Tonic engagement of nicotinic receptors bidirectionally controls striatal spiny projection neuron spike timing

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Abstract

Striatal spiny projection neurons (SPNs) transform convergent excitatory corticostriatal inputs into an inhibitory signal that shapes basal ganglia output. This process is fine-tuned by striatal GABAergic interneurons (GINs), which receive overlapping cortical inputs and mediate rapid corticostriatal feedforward inhibition of SPNs. Adding another level of control, cholinergic interneurons (CINs), which are also vigorously activated by corticostriatal excitation, can 1) disynaptically inhibit SPNs by activating α4β2 nicotinic acetylcholine receptors (nAChRs) on various GINs and 2) directly modulate corticostriatal synaptic strength via pre-synaptic α7 nAChR receptors. Measurements of the disynaptic inhibitory pathway, however, indicate that it is too slow to compete with direct GIN-mediated feed-forward inhibition. Moreover, functional nAChRs are also present on populations of GINs that do not respond to phasic activation of CINs, such as parvalbumin-positive fast-spiking interneurons (PV-FSIs), making the overall role of nAChRs in shaping striatal synaptic integration unclear. Using acute striatal slices we show that upon synchronous optogenetic activation of corticostriatal projections, blockade of α7 nAChRs delayed SPN spikes, whereas blockade of α4β2 nAChRs advanced SPN spikes and increased postsynaptic depolarizations. The nAChR-dependent inhibition was mediated by downstream GABA release, and data suggest that the GABA source was not limited to GINs that respond to phasic CIN activation. In particular, the observed spike-advancement caused by nAChR blockade was associated with a diminished frequency of spontaneous inhibitory postsynaptic currents in SPNs, and a parallel hyperpolarization of PV-FSIs. Taken together, we describe opposing roles for tonic (as opposed to phasic) engagement of nAChRs in striatal function. We conclude that tonic activation of nAChRs by CINs both sharpens the temporal fidelity of corticostriatal signaling via pre-synaptic α7 nAChRs and maintains a GABAergic brake on cortically-driven striatal output, processes that may shape SPN spike timing, striatal processing and synaptic plasticity.

Key Words: methyllycaconitine; mecamylamine; DHβE; channelrhodopsin-2; striatal microcircuitry; tonically active neurons; paired-pulse ratio; postsynaptic potential; afterdepolarization

Introduction

As the main input nucleus of the basal ganglia, the striatum receives convergent cortical synaptic information, generating a computationally transformed interpretation of this information and relaying it to intermediate and output nuclei of the basal ganglia. Striatal output is carried exclusively by GABAergic striatal spiny projection neurons (SPNs), which account for ~95% of all striatal neurons (Gerfen and Surmeier 2011, Silberberg and Bolam 2015). The remaining 5% of striatal neurons are interneurons, most of which are GABAergic interneurons (GINs). There are several molecularly distinct classes of GINs that exhibit diverse intrinsic firing patterns and patterns of intrastriatal connectivity (Tepper, Tecuapetla et al. 2010, Muñoz-Manchado, Bengtsson Gonzales et al. 2018, Assous and Tepper 2019). In the context of feedforward cortico- and thalamostriatal transmission the GINs' main function lies in the feedforward inhibition they provide to SPNs (Tepper, Koós et al. 2004, Assous, Kaminer et al. 2017). Such inhibition can fashion striatal output by perturbing the precise timing of SPNs or prevent their spiking altogether. The only non-GABAergic interneurons are the large, aspiny, cholinergic interneurons (CINs) (DiFiglia 1987, Tepper and Bolam 2004, Plotkin and Goldberg 2019, Poppi et al. 2021). Despite their small numbers (approximately 1% of striatal neurons) the acetylcholine (ACh) they release influences the entire striatal microcircuitry. A single CIN axon can cover a third of the striatum (in linear dimension) and has ACh release sites every few micrometers (DiFiglia 1987). In fact, the striatum has the highest expression of cholinergic markers in the entire CNS (Mesulam, Mash et al. 1992, Contant, Umbriaco et al. 1996). CINs are autonomous pacemakers (Bennett and Wilson 1999) that are largely identified as the tonically active neurons (TANs) of the striatum
(Kimura, Rajkowski et al. 1984, Wilson, Xu et al. 1990, Aosaki, Tsubokawa et al. 1994, Aosaki, Kimura et al. 1995, Morris, Arkadir et al. 2004), firing 3-10 spikes/s *in vivo*. On the backdrop of this ongoing discharge, the main signal they convey *in vivo* is a pause in response to primary reward or salient stimuli associated with reward (Aosaki, Tsubokawa et al. 1994, Morris, Arkadir et al. 2004, Goldberg and Reynolds 2011, Apicella 2017). The duration of the pause in response to a brief conditioned sensory stimulus is on the order of 200-300 ms (Kimura, Rajkowski et al. 1984, Raz, Feingold et al. 1996, Apicella, Legallet et al. 1997).

CINs regulate SPNs in 3 main ways. The best characterized regulation is exerted via muscarinic ACh receptors (mAChRs). Activation of presynaptic and postsynaptic mAChRs on SPNs modulates synaptic transmission, synaptic plasticity and the intrinsic excitability of SPNs by modulating various voltage-activated Ca^{2+} and K^{+} channels (Akins, Surmeier et al. 1990, Calabresi, Centonze et al. 1999, Gabel and Nisenbaum 1999, Day, Wokosin et al. 2008, Goldberg, Ding et al. 2012, Zucca, Zucca et al. 2018). CINs influence SPNs via nicotinic ACh receptors (nAChRs) as well, albeit indirectly. Activation of nAChRs on striatal dopaminergic fibers can evoke dopamine (DA) release (Zhou, Liang et al. 2001, Cachope, Mateo et al. 2012, Threlfell, Lalic et al. 2012) which, in turn, can modulate the intrinsic excitability of SPNs and synaptic transmission and plasticity at synaptic inputs. All of the above influences are mediated by GPCR-linked ACh and DA receptors, which means that they are unlikely to dynamically affect the moment-by-moment processing and transmission of excitatory inputs to SPNs. Indeed, a recent study in which CINs were silenced optogenetically *in vivo* has put a lower bound on how fast mAChR-mediated effects can come into play. When CINs are synchronously silenced for over 500 ms, SPNs begin to show signs of mAChR-dependent reductions in excitability. However, that effect comes into play only after a 400 ms delay (Zucca, Zucca et al. 2018). The only known mechanisms by which CINs can rapidly affect SPN activity (sooner than 400 ms) involve nicotinic or muscarinic modulation of synaptic glutamate release or α4β2 nAChR-dependent GABA release onto the SPNs' ionotropic $GABA_A$ receptors (Pakhotin and Bracci 2007, English, Ibanez-Sandoval et al. 2011, Faust, Assous et al. 2015, Faust, Assous et al. 2016, Assous, Kaminer et al. 2017, Assous 2021). The α4β2 nAChRs that drive disynaptic GABA release are located on GINs (English, Ibanez-Sandoval et al. 2011, Faust, Assous et al. 2015), and on dopaminergic nigrostriatal terminals (Nelson, Hammack et al. 2014).

Because CINs receive massive excitatory cortical and thalamic input (Lapper and Bolam 1992, Thomas, Smith et al. 2000) it is conceivable that their disynaptic inhibition of SPNs comes into play during bouts of cortical or thalamic activity. However, this raises a conceptual problem. Because cortex and thalamus can activate feedforward

inhibition to SPNs via various striatal GINs (e.g., cortex → GIN → SPN), what is gained by recruiting an additional pathway that is necessarily slower and less reliable due to its additional synapse (e.g., cortex → CIN → GIN → SPN)? Moreover, what is the role of nAChRs on GINs, such as the parvalbumin-positive fast-spiking interneurons (PV-FSIs) that do not respond to direct activation of CINs (English, Ibanez-Sandoval et al. 2011, Nelson, Hammack et al. 2014)? This conceptual problem exists only if we assume that the role of CINs in modulating SPN function through the activation of nAChRs is anchored around their phasic activation by excitatory afferents. In this study we demonstrate that ongoing CIN activity exerts opposing influences on SPNs via tonic engagement of nAChRs in different pathways: tonic activation of pre-synaptic α7 nAChRs sharpens the temporal fidelity of responses to cortical inputs, while tonically activated α4β2 nAChRs on GINs provide a constant brake on SPN excitation. We uncover these nicotinic effects using transgenic mice that express channelrhodopsin-2 (ChR2) in corticostriatal fibers (Arenkiel, Peca et al. 2007). Activation of these fibers creates a competition between monosynaptic cortical excitation and polysynaptic feedforward inhibition to SPNs. We show that nAChRs on distinct pre- and post-synaptic pathways are capable of bidirectionally controlling SPN spike timing with millisecond precision.

Results

To interrogate microcircuit-level responses of SPNs to cortical excitation of the striatum, we generated acute *ex vivo* brain slices from Thy1-ChR2 mice, which nominally express ChR2 in cortical neurons (Arenkiel, Peca et al. 2007, Aceves Buendia, Tiroshi et al. 2019). Full-field optogenetic stimulation with a 470 nm LED engaged corticostriatal afferents and generated in SPNs either: excitatory postsynaptic potentials (EPSPs) for low LED intensities, or – in the suprathreshold condition – an action potential (AP) followed by an after depolarization (ADP) lasting 10s of milliseconds (Fig. 1a). For the remainder of this study we tuned the LED intensity to generate a just-suprathreshold response so that AP latency and ADP amplitude could be compared among SPNs. Blockade of nAChRs with mecamylamine (mec; 10 µM) enhanced the engagement of SPN responses (Fig. 1b), as it shortened AP latency (Fig. 1c) and increased both the

ADP (Fig. 1d) and subthreshold EPSP amplitudes (Fig. 1e). These effects were replicated in the presence of the α4β2 nAChR-selective antagonist, DHβE (10 μM, AP latency: n = 8 SPNs, $p = 7.8 \cdot 10^{-3}$, SRT; ADP amplitude: n = 8 SPNs, p = 0.039, SRT; data not shown). Because the resting membrane potential of SPNs is very hyperpolarized compared to the depolarized potential from which spikes typically occur *in vivo* [the so-called "up state" (Wilson and Groves 1981, Stern, Jaeger et al. 1998)], we repeated the experiment while holding the SPN with a constant positive current injection in the just-subthreshold region (e.g., in the –55 mV to –50 mV range). Mecamylamine shortened the latency to AP during this somatically-mimicked "up state" as well (Fig. 1f-g).

Since SPNs themselves do not express nAChRs, the action of mecamylamine must be indirect. Functional nAChR are expressed at multiple loci of the striatal circuit, including afferent axonal terminals and intrastriatal GABAergic interneurons (Nelson, Hammack et al. 2014, Faust, Assous et al. 2016, Abudukeyoumu, Hernandez-Flores et al. 2019, Assous 2021). While the broad antagonistic actions of mecamylamine and DHβE should block nicotinic receptors at most of these loci, α7 nAChRs, which are predominantly expressed at corticostriatal axon terminals, are likely spared (Solinas, Scherma et al. 2007, Licheri, Lagström et al. 2018, Assous 2021). Indeed, mecamylamine did not have a significant effect on the strength or release probability of corticostriatal glutamatergic afferents, as measured by local electrical stimulation (Fig. 2a-c).

The lack of an effect of mecamylamine on presynaptic release probability does not exclude a direct influence of tonic activation of α7 nAChRs on SPN spike timing. Indeed, blockade of α7 nAChRs with (2-5 μM) MLA actually delayed AP latency (Fig. 2d,e). Thus, tonic engagement of nAChRs has opposing modulatory effects on SPN spike timing. If presynaptic corticostriatal nAChRs can advance an SPN spike, how then does mecamylamine, which has no presynaptic action, *shorten* SPN AP latency as well? Cholinergic interneurons form a disynaptic circuit with SPNs, which is mediated by neuropeptide Y–neurogliaform (NPY-NGF) GINs and perhaps other classes of nAChR-expressing GABAergic interneurons (GINs) in a nAChR-dependent manner (English,

Ibanez-Sandoval et al. 2011, Elghaba, Vautrelle et al. 2016, Faust, Assous et al. 2016, Assous, Kaminer et al. 2017, Tepper, Koós et al. 2018). Consistent with the involvement of such a polysynaptic circuit, blockade of GABAergic transmission with the $GABA_A$ and $GABA_B$ receptor antagonists SR-95531 (10 µM) and CGP-55845 (2 µM) respectively, mimicked and the effect of mecamylamine on spike timing and ADP amplitude (Fig. 3a-c). Blockade of GABA receptors fully occluded mecamylamine's effect on both spike latency and ADP amplitude (Fig. 3a-c), further implicating a CIN-GIN-SPN disynaptic circuit in mediating the actions of nAChRs. Strikingly, mecamylamine prevented GABA receptor antagonists from further advancing spike timing or enhancing ADP amplitude, suggesting that nAChR engagement can saturate GABAergic inhibition (Fig. 3d-f).

CINs receive converging excitatory inputs from the thalamus as well as the cortex (Lapper and Bolam 1992, Thomas, Smith et al. 2000). Indeed, targeted recordings from CINs confirmed that they are robustly engaged by our corticostriatal stimulation protocol, even displaying a lower stimulation threshold and shorter average delay to spike than SPNs (Fig. 4a-c). Despite the observed high fidelity and speed of cortically-evoked CIN spiking, a comparison of the temporal dynamics of synchronous cortical engagement of SPN inhibition (latency of approximately 5 ms, Fig. 5a) vs synchronous CIN engagement of GINs [latency of approximately 11 ms, (English, Ibanez-Sandoval et al. 2011, Nelson, Hammack et al. 2014)] shows that phasic activation of CINs is still not sufficiently fast to account for the mecamylamine-induced spike delay we observed in SPNs (Fig. 5b). In particular, even if there are sufficient numbers of CINs that respond *instantaneously* to cortical activation due to their ongoing activity, the earliest latency at which their phasic inhibition can influence SPNs is 11 ms later (English, Ibanez-Sandoval et al. 2011, Nelson, Hammack et al. 2014). However, SPN spikes are being delayed beginning 6 ms after stimulation (Fig. 5b magenta), which cannot be explained by phasic CIN activation.

If phasic engagement of nAChRs by synaptically evoked CIN activity cannot pragmatically account for the observed delay in SPN spike initiation, how does blocking nAChRs advance synaptically evoked SPN spiking? Given the autonomous

pacemaking nature of CINs, perhaps the explanation is that *tonic*, rather than phasic, activation of nAChRs is key to the phenomenon. There are three obvious mechanisms that could be at play in this scenario: 1) ongoing nAChR activation (either tonic or phasic activation that is not time-locked with the corticostriatal stimulation event leading to SPN spiking) may decrease SPN intrinsic excitability indirectly, likely by altering ongoing neuromodulator release (Zhou, Wilson et al. 2002, Rice and Cragg 2004) 2) the effect on SPN spiking may be a non-specific drug effect of mecamylamine (this is unlikely, since DHβE had the same effects) or 3) *tonic* engagement of somatodendritic nAChRs may produce an ongoing depolarization of GINs, ultimately priming or accentuating corticostriatal feedforward inhibition.

To test the possibility that blockade of ongoing nAChR activation increases SPN excitability, we measured the current-voltage relationship of SPNs before and during mecamylamine application. While there is no indication that our effect of mecamylamine on spike timing or ADP amplitude is bimodal and specific to direct pathway SPNs (dSPNs) or indirect pathway SPNs (iSPNs), dSPNs and iSPNs display differences in basal excitability (Gertler, Chan et al. 2008), which can complicate interpretation. We therefore performed these experiments in transgenic mice where dSPNs and iSPNs could be identified by their fluorescent label (Shuen, Chen et al. 2008, Ade, Wan et al. 2011) (Fig. 6a). Mecamylamine had no significant effect on the resting membrane potential or rheobase current of either SPN type (Fig. 6b,c). Furthermore, mecamylamine did not significantly alter the time to spike in response to somatic rheobase current injection or the latency to first spike in an AP train induced by suprathreshold somatic current injection (data not shown). Given that most data to this point came from pooled populations of SPNs, and that we observed no SPN-type specific effects of mecamylamine on SPN excitability, we performed additional analysis on pooled SPN data. While rheobase currents were unaffected, mecamylamine decreased the responsiveness to several sub-rheobase amplitude currents, arguing that if anything mecamylamine may *decrease* the excitability of SPNs in some regards (Fig. 6d). Mecamylamine had no effect on the firing rate of SPNs (Fig. 6e), nor did it alter IV properties of unidentified SPNs recorded from Thy1-ChR2 mice (n = 55 SPNs in control,

n = 9 SPNs in mecamylamine, data not shown).Taken together, spike advancement induced by nAChR blockade cannot be explained by changes in SPN intrinsic excitability.

Since mecamylamine did not increase the intrinsic excitability of SPNs, we tested if nAChR blockade attenuated the basal inhibitory GABAergic influence that they are under. Indeed, mecamylamine significantly reduced the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in a mixed population of SPNs (Fig. 7a-c). As SPNs are quiescent in acute slices and lack nAChRs, the source of these attenuated sIPSCs is likely GINs. Indeed, various classes of GINs are excited, and their spontaneous activity is elevated, by tonic activation of nAChRs (Luo, Janssen et al. 2013, Muñoz-Manchado, Foldi et al. 2014, Ibáñez-Sandoval, Xenias et al. 2015, Elghaba, Vautrelle et al. 2016, Tepper, Koós et al. 2018). Because PV-FSIs convey strong feedforward inhibition to SPNs (Koós and Tepper 1999, Tepper, Koós et al. 2004, Planert, Szydlowski et al. 2010), but do not respond to phasic CIN (English, Ibanez-Sandoval et al. 2011), we made targeted current clamp recordings from PV-Cre x Ai9-tdTomato transgenic mice (Johansson and Silberberg 2020) and tested the effect of nAChR blockade on their excitability (Fig. 7d). Indeed, blocking nAChRs by bath application of DHβE significantly hyperpolarized the resting membrane potential of PV-FSIs (Fig. 7e) and augmented AP kinetics (Fig. 7f-g). Thus, tonic activation of nAChRs depolarizes the resting membrane potential of PV-FSIs and other GINs (Elghaba 2016), continuously holding them closer to the AP threshold, and thereby priming them to transmit cortically-driven feedforward inhibition more efficiently to SPNs. Interestingly, the dependence upon trailing “primed” GIN input may explain why mecamylamine was capable of diminishing the amplitude of relatively slow rising EPSPs (Fig. 1e) but not faster peaking EPSCs (Fig. 2b). In summary, the tonic activity of CINs recruits either: i) presynaptic nAChRs to increase the fidelity of SPN responses to cortical inputs, or ii) specific GINs to provide a “nicotinic brake” on SPN responsiveness. Such mechanisms could allow SPNs to be more receptive of cortical inputs when CINs pause their firing in response to salient inputs or stimuli associated with reward.

Discussion

The present study describes a novel role for tonic nAChR activation in shaping striatal output. Decades of research have firmly established CINs as important modulators – mostly via muscarinic ACh receptors (Goldberg, Ding et al. 2012, Zucca, Zucca et al. 2018, Assous 2021) – of cortico- and thalamo- striatal synaptic integration and determinants of striatal output (Akins, Surmeier et al. 1990, English, Ibanez-Sandoval et al. 2011, Abudukeyoumu, Hernandez-Flores et al. 2019). Despite the fact that CINs are autonomous pacemakers, nearly all studies examining their influence on the precise temporal output of the striatum, particularly via nAChRs, have been centered around the consequences of their phasic engagement (English, Ibanez-Sandoval et al. 2011, Faust, Assous et al. 2016, Dorst, Tokarska et al. 2020). Here we report that ongoing, non-phasic activation of nAChRs accounts for a basal elevation in GABAergic tone, which dampens the response of SPNs to convergent cortical inputs and ultimately delays striatal output.

Basal cholinergic regulation of striatal output- a postsynaptic "nicotinic brake"?

CINs can robustly evoke GABAergic inhibition in the striatum by engaging somatodendritic nAChRs on specific classes of local GINs, in particular those expressing NPY (English, Ibanez-Sandoval et al. 2011, Luo, Janssen et al. 2013). This cell-type specific nAChR-dependent mechanism has been demonstrated using electrical or optogenetic stimulation to drive *phasic,* stimulation-locked inhibition. Somewhat puzzling, however, are reports that certain GINs, including PV-FSIs, cannot be phasically activated by CINs [or by cholinergic afferents from the midbrain (Dautan, Huerta-Ocampo et al. 2020)] even though they express the requisite somatodendritic nAChRs (Koós and Tepper 2002, English, Ibanez-Sandoval et al. 2011, Nelson, Hammack et al. 2014). Our finding that tonic engagement of nAChRs enhances the excitability of FSIs, as has been shown previously (Koós and Tepper 2002, Luo, Janssen et al. 2013), offers a physiological role for these receptors, and supports the notion that volume transmission of ACh takes place in the striatum (Picciotto, Higley et al. 2012).

Tonic nAChR excitation of GINs helps clarify another puzzling observation. It is obvious why GABAR blockers occlude the effect of mecamylamine on AP latency and ADP amplitude in SPNs- the relevant GABARs are synaptically downstream of the nAChRs on GINs. But why is it that mecamylamine seems to “saturate” the effect of GABAR inhibition on SPN AP latency and ADP? In other words, why does blockade of GABARs have no additional effect on AP latency or ADP when applied after mecamylamine, when phasic CIN activation only engages a select few types of GINs? If nAChRs were recruited solely by phasic activation by CINs, then the addition of GABAR blockers after mecamylamine should further shorten the AP latency and increase ADP amplitude by attenuating the influence of other GINs (such as PV-FSIs) that are not phasically activated by nAChRs (English, Ibanez-Sandoval et al. 2011, Nelson, Hammack et al. 2014). A “priming” effect of tonic nAChR activation on GINs that are not directly driven to spike by CINs could explain the seemingly oversized influence of nicotinic signaling on GABAergic inhibition. For this reason, determining which GINs are responsible for the effect may be ill-posed. Instead, our strategy was to demonstrate that in the presence of nAChR antagonists, SPNs receive less tonic inhibitory input, and that one of the GINs, the FSI – that has been shown to dominate the feedforward cortical inhibition (Koós and Tepper 1999, Johansson and Silberberg 2020) – is more hyperpolarized at rest (Fig. 7E). While the effect on PV-FSI resting membrane potential that we observed was modest, it is likely that multiple GINs participate in this mechanism, including low-threshold spiking (LTS) and TH^+ GINs, that are autonomously active or exhibit long plateaus (Beatty, Sullivan et al. 2012, Ibáñez-Sandoval, Xenias et al. 2015) and therefore likely (like CINs) to respond rapidly to cortical inputs (Johansson and Silberberg 2020). Indeed, LTS neurons’ spontaneous firing is slowed down considerably by mecamylamine, even though the effect on membrane voltage was on the order of what we observed in PV-FSIs (Elghaba, Vautrelle et al. 2016).Thus, various GINs are capable of laying the groundwork for a sort of ‘nicotinic brake’ present during basal circuit activity, attenuating and delaying striatal output.

Under what conditions will the 'nicotinic brake' be lifted?

Through their continuous autonomous discharge, CINs tonically-release ACh in the striatum (Zhou, Wilson et al. 2002). While a prolonged exposure of striatal nAChRs to ACh is bound to desensitize them, it has been argued that the fast action of striatal acetylcholinesterase (AChE) prevents nAChR desensitization allowing for the maintenance of tonic nAChR activation in the striatum (Zhou, Wilson et al. 2002). So if tonic nAChR activation normally puts a brake on cortical feedforward inhibition, under what circumstances is this brake removed? The tonically active neurons (TANs) of the primate striatum, which are comprised mainly of CINs (Wilson, Xu et al. 1990, Aosaki, Kimura et al. 1995), acquire a synchronized pause response (Raz, Feingold et al. 1996) in their tonic firing that lasts several hundred milliseconds in response to primary reward or, through conditioning, to salient stimuli associated with reward (Kimura, Rajkowski et al. 1984, Apicella, Legallet et al. 1997, Goldberg and Reynolds 2011, Bradfield, Bertran-Gonzalez et al. 2013). This pause duration should be sufficient – particularly while AChE rapidly clears the extracellular space from ACh – to deactivate the nAChRs, thereby removing the nicotinic brake.

Our study complements a recent study that described how CINs impact SPN neural dynamics *in vivo* (Zucca, Zucca et al. 2018). In that study it was shown that when CINs are optogenetically silenced in a synchronous-fashion for a sufficiently long period SPNs become hyperpolarized, less excitable and short-term corticostriatal plasticity is dampened. However, these effects commence only 400 ms after CINs are silenced. Until that point SPN excitability is unaffected. Thus, that study remained agnostic about the effect of CIN pauses that are shorter than 400 ms long on SPNs. Because physiological TAN pauses in response to sensory cues are typically over after 300 ms (Kimura, Rajkowski et al. 1984, Apicella, Legallet et al. 1997), the mAChR-mediated curbing of SPN excitability and responsiveness revealed in that and other studies (Ebihara, Yamamoto et al. 2013) may not be relevant to the role of the shorter, more physiological CIN pauses. Assuming that the nAChR-dependent mechanism we revealed can come into play sooner, our findings suggest that there are two dichotomous phases to the effect of synchronous CIN pauses: an 'early' period that is

dominated by the consequences of removing tonic nAChR engagement, and a more delayed period driven by diminished activation of mAChRs, where the responsiveness of SPNs to incoming cortical input is weakened, as shown previously (Zucca, Zucca et al. 2018).

Implications for tuning striatal circuit activity

Our data suggest that as CIN pauses develop several things will happen: tonic nAChR-driven boosting of corticostriatal synaptic inputs will be lost, but ongoing GABAergic inhibition will be relieved. This could have the effect of raising the threshold for spike generation, but sharpening the timing of spiking by several milliseconds once that threshold is reached, ultimately shaping the moment-by-moment processing of striatal output. Because the TAN pause usually coincides with the arrival of a salient stimulus, it is precisely a moment when SPNs need to be more attuned to cortical input and respond more reliably. Given the relatively higher affinity of α4β2 vs α7 nAChRs (Albuquerque, Pereira et al. 2009), it is feasible that the initial response to a salient stimulus (and drop in ACh levels) would be a rapid overall reduction in the responsivity of SPNs to cortical inputs, followed by a temporal sharpening of any proceeding spikes that are induced. Moreover, seminal experiments in primates have shown that CIN pauses become more prominently and reliably evoked by sensory stimuli over the course of Pavlovian conditioning (Aosaki, Tsubokawa et al. 1994, Aosaki, Kimura et al. 1995). Together, this implies that removal of the 'nicotinic brake' may be a crucial component of striatal learning. While a several milliseconds advancement in spike timing may not seem like much in the context of 100s of milliseconds long CIN pauses or SPN state transitions, it may be ample time to move a spike into or out of the time window for NMDA receptor-dependent coincidence detection to occur (Hao and Oertner 2012).

Both dSPNs and iSPNs robustly support spike timing dependent plasticity (STDP) (Pawlak and Kerr 2008, Shen, Flajolet et al. 2008). Under permissive conditions, constraining the relative timing of SPN APs to occur 5 milliseconds after a corticostriatal synaptic burst efficiently potentiates the active synapses (Shen, Flajolet et al. 2008). In

this view, spike advancement on the time scale we observe may be sufficient to bring the spike temporally close enough to NMDA receptor activation to engage the signaling cascades that are crucial for long term potentiation. Such subtle nAChR-dependent tuning could be a way of 'distancing' those same cortical afferents from SPN activity under conditions of basal activity, allowing for the synapses encoding a particular sensorimotor cue to become potentiated only when deemed appropriate by a learned cholinergic signal.

The extent to which tonic engagement of nAChRs will be capable of influencing GABA release and SPN responses to cortical inputs will likely depend on the capacity of AChE activity to both allow for the detection of a CIN pause, and minimize nAChR desensitization (Zhou, Wilson et al. 2002). This brings up an important corollary of the 'nicotinic brake' hypothesis: the putative mechanism by which CIN pauses remove the nicotinic brake may be most robust in the AChE-enriched compartment of the striatum, also known as the matrix (as opposed to striosomes) (Graybiel and Ragsdale 1978, Brimblecombe and Cragg 2017, Prager and Plotkin 2019). Since the matrix constitutes ~85% of the striatum, our data likely reflect the situation in that compartment (Prager and Plotkin 2019). While coordinated CIN activation can interrupt the timing of ongoing APs in both matrix and striosome SPNs (Crittenden, Lacey et al. 2017), the degree of tonic nAChR engagement and existence of a 'nicotinic brake' in striosomes is unclear.

Taken together, our data suggest an additional role for cholinergic signaling in shaping striatal activity. While a great deal is known about how ACh can impact SPN function through metabotropic muscarinic receptors, nearly all studies of the 'faster' nAChR arm of ACh signaling have focused on its phasic activation. The data in this study demonstrate that nAChR signaling is not constrained to times of phasic CIN engagement, and tonic activation of nAChRs places a 'brake' on SPN activity that may help guide the fine tuning of striatal output.

Materials and Methods

Animals

All experimental procedures on mice adhered to and received prior written approval from the Institutional Animal Care and Use Committees of the Hebrew University of Jerusalem and of Stony Brook University and of the local ethics committee of Stockholm, Stockholms Norra djurförsöksetiska nämnd. Experiments were conducted on various transgenic mice. For optogenetic activation of corticostriatal fibers, we used 1-4 months old male and female homozygous transgenic Thy1-ChR2 mice [B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J] that express channelrhodopsin-2 under the Thy1 promoter were used. Thy1-ChR2 mice express ChR2 in cortical afferents under the thymus cell antigen 1 (Thy1) promoter. Thy1 is generally expressed in the axons of layer V pyramidal neurons, limbic system, midbrain and brainstem (Arenkiel, Peca et al. 2007) . For experiments measuring paired pulse ratios, neuronal excitability and spontaneous GABAergic events, we used 1.5-4 month old male and female C57bl/6 mice crossed with one of two BAC transgenic lines (where indicated): drd1-tdTomato (labeling dSPNs) or drd2-eGFP (labeling iSPNs) (Shuen, Chen et al. 2008, Ade, Wan et al. 2011). For PV-FSI recordings, both male and female mice (p59-p65) were used in this study. Mice were group-housed under a 12 hr light / dark schedule and given ad libidum access to food and water. The PV-Cre (stock #017320, the Jackson laboratory) mouse line was crossed with a homozygous tdTomato reporter mouse line ('Ai9', stock #007909, the Jackson laboratory) to allow identification of FSIs based on the expression of a fluorescent marker protein. The Cre line was heterozygous and maintained on a wild-type C57BL/6J background (stock # 000664, the Jackson Laboratory).

Slice preparation

For optogenetic activation experiments, PPR, excitability and spontaneous GABAergic event experiments: Mice were deeply anesthetized with ketamine–xylazine and perfused transcardially with ice-cold modified artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2–5% CO_2, and containing (in mM): 2.5 KCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 0.5 $CaCl_2$, 10 $MgSO_4$, 0.4 ascorbic acid, 10 glucose, and 210 sucrose (optogenetic activation studies) or 3 KCl, 26 $NaHCO_3$, 1 NaH_2PO_4, 1 $CaCl_2$, 1.5 $MgCl_2$,

124 NaCl and 14 glucose (PPR, excitability and spontaneous GABAergic event measures). The brain was removed and sagittal or coronal slices (sectioned at a thickness of 275 µm were obtained in ice-cold modified ACSF. Slices were then submerged in ACSF, bubbled with 95% O_2–5% CO_2, and containing (in mM): 2.5 KCl, 126 NaCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 2 $CaCl_2$, 2 $MgSO_4$, and 10 glucose and stored at room temperature for at least 1 h prior to recording (optogenetic activation studies), or 3 KCl, 26 $NaHCO_3$, 1 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 124 NaCl and 14 glucose, incubated at 32°C for 45 then held at room temperature until recording (PPR, excitability and spontaneous GABAergic event measures).

For PV-FSI experiments: Mice were deeply anaesthetized with isoflurane and decapitated. The brain was removed and immersed in ice-cold cutting solution containing 205 mM sucrose, 10 mM glucose, 25 mM NaHCO3, 2.5 mM KCl, 1.25 mM NaH2PO4, 0.5 mM CaCl2 and 7.5 mM MgCl2. Parasagittal brain slices (thickness 250 mm) were prepared with a Leica VT 1000S vibratome and incubated for 30 – 60 min at 34˚C in a submerged chamber filled with artificial cerebrospinal fluid (ACSF) saturated with 95% oxygen and 5% carbon dioxide. ACSF was composed of 125 mM NaCl, 25 mM glucose, 25 mM NaHCO3, 2.5 mM KCl, 2 mM CaCl2, 1.25 mM NaH2PO4, 1 mM MgCl2. Subsequently, slices were kept for at least 30 min at room temperature before recording.

Slice visualization, electrophysiology and optogenetic stimulation

For optogenetic activation experiments: The slices were transferred to the recording chamber mounted on an Olympus BX51 upright, fixed-stage microscope and perfused with oxygenated ACSF at room temperature. A 60X, 0.9 NA water-immersion objective was used to examine the slice using Dodt contrast video microscopy. Patch pipette resistance was typically 3-4 MΩ when filled with recording solutions. In voltage-clamp experiments of IPSCs in SPNs the intracellular solution contained (in mM): 127.5 $CsCH_3SO_3$, 7.5 CsCl, 10 HEPES, 10 TEA-Cl, 4 phosphocreatine disodium, 0.2 EGTA, 0.21 Na_2GTP, and 2 $Mg_{1.5}ATP$ (pH=7.3 with CsOH, 280-290 mOsm/kg). For whole-cell current clamp recordings from SPNs and cell-attached recordings from CINs the pipette contained (in mM): 135.5 KCH_3SO_4, 5 KCl, 2.5 NaCl, 5 Na-phosphocreatine, 10

HEPES, 0.2 EGTA, 0.21 Na_2GTP, and 2 $Mg_{1.5}ATP$ (pH=7.3 with KOH, 280-290 mOsm/kg). Electrophysiological recordings were obtained with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Junction potential, which was 7-8 mV, was not corrected. Signals were digitized at 10 kHz and logged onto a personal computer with the Winfluor software (John Dempster, University of Strathclyde, UK). Blue light LED (470 nm, Mightex, Toronto, Ontario, Canada) was used for full-field illumination via the objective. Single pulses were 1 ms long. In order to compare action potential (AP) latency and afterdepolarization (ADP) amplitude among SPNs the LED intensity was set to generate a just suprathreshold response in each SPN recorded.

For PPR, excitability and spontaneous GABAergic event experiments: The slices were transferred to the recording chamber mounted on a modified Ultima laser scanning microscope system (Bruker Nano) (excitability and spontaneous GABAergic event experiments) or an Olympus BX51W1 microscope (PPR experiments) and perfused with oxygenated ACSF at room temperature. A 60X, 1.0 NA Olympus LUMPFL water-immersion objective was used to visualize slices, and fluorescently labeled neurons identified for recordings with the aid of a Dodt contrast image displayed in registration with the fluorescence image for experiments requiring identification of dSPNs vs iSPNs. Patch pipette resistance was typically 3-6 MΩ when filled with recording solutions. For voltage-clamp recordings the intracellular solution contained (in mM): 120 $CsMeSO_3$, 5 NaCl, 10 TEA-Cl (tetraethylammonium-Cl), 10 HEPES, 5 Qx-314, 4 ATP-Mg^{2+}, 0.3 GTP-Na^+, 0.25 EGTA, and 0.05 Alexa Fluor 568 hydrazide Na^+salt (Alexa Fluor was omitted in PPR experiments). For current clamp recordings the internal solution contained (in mM): 135 $KMeSO_4$, 5 KCl, 10 HEPES, 2 ATP-Mg^{2+}, 0.5 GTP-Na^+, 5 phosphocreatine-tris, 5 phosphocreatine-Na^+, 0.1 Fluo-4 pentapotassium salt, and 0.05 Alexa Fluor 568 hydrazide Na^+ salt. Patched SPNs were allowed to equilibrate for 10 min after rupture. Recordings were made using a Multiclamp 700B amplifier and either PrairieView 5.0 software (Bruker) or custom MATLAB protocols. sIPSCs were recorded in voltage-clamp mode at +10 mV for 180 seconds before and after bath application of drug. Access resistance and holding current were monitored throughout experiments and cells were excluded if these values changed by more than 20%. Local electrical stimulation was evoked using a concentric bipolar stimulation electrode (FHC, Inc.,

Bowdoin, ME) placed near the cell using a stimulating amplitude of ~1-3 mV and 50 ms between stimuli pulses; 10 sweeps (10 s inter-sweep interval) were averaged per cell; cells were held at -70mV. PPR experiments were performed at 26-29°C. Rheobase & action potential firing frequencies were determined in current-clamp mode via a step series of 500 ms current injections starting at -200 pA and increasing by 25 pA with each step. Resting membrane potential & cell morphology were monitored throughout experiments and cells were excluded if resting at a more depolarized potential than -77.0 mV.

For PV-FSI experiments: Whole-cell patch clamp recordings were obtained in oxygenated ACSF at 35˚C. Neurons were visualized using infrared differential interference contrast (IR-DIC) microscopy (Zeiss FS Axioskop, Oberkochen, Germany). tdTomato expressing neurons were identified by switching to epifluorescence using a mercury lamp (X-cite, 120Q, Lumen Dynamics). Borosilicate pipettes of 5 – 8 MOhm resistance were pulled with a Flaming / Brown micropipette puller P-1000 (Sutter instruments). All recordings were done in current clamp with an intracellular solution containing 130 mM K-gluconate, 5 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM GTP, 10 mM Na2-phospho- creatine (pH 7.25, osmolarity 285 mOsm). Recordings were amplified using a MultiClamp 700B amplifier (Molecular Devices, CA, USA), filtered at 2 kHz, digitized at 10-20 kHz using ITC-18 (HEKA Elektronik, Instrutech, NY, USA), and acquired using custom-made routines running on Igor Pro (Wavemetrics, OR, USA). Liquid junction potential was not corrected. Throughout all recordings pipette capacitance and access resistance were compensated for and data were discarded when access resistance increased beyond 30 MOhm. The intrinsic properties of the neurons were determined by a series of hyperpolarizing and depolarizing current steps and ramps, enabling the extraction of sub- and suprathreshold properties. All neurons were recorded in control conditions and after >5 min of bath application of 1 μM DHβE (Tocris).

Drugs and Reagents

Experiments in Thy1-ChR2 mice were performed in the presence or absence of synaptic receptor blockers including 10 μM SR-95531 (gabazine) to block $GABA_A$

receptors, 2 µM CGP-55845 to block $GABA_B$ receptors, 10 µM mecamylamine, a non-selective nAChR-antagonist and 1 or 10 µM Dihydro-β-erythroidine hydrobromide (DHβE), a competitive α4β2 nAChR antagonist. Experiments were conducted with various combinations of the blockers. The acute effects of solution exchanges were measured at least 5 min after wash on. All drugs and reagents were acquired from Tocris (Ellisville, MO, USA) or HelloBio (Bristol, Avonmouth, UK).

Data Analysis and Statistics

Data analysis was performed using custom-made code in MATLAB (MathWorks, Natick, MA, USA) or Python; sIPSCs were analyzed using the Mini Analysis program (Synaptosoft). Two-tailed Wilcoxon signed-rank test (SRT) was used to test for changes of medians in matched-paired comparisons. The null hypotheses were rejected if the P values were below 0.05. Boxplots represent range (whiskers), median (thick bar) and lower and upper quartiles. To analyze responses to stimulation, peristimulus time histograms (PSTH) were generated. PSTH were estimated using 20 ms wide bins. IV relationships, sIPSC frequencies and inter-event intervals were analyzed by two-way ANOVA.

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Figure Captions

Fig. 1. nAChR-dependent inhibition and delay of spike latency in spiny projection neurons (SPNs) activated by corticostriatal fibers. A. Left: diagram of recording configuration. An SPN is patched in an acute slice from a ChAT-ChR2 mouse. Right: 1-ms-long 470 nm LED pulse of increasing intensity generates excitatory postsynaptic potentials (EPSPs), or an AP followed by an after depolarization (ADP) in an SPN. **B.** Examples of the effect of 10 µM mecamylamine (mec), an nAChR antagonist, on EPSP

amplitude (dashed) or spike latency and ADP amplitude (solid). Mecamylamine significantly shortens spike latencies ($p = 4.8 \cdot 10^{-4}$, n = 12 SPNs, SRT) (**C**), as well as ADP ($p = 2.4 \cdot 10^{-3}$, n = 12 SPNs, SRT) (**D**) and EPSP ($p = 0.016$, n = 7 SPNs, SRT) (**E**) amplitudes. **F**. Examples of the effect of mecamylamine on latency of a spike triggered synaptically from a depolarized ("up-state") potential with an LED pulse. **G**. Mecamylamine significantly shortens "up-state" spike latencies ($p = 0.03$, n = 6 SPNs, SRT) - two-sided Wilcoxon Signed-Rank test (SRT). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Fig. 2. Blockade of mecamylamine-insensitive α7 **nAChRs with MLA increases SPN AP latency. A.** Example responses of an SPN to paired pulse stimulation (evoked by two local electrical stimuli separated by 50 ms), before and in the presence of mecamylamine (10 µM). **B.** Mecamylamine had no effect on the EPSC amplitude evoked by the first paired stimulus (P1) ($p = 0.59$, n = 15 SPNs, SRT). **C.** Mecamylamine had no effect on the paired pulse ratio (P2/P1, $p = 0.33$, n = 15 SPNs, SRT). **D.** Example responses of MLA (2-5 µM) on optogenetically-evoked AP latency. **E.** MLA delayed AP latency in SPNs after full field optogenetic stimulation of corticostriatal inputs ($p = 0.039$, n= 9, SRT).

Fig. 3. The nAChR-dependent inhibition of corticostriatal SPN activation is mediated through and saturates GABAergic inhibition. A. Example of the occlusion of the mecamylamine effect on optogenetic synaptic activation of SPNs by receptor (GABAR) antagonists, 10 µM SR-95531 ($GABA_AR$ antagonist) and 2 µM CGP-55845 ($GABA_BR$ antagonist). Distribution of spike latencies (**B**) and ADP amplitude (**C**) in response to application of GABAR antagonists followed by mecamylamine, show that application of GABAR antagonist significantly shortens the AP latency ($p = 4.6 \cdot 10^{-3}$, n = 13 SPNs, SRT) and enhancement of the ADP amplitude ($p = 2.4 \cdot 10^{-4}$, n = 13 SPNs, SRT). In contrast, the subsequent mecamylamine application fails to further shorten the AP latency ($p = 1/16$, n = 5 SPNs, SRT) or further enhance the ADP amplitude ($p = 1$, n = 5 SPNs, SRT). **D.** Example of how the mecamylamine effect saturates the GABAergic inhibition

of the optogenetic synaptic activation of SPNs. **E-F.** Distribution of spike latencies (**E**) and ADP amplitude (**F**) in response to application of mecamylamine followed by GABAR antagonists, showed that the subsequent application of GABAR antagonists fails to further shorten the AP latency ($p = 1/4$, n = 5 SPNs, SRT) or further enhance the ADP amplitude ($p = 0.44$, n = 5 SPNs, SRT).

Fig. 4. The autonomous firing of cholinergic interneurons (CINs) enables them to respond more rapidly and vigorously than SPNs to feedforward corticostriatal excitation. A. Example of the autonomous discharge of a CIN recorded in cell-attached configuration (top) and the distribution of firing rates (bottom, n = 15 CINs) in an acute striatal slice. **B**. Peristimulus histograms (PSTHs) of a CIN in response to optogenetic activation of corticostriatal fibers at various 470 nm LED intensities as compared to the amplitude of the EPSPs evoked in a nearby SPN (insets), demonstrates that CINs are much more sensitive than SPNs to feedforward cortical activation. **C**. Cumulative distribution of the latency to first spike of CINs recorded in cell-attached mode (green, n = 8 CINs) as compared to the latency to first spike in SPNs recorded (from a resting-state) in whole-cell mode (n **=** 12) before (black) and after (magenta) application of mecamylamine.

Fig. 5. Latency of feedforward GABAergic IPSCs in SPNs activated synaptically by corticostriatal fibers. A. Left: IPSCs recorded in an SPN held at +10 mV before (black) and after (red) application of GABAR antagonists, reveals GABA-sensitive (purple) IPSC. Right: Zoom in of the GABAergic IPSCs enables estimation (fit of diagonal black line) of the latency of the feed-forward GABA-sensitive inhibition. **B.** Distribution of the latency of feedforward GABAergic inhibition to SPNs in the Thy1-ChR2 mouse (n = 7 SPNs). **C**. Schematic demonstrating that the feedforward GABAergic inhibition precedes the earliest timing of feedforward cholinergic di-synaptic inhibition of SPNs, indicating that phasic activation of CINs cannot explain the nAChR-dependent delay of spike latency.

Fig 6. SPN intrinsic excitability is not increased by nAChR-blockade. A. Example IV traces of dSPNs and iSPNs in the absence and presence of 10 µM mecamylamine. **B.** Left: resting membrane potential of dSPNs (black) and iSPNs (blue) in the absence and presence of mecamylamine (all SPNs: $p = 0.43$, n = 13; dSPNs: $p = 0.16$, n = 7; iSPNs: $p = 0.87$, n = 6; SRT). Right: percent change in resting membrane potential after mecamylamine application. **C.** Left: rheobase current of dSPNs and iSPNs in the absence and presence of mecamylamine (all SPNs: $p = 0.075$, n = 13; dSPNs: $p = 0.5$, n = 7; iSPNs: $p = 0.095$, n = 6; SRT). Right: percent change in rheobase current after mecamylamine application. **D.** Voltage responses to subthreshold current injections are not enhanced by mecamylamine ($F_{11,120} = 0.86$ $p = 0.58$; n = 11; two-way ANOVA), though a post-hoc Bonferroni test reveals a significant mecamylamine-induced decrease at 50 pA ($p < 0.05$) and 75 pA ($p < 0.01$) current injections. **E.** AP firing frequencies in response to suprathreshold somatic current injections were unaffected by mecamylamine ($F_{20,231} = 0.43$, $p = 0.99$; n = 12; two-way ANOVA).

Fig 7. nAChR-blockade hyperpolarizes PV-FSI resting membrane potential and reduces the frequency of sIPSCs in SPNs. A. Example recordings of sIPSCs from SPNs voltage-clamped at +10mV, before and during mecamylamine (10 µM) application. **B.** Mecamylamine significantly enhanced sIPSC frequency in SPNs ($F_{8,108} = 2.607$, $p = 0.012$; n = 13; two-way ANOVA). A post-hoc Bonferroni test revealed that this decrease was limited to low amplitude sIPSCs (20 pA bin: $p < 0.001$; 25 pA bin: $p < 0.01$). **C.** Mecamylamine caused a rightward shift in the cumulative probability distribution of sIPSC interevent intervals in SPNs ($F_{20,252} = 2.10$, $p = 4.7 \cdot 10^{-3}$; n = 13; two-way ANOVA). **D.** Example traces of a PV-FSI before and after wash-in of 1 µM DHβE. **E.** Resting membrane potentials of PV-FSIs before and after wash-in of DHβE (n = 12; $p = 9.8 \cdot 10^{-4}$; SRT). **F.** AP durations of PV-FSIs in the absence and presence of DHβE (n = 10; p = 0.03; SRT). **G.** AP rise times of PV-FSIs in the absence and presence of DHβE (n = 10; p = 0.02; SRT).

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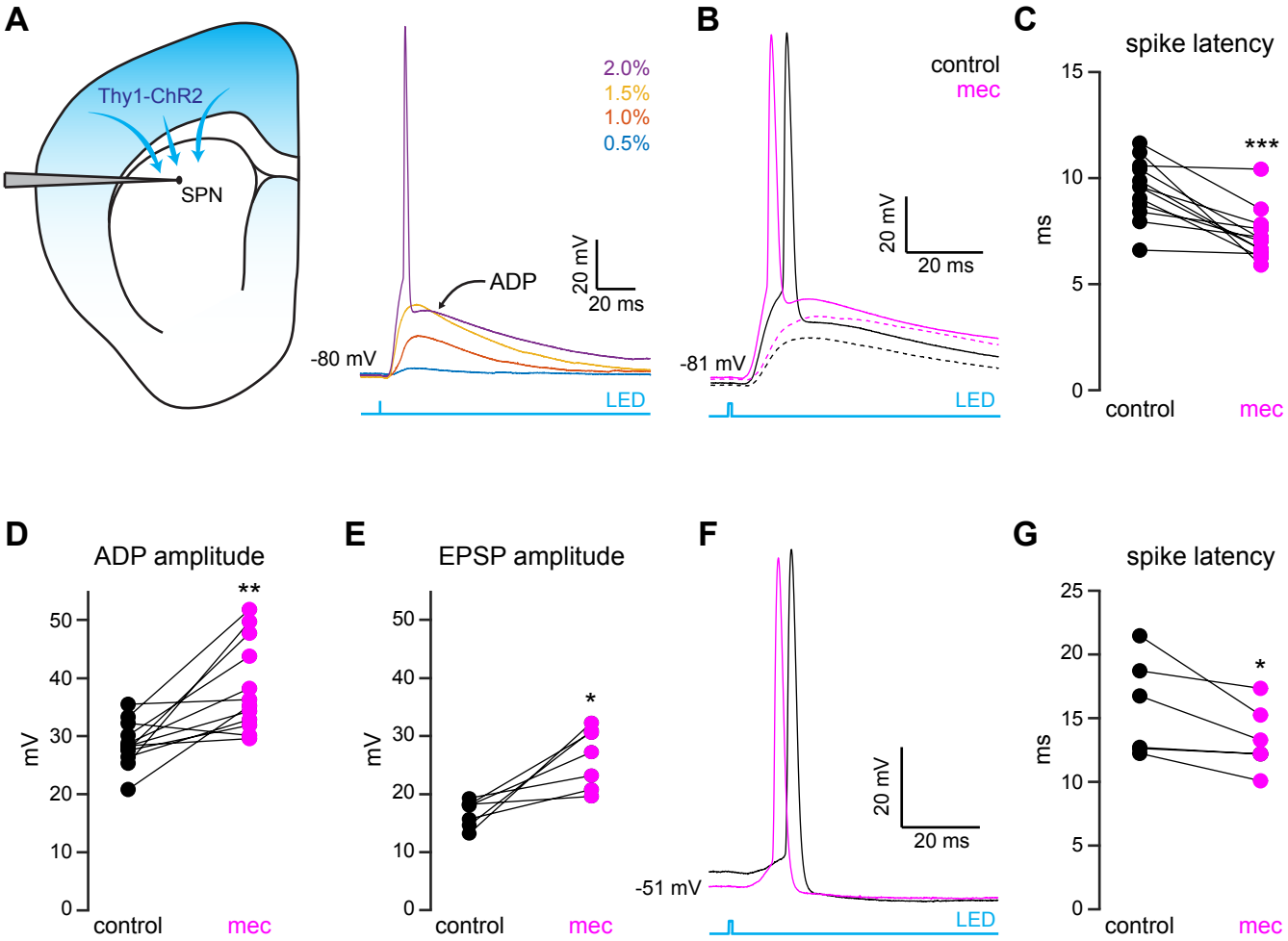

Fig. 1. nAChR-dependent inhibition and delay of spike latency in spiny projection neurons (SPNs) activated by corticostriatal fibers. A. Left: diagram of recording configuration. An SPN is patched in an acute slice from a ChAT-ChR2 mouse. Right: 1-ms-long 470 nm LED pulse of increasing intensity generates excitatory postsynaptic potentials (EPSPs), or an AP followed by an after depolarization (ADP) in an SPN. **B.** Examples of the effect of 10 μM mecamylamine (mec), an nAChR antagonist, on EPSP amplitude (dashed) or spike latency and ADP amplitude (solid). Mecamylamine significantly shortens spike latencies ($p = 4.8 \cdot 10\text{-}4$, n = 12 SPNs, SRT) (**C**), as well as ADP ($p = 2.4 \cdot 10\text{-}3$, n = 12 SPNs, SRT) (**D**) and EPSP ($p = 0.016$, n = 7 SPNs, SRT) (**E**) amplitudes. **F.** Examples of the effect of mecamylamine on latency of a spike triggered synaptically from a depolarized ("up-state") potential with an LED pulse. **G.** Mecamylamine significantly shortens "up-state" spike latencies ($p = 0.03$, n = 6 SPNs, SRT) SRT - two-sided Wilcoxon Signed-Rank test (SRT). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

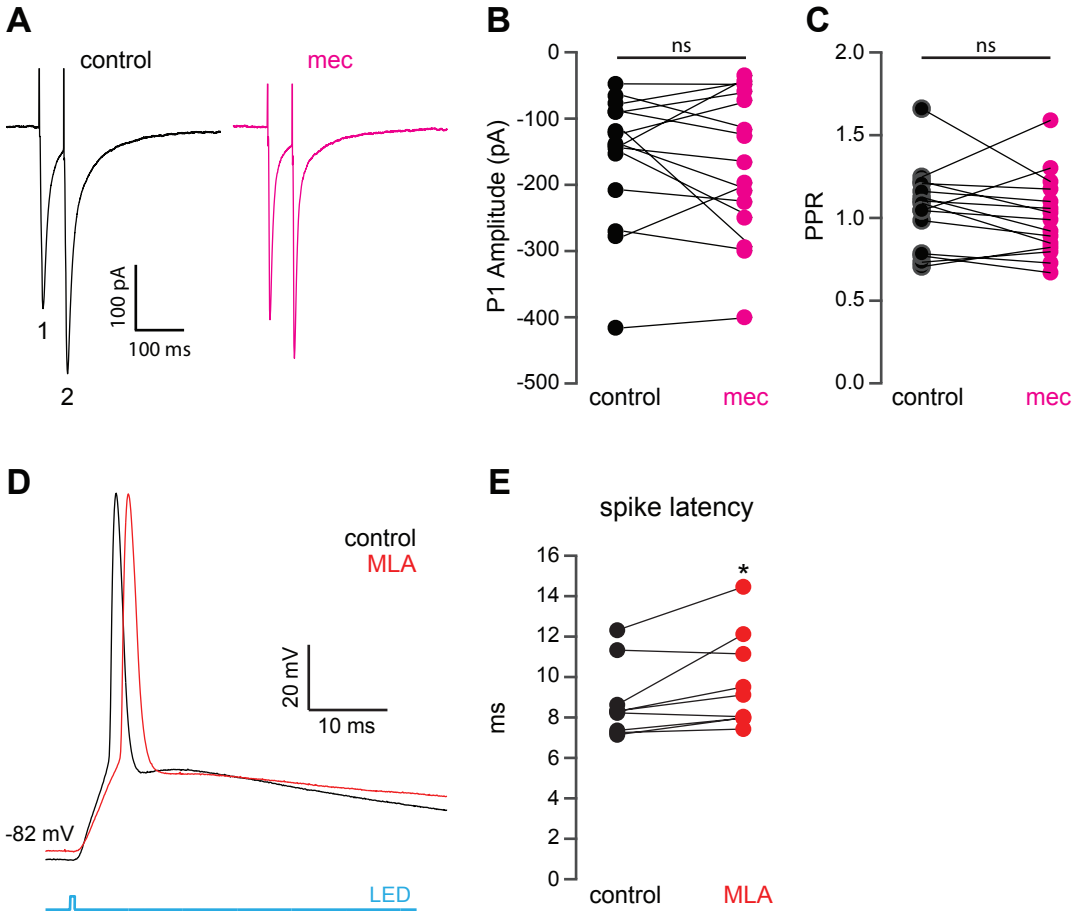

Fig. 2. Blockade of mecamylamine-insensitive α7 nAChRs with MLA increases SPN AP latency. A. Example responses of an SPN to paired pulse stimulation (evoked by two local electrical stimuli separated by 50 ms), before and in the presence of mecamylamine (10 μM). **B.** Mecamylamine had no effect on the EPSC amplitude evoked by the first paired stimulus (P1) ($p = 0.59$, n = 15 SPNs, SRT). **C.** Mecamylamine had no effect on the paired pulse ratio (P2/P1, $p = 0.33$, n = 15 SPNs, SRT). **D.** Example responses of MLA (2-5 μM) on optogenetically-evoked AP latency. **E.** MLA delayed AP latency in SPNs after full field optogenetic stimulation of corticostriatal inputs ($p = 0.039$, n= 9, SRT).

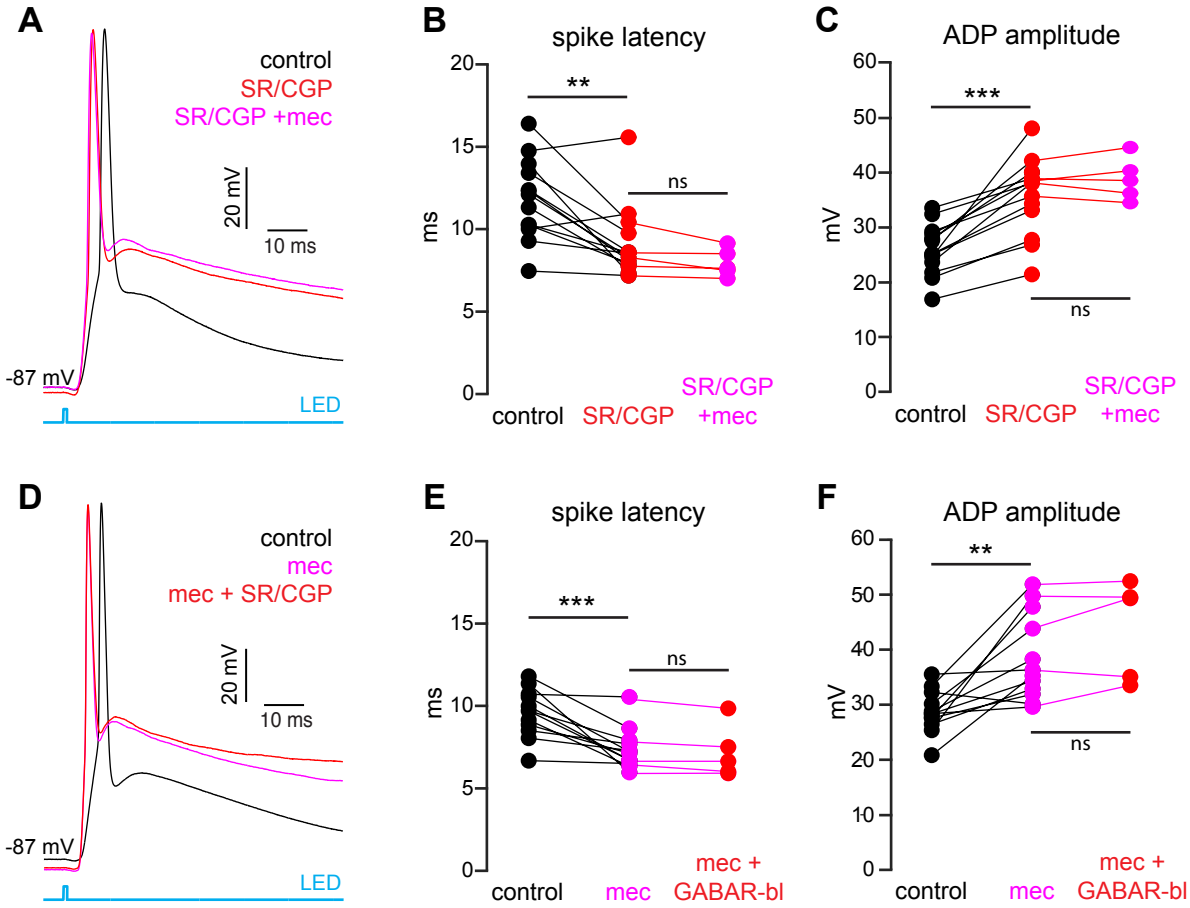

Fig. 3. The nAChR-dependent inhibition of corticostriatal SPN activation is mediated through and saturates GABAergic inhibition. A. Example of the occlusion of the mecamylamine effect on optogenetic synaptic activation of SPNs by receptor (GABAR) antagonists, 10 μM SR-95531 (GABAAR antagonist) and 2 μM CGP-55845 (GABABR antagonist). Distribution of spike latencies (**B**) and ADP amplitude (**C**) in response to application of GABAR antagonists followed by mecamylamine, show that application of GABAR antagonist significantly shortens the AP latency (p = 4.6·10-3, n = 13 SPNs, SRT) and enhancement of the ADP amplitude (p = 2.4·10-4, n = 13 SPNs, SRT). In contrast, the subsequent mecamylamine application fails to further shorten the AP latency (p = 1/16, n = 5 SPNs, SRT) or further enhance the ADP amplitude (p =1, n = 5 SPNs, SRT). **D.** Example of how the mecamylamine effect saturates the GABAergic inhibition of the optogenetic synaptic activation of SPNs. E-F. Distribution of spike latencies (**E**) and ADP amplitude (**F**) in response to application of mecamylamine followed by GABAR antagonists, showed that the subsequent application of GABAR antagonists fails to further shorten the AP latency (p = 1/4, n = 5 SPNs, SRT) or further enhance the ADP amplitude (p = 0.44, n = 5 SPNs, SRT).

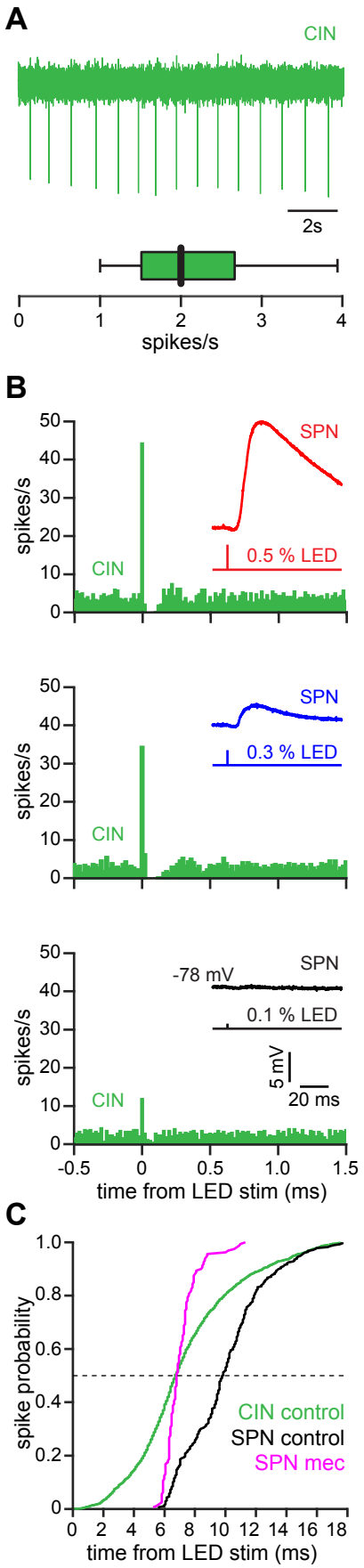

Fig. 4. The autonomous firing of cholinergic interneurons (CINs) enables them to respond more rapidly and vigorously than SPNs to feedforward corticostriatal excitation. A. Example of the autonomous discharge of a CIN recorded in cell-attached configuration (top) and the distribution of firing rates (bottom, n = 15 CINs) in an acute striatal slice. **B.** Peristimulus histograms (PSTHs) of a CIN in response to optogenetic activation of corticostriatal fibers at various 470 nm LED intensities as compared to the amplitude of the EPSPs evoked in a nearby SPN (insets), demonstrates that CINs are much more sensitive than SPNs to feedforward cortical activation. **C.** Cumulative distribution of the latency to first spike of CINs recorded in cell-attached mode (green, n = 8 CINs) as compared to the latency to first spike in SPNs recorded (from a resting-state) in whole-cell mode (n=12) before (black) and after (magenta) application of mecamylamine.

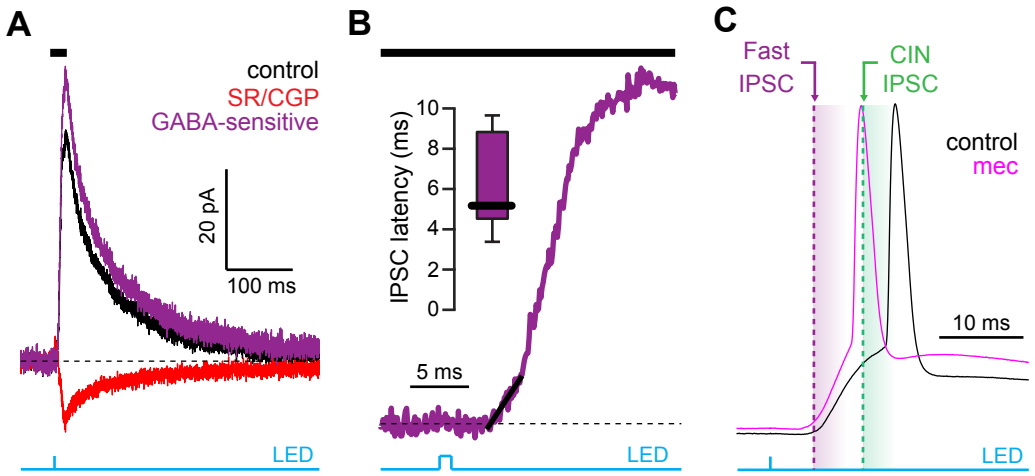

Fig. 5. Latency of feedforward GABAergic IPSCs in SPNs activated synaptically by corticostriatal fibers. A. Left: IPSCs recorded in an SPN held at +10 mV before (black) and after (red) application of GABAR antagonists, reveals GABA-sensitive (purple) IPSC. Right: Zoom in of the GABAergic IPSCs enables estimation (fit of diagonal black line) of the latency of the feed-forward GABA-sensitive inhibition. **B.** Distribution of the latency of feedforward GABAergic inhibition to SPNs in the Thy1-ChR2 mouse (n = 7 SPNs). **C.** Schematic demonstrating that the feedforward GABAergic inhibition precedes the earliest timing of feedforward cholinergic di-synaptic inhibition of SPNs, indicating that phasic activation of CINs cannot explain the nAChR-dependent delay of spike latency.

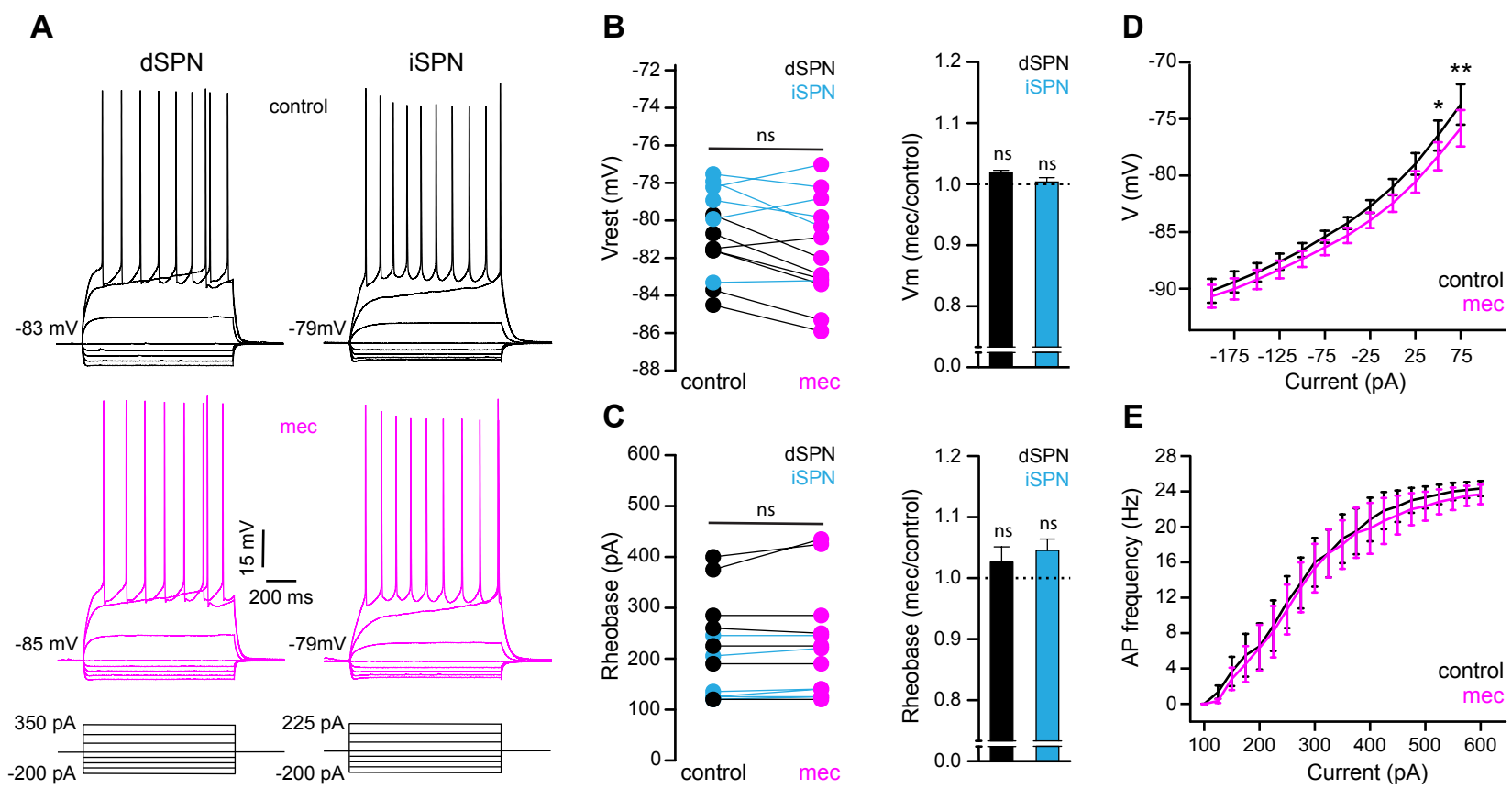

Fig 6. SPN intrinsic excitability is not increased by nAChR-blockade. A. Example IV traces of dSPNs and iSPNs in the absence and presence of 10 μM mecamylamine. **B.** Left: resting membrane potential of dSPNs (black) and iSPNs (blue) in the absence and presence of mecamylamine (all SPNs: $p = 0.43$, $n = 13$; dSPNs: $p = 0.16$, $n = 7$; iSPNs: $p = 0.87$, $n = 6$; SRT). Right: percent change in resting membrane potential after mecamylamine application. **C.** Left: rheobase current of dSPNs and iSPNs in the absence and presence of mecamylamine (all SPNs: $p = 0.075$, $n = 13$; dSPNs: $p = 0.5$, $n = 7$; iSPNs: $p = 0.095$, $n = 6$; SRT). Right: percent change in rheobase current after mecamylamine application. **D.** Voltage responses to subthreshold current injections are not enhanced by mecamylamine ($F_{11,120} = 0.86$ $p = 0.58$; $n = 11$; two-way ANOVA), though a post-hoc Bonferroni test reveals a significant mecamylamine-induced decrease at 50 pA ($p < 0.05$) and 75 pA ($p < 0.01$) current injections. **E.** AP firing frequencies in response to suprathreshold somatic current injections were unaffected by mecamylamine ($F_{20,231} = 0.43$, $p = 0.99$; $n = 12$; two-way ANOVA).

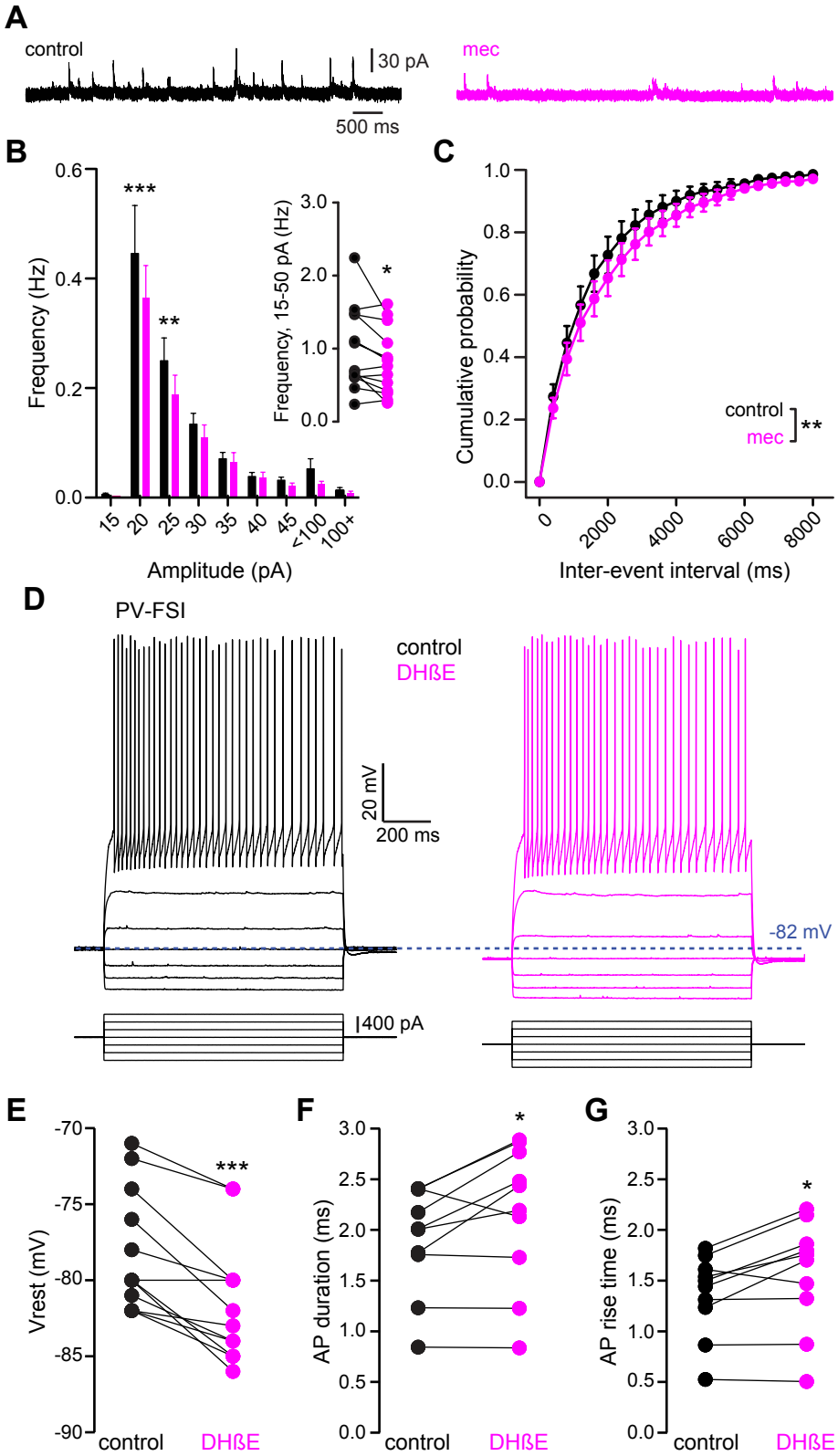

Fig 7. nAChR-blockade hyperpolarizes PV-FSI resting membrane potential and reduces the frequency of sIPSCs in SPNs. A. Example recordings of sIPSCs from SPNs voltage-clamped at +10mV, before and during mecamylamine (10 μM) application. **B.** Mecamylamine significantly enhanced sIPSC frequency in SPNs ($F_{8,108} = 2.607$, $p = 0.012$; n = 13; two-way ANOVA). A post-hoc Bonferroni test revealed that this decrease was limited to low amplitude sIPSCs (20 pA bin: $p < 0.001$; 25 pA bin: $p < 0.01$). **C.** Mecamylamine caused a rightward shift in the cumulative probability distribution of sIPSC interevent intervals in SPNs ($F_{20,252} = 2.10$, $p = 4.7 \cdot 10\text{-}3$; n = 13; two-way ANOVA). **D.** Example traces of a PV-FSI before and after wash-in of 1 μM DHβE. **E.** Resting membrane potentials of PV-FSIs before and after wash-in of DHβE (n = 12; $p = 9.8 \cdot 10\text{-}4$; SRT). **F.** AP durations of PV-FSIs in the absence and presence of DHβE (n = 10; p = 0.03; SRT). G. AP rise times of PV-FSIs in the absence and presence of DHβE (n = 10; p = 0.02; SRT).