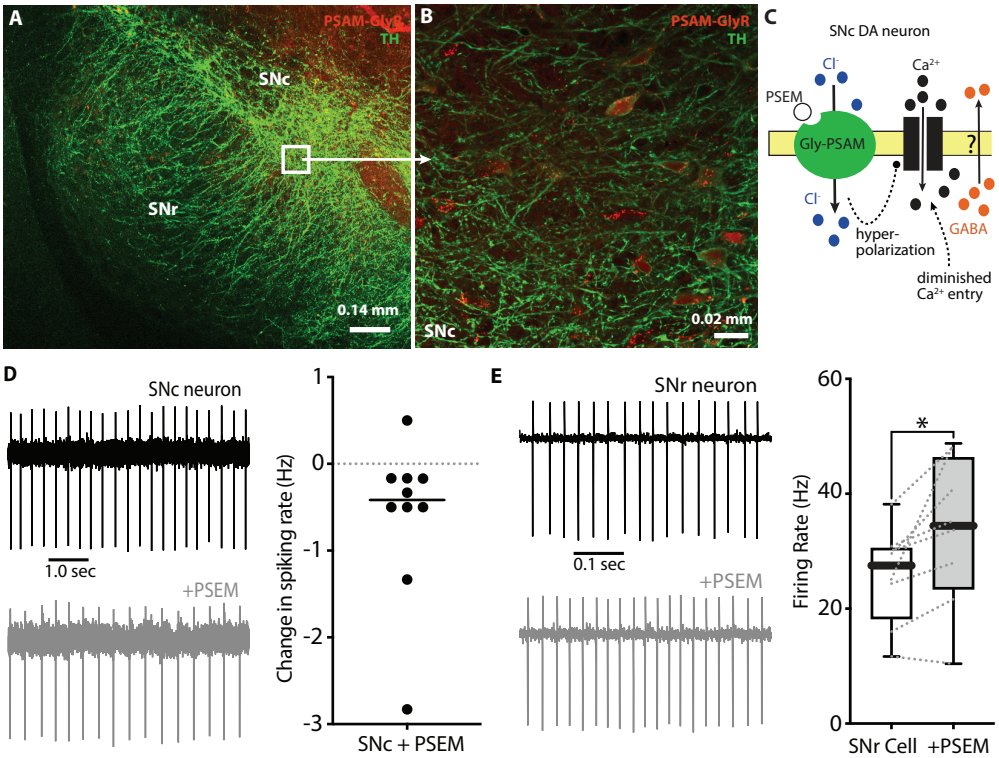
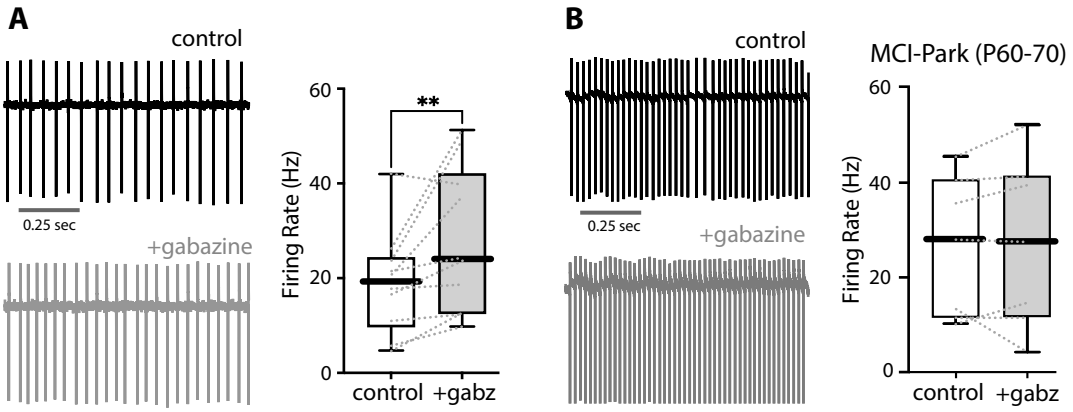


**Figure 7. Chemogenetic inhibition of SNc disinhibits SNr.**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