

Striatal cholinergic interneuron pause response requires Kv1 channels, is absent in dyskinetic mice, and is restored by dopamine D5 receptor inverse agonism

Reviewed Preprint

v2 • February 3, 2025

Revised by authors

Reviewed Preprint

v1 • October 29, 2024

Cecilia Tubert, Rodrigo M Paz, Agostina M Stahl, Lorena Rela, Mario G Murer 

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina • Department of Neurology, University of California, San Francisco, San Francisco, United States • Universidad de Buenos Aires, Facultad de Medicina, Departamento de Ciencias Fisiológicas, Buenos Aires, Argentina

 https://en.wikipedia.org/wiki/Open_access
 Copyright information

eLife Assessment

The authors investigated the mechanisms underlying the pause in striatal cholinergic interneurons (SCINs) induced by thalamic input, identifying that Kv1 channels play a key role in this burst-dependent pause. The **valuable** study provides mechanistic insights into how burst activity in SCINs leads to a subsequent pause, highlighting the involvement of D1/D5 receptors. The experimental evidence is **solid**; however, the reviewers suggest further clarifying the mechanism by which clozapine reduces D5R ligand-independent activity in the L-DOPA-off state.

<https://doi.org/10.7554/eLife.102184.2.sa4>

Abstract

Striatal cholinergic interneurons (SCIN) exhibit pause responses conveying information about rewarding events, but the mechanisms underlying them remain elusive. Thalamic inputs induce a pause mediated by intrinsic mechanisms and regulated by dopamine D2 receptors, though the underlying membrane currents are unknown. Moreover, the role of D5 receptors (D5R) has not been addressed so far. We performed *ex vivo* studies that show that glutamate released by thalamic inputs in the dorsolateral striatum induces a burst in SCIN, followed by the activation of a Kv1-dependent delayed rectifier current responsible for the pause. Endogenous dopamine promotes the pause through D2R stimulation, while pharmacological stimulation of D5R suppresses it. Remarkably, the pause response is absent in parkinsonian mice rendered dyskinetic by chronic L-DOPA treatment but can be reinstated acutely by the inverse D5R agonist clozapine. Blocking the Kv1 current eliminates the pause reinstated by the D5R inverse agonist. In conclusion, the pause response is mediated by delayed rectifier Kv1 channels, which are tonically blocked in dyskinetic mice by a

mechanism depending on D5R ligand-independent activity. Targeting these alterations may have therapeutic value in Parkinson's disease.

Highlights

- In brain slices, thalamostriatal input triggers a burst followed by a pause in SCIN.
- Kv1, but not Kv7 or Kir2.2 channels, are necessary for the expression of the pause.
- D2R stimulation promotes, and D5R stimulation inhibits the pause.
- Thalamic bursts are not followed by a pause in SCIN from dyskinetic mice.
- D5R inverse agonism restores a Kv1-dependent pause response in dyskinetic mice.

Introduction

Dopamine (DA), released by neurons from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), and acetylcholine (ACh), mainly released by striatal cholinergic interneurons (SCIN), are the main modulators of cortico-striatal circuits. Both types of neurons are tonically active, and their activity is altered by unexpected rewards or by the sensory cues that predict those rewards. These events evoke a burst in dopaminergic neurons coincident with a pause response in SCIN. The resulting increase in DA and decrease in ACh are critical for striatal-dependent learning and decision-making through modulatory effects on striatal “medium spiny” projection neurons (MSNs) (Cragg, 2006 [↗](#); Ding et al., 2010 [↗](#); Reynolds et al., 2022 [↗](#)).

In vivo recordings show that the phasic response of striatal tonically active neurons (TANs, putative SCIN) to salient events typically involves a brief burst of action potentials followed by a short pause (Kimura et al., 1984 [↗](#); Aosaki et al., 1994a [↗](#), 1994b [↗](#); Apicella, 2007 [↗](#)). Recent *in vivo* studies in rodents show variations in the SCIN pause response across striatal subregions, where the initial excitation preceding the pause is typically seen in the dorsolateral striatum but may be minimal or absent in the dorsomedial region (Duhne et al., 2024 [↗](#)). The precise mechanisms underlying this response remain elusive. Evidence suggests the involvement of both dopaminergic and thalamic inputs in the generation of the pause. Neurons of the intralaminar nuclei of the thalamus, which send excitatory projections to the striatum, also respond to reward and salient stimuli, and the TANs pause is absent when the thalamus is locally inhibited (Matsumoto et al., 2001 [↗](#)). Dopamine D2 receptor (D2R) antagonists or genetic elimination of D2R from cholinergic neurons have been shown to reduce the duration of the pause (Watanabe and Kimura, 1998 [↗](#); Ding et al., 2010 [↗](#); Kharkwal et al., 2016 [↗](#)), implicating a direct contribution of D2R-mediated inhibition of SCIN. Additionally, *ex vivo* studies reveal heterogeneous responses of SCIN to stimulation of dopaminergic axons, with a more pronounced dopaminergic influence on the pause response in the dorsal versus the ventral striatum (Chuhma et al., 2014 [↗](#)). Further evidence suggests that the pause in SCIN arises from intrinsic mechanisms activated by depolarization (Reynolds et al., 2004 [↗](#); Wilson and Goldberg, 2006 [↗](#); Sanchez et al., 2011 [↗](#)). Experiments conducted in brain slices demonstrate that the pause that follows stimulation of SCIN excitatory inputs persists even after blocking GABA-A receptors, indicating the involvement of voltage (Kv) or calcium-dependent (KCa) potassium currents, with dopamine likely modulating these currents (Ding et al., 2010 [↗](#); Schulz and Reynolds, 2013 [↗](#); Zhang et al., 2018 [↗](#); McGuirt et al., 2022 [↗](#)).

The importance of dopamine is underscored by the absence of pause in animals with chronic nigrostriatal lesions (Aosaki et al., 1994a [↗](#)). While reduced D2R stimulation may explain this, genetic elimination of D2R from cholinergic neurons reduces but does not eliminate the pause (Kharkwal et al., 2016 [↗](#)), suggesting that additional factors are involved. SCIN also express dopamine D5 receptors (D5R), which have been recently linked to SCIN functional changes in parkinsonian animals (Paz et al., 2022 [↗](#)). However, their role in regulating phasic SCIN responses remains unaddressed, and whether alterations in D5R signaling contribute to the absence of pause in dopamine-depleted animals remains unclear.

Here, we report *ex vivo* studies showing that specific activation of intralaminar thalamostriatal inputs triggers a burst followed by a pause in SCIN recorded from the dorsolateral striatum. This pause requires the activation of an intrinsic delayed-rectifier current mediated by Kv1 channels, and is modulated by D2R and D5R. Furthermore, we found that the pause response is absent in parkinsonian mice with chronic L-DOPA treatment, but can be acutely reinstated by the inverse D5R agonist clozapine. Finally, the pause response reinstated by clozapine is abolished by blocking the Kv1 current, indicating that inhibition of constitutive D5R signaling releases this current from a tonic restraining effect, aligning with our prior findings (Paz et al., 2022 [↗](#)).

Results

The timing of thalamic input interacts with SCIN intrinsic mechanisms to determine the duration of the pause response

To induce a pause in SCIN in response to thalamic activation, we injected an AAV2-CamKII-ChR2-YFP viral vector into the intralaminar nuclei of the thalamus in adult ChAT-Cre;tdTomato mice. We used these mice to facilitate the localization of SCIN in brain slices (Tubert et al., 2016 [↗](#)). Four weeks later, we sacrificed the animals and made coronal slices of the striatum for *ex vivo* patch clamp recordings (Figure 1A [↗](#)). Histological analysis confirmed the site of AAV injection and the labeling of thalamostriatal projections (Figure 1B [↗](#)). We recorded SCIN in the cell-attached configuration and stimulated thalamic axons with 2 ms-long pulses of blue light at 20 Hz and 10 mW (Figure 1C [↗](#)). All the experiments were performed in the presence of picrotoxin (PIC, 100 μ M) in the bath. When the stimulation elicited a minimal response (1 spike), the latency of the evoked spike decreased with the time separating the stimulus from the preceding spontaneous spike (Figure 1D-E [↗](#)). Thus, a stimulus arriving after an average baseline interspike interval (ISI) has elapsed produces a shorter latency response than a stimulus arriving shortly after the preceding spontaneous spike (Figure 1E [↗](#)), as expected given the strong after-hyperpolarization (AHP) that follows SCIN spikes (Reynolds et al., 2004 [↗](#); Wilson and Goldberg, 2006 [↗](#)). While a similar inverse relationship is observed under conditions of “sham stimulation” (Figure 1E [↗](#)), after optical stimulation the response latencies were significantly smaller at intervals shorter and longer than the average ISI, indicating that the stimulation increased spike probability (Figure 1F [↗](#)). Remarkably, the interval that followed these minimal responses was longer than the average ISI (baseline ISI: 464.61 $\pm$ 42.44 ms; pause after 1 elicited spike: 609.3 $\pm$ 59.5; $p=0.0181$, paired *t* test).

By increasing the number of pulses in the stimulation train, we obtained responses of 2 to 4 spikes (“bursts”) followed by an interruption of SCIN firing, which were abolished by application of an AMPA receptor antagonist in the bath (firing rate during stimulus/baseline firing rate: PIC=3.611; PIC+CNQX=1.343; paired *t* test $p=0.0409$; $n=5$ cells from 5 animals) (Figure 1G [↗](#)). Different train durations were required to evoke a given burst response across SCIN, probably relating to variability in the density of ChR2-expressing afferent fibers across experiments. To minimize the effect of this source of variability, we studied the pause duration as a function of the number of spikes in the burst evoked by thalamic stimulation. The duration of the pause response increased with the number of spikes in the burst (Figure 1H [↗](#)), without changes in baseline ISI (Figure

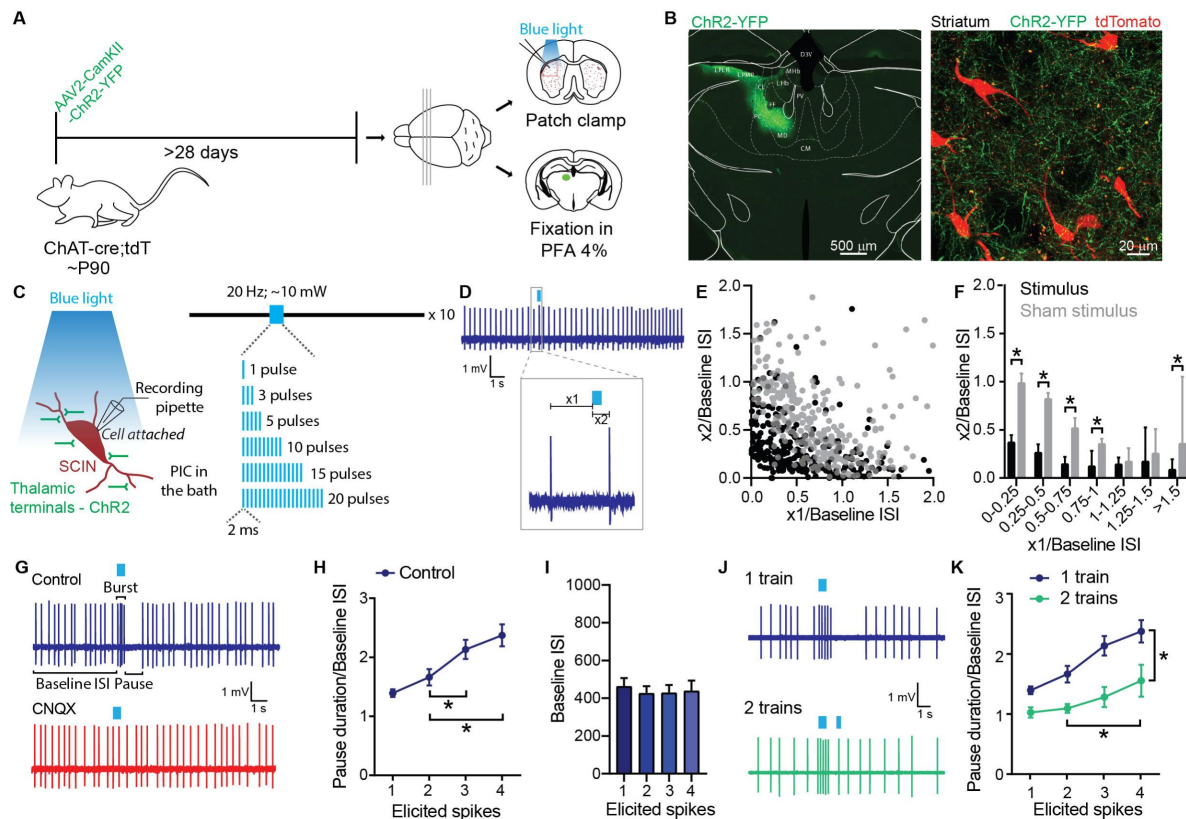


Figure 1

SCIN burst-pause response to activation of thalamic terminals.

A. Experimental design. **B.** Micrograph showing the thalamic region transfected with ChR2-YFP (left) and a striatal region (z-stack maximal projection) showing tdTomato-reported SCIN and thalamic terminals expressing ChR2-YFP (right). **C.** Schematic representation of optogenetic stimulation of thalamic inputs to a SCIN recorded in cell-attached configuration. Picrotoxin (PIC, 100 μ M; GABA-A antagonist) was present in the bath in all experiments. **D.** Representative recording of a SCIN that responded to optogenetic activation of thalamic terminals with 1 spike. Inset: Detail of the measurements of x1 and x2 values. x1: time from the last spike before the optogenetic stimulation and the start of the stimulation; x2: time from the initiation of stimulation to the following spike. **E.** x2/Baseline ISI vs x1/Baseline ISI for single spike responses to optogenetic activation of thalamic terminals (black dots) and for sham stimulation (grey dots). **F.** Data shown in E were binned and expressed as median and 95% confidence interval. * $p < 0.05$, Mann Whitney U test; **G.** Representative recordings of a SCIN to optogenetic stimulation of thalamic terminals in cell-attached configuration before and after bath application of CNQX (20 μ M; AMPA receptor antagonist). **H.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals (One-way RM ANOVA, treatment 2 vs 3 spikes: * $p = 0.0032$; 2 vs 4 spikes: * $p = 0.0017$). **I.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals (One-way RM ANOVA, ns). **J.** Representative recordings of SCIN stimulated with one train of optogenetic activation of thalamic terminals (top) or with two trains (bottom). **K.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, with one or two trains of stimulation pulses. The second train occurs 350 ms after the first train. (Two-way RM ANOVA, interaction ns; treatment * $p < 0.0001$; Elicited spikes * $p < 0.0001$; 2 vs 4 spikes * $p = 0.0042$). Mean $\pm$ SEM; $n = 11-18$ cells per group, from >5 mice.

11 [↗](#)), as expected if the pause depended on the recruitment of a depolarization-activated hyperpolarizing current (Average pause durations: 1 elicited spike = 609.3 +/- 59.5; 2 elicited spikes = 744.2 +/- 92.4 ms; 3 elicited spikes = 964.7 +/- 92.3 ms; 4 elicited spikes = 1065.7 +/- 136.5 ms).

A pioneering *in vivo* study showed that the thalamic neurons presumptively driving the phasic response of SCIN show a burst followed by a pause that ends with a rebound of activity, in connection with reward-related events. This thalamic burst-pause response temporally coincides with the burst-pause response of the presumptive SCIN (Matsumoto et al., 2001 [↗](#)). This suggests that a decrease of excitatory afferent activity may be necessary for the expression of the SCIN pause. To evaluate the effect of a second volley of excitatory inputs on the SCIN pause duration, we delivered a second thalamic stimulus train 350 ms after the end of the first stimulus. We found that the second stimulus train could terminate the pause, although its effect is delayed as more spikes are recruited in the burst by the first stimulus (Figure 1J-K [↗](#)). This is consistent with a graded recruitment of an intrinsic current that reduces the excitability of SCIN, delaying their response to the second stimulus.

The pause response to thalamic stimulation requires activation of Kv1 channels

In previous work we found that Kv delayed rectifier channels conformed by Kv1.3 and Kv1.1 subunits contribute to the sAHP current in SCIN of adult mice (Tubert et al., 2016 [↗](#)). Moreover, a train of EPSPs evoked by thalamic afferents stimulation is followed by an AHP, which persists in the presence of the GABA-A receptor antagonist picrotoxin and is blocked by the Kv1.3 channel blocker margatoxin (Tubert et al., 2016 [↗](#)). We asked whether this Kv1 current is recruited during the SCIN pause response. Both margatoxin (MgTx) at 30 nM – but not at 3 nM – and dendrotoxin (DTx, 100 nM, a Kv1.1 and Kv1.6 channel blocker) abolished the pause response without changing the spontaneous firing rate or the burst duration (Figure 2A-E [↗](#)). Previous studies showed that Kv1.1 and Kv1.3 mRNAs are expressed in SCIN but not in striatal tissue samples lacking SCIN (Tubert et al., 2016 [↗](#)), consistent with independent findings showing that Kv1.3 mRNA is absent in MSNs (Shen et al., 2004 [↗](#)). These findings suggest that the effect of MgTx on the pause response is likely mediated by direct actions on SCIN.

SCIN have a high reserve of Kv7 channels that limit EPSP summation but do not seem to contribute to the sAHP (Paz et al., 2018 [↗](#)) or pause response (McGuirt et al., 2022 [↗](#)) in adult mice. Consistent with these findings, the pause induced by thalamic stimulation was not reduced by the Kv7 channel blocker XE-991 (10 uM) (Figure 2F-I [↗](#)). Moreover, SCIN hyperpolarizations can be amplified by the recruitment of inwardly rectifying Kir2 channels, presumptively of the Kir2.2 subtype (Paz et al., 2021 [↗](#)), which could then contribute to the pause response (Figure 2A [↗](#)). However, a low concentration of barium that preferentially blocks Kir2.2 channels (10 uM) had no effect on firing frequency, burst duration, or pause response of SCIN to thalamic stimulation (Figure 2F-I [↗](#)). Overall, the data show that the Kv1 current is necessary for the expression of the pause that follows stimulation of thalamic inputs in SCIN of adult mice.

Activation of D5R reduces the pause generated in response to thalamic stimulation

Previous *in vivo* and *ex vivo* studies have shown that dopamine promotes the pause response through D2R-mediated actions (Aosaki et al., 1994a [↗](#); Watanabe and Kimura, 1998 [↗](#); Deng et al., 2007 [↗](#); Ding et al., 2010 [↗](#)), including inhibitory effects on Ih, which could be activated by the pause and contribute to its end (Deng et al., 2007 [↗](#); McGuirt et al., 2022 [↗](#)). The pause shortens when D2R endogenous activation is blocked with D2-type receptor antagonists (Aosaki et al., 1994a [↗](#); Watanabe and Kimura, 1998 [↗](#); Ding et al., 2010 [↗](#)) or D2R are genetically eliminated (Kharkwal et al., 2016 [↗](#)), and lengthens when D2R are overexpressed in SCIN (Gallo et al., 2022 [↗](#)). In addition to D2R, SCIN express D5R that increase SCIN excitability by reducing the Kv1-mediated

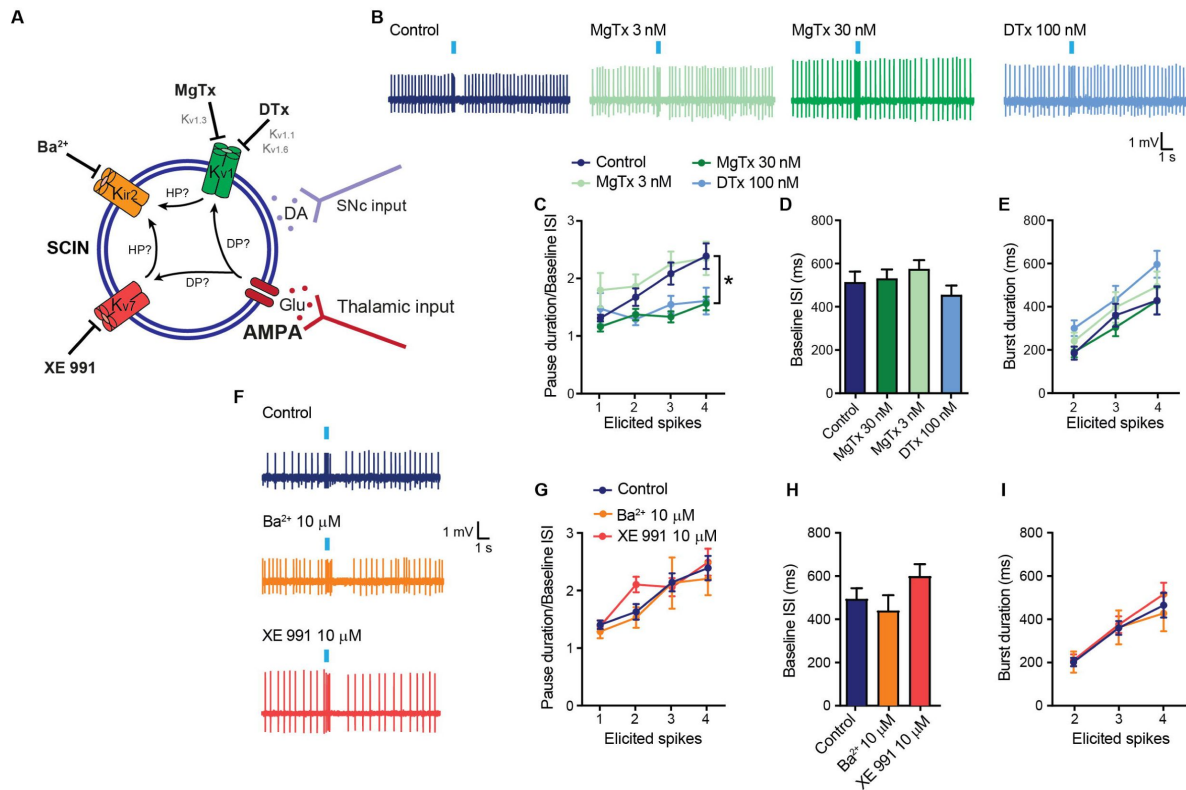


Figure 2

Contribution of the Kv1 current to the pause response.

A. Schematic representation of the tested hypotheses. DP: depolarization; HP: hyperpolarization; Glu: glutamate; DA: dopamine. **B.** Representative recordings of SCIN in response to optogenetic activation of thalamic terminals, with or without Margatoxin (MgTx; Kv1.3 channel blocker) or dendrotoxin (DTx; Kv1.1 and Kv1.6 channels blocker) in the bath. **C.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, with or without MgTx 3nM, 30 nM, or DTx 100 nM, in the bath (Two-way RM ANOVA interaction ns, treatment *p=0.0051; Control-MgTx 30nM: *p=0.0005; Control-MgTx 3nM: ns; Control-DTx: *p=0.0462; MgTx 30nM-MgTx 3nM: *p<0.0001; MgTx 30nM-DTx: ns; MgTx 3nM-DTx: *p=0.0027). **D.** Baseline ISI of SCIN recorded with or without MgTx 3nM, 30 nM, or DTx (One-way ANOVA, ns). **E.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, with or without MgTx 3nM, 30 nM or DTx 100 nM, in the bath (Two-way RM ANOVA, ns). **F.** Representative recordings of SCIN in response to optogenetic activation of thalamic terminals, with or without Ba²⁺ (10 μ M; Kir2.2 channel blocker at this concentration) or XE 991 (10 μ M; Kv7 channel blocker) in the bath. **G.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, with or without 10 μ M XE 991 or 10 μ M Ba²⁺ (Two-way RM ANOVA, ns). **H.** Baseline ISI of SCIN recorded with or without Ba²⁺ or XE 991 (One-way ANOVA, ns). **I.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, for the above conditions (Two-way RM ANOVA, ns). Mean $\pm$ SEM; n=7-16 cells per group, from >5 mice.

delayed rectifier current (Paz et al., 2021 [↗](#), 2022 [↗](#)), shown above to be involved in the pause response. To assess whether D5R modulates the pause response induced by thalamic stimulation, we tested the effect of the D1/D5 receptor selective agonist SKF81297 in our *ex vivo* preparation. SKF81297 suppressed the pause without changing SCIN spontaneous firing rate (Figure 3A-E [↗](#)). The effect of SKF81297 was prevented by co-application of the selective D1/D5 receptor antagonist SCH23390, which had no effects by itself, ruling out tonic effects of DA through D5R (Figure 3A-E [↗](#)). Furthermore, we found a small effect of the selective D2R antagonist sulpiride, limited to the pauses that followed 4-spike bursts, supporting a modulatory role of D2R (Figure 3F-I [↗](#)). Previous studies suggested that synchronized SCIN bursts induce dopamine release through local effects mediated by nicotinic receptors located in dopaminergic terminals (Threlfell et al., 2012 [↗](#)). Accordingly, the nicotinic receptor antagonist mecamylamine also reduced the pause that followed 4-spike bursts (Figure 3F-I [↗](#)). Presumably, endogenous levels of dopamine in the slice, produced by tonic release mechanisms or phasic cholinergic activation of dopaminergic terminals, are sufficient to stimulate D2R but not D5R.

Reduction of I_h current does not block the pause generated in response to thalamic activation

To evaluate if D5R stimulation reduces the pause response through effects on I_h current, we assessed if SKF81297 effect persisted in the presence of the HCN channel blocker ZD7288 (Figure 4A [↗](#)). We performed whole cell patch clamp recordings including ZD7288 in the recording pipette (Paz et al., 2021 [↗](#)). ZD7288 blocked HCN channels as shown by the disappearance of the sag induced by hyperpolarizing steps (Figure 4B-C [↗](#)). Because HCN channel block also stopped spontaneous activity, we injected current through the electrode to induce spiking. In these conditions, thalamic input stimulation induced a burst-pause response. Additionally, the suppression of the pause by stimulation of D5R with SKF81297 was not prevented by I_h block (Figure 4D-G [↗](#)).

Our results, so far, extend previous work by showing that D5R can inhibit the pause response through effects that are independent of GABA-A receptors, burst amplitude, and I_h current, likely involving Kv1 current inhibition.

SCIN from L-DOPA-treated parkinsonian mice show reduced pause responses to thalamic activation

Previous *in vivo* work has shown that the pause response in SCIN is absent in an animal model of Parkinson's disease and is partially restored by acute administration of the dopamine receptor agonist apomorphine (Aosaki et al., 1994a [↗](#)). Chronic administration of L-DOPA to parkinsonian animals produces lasting changes in SCIN membrane excitability involving Kv1, Kir2, and likely, other currents (McKinley et al., 2019 [↗](#); Choi et al., 2020 [↗](#); Paz et al., 2021 [↗](#), 2022 [↗](#)). Yet, whether chronic L-DOPA modifies the SCIN pause response has not been addressed before. We studied the pause response induced by thalamic afferents stimulation in SCIN from parkinsonian mice treated for 14 consecutive days with L-DOPA (12 mg/kg) or vehicle (Figure 5A [↗](#)). Mice unilaterally lesioned with 6-OHDA showed extensive loss of TH-positive neurons in substantia nigra pars compacta (Figure 5B [↗](#)), accompanied by marked parkinsonian-like motor impairment (Figure 5C-E [↗](#)). Moreover, these mice developed dyskinesia in response to L-DOPA administration, characterized by an abnormal involuntary movements score (AIM) that worsens after day 4 of treatment (Figure 5F [↗](#)). In previous studies, we have shown that the Kv1 current diminishes after lesioning nigral dopaminergic neurons and further suppressed when recorded 24 hours after the end of a chronic L-DOPA treatment (Paz et al., 2022 [↗](#)). Cell-attached recordings performed 24 hours after the last L-DOPA challenge (OFF L-DOPA condition) showed that the pause is absent in SCIN from dyskinetic mice (Figure 5G-H [↗](#)), regardless of the burst duration (Figure 5J [↗](#)). Consistent with previous findings (Paz et al., 2021 [↗](#)), the spontaneous firing rate was higher in

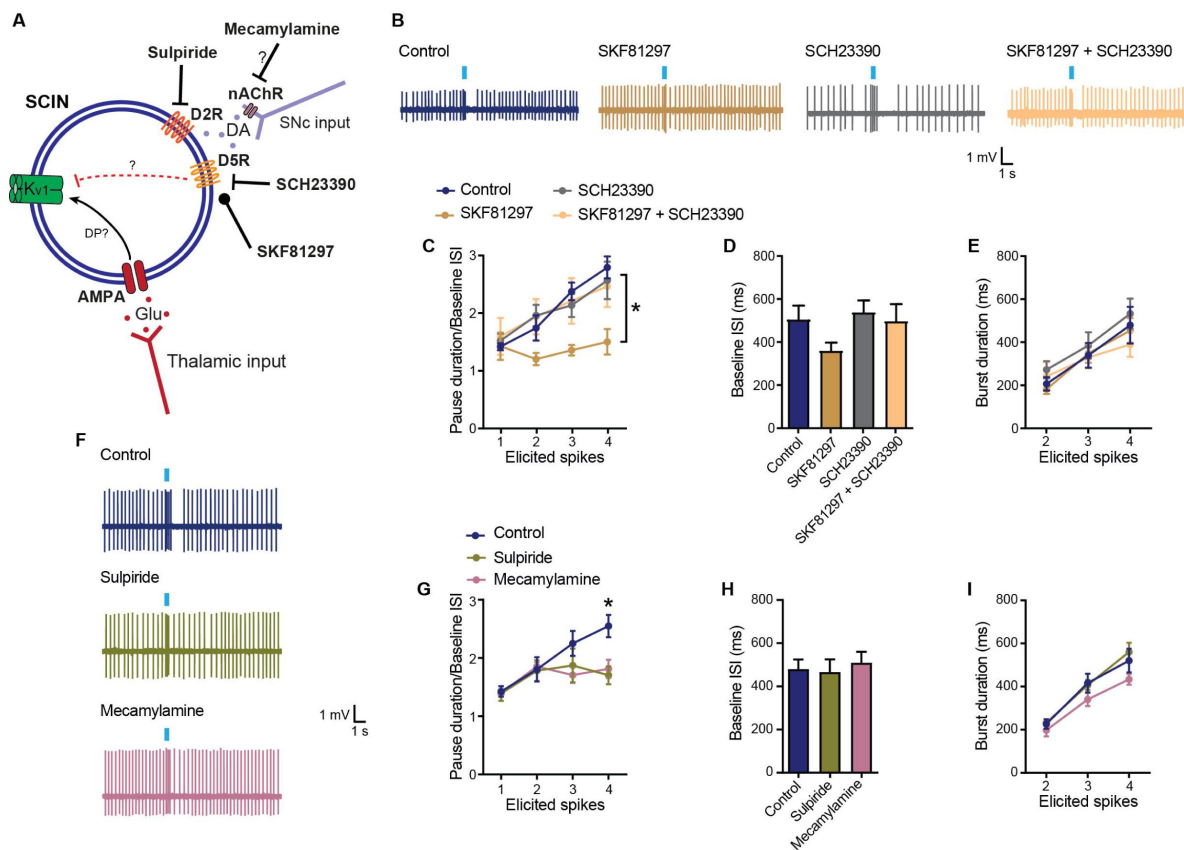


Figure 3

Modulation of the pause response by dopamine receptors.

A. Schematic representation of the tested hypotheses. DP: depolarization. **B.** Representative responses of SCIN to optogenetic activation of thalamic terminals, with SKF81297 (2 μ M; D1/D5 selective agonist), SCH23390 (10 μ M; D1/D5 selective antagonist) or SKF81297 + SCH23390 in the bath. **C.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, interaction $p=0.0381$, Dunnett's multiple comparisons test: Control vs SKF81297: 3 spikes $*p=0.0001$; 4 spikes $*p=0.0010$). **D.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (One-way ANOVA, $*p=0.0906$). **E.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, ns). **F.** Representative responses of SCIN to optogenetic activation of thalamic terminals, with Sulpiride (10 μ M; D2-type receptor selective antagonist) or Mecamylamine (10 μ M; nicotinic receptors antagonist) in the bath. **G.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, interaction $p=0.0369$, Dunnett's multiple comparisons test: Control vs Sulpiride: 4 spikes $*p=0.0056$; Control vs Mecamylamine: 4 spikes $*p=0.0167$). **H.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (One-way ANOVA, ns). **I.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, ns). Mean $\pm$ SEM; $n=7-15$ cells per group, from >5 mice.

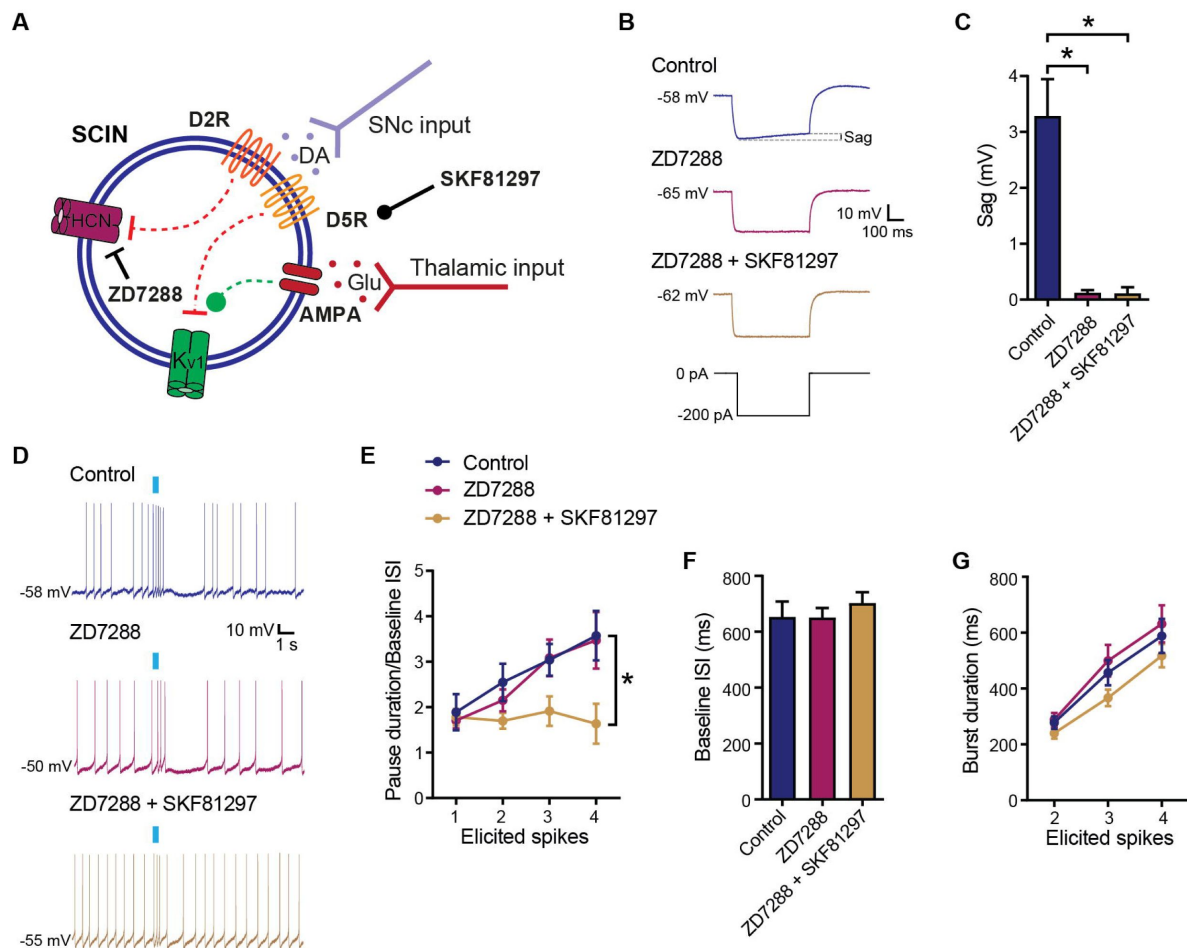


Figure 4

D5R modulation independent of I_h current.

A. Schematic representation of the tested hypotheses. **B.** Time matched representative responses of SCIN to hyperpolarizing current steps with or without ZD7288 (30 μ M; HCN channel blocker) in the recording pipette, and with or without SKF81297 (2 μ M; D1/D5 selective agonist) in the bath. **C.** Sag evaluated in the hyperpolarizing step, under the above conditions (One-way ANOVA, $p < 0.0001$; Tukey's multiple comparisons test: Control vs ZD7288 $*p < 0.0001$; Control vs ZD7288+SKF81297 $*p < 0.0001$). **D.** Representative responses in whole cell configuration to optogenetic activation of thalamic terminals, under the above conditions. **E.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, interaction ns, treatment $p = 0.0373$; Control vs ZD7288+SKF81297: $*p = 0.0005$; ZD7288 vs ZD7288+SKF81297: $*p = 0.0067$). **F.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (One-way ANOVA, ns). **G.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, ns). Mean $\pm$ SEM; $n = 13/14$ cells per group, from >5 mice.

SCIN recorded from mice in the OFF L-DOPA condition compared with sham mice (**Figure 5I**). In addition, inclusion of MgTx in the bath did not have any effect on pause duration, baseline firing rate and burst duration (**Figure 5G-J**), as expected following previous findings showing a lack of effect of MgTx on membrane excitability in the same animal model (Paz et al., 2022). These results support that a reduction of the Kv1 current is involved in the loss of the pause response in dyskinetic mice.

Reducing D5R ligand-independent activity restores the SCIN pause response

In a previous study, we found that the physiological changes observed in SCIN of dyskinetic mice depend on an increase of ligand-independent activity of D5R (Paz et al., 2022). Paz et al. (2022) recorded SCIN in the parkinsonian OFF-L-DOPA condition and showed an increase of membrane excitability that mimics changes acutely induced by SKF81297 in control mice. This hyperexcitability phenotype was reverted by acute administration of drugs that reduce D5R ligand-independent activity (i.e., inverse agonists, like clozapine or flupentixol), and by acutely reducing intracellular cAMP signaling (Paz et al., 2022). Interestingly, the hyperexcitability of SCIN recorded in the OFF L-DOPA condition was not further enhanced by SKF81297 or MgTx (Paz et al., 2022), suggesting D5R-cAMP-Kv1 inhibitory signaling is already working at full steam in these cells. Therefore, we speculated that, if suppression of the Kv1 current in parkinsonian mice causes the lack of pause response, D1/D5 inverse agonists should reinstate the pause. The atypical antipsychotic clozapine behaves as an antagonist of D1/D5 and other dopamine receptors when an agonist is present, but has inverse agonist effects on D5R in the absence of an agonist (Zhang et al., 2014). Remarkably, clozapine restores the pause in SCIN from dyskinetic mice without changing their firing rate and regardless of the burst duration (**Figure 6A-E**). As clozapine is not selective for D5R, we bathed the slices with the D1/D5 selective antagonist SCH23390, and with clozapine + SCH23390, to assess the involvement of D1/D5 dopamine receptors. We found that SCH23390 had no effect per se in the SCIN response to thalamic stimulation but prevented the restoration of the pause response by clozapine (**Figure 6A-E**). Moreover, to confirm that the pause restored by clozapine involves Kv1 channels, as is the case for naïve animals, we added clozapine and MgTx to the bath (**Figure 6A**).

MgTx occluded the pause-restoring effect of clozapine (**Figure 6B-E**), indicating that the reduction of ligand-independent activity of D5R reinstates the Kv1 current in SCIN from OFF L-DOPA parkinsonian mice.

Discussion

While previous studies support the role of intrinsic membrane currents in the pause response of SCIN, the specific currents involved remain debated. Theories implicating deactivation of Ih current as its primary cause were refuted by experiments demonstrating pause induction after Ih current blockade when the resulting hyperpolarization is corrected by current injection (Zhang et al., 2018; present findings). Although Ih likely contributes to a rebound depolarization limiting pause duration (Deng et al., 2007; McGuirt et al., 2022). Further work suggested the involvement of a slow current mediated by Kv7 channels in juvenile mice (Zhang et al., 2018), which is developmentally downregulated and seems to not contribute significantly in the adult (McGuirt et al., 2022; present findings), though SCIN retain a high reserve of Kv7 channels sensitive to activators (Paz et al., 2018). Whether this Kv7 channel reserve is recruited under particular conditions not captured in our slice preparation remains to be determined. Previously, we found that Kv1 channels expressed by SCIN markedly modulate their excitability (Tubert et al., 2016). Heteromeric channels constituted by Kv1.3 and Kv1.1 subunits can be blocked by the binding of a single MgTx molecule (Hopkins, 1998). In SCIN, MgTx enhances subthreshold EPSP

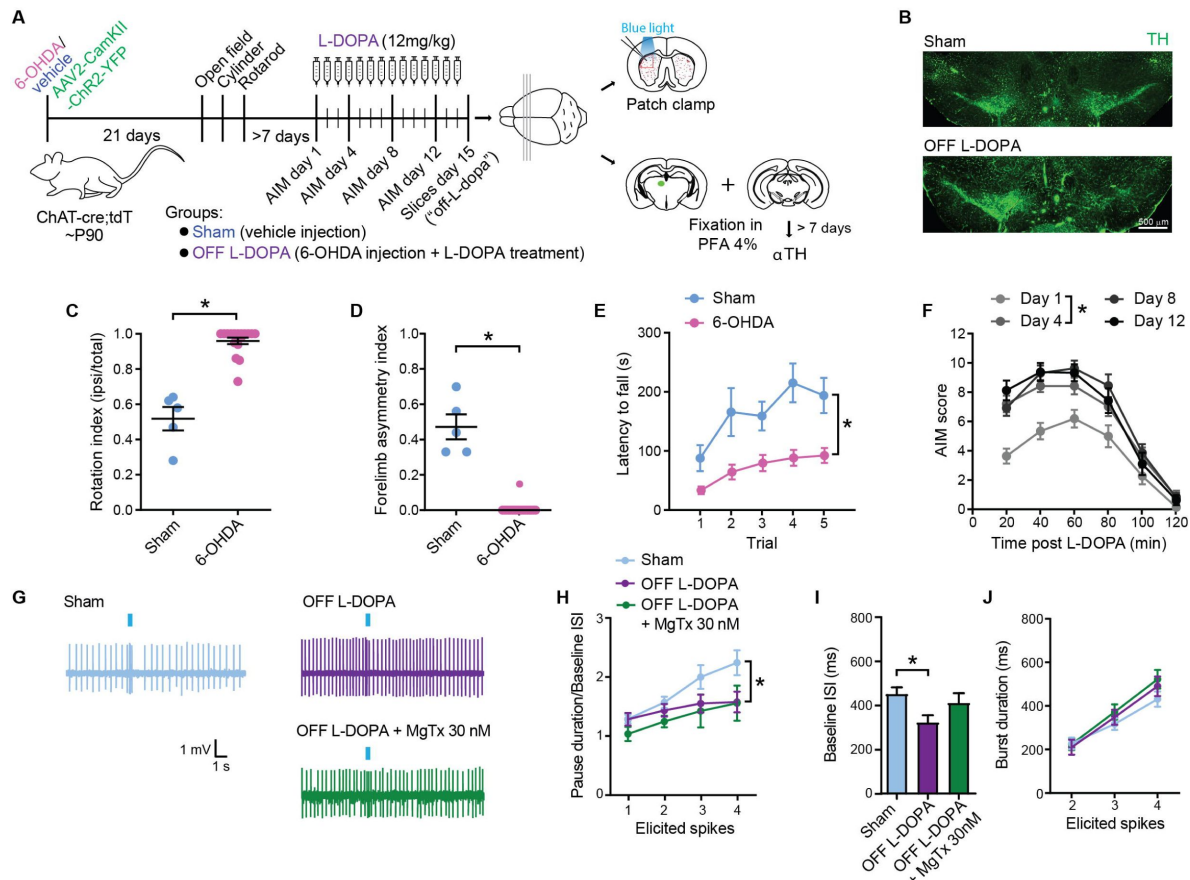


Figure 5

SCIN from L-DOPA-treated parkinsonian mice show a reduced pause response.

A. Experimental design. **B.** Micrographs showing TH immunostaining at the level of the substantia nigra. **C-E.** Rotation index (C), forelimb asymmetry (D) and latency to fall from the rotarod (E) for sham and 6-OHDA mice (C-D: unpaired t test, $**p < 0.0001$; E: Two-way RM ANOVA, interaction: $*p = 0.0284$, $n = 5$ sham and 22 6-OHDA mice). **F.** Abnormal involuntary movement (AIM) score of chronically L-DOPA-treated 6-OHDA mice. (Two-way RM ANOVA, interaction: $p = 0.0081$; post-hoc: Day 1 vs Day 4 $*p < 0.03$). **G.** Representative cell-attached recordings of the pause response of SCIN to optogenetic stimulation of thalamic terminals, in a sham and a dyskinetic mouse in the OFF L-DOPA condition, with or without MgTx 30 nM in the bath. **H.** Pause duration/Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals (Two-way RM ANOVA, interaction ns, treatment $*p = 0.0227$; Sham vs OFF L-DOPA: $*p = 0.0301$; OFF L-DOPA vs OFF L-DOPA+MgTx: ns; Sham vs OFF L-DOPA+MgTx: $*p = 0.0032$). **I.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals under the above conditions (One-way ANOVA, $*p = 0.0254$; Sham vs OFF L-DOPA: $*p = 0.0201$; OFF L-DOPA vs OFF L-DOPA+MgTx: ns; Sham vs OFF L-DOPA+MgTx: ns). **J.** Burst duration of SCIN that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals under the above conditions (Two-way RM ANOVA, ns). Mean $\pm$ SEM; $n = 14-27$ cells per group, from > 5 mice.

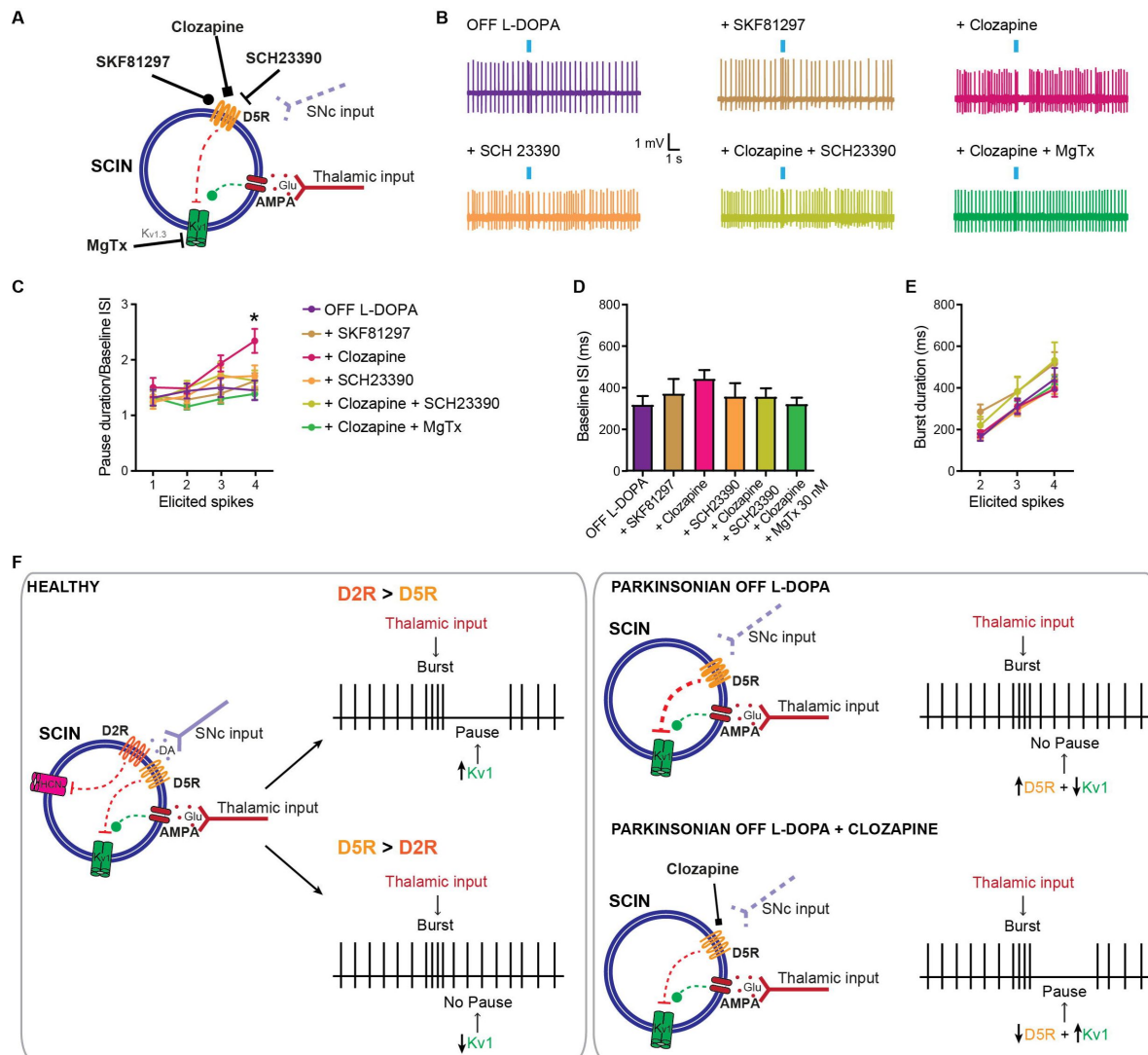


Figure 6

D1-like receptors inverse agonist restores the pause response in SCIN from OFF L-DOPA parkinsonian mice.

A. Schematic representation of the tested hypothesis. **B.** Representative responses of SCIN from OFF L-DOPA dyskinetic mice to optogenetic activation of thalamic terminals, with or without SKF81297, Clozapine (10 μ M; D1/D5 receptor inverse agonist), SCH23390 (10 μ M; D1/D5 selective antagonist), Clozapine + SCH23390 or Clozapine + MgTx, in the bath. **C.** Pause duration/Baseline ISI of SCIN from OFF L-DOPA dyskinetic mice that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals (Two-way RM ANOVA, interaction $*p=0.0489$, Dunnett's multiple comparisons test: 4 elicited spikes: OFF L-DOPA vs Clozapine: $*p=0.0181$). **D.** Baseline ISI of SCIN that responded with 1, 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (One-way ANOVA, ns). **E.** Burst duration of SCIN from dyskinetic mice OFF L-DOPA that responded with 2, 3 or 4 spikes to optogenetic activation of thalamic terminals, under the above conditions (Two-way RM ANOVA, interaction ns, treatment ns). Mean $\pm$ SEM; $n=6-17$ cells per group, from >5 mice. **F.** Schematic representation of conclusion. Left, in physiologic conditions the balance of D2 versus D5 activation emerges as a determinant of the Kv1-dependent pause expression; right, in parkinsonian mice an imbalance towards D5 signaling abolishes the pause (top), which can be reset with D5 inverse agonism (bottom).

summation and blocks the ensuing AHP (Tubert et al., 2016). The present study extends these findings by showing that specific blockers of channels constituted by Kv1.3 and Kv1.1 subunits suppress the pause that follows action potential bursts induced by thalamostriatal afferents in adult mice. Although SCIN express inwardly rectifying Kir2 channels capable of amplifying hyperpolarizations (Wilson, 2005; Paz et al., 2021), these channels do not contribute to the pause, likely because of insufficient membrane hyperpolarization to recruit Kir currents. Finally, *in vivo*, SCIN spontaneous firing rate synchronizes with excitatory synaptic input fluctuations (Krok et al., 2023). The SCIN phasic response could reflect burst-pause discharge synchronization with thalamic afferents, known to exhibit a burst-pause-rebound pattern in response to reward stimuli *in vivo* (Matsumoto et al., 2001). The effect of thalamic rebound activity on pause duration has not been examined before in brain slices. We observed that a second round of stimulation of thalamic afferents reduces the duration of the pause, although its impact is delayed by additional spikes in the initial SCIN burst, likely due to recruitment of a more robust Kv1 current.

Recent *in vivo* studies showed anticorrelated changes of DA and ACh striatal levels, not only during rewarded tasks but also during spontaneous behavior (Chantranupong et al., 2023; Krok et al., 2023). Several studies support that dopamine positively modulates the pause response of SCIN through D2R-mediated effects (Watanabe and Kimura, 1998; Ding et al., 2010; Kharkwal et al., 2016) and constitutive ablation of D2R from cholinergic neurons disrupts the anticorrelated DA-ACh signal during specific portions of an operant task (Chantranupong et al., 2023). However, striatal ACh transients persist and even increase after blockade of striatal DA receptors or lesion of nigrostriatal neurons, while they are reduced after intrastriatal infusion of glutamate receptor antagonists (Krok et al., 2023). Although these findings suggest that slow variations of striatal glutamate, rather than DA, control ACh release from SCIN at the space and time scales resolved by fiber photometry (Krok et al., 2023), they do not rule out a fine dopaminergic regulation of phasic SCIN responses to glutamatergic inputs, which could be embedded within broader modulations of DA and ACh levels. Our *ex vivo* data support that endogenous activation of D2R modulates the pause induced by thalamic afferents in SCIN. Moreover, we found that ambient DA in the slice does not modulate SCIN activity through D5R, but pharmacological stimulation of D1/D5 receptors markedly inhibited the pause response, likely through inhibitory effects of D5R on Kv1 channels. Overall, our data show that, in physiological conditions, Kv1 channels are necessary for the expression of the pause response in SCIN of adult mice, and that the pause can be modulated by D5R in addition to D2R. This D5R modulation may be relevant under conditions of phasic dopamine release producing synaptic concentrations of DA capable of activating the low affinity D5R (Figure 6F).

Learning deficits are believed to play a role in the development of Parkinson's disease symptoms. A recent study demonstrates that practice worsens the performance of previously learned motor skills in parkinsonian mice, while practice combined with therapeutic doses of L-DOPA prevents skill deterioration (Cheung et al., 2023). While this learning deficit may be due to the loss of dopamine, a possible contribution of SCIN dysfunction cannot be dismissed given that, in parkinsonian monkeys, the pause response of presumptive SCIN is lost and can be partially restored with apomorphine (Aosaki et al., 1994a). Our *ex vivo* studies provide a possible explanation for this pause response loss. We reported that the Kv1 current is diminished in parkinsonian mice and further suppressed 24 hours after the termination of a dyskinetogenic L-DOPA treatment (Tubert et al., 2016; Paz et al., 2022). Consistent with a key role of this current in pause generation, we find that the pause that follows a burst of spikes induced by stimulation of thalamostriatal afferents is markedly diminished in this animal model. Importantly, in previous work we reported that suppression of the Kv1 current in dyskinetic mice is caused by sustained cAMP signaling, likely due to enhanced ligand-independent activity of D5R, as demonstrated by showing that inverse agonists of D5R acutely restore the Kv1 current (Paz et al., 2022). In the current study, we find that clozapine, an atypical antipsychotic that behaves as an inverse agonist of D5R in the absence of an agonist (Zhang et al., 2014), reinstates the pause response of SCIN in

dyskinetic mice. Although clozapine also has D1/D5 receptor antagonist properties, these effects would not manifest when dopamine has been depleted, as it is the case in the OFF L-DOPA condition. Consistently, the prototypical D1/D5 receptor antagonist SCH23390, which lacks inverse agonist activity on these receptors (Zhang et al., 2014), neither reinstates the pause response nor alters SCIN spontaneous firing. However, SCH23390 blocks clozapine's effect, supporting the conclusion that clozapine restores the pause response by reducing ligand-independent D5R activity.

Remarkably, while the Kv1.3 blocker MgTx has no effect on SCIN in dyskinetic mice, as expected given the loss of Kv1 currents in this animal model (Paz et al., 2022), the pause reinstated by clozapine is suppressed by MgTx. This is consistent with previous findings showing that D5R inverse agonists reinstate the Kv1 current in dyskinetic mice (Paz et al., 2022), and supports the key role of the Kv1 current in the pause response and in the dysfunction observed in SCIN after DA neuron lesion and L-DOPA treatment (**Figure 6F**). While our prior work has implicated sustained cAMP signaling in the suppression of the Kv1 current in dyskinetic animals (Paz et al., 2022), further work is needed to link cAMP levels to the loss of the pause response.

In conclusion, our study demonstrates that Kv1 channels are essential for the pause response of SCIN. We also find that D1/D5 receptor dysregulation of Kv1 channels may contribute to the loss of this pause response in parkinsonian animals chronically treated with L-DOPA. Previously, in the same animal model, we showed that D1/D5 inverse agonists normalize the spontaneous firing pattern and membrane excitability of SCIN by restoring various membrane currents including Kv1 (Paz et al., 2022). While antipsychotics having D5R inverse agonist properties bind to multiple metabotropic receptors that could contribute to their effects, our data highlight a key role of ligand-independent activity of D5R in the absence of the pause response in the OFF L-DOPA condition.

Methods

Animals

Adult male and female mice (P60-250) allowing the identification of SCIN through cell type-specific expression of the fluorescent protein tdTomato (ChAT-Cre;tdT, for simplicity) were obtained as previously reported (Tubert et al., 2016; Paz et al., 2021). Briefly, ChAT-Cre;tdT mice were ChAT-Cre⁺/⁻ and tdT⁺/⁻, and were obtained by crossing ChAT-Cre⁺/⁺ (B6;129S6-Chat tm2(cre)Lowl, stock 6410, The Jackson Laboratories; Rossi et al., 2011) and Rosa-CAG-LSL-tdTomato-WPRE⁺/⁺ mice (B6.CgGt(ROSA)26Sor tm14(CAG-tdTomato)Hze, stock 7914, The Jackson Laboratories). Up to six mice were housed per cage with water and food *ad libitum* and a 12:12 h light/dark cycle (lights on at 7:00 A.M.). Animals were cared for in accordance with institutional regulations (IACUC of the School of Medicine, University of Buenos Aires, 2023-891).

6-Hydroxydopamine Lesion

Under deep surgical anesthesia (isoflurane, 3% in O₂ for induction, 0.5-1% for maintenance) each mouse was mounted in a stereotaxic frame (Kopf Instruments; USA). The neurotoxin 6-hydroxydopamine-HBr (6-OHDA; Sigma Aldrich AB) was dissolved in 0.1% ascorbate-saline at the concentration 4 µg/µl (freebase). The skull was exposed, and a small hole was drilled at the desired injection site. The following stereotaxic coordinates were used to target the left medial forebrain bundle (MFB): 1 mm posterior (from Bregma), 1.1 mm lateral and 4.8 mm ventral from dura (Paxinos and Franklin, 2001). 1 µl was injected in adult mice (P90-110) at a rate of 0.5 µl/min using a 300 µm diameter cannula attached to a 25 µl Hamilton syringe controlled by a motorized pump (Bioanalytical Systems, USA). The injection cannula was left in place for six additional minutes before slowly retracting it. Littermates of lesioned mice received an injection of 0.1% ascorbate-saline in the MFB (sham group). After surgery, each mouse was daily weighted and

received a subcutaneous injection of saline and an enriched diet. These cares continued until animals began to regain weight (7 to 20 days after surgery). To assess behavioral signs of parkinsonism, animals were subjected to extensive behavioral testing three weeks after 6-OHDA injection in three non-consecutive days (**Figure 4A**), during the light phase, by an investigator blind to treatment, as described in Escande et al., 2016. Briefly, spontaneous ipsilateral rotation in a novel open field (40 x 40 cm) is assessed using an automated video-tracking system (Anymaze). A rotation asymmetry index is computed by expressing the number of ipsilateral rotations relative to the total number of rotations. In the “cylinder test”, each mouse is placed in a transparent acrylic cylinder (10 cm diameter and 14 cm high) and videotaped for five minutes. A limb use asymmetry score is computed by expressing the number of wall contacts performed with the forepaw contralateral to the lesion relative to the total number of wall contacts performed with the forepaws. Finally, motor coordination is assessed in an accelerating rotarod (from 4 to 40 rpm in five minutes; Ugo Basile, Italy). The latency to fall from the rod is automatically recorded with a cut-off time of five minutes. Each animal was assessed five times with five-minute intertrial intervals in a single day.

L-DOPA Treatment

Mice were treated with 12 mg/kg of L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA, Sigma Aldrich) combined with 12 mg/kg of benserazide (Sigma Aldrich). L-DOPA and benserazide were diluted in 0.9% saline solution. Abnormal involuntary movements (AIMs) were rated as previously described (Keifman et al., 2019). Briefly, AIMs rating started after 20 minutes of L-DOPA injection and mice were rated for 1 minute every 20 minutes during 2 hours. Each subtype of dyskinesia (oral, axial and forelimb) was scored on a scale ranging from 0 to 4 (where 0 = no dyskinesia; 1 = occasional dyskinesia displayed for <50% of the observation time; 2 = sustained dyskinesia displayed for >50% of the observation time; 3 = continuous dyskinesia; 4 = continuous dyskinesia not interruptible by external stimuli).

ChR2-EYFP adeno-associated vector injection

Anesthesia and surgical procedures were as indicated for the nigrostriatal lesions. A CaMKII-driven ChR2-EYFP expressing adeno-associated virus (rAAV2/CaMKII-hChR2(H134R)-EYFP-WPRE-PA) was injected in the thalamus at the following coordinates: -1.3 mm posterior from Bregma, 0.8 mm lateral and -3 mm ventral from dura (Paxinos and Franklin, 2001). 0.5 μ l of viral vector was delivered using a glass micropipette (World Precision Instruments) pulled with a vertical glass puller (Narishige). Experiments were performed after at least 28 postoperative days (**Figure 1A-B**).

Patch Clamp Recordings

Twenty-four hours after the last L-DOPA shot, mice were anesthetized with isoflurane and decapitated for brain slicing. The brain was quickly removed, chilled in ice-cold low- Ca^{2+} /high- Mg^{2+} ACSF, and prepared for slicing. Coronal slices (300 μ m) at the level of the striatum were cut with a vibratome (Pelco T series 1000, Ted Pella) and were incubated in low- Ca^{2+} /high- Mg^{2+} artificial cerebrospinal fluid (ACSF) at 34°C for 30 minutes and then at room temperature. ACSF composition was as follows (in mM): 125 NaCl, 2.5 KCl, 1.3 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 26 NaHCO_3 , 2 CaCl_2 , 1 MgCl_2 , and 10 glucose. For the low- Ca^{2+} /high- Mg^{2+} ACSF, 0.5 mM CaCl_2 and 2.5 mM MgCl_2 were used. Slices were transferred to a submersion-type chamber perfused by a peristaltic pump (Ismatec, Germany) with ACSF at a constant rate of 3ml/min; temperature in the recording chamber was set at 32°C with a TC-344B temperature controller (Warner Instruments). Cells were visualized using an upright microscope (Nikon Eclipse) equipped with a 40X water-immersion objective, DIC and fluorescence optics, and an infrared camera connected to a monitor and computer. Recording electrodes were made with borosilicate glass capillaries shaped with a puller (P-97, Sutter Instruments). Dorsolateral SCINs were recorded in cell-attached configuration with patch pipettes filled with intracellular solution (in mM): 20 KCl, 120 K-gluconate, 10 HEPES, 3

Na₂ATP, 0.3 NaGTP, 0.1 EGTA, 10 phosphocreatine and 2 MgCl₂, pH 7.3 adjusted with KOH. Recordings were amplified (Axopatch-1D; Molecular Devices), sampled at 20 kHz (Digidata 1322A, Molecular Devices) and acquired on a PC running pClamp 9.2 (Molecular Devices). In all the experiments, picrotoxin (100 μM) was always present in the ACSF to block GABAergic transmission. Optogenetic stimulation was generated with a 447-nm light-emitting diode (Tolket, Argentina) and delivered through an optic fiber placed <300 μm from the recorded cell. One to 20, 2-ms long pulses, at 20 Hz and 10 mW, were delivered 5 s after the initiation of the sweep, for 10 consecutive sweeps (**Figure 1C**). For experiments with a second pulse train, the second train was delivered 350 ms after the end of the first train. The second train contained 1-3 pulses (**Figure 1I**).

Pharmacological manipulations

Unless otherwise stated, reagents were purchased from Sigma (Argentina). Salts were purchased from Baker (Research AG, Argentina). Drugs were prepared as stock solutions, diluted in ACSF immediately before use, and applied through the perfusion system. The following stock solvents and final concentrations were used: distilled H₂O for BaCl (10 μM), ZD7288 (30 μM) and mecamlamine (10 μM, RBI); DMSO for CNQX (40 μM, Tocris), picrotoxin (100 μM), SKF81297 (2 μM), SCH23390 (10 μM), Sulpiride (10 μM, Santa Cruz Biotechnology), XE991 (10 μM) and clozapine (10 μM, Rospaw Laboratory); and the manufacturer's recommended storage buffer (0.1% BSA, 100 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA) for margatoxin (MgTx 3 nM and 30 nM, Alomone Labs) and g-Dendrotoxin (100 nM, Alomone labs).

Acquisition and analysis of electrophysiological data

Data acquisition and analysis were performed with ClampFit (Molecular Devices, RRID: SCR_011323). GraphPad Prism version 8.00 for Windows (GraphPad Software, RRID:SCR_002798) and custom-made MATLAB (Mathworks, RRID: SCR_001622) routines were used for data analysis.

For each cell, 3 to 5 recordings were made, varying the number of light pulses delivered during the stimulation train. Each recording consisted of 10 sweeps with the same stimulus. Then, the recordings were analyzed by merging all the sweeps where the stimulation elicited the same number of spikes, regardless of the stimulation protocol. Only sweeps that triggered 1 to 4 spikes in response to the light were included in the analysis. The number of spikes in the burst was determined by the number of spikes fired during the length of the stimulus plus 250 ms after the end of the stimulus. The pause was calculated as the time from the last spike of the burst triggered by the thalamostriatal input activation, and the following spike. The average baseline inter-spike-interval (ISI) was calculated taking into account all ISI collected during the five seconds preceding each stimulus. The burst duration was calculated as the time from the first to the last spike in the burst. To determine the influence of spontaneous spikes on evoked spiking, we plotted the time from the stimulus onset to the first spike in the burst (x2, for stimulations that evoked one spike) as a function of the time from the spike preceding the stimulus to the stimulus onset (x1). Each value was normalized to the average baseline ISI. For sham stimulation we performed the same analysis at a random time point during baseline, for the same sweeps used to analyze light stimulus responses.

Each cell was recorded with only one pharmacological treatment. Most animals contributed with at least two neurons that received different drug treatments. For each experiment, control neurons were obtained from the same animals that contributed treated cells, plus a subset of cells from animals in which no cells were treated.

Perfusion

For confirmation of thalamostriatal projections to the striatum, ChAT-Cre;tdT mice were anesthetized with pentobarbital (100 mg/kg, i.p.), perfused through the ascending aorta with saline solution supplemented with heparin (2 U/ml, Rivero, Argentina) followed by 4% paraformaldehyde

in 0.1 M phosphate buffer saline 0.1 M, pH 7.4 (PBS). Brains were removed, post-fixed for 24 hr in the same fixative, cryoprotected in 30% sucrose/PBS and cut. Sections containing the striatum and the thalamus were preserved in PBS with 0.1% sodium azide.

Immunohistochemistry

To confirm nigral dopaminergic lesions and the site of the AAV injection into the thalamus in mice used for acute slicing, tissue blocks containing thalamus and substantia nigra obtained after striatal slicing were fixed overnight in 4% paraformaldehyde in PBS, cryoprotected in 30% sucrose/PBS and cut at 30 μ m with a sliding-freezing microtome (Leica). Sections containing the substantia nigra pars compacta were preserved in PBS with 0.1% sodium azide. For TH-immunoreactivity, sections were blocked for 2 hours at room temperature (PBS containing 5% NGS and 0.3% Triton X-100), incubated overnight with primary antibodies (mouse anti-TH, Chemicon, 1:1000, RRID: AB_2201528) at 4°C, washed 3 times in 0.15% Triton X-100/PBS (PBS-T) and incubated with secondary antibodies conjugated to Alexa Fluor488 (Goat anti-mouse IgG, Invitrogen, 1:500, RRID: AB_138404) for 2 hours at room temperature. Sections were then washed 3 times in PBS-T and one time in PBS, mounted on glass slides with Vectashield (Vector) and coverslipped. For confirmation of the site of AAV injection, sections containing the thalamus were washed in PBS, mounted on glass slides with Vectashield (Vector) and coverslipped.

Image acquisition and analysis

Images of mounted sections were acquired with a Zeiss AxioImager M2 microscope with NeuroLucida software (MFB Bioscience), with 10X, 20X or 40X objectives. Images were not modified after acquisition.

Statistical Analyses

Data were tested for normality and homoscedasticity with Anderson-Darling test and Spearman's rank correlation test respectively. Non-parametric tests were used when these criteria were not met. Statistical analysis details can be found in the figure legends and wherever numerical data is presented in the text. Significance level was set at $p < 0.05$, and all data are expressed as mean $\pm$ SEM unless otherwise specified. GraphPad Prism and MATLAB routines were used for data analysis. Sidak or Dunn's corrections were applied for multiple comparisons.

Acknowledgements

The authors thank Graciela Ortega, Analía López Díaz, Agostina Presta, Verónica Riso, Lucía Garbini and Micaela Buscema for expert technical assistance in animal genotyping, histology and animal care and Juan Belforte for helpful discussions. We also thank the Rospaw Laboratory for their kind donation of clozapine. This study was supported by Fondo para la Investigación Científica y Tecnológica (FONCYT; Proyecto de Investigación Científica y Tecnológica [PICT] 2018-2738, 2019-2416, 2020-325 and 2022-42) and Universidad de Buenos Aires (UBACYT2018-305).

Additional information

Author contributions

L.R., M.G.M., C.T. and R.M.P. designed the study. C.T. and R.M.P. performed the experiments. A.M.S. helped with the surgeries and animal care after the lesion. C.T., R.M.P., L.R., and M.G.M. wrote the manuscript.

References

- Aosaki T, Graybiel AM, Kimura M 1994a) **Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys** *Science* **265**:412–415
- Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M 1994b) **Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning** *J Neurosci* **14**:3969–3984
- Apicella P 2007) **Leading tonically active neurons of the striatum from reward detection to context recognition** *Trends Neurosci* **30**:299–306
- Chantranupong L, Beron CC, Zimmer JA, Wen MJ, Wang W, Sabatini BL 2023) **Dopamine and glutamate regulate striatal acetylcholine in decision-making** *Nat* **2023**:577–585 <https://www.nature.com/articles/s41586-023-06492-9>
- Cheung THC, Ding Y, Zhuang X, Kang UJ 2023) **Learning critically drives parkinsonian motor deficits through imbalanced striatal pathway recruitment** *Proc Natl Acad Sci U S A* **120**
- Choi SJ, Ma TC, Ding Y, Cheung T, Joshi N, Sulzer D, Mosharov E V., Kang UJ 2020) **Alterations in the intrinsic properties of striatal cholinergic interneurons after dopamine lesion and chronic L-DOPA** *Elife* **9**:1–24
- Chuhma N, Mingote S, Moore H, Rayport S 2014) **Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling** *Neuron* **81**:901–912
- Cragg SJ 2006) **Meaningful silences: how dopamine listens to the ACh pause** *Trends Neurosci* **29**:125–131
- Deng P, Zhang Y, Xu ZC 2007) **Involvement of I(h) in dopamine modulation of tonic firing in striatal cholinergic interneurons** *J Neurosci* **27**:3148–3156
- Ding JB, Guzman JN, Peterson JD, Goldberg JA, Surmeier DJ 2010) **Thalamic gating of corticostriatal signaling by cholinergic interneurons** *Neuron* **67**:294–307
- Duhne M, Mohebi A, Kim K, Pelattini L, Berke JD 2024) **A mismatch between striatal cholinergic pauses and dopaminergic reward prediction errors** *Proc Natl Acad Sci U S A* **121**
- Escande MV, Taravini IRE, Zold CL, Belforte JE, Murer MG 2016) **Loss of homeostasis in the direct pathway in a mouse model of asymptomatic parkinson's disease** *J Neurosci* **36**
- Gallo EF, Greenwald J, Yeisley J, Teboul E, Martyniuk KM, Villarin JM, Li Y, Javitch JA, Balsam PD, Kellendonk C 2022) **Dopamine D2 receptors modulate the cholinergic pause and inhibitory learning** *Mol Psychiatry* **27**:1502–1514
- Hopkins W 1998) **Toxin and subunit specificity of blocking affinity of three peptide toxins for heteromultimeric, voltage-gated potassium channels expressed in Xenopus oocytes** - *PubMed J Pharmacol Exp Ther* **285**:1051–1060

- Keifman E, Ruiz-DeDiego I, Pafundo DE, Paz RM, Solís O, Murer MG, Moratalla R 2019) **Optostimulation of striatonigral terminals in substantia nigra induces dyskinesia that increases after L-DOPA in a mouse model of Parkinson's disease** *Br J Pharmacol* **176**:2146–2161 <https://onlinelibrary.wiley.com/doi/abs/10.1111/bph.14663>
- Kharkwal G, Brami-Cherrier K, Lizardi-Ortiz JE, Nelson AB, Ramos M, Del Barrio D, Sulzer D, Kreitzer AC, Borrelli E 2016) **Parkinsonism Driven by Antipsychotics Originates from Dopaminergic Control of Striatal Cholinergic Interneurons** *Neuron* **91**:67–78 <http://linkinghub.elsevier.com/retrieve/pii/S0896627316302677>
- Kimura M, Rajkowski J, Evarts E 1984) **Tonically discharging putamen neurons exhibit set-dependent responses** *Proc Natl Acad Sci U S A* **81**:4998–5001
- Krok AC, Maltese M, Mistry P, Miao X, Li Y, Tritsch NX 2023) **Intrinsic dopamine and acetylcholine dynamics in the striatum of mice** *Nature* **621**:543–549
- Matsumoto N, Minamimoto T, Graybiel AM, Kimura M 2001) **Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events** *J Neurophysiol* **85**:960–976
- McGuirt A, Pigulevskiy I, Sulzer D 2022) **Developmental regulation of thalamus-driven pauses in striatal cholinergic interneurons** *iScience*
- McKinley JW, Shi Z, Kawikova I, Hur M, Bamford IJ, Sudarsana Devi SP, Vahedipour A, Darvas M, Bamford NS 2019) **Dopamine Deficiency Reduces Striatal Cholinergic Interneuron Function in Models of Parkinson's Disease** *Neuron* **103**:1056–1072
- Paxinos G, Franklin K 2001) **The mouse brain in stereotaxic coordinates** San Diego: Academic Press
- Paz RM, Stahl AM, Rela L, Murer MG, Tubert C 2022) **D1/D5 Inverse Agonists Restore Striatal Cholinergic Interneuron Physiology in Dyskinetic Mice** *Mov Disord* **37**:1693–1706
- Paz RM, Tubert C, Stahl A, Díaz AL, Etchenique R, Murer MG, Rela L 2018) **Inhibition of striatal cholinergic interneuron activity by the Kv7 opener retigabine and the nonsteroidal anti-inflammatory drug diclofenac** *Neuropharmacology* **137**
- Paz RM, Tubert C, Stahl AM, Amarillo Y, Rela L, Murer MG 2021) **Levodopa Causes Striatal Cholinergic Interneuron Burst-Pause Activity in Parkinsonian Mice** *Mov Disord*
- Reynolds JNJ, Avvisati R, Dodson PD, Fisher SD, Oswald MJ, Wickens JR, Zhang YF 2022) **Coincidence of cholinergic pauses, dopaminergic activation and depolarisation of spiny projection neurons drives synaptic plasticity in the striatum** *Nat Commun* **13**
- Reynolds JNJ, Hyland BI, Wickens JR 2004) **Modulation of an Afterhyperpolarization by the Substantia Nigra Induces Pauses in the Tonic Firing of Striatal Cholinergic Interneurons** *J Neurosci* **24**:9870–9877
- Sanchez G, Rodriguez MJ, Pomata P, Rela L, Murer MG 2011) **Reduction of an afterhyperpolarization current increases excitability in striatal cholinergic interneurons in rat parkinsonism** *J Neurosci* **31**:6553–6564
- Schulz JM, Reynolds JNJ 2013) **Pause and rebound: sensory control of cholinergic signaling in the striatum** *Trends Neurosci* **36**:41–50

- Shen W, Hernandez-Lopez S, Tkatch T, Held JE, Surmeier DJ (2004) **Kv1.2-containing K⁺ channels regulate subthreshold excitability of striatal medium spiny neurons** *J Neurophysiol* **91**:1337–1349
- Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ (2012) **Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons** *Neuron* **75**:58–64
- Tubert C, Taravini IRE, Flores-Barrera E, Sánchez GM, Prost MA, Avale ME, Tseng KY, Rela L, Murer MG (2016) **Decrease of a Current Mediated by K 1.3 Channels Causes Striatal Cholinergic Interneuron Hyperexcitability in Experimental Parkinsonism** *Cell Rep*
- Watanabe K, Kimura M (1998) **Dopamine Receptor-Mediated Mechanisms Involved in the Expression of Learned Activity of Primate Striatal Neurons** *J Neurophysiol* **79**:2568–2580
- Wilson CJ (2005) **The mechanism of intrinsic amplification of hyperpolarizations and spontaneous bursting in striatal cholinergic interneurons** *Neuron* **45**:575–585
- Wilson CJ, Goldberg JA (2006) **Origin of the slow afterhyperpolarization and slow rhythmic bursting in striatal cholinergic interneurons** *J Neurophysiol* **95**:196–204
- Zhang B, Albaker A, Plouffe B, Lefebvre C, Tiberi M (2014) **Constitutive activities and inverse agonism in dopamine receptors** *Adv Pharmacol* **70**:175–214
- Zhang YF, Reynolds JNJ, Cragg SJ (2018) **Pauses in Cholinergic Interneuron Activity Are Driven by Excitatory Input and Delayed Rectification, with Dopamine Modulation** *Neuron* **98**:918–925

Author information

Cecilia Tubert^{*}

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina
ORCID iD: [0000-0001-6766-5849](https://orcid.org/0000-0001-6766-5849)

^{*}These authors contributed equally

Rodrigo M Paz^{*†}

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina, Department of Neurology, University of California, San Francisco, San Francisco, United States

^{*}These authors contributed equally

[†]Present address: Department of Neurology, UCSF, San Francisco, USA

Agostina M Stahl

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina

Lorena Rela

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina, Universidad de Buenos Aires, Facultad de Medicina, Departamento de Ciencias Fisiológicas, Buenos Aires, Argentina

Mario G Murer

Universidad de Buenos Aires, CONICET, Instituto de Fisiología y Biofísica Bernardo Houssay, Grupo de Neurociencia de Sistemas, Buenos Aires, Argentina, Universidad de Buenos Aires, Facultad de Medicina, Departamento de Ciencias Fisiológicas, Buenos Aires, Argentina

For correspondence: gmurer@fmed.uba.ar

Editors

Reviewing Editor

Jun Ding

Stanford University, Stanford, United States of America

Senior Editor

Michael Frank

Brown University, Providence, United States of America

Reviewer #1 (Public review):

Summary:

Tubert C. et al. investigated the role of dopamine D5 receptors (D5R) and their downstream potassium channel, Kv1, in the striatal cholinergic neuron pause response induced by thalamic excitatory input. Using slice electrophysiological analysis combined with pharmacological approaches, the authors tested which receptors and channels contribute to the cholinergic interneuron pause response in both control and dyskinetic mice (in the L-DOPA off state). They found that activation of Kv1 was necessary for the pause response, while activation of D5R blocked the pause response in control mice. Furthermore, in the L-DOPA off state of dyskinetic mice, the absence of the pause response was restored by the application of clozapine. The authors claimed that 1) the D5R-Kv1 pathway contributes to the cholinergic interneuron pause response in a phasic dopamine concentration-dependent manner, and 2) clozapine inhibits D5R in the L-DOPA off state, which restores the pause response.

Strengths

The electrophysiological and pharmacological approaches used in this study are powerful tools for testing channel properties and functions. The authors' group has well-established these methodologies and analysis pipelines. Indeed, the data presented were robust and reliable.

Weaknesses:

Although the paper has strengths in its methodological approaches, there is a significant gap between the presented data and the authors' claims.

The authors answered the most of concerns I raised. However, the critical issue remains unresolved.

I am still not convinced by the results presented in Fig. 6 and their interpretation. Since Clozapine acts as an agonist in the absence of an endogenous agonist, it may stimulate the D5R-cAMP-Kv1 pathway. Stimulation of this pathway should abolish the pause response mediated by thalamic stimulation in SCINs, rather than restoring the pause response. Clarification is needed regarding how Clozapine reduces D5R-ligand-independent activity in the absence of dopamine (the endogenous agonist). In addition, the author's argued that D5R antagonist does not work in the absence of dopamine, therefore solely D5R antagonist didn't restore the pause response. However, if D5R-cAMP-Kv1 pathway is already active in L-DOPA off state, why D5R antagonist didn't contribute to inhibition of D5R pathway? Since Clozapine is not D5 specific and Clozapine experiments were not concrete, I recommend testing whether other receptors, such as the D2 receptor, contribute to the Clozapine-induced pause response in the L-DOPA-off state.

<https://doi.org/10.7554/eLife.102184.2.sa3>

Reviewer #2 (Public review):

Summary:

This manuscript by Tubert et al. presents the role of D5 receptors (D5R) in regulating the striatal cholinergic interneuron (CIN) pause response through D5R-cAMP-Kv1 inhibitory signaling. Their findings provide a compelling model explaining the "on/off" switch of the CIN pause, driven by the distinct dopamine affinities of D2R and D5R. This mechanism, coupled with varying dopamine states, is likely critical for modulating synaptic plasticity in cortico-striatal circuits during motor learning and execution. Furthermore, the study bridges their previous finding of CIN hyperexcitability (Paz et al., Movement Disorder 2022) with the loss of the pause response in LID mice and demonstrates the restore of the pause through D1/D5 inverse agonism.

Strengths:

The study presents solid findings, and the writing is logically structured and easy to follow. The experiments are well-designed, properly combining ex vivo electrophysiology recording, optogenetics, and pharmacological treatment to dissect / rule out most, if not all, alternative mechanisms in their model.

Weaknesses:

While the manuscript is overall satisfying, one conceptual gap needs to be further addressed or discussed: the potential "imbalance" between D2R and D5R signaling due to the ligand-independent activity of D5R in LID. Given that D2R and D5R oppositely regulate CIN pause responses through cAMP signaling, investigating the role of D2R under LID off L-DOPA (e.g., by applying D2 agonists or antagonists, even together with intracellular cAMP analogs or inhibitors) could provide critical insights. Addressing this aspect would strengthen the manuscript in understanding CIN pause loss under pathological conditions.

<https://doi.org/10.7554/eLife.102184.2.sa2>

Reviewer #3 (Public review):

Summary:

Tubert et al. investigate the mechanisms underlying the pause response in striatal cholinergic interneurons (SCINs). The authors demonstrate that optogenetic activation of thalamic axons

in the striatum induces burst activity in SCINs, followed by a brief pause in firing. They show that the duration of this pause correlates with the number of elicited action potentials, suggesting a burst-dependent pause mechanism. The authors demonstrated this burst-dependent pause relied on Kv1 channels. The pause is blocked by a SKF81297 and partially by sulpiride and mecamylamine, implicating D1/D5 receptor involvement. The study also shows that the ZD7288 does not reduce the duration of the pause, and that lesioning dopamine neurons abolishes this response, which can be restored by clozapine.

Weaknesses:

While this study presents an interesting mechanism for SCIN pausing after burst activity, there are several major concerns that should be addressed:

(1) Scope of the Mechanism: It is important to clarify that the proposed mechanism may apply specifically to the pause in SCINs following burst activity. The manuscript does not provide clear evidence that this mechanism contributes to the pause response observed in behavioral animals. While the thalamus is crucial for SCIN pauses in behavioral contexts, the exact mechanism remains unclear. Activating thalamic input triggers burst activity in SCINs, leading to a subsequent pause, but this mechanism may not be generalizable across different scenarios. For instance, approximately half of TANs do not exhibit initial excitation but still pause during behavior, suggesting that the burst-dependent pause mechanism is unlikely to explain this phenomenon. Furthermore, in behavioral animals, the duration of the pause seems consistent, whereas the proposed mechanism suggests it depends on the prior burst, which is not aligned with in vivo observations. Additionally, many in vivo recordings show that the pause response is a reduction in firing rate, not complete silence, which the mechanism described here does not explain. Please address these in the manuscript.

(2) Terminology: The use of "pause response" throughout the manuscript is misleading. The pause induced by thalamic input in brain slices is distinct from the pause observed in behavioral animals. Given the lack of a clear link between these two phenomena in the manuscript, it is essential to use more precise terminology throughout, including in the title, bullet points, and body of the manuscript.

(3) Kv1 Blocker Specificity: It is unclear how the authors ruled out the possibility that the Kv1 blocker did not act directly on SCINs. Could there be an indirect effect contributing to the burst-dependent pause? Clarification on this point would strengthen the interpretation of the results.

(4) Role of D1 Receptors: While it is well-established that activating thalamic input to SCINs triggers dopamine release, contributing to SCIN pausing (as shown in Figure 3), it would be helpful to assess the extent to which D1 receptors contribute to this burst-dependent pause. This could be achieved by applying the D1 agonist SKF81297 after blocking nAChRs and D2 receptors.

(5) Clozapine's Mechanism of Action: The restoration of the burst-dependent pause by clozapine following dopamine neuron lesioning is interesting, but clozapine acts on multiple receptors beyond D1 and D5. Although it may be challenging to find a specific D5 antagonist or inverse agonist, it would be more accurate to state that clozapine restores the burst-dependent pause without conclusively attributing this effect to D5 receptors.

Comments on revisions:

The authors have addressed many of my concerns. However, I remain unconvinced that adding an 'ex vivo' experiment fully resolves the fundamental differences between the burst-dependent pause observed in slices - defined by the duration of a single AHP - and the pause response in CHINs observed in vivo, which may involve contributions from more than one prolonged AHP. In vivo, neurons can still fire action potentials during the pause, albeit at a

lower frequency. Moreover, in behaving animals, pause duration does not vary with or without initial excitation. The mechanism proposed demonstrates that the pause duration, defined by the length of a single AHP, is positively correlated with preceding burst activity.

To improve clarity, I recommend using the term 'SCIN pause' to describe the ex vivo findings, distinguishing them more explicitly from the 'pause response' observed in behaving animals. This distinction would help contextualize the ex vivo findings as potentially contributing to, but not fully representing, the pause response in vivo.

Again, it would be helpful to present raw data for pause durations rather than relying solely on ratios. This approach would provide the audience with a clearer understanding of the absolute duration of the burst-dependent pause and allow for better comparison to the ~200 ms pause observed in behaving animals.

<https://doi.org/10.7554/eLife.102184.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public review):

Summary:

Tubert C. et al. investigated the role of dopamine D5 receptors (D5R) and their downstream potassium channel, Kv1, in the striatal cholinergic neuron pause response induced by thalamic excitatory input. Using slice electrophysiological analysis combined with pharmacological approaches, the authors tested which receptors and channels contribute to the cholinergic interneuron pause response in both control and dyskinetic mice (in the LDOPA off state). They found that activation of Kv1 was necessary for the pause response, while activation of D5R blocked the pause response in control mice. Furthermore, in the LDOPA off-state of dyskinetic mice, the absence of the pause response was restored by the application of clozapine. The authors claimed that (1) the D5R-Kv1 pathway contributes to the cholinergic interneuron pause response in a phasic dopamine concentration-dependent manner, and (2) clozapine inhibits D5R in the L-DOPA off state, which restores the pause response.

Strengths:

The electrophysiological and pharmacological approaches used in this study are powerful tools for testing channel properties and functions. The authors' group has well-established these methodologies and analysis pipelines. Indeed, the data presented were robust and reliable.

Thank you for your comments.

Weaknesses:

Although the paper has strengths in its methodological approaches, there is a significant gap between the presented data and the authors' claims.

There was no direct demonstration that the D5R-Kv1 pathway is dominant when dopamine levels are high. The term 'high' is ambiguous, and it raises the question of

whether the authors believe that dopamine levels do not reach the threshold required to activate D5R under physiological conditions.

We acknowledge that further work is necessary to clarify the role of the D5R in physiological conditions. While we haven't found effects of the D1/D5 receptor antagonist SCH23390 on the pause response in control animals (Fig. 3), it is still possible that dopamine levels reach the threshold to stimulate D5R when burst firing of dopaminergic neurons contributes to dopamine release. We believe the pause response depends, among other factors, on the relative stimulation levels of SCIN D2 and D5 receptors, which is likely not an all-or-nothing phenomenon. To reduce ambiguity, we have eliminated the labels referring to dopamine levels in Figure 6F.

Furthermore, the data presented in Figure 6 are confusing. If clozapine inhibits active D5R and restores the pause response, the D5R antagonist SCH23390 should have the same effect. The data suggest that clozapine-induced restoration of the pause response might be mediated by other receptors, rather than D5R alone.

Thank you for letting us clarify this issue. Please note that the levels of endogenous dopamine 24 h after the last L-DOPA challenge in severe parkinsonian mice are expected to be very low. In the absence of an agonist, a pure D1/D5 antagonist would not exert an effect, as demonstrated with SCH23390 alone, which did not have an impact on the SCIN response to thalamic stimulation (Fig. 6). While clozapine can also act as a D1/D5 receptor antagonist, its D1/D5 effects in absence of an agonist are attributed to its inverse agonist properties (PMID: 24931197). Notably, SCH23390 prevented the effect of clozapine, allowing us to conclude that ligand-independent D1/D5 receptor-mediated mechanisms are involved in suppressing the pause response in dyskinetic mice. We now made it clearer in the third paragraph of the Discussion.

Reviewer #2 (Public review):

Summary:

This manuscript by Tubert et al presents the role of the D5 receptor in modulating the striatal cholinergic interneuron (CIN) pause response through D5R-cAMP-Kv1 inhibitory signaling. Their model elucidates the on / off switch of CIN pause, likely due to the different DA affinity between D2R and D5R. This machinery may be crucial in modulating synaptic plasticity in cortical-striatal circuits during motor learning and execution. Furthermore, the study bridges their previous finding of CIN hyperexcitability (Paz et al., Movement Disorder 2022) with the loss of pause response in LID mice.

Strengths:

The study had solid findings, and the writing was logically structured and easy to follow. The experiments are well-designed, and they properly combined electrophysiology recording, optogenetics, and pharmacological treatment to dissect/rule out most, if not all, possible mechanisms in their model.

Thank you for your comments.

Weaknesses:

The manuscript is overall satisfying with only some minor concerns that need to be addressed. Manipulation of intracellular cAMP (e.g. using pharmacological analogs or inhibitors) can add additional evidence to strengthen the conclusion.

Thank you for the suggestion. While we acknowledge that we are not providing direct evidence of the role of cAMP, we chose not to conduct these experiments because cAMP levels influence several intrinsic and synaptic currents beyond Kv1, significantly affecting membrane oscillations and spontaneous firing, as shown in Paz et al. 2021. However, we are modifying the fourth paragraph of the Discussion so there is no misinterpretation about our findings in the current work.

Reviewer #3 (Public review):

Summary:

Tubert et al. investigate the mechanisms underlying the pause response in striatal cholinergic interneurons (SCINs). The authors demonstrate that optogenetic activation of thalamic axons in the striatum induces burst activity in SCINs, followed by a brief pause in firing. They show that the duration of this pause correlates with the number of elicited action potentials, suggesting a burst-dependent pause mechanism. The authors demonstrated this burst-dependent pause relied on Kv1 channels. The pause is blocked by an SKF81297 and partially by sulpiride and mecamylamine, implicating D1/D5 receptor involvement. The study also shows that the ZD7288 does not reduce the duration of the pause and that lesioning dopamine neurons abolishes this response, which can be restored by clozapine.

Weaknesses:

While this study presents an interesting mechanism for SCIN pausing after burst activity, there are several major concerns that should be addressed:

(1) Scope of the Mechanism:

It is important to clarify that the proposed mechanism may apply specifically to the pause in SCINs following burst activity. The manuscript does not provide clear evidence that this mechanism contributes to the pause response observed in behavioral animals. While the thalamus is crucial for SCIN pauses in behavioral contexts, the exact mechanism remains unclear. Activating thalamic input triggers burst activity in SCINs, leading to a subsequent pause, but this mechanism may not be generalizable across different scenarios. For instance, approximately half of TANs do not exhibit initial excitation but still pause during behavior, suggesting that the burst-dependent pause mechanism is unlikely to explain this phenomenon. Furthermore, in behavioral animals, the duration of the pause seems consistent, whereas the proposed mechanism suggests it depends on the prior burst, which is not aligned with in vivo observations. Additionally, many in vivo recordings show that the pause response is a reduction in firing rate, not complete silence, which the mechanism described here does not explain. Please address these in the manuscript.

Thank you for your valuable feedback. While the absence of an initial burst in some TANs in vivo may suggest the involvement of alternative or additional mechanisms, this does not exclude a participation of Kv1 currents. We have seen that subthreshold depolarizations induced by thalamic inputs are sufficient to produce an afterhyperpolarization (AHP) mediated by Kv1 channels (see Tubert et al., 2016, PMID: 27568555). Although such subthreshold depolarizations are not captured in current recordings from behaving animals, intracellular in vivo recordings have demonstrated an intrinsically generated AHP after subthreshold depolarization of SCIN caused by stimulation of excitatory afferents (PMID: 15525771). Additionally, when pause duration is plotted against the number of spikes elicited by thalamic input (Fig. 1G), we found that one elicited spike is followed by an interspike interval 1.4 times longer than the average spontaneous interspike interval. We acknowledge

the potential involvement of additional factors, including a decrease of excitatory thalamic input coinciding with the pause, followed by a second volley of thalamic inputs (Fig. 1J-K, after observations by Matsumoto et al., 2001- PMID: 11160526), as well as the timing of elicited spikes relative to ongoing spontaneous firing (Fig. 1D-E). Dopaminergic modulation (Fig. 3) and regional differences among striatal regions (PMID: 24559678) may also contribute to the complexity of these dynamics.

(2) Terminology:

The use of "pause response" throughout the manuscript is misleading. The pause induced by thalamic input in brain slices is distinct from the pause observed in behavioral animals. Given the lack of a clear link between these two phenomena in the manuscript, it is essential to use more precise terminology throughout, including in the title, bullet points, and body of the manuscript.

While we acknowledge that our study does not include in vivo evidence, we believe ex vivo preparations have been instrumental in elucidating the mechanisms underlying the responses observed in vivo. We also agree with previous ex vivo studies in using consistent terminology. However, we will clarify the ex vivo nature of our work in the abstract and bullet points for greater transparency.

(3) Kv1 Blocker Specificity:

It is unclear how the authors ruled out the possibility that the Kv1 blocker did not act directly on SCINs. Could there be an indirect effect contributing to the burst-dependent pause? Clarification on this point would strengthen the interpretation of the results.

Thank you for letting us clarify this issue. In our previous work (Tubert et al., 2016) we showed that the Kv1.3 and Kv1.1 subunits are selectively expressed in SCIN throughout the striatum. Moreover, gabaergic transmission is blocked in our preparations. We are including a phrase to make it clearer in the manuscript (Results section, subheading "The pause response to thalamic stimulation requires activation of Kv1 channels").

(4) Role of D1 Receptors:

While it is well-established that activating thalamic input to SCINs triggers dopamine release, contributing to SCIN pausing (as shown in Figure 3), it would be helpful to assess the extent to which D1 receptors contribute to this burst-dependent pause. This could be achieved by applying the D1 agonist SKF81297 after blocking nAChRs and D2 receptors.

Thank you for letting us clarify this point. We show that blocking D2R or nAChR reduces the pause only for strong thalamic stimulation eliciting 4 SCIN spikes (Figure 3G), whereas the D1/D5 agonist SKF81297 is able to reduce the pause induced by weaker stimulation as well (Figure 3C). In addition, the D1/D5 receptor antagonist SCH23390 does not modify the pause response (Figure 3C). This may indicate that nAChR-mediated dopamine release induced by thalamic-induced bursts more efficiently activates D2R compared to D5R. We speculate that, in this context, lack of D5R activation may be necessary to keep normal levels of Kv1.3 currents necessary for SCIN pauses.

(5) Clozapine's Mechanism of Action:

The restoration of the burst-dependent pause by clozapine following dopamine neuron lesioning is interesting, but clozapine acts on multiple receptors beyond D1 and D5.

Although it may be challenging to find a specific D5 antagonist or inverse agonist, it would be more accurate to state that clozapine restores the burst-dependent pause without conclusively attributing this effect to D5 receptors.

Thank you for your insightful observation. We acknowledge the difficulty of targeting dopamine receptors pharmacologically due to the lack of highly selective D1/D5 inverse agonists. We used SCH23390, which is a highly selective D1/D5 receptor antagonist devoid of inverse agonist effects, to block clozapine's ability to restore SCIN pauses (Figure 6C). This indicates that the restoration of SCIN pauses by clozapine depends on D1/D5 receptors. Furthermore, in a previous study, we demonstrated that clozapine's effect on restoring SCIN excitability in dyskinetic mice (a phenomenon mediated by Kv1 channels in SCIN; Tubert et al., 2016) was not due to its action on serotonin receptors (Paz, Stahl et al., 2022). While our data do not rule out the potential contribution of other receptors, such as muscarinic acetylcholine receptors, we believe they strongly support the role of D1/D5 receptors. To reflect this, we added a statement discussing the potential contribution of receptors beyond D1/D5 in the last paragraph of the Discussion.

Recommendations for the authors:

Reviewer #1 (Recommendations for the authors):

(1) The effect of MgTx was not consistent with the previous study (Tubert, 2016). I expected MgTx to increase the basal firing rate of cholinergic interneurons.

Thank you for highlighting this. In our previous study we used ACSF in the recording pipette, instead of the intracellular solution -higher in potassium- used in the present study. This is likely related to the higher spontaneous firing rates of SCIN observed in the present study, which made the SCIN response stand out. In addition, our previous study analyzed the effect of MgTx on spontaneous firing frequency of SCIN isolated from major circuit regulation by adding CNQX and picrotoxin to the bath, while in this study we needed to preserve the thalamic input and only picrotoxin in the bath was used. Given these differences, the two conditions are not strictly comparable but rather give complementary information.

(2) In the text, the authors claim that "SCINs recorded in the parkinsonian OFF-L-DOPA condition show an increase in membrane excitability that mimics changes acutely induced by SKF81297 in SCINs from control mice." However, the data for SKF81297 do not support this claim.

We modified the text to make it clearer that the cited phrase refers to a previous publication (PMID: 35535012) in which SCIN intrinsic excitability was characterized via analysis of responses to somatic current injection in whole-cell recordings. In the present study Fig. 3D shows SKF81297 effects on interspike intervals during spontaneous activity with a trend towards increased firing, and Fig. 4E a lack of effect on "burst duration" for responses with different numbers of spikes elicited by thalamic afferent stimulation.

(3) I recommend testing whether other receptors, such as D2R, contribute to the clozapine-induced pause response in the L-DOPA off state.

Thank you for your suggestion. We acknowledge that studying the role of D2R is important. However, our preliminary data suggest that a comprehensive follow up study, which is beyond the scope of this manuscript, is necessary to understand their role.

Reviewer #2 (Recommendations for the authors):

(1) For Figure 1D-E, I understand that the authors are trying to state that the previous spontaneous spike contributes to a hyperpolarized window that induces a delay in the evoked spikes. However, it is almost impossible to discriminate between spontaneous and evoked spikes in this experiment. Furthermore, considering the tonic firing property, I highly suspect that even a sham control design (no optogenetic light) will give you a similar distribution as in Figure 1E (the longer IN X1, the shorter in X2).

We agree that some spikes following stimulus onset may have occurred independently of the light stimulus, as it is also possible during behavioral tasks. We used the baseline recordings to estimate the effects of a sham stimulus as requested and included the data in Fig. 1E-F. As expected, the sham stimulation data showed a similar inverse relationship with the time elapsed from the preceding spike, but latencies were longer than with the stimulus (except for times close to the average ISI), suggesting that the optical stimulation increased the probability of evoking a spike (Fig. 1F). Remarkably, the pause following this threshold stimulation was significantly longer than the baseline ISI, as reported in the main text (Results section, last sentence of first paragraph).

(2) The authors used optogenetics to induce thalamic inputs to induce the pause after bursts. Considering CINs also receive inputs from different brain regions, e.g. cortex, does this phenomena/pause after bursts also exist following cortical inputs?

We did not study the SCIN response to cortical inputs, but both thalamic and cortical inputs seem to drive SCIN pause-responses as observed by others (PMID: 24553950).

(3) The effect of the D5R inverse agonism, and the further combined with D5R agonist and antagonist, faithfully reveal/confirm the increase of ligand-independent activity of D5R in LID reported previously. It would be ideal to also directly modulate intracellular cAMP (as in the 2022 paper) to confirm the rescue effects on the CIN pause response.

Please, see our response in the public review.

(4) In healthy conditions, the balance between D2R and D5R signaling (shown in Figure 6F left) switches the pause and no pause modes which potentially contributes to cortical-striatal plasticity. How about in LID off L-DOPA condition? Is it possible to rescue/modulate the pause on/off mode by D2R agonism in LID?

We haven't tested the effect of D2 agonists yet, but this is scheduled for follow up studies.

Reviewer #3 (Recommendations for the authors):

(1) The authors use the ratio of pause duration to baseline ISI to describe the pause, which is useful for detecting significant differences. However, it would be beneficial to also report the actual duration of the burst-dependent pause to provide readers with a clearer understanding of the variation in pauses.

In all figures we report the average baseline ISI duration for each experiment / experimental condition, allowing readers to estimate actual pause durations. We added in the main text actual average pause durations corresponding to Fig. 1H, which are representative of those observed along the study.

(2) In Figure 3D, a more detailed comparison would be helpful, as there appears to be a significant difference between the SKF81297 group and others.

We acknowledge that there might be a trend towards reduced ISIs, however, it was statistically non-significant (see legend of figure 3). In addition, the effect of SKF81297 seems unrelated to this trend, as its effect is also seen under the effect of ZD7288, which substantially prolongs the baseline ISI (Fig. 4E-F).

<https://doi.org/10.7554/eLife.102184.2.sa0>