The glutamatergic subthalamic nucleus (STN) exerts control over motor output through nuclei of the basal ganglia. High-frequency electrical stimuli in the STN effectively alleviate motor symptoms in movement disorders, and cholinergic stimulation boosts this effect. To gain knowledge about the mechanisms of cholinergic modulation in the STN, we studied cellular and circuit aspects of nicotinic acetylcholine receptors (nAChRs) in mouse STN. We discovered two largely divergent microcircuits in the STN; these are regulated in part by either α4β2 or α7 nAChRs. STN neurons containing α4β2 nAChRs (α4β2 neurons) received more glutamatergic inputs, and preferentially innervated GABAergic neurons in the substantia nigra pars reticulata. In contrast, STN neurons containing α7 nAChRs (α7 neurons) received more GABAergic inputs, and preferentially innervated dopaminergic neurons in the substantia nigra pars compacta. Interestingly, local electrical stimuli excited a majority (79%) of α4β2 neurons but exerted strong inhibition in 58% of α7 neurons, indicating an additional diversity of STN neurons: responses to electrical stimulation. Chronic exposure to nicotine selectively affects α4β2 nAChRs in STN: this treatment increased the number of α4β2 neurons, upregulated α4-containing nAChR number and sensitivity, and enhanced the basal firing rate of α4β2 neurons both ex vivo and in vivo. Thus, chronic nicotine enhances the function of the microcircuit involving α4β2 nAChRs. This indicates chronic exposure to nicotinic agonist as a potential pharmacological intervention to alter selectively the balance between these two microcircuits, and may provide a means to inhibit substantia nigra dopaminergic neurons.

**Key words:** alpha4beta2; alpha7; chronic nicotine; Parkinson’s disease; substantia nigra; upregulation

**Introduction**

The subthalamic nucleus (STN), through glutamatergic synapses, governs two output nuclei of the basal ganglia: the internal segment of the globus pallidus and the substantia nigra pars reticulata (SNr) (Smith and Parent, 1988; Heida et al., 2008; Marani et al., 2008). Parkinson’s disease (PD) is caused by the death of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and is characterized by motor symptoms, such as resting tremor, rigidity, bradykinesia, and akinesia. STN neurons in PD show abnormal activity, including accelerated, burst, and synchronized firing, which are correlated with motor symptoms (Bergman et al., 1994; Wichmann et al., 1994; Kreiss et al., 1997; Magnen et al., 2000; Perlmutter and Mink, 2006; Weinberger et al., 2009). Dopamine compensation with l-dopa or apomorphine relieves motor symptoms and restores normal activity in STN neurons (Kreiss et al., 1997; Levy et al., 2001, 2002; Weinberger et al., 2006). As PD progresses, l-dopa efficacy decreases; however, increasing l-dopa dosage causes motor fluctuations and dyskinesia (Perlmutter and Mink, 2006; Cenci, 2007; Poewe, 2009). Therefore, it is important to identify other therapies aiming to enhance or maintain l-dopa efficacy to minimize these side effects.

Deep brain stimulation (DBS) in the STN not only improves the major motor symptoms of PD but also reduces required doses of l-dopa and mitigates motor fluctuations and dyskinesia (Nutt et al., 2001; Breit et al., 2004; Perlmutter and Mink, 2006; Benabid et al., 2009; Gradinaru et al., 2009). Cholinergic neurons in the pedunculopontine tegmental nucleus (PPTg) project to STN (Ichinohe et al., 2000; Benarroch, 2008), and clinical data show that stimulating these neurons boosts the therapeutic effects of STN DBS (Stefani et al., 2007). Additionally, long-term use of nicotinic agonists potentiates the ability of l-dopa to improve motor symptoms (Schneider et al., 1998; Domino et al., 1999) and attenuates l-dopa-induced dyskinesia (Quik et al., 2007; Bordia et al., 2008; Huang et al., 2011). These facts suggest that cholinergic agonists and DBS may target the same neuron types in STN.

Nicotinic acetylcholine receptors (nAChRs) in the basal ganglia help to govern voluntary movement (Dani and Bertrand, 2007; Miwa et al., 2011; Quik and Wonnacott, 2011). Mice carrying gain-of-function or null mutations in nAChR subunits al-
low-selective activation or deletion of certain nAChR subtypes in vivo (Champiaux and Changeux, 2002; Lester et al., 2003). Studies using these mouse lines implicate nAChRs containing α4, α6, and β2 subunits in the control of movement (Labarca et al., 2001; Tapper et al., 2004; Drenan et al., 2008, 2010; Drenan and Lester, 2012). It is well established that these nAChRs modulate neuronal activity and neurotransmitter release in the SN and the striatum (Nashmi et al., 2007; Drenan et al., 2008, 2010; Xiao et al., 2009a; Quik and Wonnacott, 2011). However, how these nAChRs regulate other nuclei in the basal ganglia is understood poorly.

Although several nAChR subunit mRNAs and binding sites have been detected in the SN (Cimino et al., 1992; Quik et al., 2000; Pimlott et al., 2004), it remains unclear whether nAChRs are differentially expressed in the SN and the striatum. In this study, we addressed these questions.

### Materials and Methods

The care and use of animals and the experimental protocol of this study were approved by the Institutional Animal Care and Use Committee of the California Institute of Technology. Efforts were made to minimize animal suffering and to minimize the number of animals used.

Electrophysiological recordings: brain slice preparation. The recordings were performed using brain slices, which were prepared from 7- to 11-week-old C57BL/6 (wild-type) mice, nAChR α4-YFP, or α4 subunit knock-out mice, using the protocol described with some modifications (Ye et al., 2006; Xiao et al., 2009a, b). Both α4-KO and α4-YFP mice were backcrossed > N10 to C57BL/6. In brief, the mice were killed with CO2 and were subject to cardiac perfusion with ice-cold modified glycerol-based artificial CSF, containing the following (in mM): 250 glycerol, 2.5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 26 NaHCO3, and 11 glucose. The brain was subsequently removed and sliced with a microslicer (DTK-1000, Ted Pella) or a Compressotive (VH-200, Precisionary) while immersed in modified glycerol-based artificial CSF. To retain the connection between STN and the substantia nigra (SN), we cut parasaggital slices (350 μm) with an angle of 10°-14° toward the forebrain. Brain slices, containing STN and the SN, were allowed to recover at 32°C in a holding chamber filled with carbenogated artificial CSF, containing the following (in mM): 125 NaCl, 2.5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 26 NaHCO3, and 11 glucose. One hour later, the holding chamber with slices was placed at room temperature. One of the slices was transferred into the recording chamber and superfused (1.5–2.0 ml/min) with carbenogated artificial CSF at 32 ± 0.5°C. Three or four slices per mouse were used for recordings.

Patch-clamp recording. The neurons were visualized with an upright microscope (BX50WI; Olympus) and near infrared or blue illumination (the latter for visualizing fluorescent proteins). Single or dual whole-cell patch-clamp techniques were used to record electrophysiological signals from neurons with MultiClamp 700B amplifiers (Molecular Devices), Digidata 1322 analog-to-digital converters (Molecular Devices), and pClamp 9.2 software (Molecular Devices). Data were sampled at 10 kHz and filtered at 2 kHz. A patch electrode had a resistance of 4–6 MΩ when filled with intrapipette solution: solution 1 (in mM, for most experiments): 135 Kgluconate, 5 KCl, 5 EGTA, 0.5 CaCl2, 10 HEPES, 2 Mg-ATP, and 0.1 GTP; solution 2 (in mM, for particular experiments as stated): 70 K gluconate, 65 KCl, 5 EGTA, 0.5 CaCl2, 10 HEPES, 2 Mg-ATP, 0.1 GTP, and 2 mM QX-314. The pH of these solutions was adjusted to 7.2 with Tris-base, and their osmolality was adjusted to 300 mOsm with sucrose. The junction potential between patch pipette and bath solutions was nulled just before gigascale formation. Series resistance was monitored without compensation throughout the experiment using the Multiclamp 700B. The data were discarded if the series resistance (15–30 MΩ) changed by >20% during whole-cell recordings.

The bath was continually perfused with artificial CSF. All recordings were done at a temperature of 32 ± 1°C.

### Anterograde neuronal tracing

Male adult C57BL/6 mice (3–4 months old) were anesthetized with isoflurane (4% for induction, 1.5%–2% for maintenance), placed on a heating pad, and immobilized on a stereotaxic frame (Stoeling) (Xiao et al., 2009a). An incision was made in the skin of the head to expose the skull, and a 0.4-mm-diameter hole, centered at 1.5 mm caudal to bregma and 1.5 mm right from midline, was drilled on the skull. The dura was torn with a sharp needle. A glass pipette with 50–60 μm tip was filled with 3% biotinylated-dextran amine (BDA-10,000, in saline), an anterograde tracer (InVitrogen), was held on a Hamilton syringe and mounted onto a microinjector (WPI). BDA (0.1 μl) was bilaterally injected in a spot (anteroposterior 1.5 mm; lateral 1.5 mm; dorsoventral 4.8 mm) in 0 min, and the glass pipette was gradually lifted up after injection. Each mouse was allowed to recover for 21 d before histology assay.

In vivo single-unit recording. Male adult C57BL/6 mice (3–4 months old) were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed on a heating pad, and mounted on a stereotaxic frame (Stoeling) (Xiao et al., 2009a). The skin of the head was opened to expose the skull, and a 2-mm-diameter hole, centered at 1.5 mm caudal to bregma and 1.5 mm right from midline, was drilled in the skull. The dura was torn with a sharp needle. Chloral hydrate was intraperitoneally injected at 100 mg/kg/30 min to maintain deep anesthesia (loss of toe pinch reflex).

Electrodes with a 3 μm tip were pulled from thick-wall borosilicate glass (WPI) with a horizontal micropipette puller (Sutter Instruments). When filled with 2 mM NaCl, the electrodes had resistance of 2–5 MΩ. The signals were amplified with Axoclamp 2A (Molecular Devices) and Brownlee Precision (model 440) amplifiers, sampled at 10 kHz with Digidata 1200 (Molecular Devices), controlled by pClamp 9.2 (Molecular Devices) software. We searched for spikes at the coordinates (lateral: 1.3, 1.5, 1.7 mm; bregma: −1.6, −1.8, −2.0 mm; ventral from bregma: 4.3–4.75 mm), according to the parasagittal planes in a mouse brain atlas (Paxinos and Franklin, 2001).

To confirm the recording sites, we added 0.2% neurobiotin in the intrapipette solution (2 μM NaCl), and injected 10 nA (5 min) using an Axoclamp 2A amplifier, at the end of each experiment. We performed histology to visualize neurobiotin (see Fig. 5A). The neurobiotin location was defined according to the mouse brain atlas and was used to calibrate our recording coordinates.

In some experiments, we applied acetylicholine (ACh) by iontophoresis. The recording/stimulating electrode contained 0.5 μM ACh in 2 μM NaCl. We applied −5 nA current to prevent ACh from leaking, and ACh was applied by 30 nA current injection (Armstrong and Lester, 1979).

### Immunohistochemistry

The C57BL/6 mice and α4-YFP mice were killed with CO2 and subject to cardiac perfusion with 7 ml PBS with heparin, and then with 35 ml 4% PFA in PBS. The mouse brain was removed, postfixed in 4% PFA in PBS at 4°C for 2 h, and sectioned into 40 μm slices with a Compressethe VH-200 (Precisionary). The slices were mounted onto microscope slides (Fisher Scientific), dried at room temperature, and frozen at −20°C. GFP antibody was used to detect the expression levels of α4-YFP or α4-GFP nAChRs, and Alexa-555-conjugated streptavidin was used to visualize neurobiotin. The brain sections were thawed and washed twice (10 min each) with cold PBS (4°C), permeabilized for 45 min in PBS/0.5% saponin, blocked for 45 min in PBS/10% donkey serum, incubated in primary antibodies (1:500, rabbit anti-GFP IgG, Invitrogen) or streptavidin (1:50) in PBS/4% donkey serum at 4°C for 18 h, washed 3 times (15 min each) in PBS, incubated in secondary antibody (1:500, Alexa-488-conjugated donkey anti-rabbit IgG, Invitrogen) in PBS/4% donkey serum at room temperature for 1 h. Samples were washed 3 times (10 min each) in PBS, dried at room temperature, and coverslipped with mounting medium (Vector Laboratories).

### Chronic nicotine treatment

Nicotine or vehicle (saline) was administered to mice (5- to 6-week-old) by subcutaneously implanted osmotic minipumps (model 1002 or 2002, Alzet) for 7 or 14 d to avoid repetitive handling, which causes stress (Marks et al., 1992; Collins et al., 1994; Marks et al., 2004; Nashmi et al., 2007). On the day of minipump implantation, vehicle or (−) nicotine hydrogen tartrate was prepared
were and increase of firing rate) between different groups of neurons.

Incidence of drug responsiveness (e.g., occurrence of inward currents parasagittal mouse brain slices (Fig. 1). We added 0.5 Li DL-2-amino-5-phosphonovalerate; methyl-erythroidine hydrobromide (DH

To investigate the heterogeneity of STN neurons, we examined 0.5 ACh experiments, by applying TTX and 20 mM CNQX did not change the amplitude of IACh (faster component: 99 ± 4% of control, n = 5, p = 0.83; slower component: 102 ± 5% of control, n = 7, p = 0.51). This indicates that these currents are mainly mediated by somatodendritic nAChRs, but not by presynaptic nAChRs in axonal terminals, which often facilitate neurotransmitter release (Dani and Bertrand, 2007; Exley and Cragg, 2008; Miwa et al., 2011).

A current decay that begins during the agonist application, then, outlasts the application, may occur by three mechanisms: receptor desensitization, deactivation of receptors due to agonist dissociation, agonist diffusion, or by a combination of these factors. Therefore, we sought to exclude the contribution of agonist dissociation or diffusion in the acute ACh experiments, by applying ACh for 3 s (Fig. 1C,D), so that the desensitization of nAChRs can be assessed in the sustained presence of ACh. Extending ACh application from 0.1 to 3 s prolonged the current decay by twofold to threefold, and also magnified the contrast between decay times of the two distinct neuronal populations that exhibit the faster and slower ACh currents. With prolonged agonist application, the two average time constants differed by ~9-fold (Fig. 1E).

nAChRs are pentameric ligand-gated ion channels and are composed of various combinations of subunits (Wooltorton et al., 2003; Xiao et al., 2009a; Changeux, 2010). The nAChRs containing α4β2 or α7 subunits are widely expressed in the CNS. They have different decay times, sensitivities to nicotine, and desensitization properties. To determine whether α4β2 or α7-containing nAChRs mediate the slower or faster-decaying IACh
in STN neurons, we tested the pharmacological properties of these currents (Fig. 2).

The slower-decaying I_{ACh} was blocked completely by 200 nm DHβE, a selective antagonist of β2-containing nAChRs (Fig. 2B). The neurons with slower-decaying I_{ACh} responded to RJR-2403, a somewhat selective antagonist for α4β2 nAChRs, but not to PNU-282987, a selective agonist for α7 nAChRs (Fig. 2C). In contrast, the faster-decaying I_{ACh} was inhibited by 10 nm MLA (Fig. 2B). These same neurons responded to PNU-282987, but not to RJR-2403 (Fig. 2C). These data indicate that α4β2 nAChRs predominantly mediated the slower-decaying I_{ACh}, whereas α7 nAChRs predominantly mediated the faster-decaying I_{ACh}.

We verified the presence of α4-containing (α4*) nAChRs in the STN by taking advantage of a knock-in mouse line in which fully functional α4 nAChR subunits contain yellow fluorescent protein (YFP) (α4-YFP) (Nashmi et al., 2007). We conducted imaging in live brain slices from these mice and observed YFP fluorescence in ~15% of STN neurons (see Fig. 5C), verifying the presence of α4* nAChRs in a subset of STN neurons. To further characterize α4* nAChR expression, we performed immunostaining of the α4* nAChR protein, using the antibody to a closely related protein to YFP, GFP, in serial parasagittal sections from fixed brains of α4-YFP mice. Using confocal microscopy, we detected α4* nAChRs in some STN neurons (Fig. 2A1–A3), and the numbers of YFP-positive STN neurons were comparable among parasagittal sections in series from medial to lateral.

To confirm the involvement of the α4 subunit in nAChRs mediating the slower-decaying I_{ACh}, we tested ACh responses in STN neurons of nAChR α4 subunit knock-out (α4-KO) mice. In 25 neurons from such mice, we detected only faster-decaying I_{ACh} but no slower-decaying current. Furthermore, the faster-decaying I_{ACh} occurred in a proportion (44%, 11 neurons, amplitude: 138 ± 38 pA; τ: 54 ± 10 ms) of neurons in α4-KO mice similar to that observed in wild-type mice (~40%). These data strongly suggest that the slower-decaying I_{ACh} in STN were predominantly mediated by α4* nAChRs.

Because nicotine induces desensitization of nAChRs on various time scales, we also tested the desensitization of I_{ACh} in the presence of 0.2 and 1 μM nicotine. Repetitive ACh (300 μM) applications at >3 min intervals evoked similar currents with <10% variation in peak amplitude (Fig. 2D), indicating that this protocol could prevent rundown of ACh responses. In the presence of nicotine, α4β2 nAChR-mediated I_{ACh} desensitized more quickly and more completely than α7 nAChR-mediated ones (Fig. 2D). The effects are concentration-dependent. Interestingly, after 15 min washout, α7 nAChR-mediated I_{ACh} recovered to >82% of initial level, but α4β2 nAChR currents showed little or no recovery (Fig. 2D).

These data indicated that α4β2* and α7 nAChRs predominantly mediated I_{ACh} in separate groups of STN neurons and might modulate neuronal activity in different patterns due to their deactivation and desensitization properties. These findings extend previous binding assays (Schulz et al., 1991; Pimlott et al., 2004).

We did not find significant difference in the membrane properties of STN neurons expressing α4β2*, α7, or no nAChRs (Table 1). In most STN neurons (no nAChRs: 73%, 16 of 22; α7: 70%, 14 of 20; α4β2: 75%, 15 of 20, \( x^2 = 0.0003, p = 0.98 \)), injection of a negative
current induced a hyperpolarization with a transient component, called voltage sag, due to hyperpolarization-induced inward current ($I_h$). The three types of neurons had comparable voltage sag. After the end of the hyperpolarizing pulse, we observed that the membrane potential transiently relaxed to a level more positive than the previous resting potential. This depolarization lasted a few hundred milliseconds in most STN neurons, as $I_h$ deactivated (no nAChRs: 86%, 19 of 22; α7: 70%, 14 of 20; α4β2: 65%, 13 of 20; $\chi^2 = 3.1$, $p = 0.08$). The time course of the relaxation did not differ among the three types of neurons.

To further address the modulation of microcircuits in STN by nAChRs, we next examined GABAergic and glutamatergic synaptic inputs and synaptic targets (the SN) of neurons that express either α4β2 or α7 nAChRs predominantly. To simplify the description, hereafter we refer to the two neuron types as α4β2 and α7 neurons, respectively.

### Table 1. Membrane properties of STN neurons expressing different nAChRs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No nAChRs</th>
<th>α7 nAChRs</th>
<th>α4β2 nAChRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mV)</td>
<td>n</td>
<td>Value</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>$-47.2 \pm 1.4$</td>
<td>20</td>
</tr>
<tr>
<td>Rm (MΩ)</td>
<td>22</td>
<td>863 ± 98</td>
<td>20</td>
</tr>
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| Action potential            | Frequency (Hz) | 18 | 4.5 ± 0.7 | 18 | 6.6 ± 0.8 | 18 | 4.3 ± 0.7 | 3.0 | 0.06 |
|                            | Half-width (ms) | 22 | 1.4 ± 0.1 | 20 | 1.1 ± 0.1 | 19 | 1.1 ± 0.1 | 1.97 | 0.14 |
|                            | Variability    | 22 | 0.26 ± 0.06 | 20 | 0.25 ± 0.03 | 19 | 0.20 ± 0.04 | 0.45 | 0.6 |
|                            | AHP (mV)       | 22 | $-14.8 \pm 0.6$ | 19 | $-17.8 \pm 1.1$ | 19 | $-17.1 \pm 0.8$ | 3.6 | 0.03 |

| Hyperpolarizing response    | Voltage sag (mV) | 16 | 10.4 ± 1.6 | 14 | 9.5 ± 1.8 | 15 | 9.5 ± 1.2 | 0.13 | 0.82 |
|                            | Rebound length (ms) | 19 | 447 ± 106 | 14 | 568 ± 87 | 13 | 331 ± 105 | 1.2 | 0.32 |

*Values are mean ± SE. Vm, Membrane potential; Rm, membrane resistance; AHP, afterhyperpolarization. Firing variability is the ratio of SD to average of firing rate. To measure Rm, we applied a 5 mV hyperpolarization (0.5 s) from a holding potential of $-50$ mV, and Rm was calculated from Ohm’s law. Voltage sag, transient component of the hyperpolarization in response to $-80$ nA current injection, caused by hyperpolarization-induced inward currents ($I_h$). In some neurons, a robust transient depolarization (rebound) occurred immediately after the end of the hyperpolarizing pulse. The duration of the depolarization is rebound length.

Synaptic inputs onto STN neurons are distinct between neuronal subtypes

STN neurons are influenced by both glutamatergic and GABAergic inputs. To test whether these synaptic inputs onto the STN are diverse among subtypes of STN neurons, we recorded spontaneous inward and outward PSCs ($V_T = -50$ mV), which were respectively blocked by 20 μM CNQX, an antagonist of non-NMDA glutamate receptors, and by GABAzine, an antagonist of GABA_A receptors (Fig. 3A). At the beginning of each experiment, we applied ACh onto the recorded neuron to identify the neuron type. Interestingly, α4β2 neurons exhibited a twofold higher frequency of sEPSCs ($U = 62$, $n_1 = 9$, $n_2 = 16$, $p = 0.006$, Mann–Whitney Rank Sum Test), but a threefold lower frequency of sIPSCs than α7 neurons ($U = 62$, $n_1 = 9$, $n_2 = 16$, $p < 0.001$, Mann–Whitney Rank Sum Test) (Fig. 3B).

We attempted to mitigate any potential recording artifacts that could arise from our recording conditions. The Nerst potential of $\text{Cl}^-$ is $-83$ mV, if we use a low $\text{Cl}^-$ (5 mM) intrappetite solution (see Materials and Methods). The electrochemical gradient, or driving force, of $\text{Cl}^-$ in this recording condition might be too small that some sIPSCs could be obscured in noise. This may result in underestimation of the sIPSC frequency. To confirm the reliability of the results, we increased the driving force for IPSCs with a higher $\text{Cl}^-$ (65 mM) intrappetite solution (see Materials and Methods). The sIPSCs were recorded from voltage-clamped STN neurons ($V_T = -65$ mV) in the presence of 20 μM CNQX and were blocked by 10 μM bicuculline, a GABA_A receptor antagonist. Under these recording conditions, we confirmed that sIPSC frequency in α4β2 neurons was threefold lower than that in α7 neurons (α4β2: $1.3 \pm 0.4$ Hz, $n = 7$; α7: $5.1 \pm 1.5$ Hz, $n = 20$, $U = 23$, $p = 0.01$, Mann–Whitney Rank Sum Test). These data suggest that α4β2 and α7 neurons differ in the strength of both GABAergic and glutamatergic inputs impinging on them.

To determine whether these differences were driven by differential firing patterns in presynaptic neurons, or by differential synaptic release properties, we next examined miniature IPSCs (mIPSCs) and miniature EPSCs (mEPSCs) when neuronal firing was eliminated by 0.5 μM TTX. We found that in both neuron types, mEPSCs or mIPSCs did not differ from sEPSCs or sIPSCs, respectively, in either frequency or amplitude (Fig. 3C). These data indicate that varying spike frequency in the axonal terminals did not appear to contribute to the difference in synaptic strength between α4β2 and α7 neurons.

We electrically stimulated the dorsal part of the STN and recorded EPSCs or IPSCs in STN neurons at the reversal potential of $\text{Cl}^-$ ($-80$ mV) or of cations (0 mV) (Fig. 3D). Using electrical stimulation giving maximal responses (0.2 ms, 0.5 mA), we quantified the excitatory and inhibitory inputs to the STN neurons when we clamped membrane potential at $-80$ and 0 mV, respectively. We observed larger evoked EPSCs but smaller evoked IPSCs in α4β2 neurons than in α7 neurons (Fig. 3E). The data suggest that α4β2 neurons received stronger action potential-dependent glutamatergic inputs but less GABAergic inputs than α7 neurons.

To address the circuit integration of glutamatergic and GABAergic inputs onto STN neuron subsets, we recorded evoked PSCs (ePSCs) at $-50$ mV, which was close to the baseline membrane potentials of STN neurons. We observed PSCs that were outward in some cells (e.g., Fig. 3F1) and inward in others (Fig. 3G1); these were apparently compound, with two peaks (Fig. 3F1) or a shoulder (Fig. 3G1). For cells where electrical stimulation evoked an outward eEPSC in the voltage-clamp mode, electrical stimulation delayed spontaneous spikes (lengthened interspike intervals) (Fig. 3F2). Conversely, for cells where it evoked an inward ePSC in voltage-clamp mode, the electrical stimulation accelerated spontaneous spikes (shortened interspike intervals) (Fig. 3G2).

The electrical stimulation-induced outward currents were rendered inward by 10 μM bicuculline, and the waveform became monophasic. These remaining inward currents were blocked by 20 μM CNQX (Fig. 3F1). These data suggest that the outward ePSCs indicated predominance of GABAergic inputs, whereas the inward ePSCs indicated predominance of glutamatergic inputs. Indeed, the resulting compound ePSCs was significantly correlated with eEPSCs, measured at $-80$ mV ($r = 0.56$, $n = 25$, $p = 0.001$, Spearman rank order correlation), and with eIPSCs,
measured at 0 mV (r = 0.55, n = 25, p = 0.0008, Spearman rank order correlation). The ePSC amplitude was significantly correlated with the alteration of interspike intervals (r = 0.74, n = 23, p < 0.0001; Spearman rank order correlation).

We observed that local electrical stimulation led to more frequent and stronger excitation of α4β2 neurons but caused more frequent inhibition of α7 neurons (Fig. 3H1, H2). Thus, local stimulation caused excitation in a higher percentage of α4β2 neurons (11 of 14, 79%) than of α7 neurons (8 of 19, 42%) (χ² = 4.39, p = 0.04). Furthermore, instantaneous firing rates were increased to 262 ± 35% of control in α4β2 neurons, and to 146 ± 10% of control in α7 neurons (p = 0.001, α4β2 vs α7 neurons, Mann–Whitney Rank Sum Test) (Fig. 3H1). In contrast, local stimulation led to inhibition in a lower percentage of α4β2 neurons (3 of 14, 21%) than of α7 neurons (11 of 19, 58%) (χ² = 4.39, p = 0.04). The α4β2 and α7 neurons were similarly inhibited to 52 ± 7% and 56 ± 5% of control firing rates, respectively (not significantly different) (Fig. 3H2). These results support the notion that α4β2 neurons receive a greater proportionate share of excitation, and α7 neurons receive greater inhibition.

**α4β2 and α7 nAChRs in STN neurons differentially modulate glutamate release onto SN neurons**

We next wanted to understand whether the differences in the two STN populations had functional consequences downstream to their targets. We therefore turned our investigations to the targets of STN neurons, the SN. We stereotaxically injected 0.1 μl BDA (10,000, 3% in saline), an anterograde tracer, in mouse STN. After 3 weeks recovery, we detected BDA-containing fibers in both the SNr and SNc (Fig. 4A, A2), consistent with previous studies showing that STN neurons project to the SN.

As mentioned previously, STN neurons are mostly glutamatergic (Smith and Parent, 1988). To test whether synaptic projection of STN neurons to SNr and SNc are preserved in the slice preparation, we recorded evoked-EPSCs from either SNr
GABAergic or SNc DA neurons by electrically stimulating STN in the presence of 10 μM bicuculline. Evoked EPSCs were detected in all tested neurons in SNr (n = 4) and SNc (n = 3), and these EPSCs were blocked by adding 20 μM CNQX (Fig. 4B2, B3). These results confirmed that STN glutamatergic projections to the SN were at least partially preserved in the parasagittal slices.

We activated either α4β2 or α7 neurons with specific agonists and recorded responses in the SN. We focally ejected 50 μM RJR-2403 in STN for 5 s to stimulate α4β2 neurons, or 20 μM PNU-282987 in STN for 5 s to stimulate α7 neurons. We recorded sEPSCs from voltage-clamped SNc dopaminergic neurons or SNr GABAergic neurons (VTH = −65 mV). The frequency of sEPSCs in SNr GABAergic neurons (4.6 ± 0.7, n = 14) is 2.5-fold higher than in SNc DA neurons (1.9 ± 0.3, n = 11) (df = 23, t = 3.57, p = 0.002, unpaired t test). Local ejection of RJR-2403 in STN increased sEPSC frequency in SNr GABAergic neurons to 157 ± 6% of baseline (n = 17, p < 0.0001; Fig. 4D1, F1), but in SNc DA neurons to only 113 ± 7% of baseline (n = 15, p = 0.6; Fig. 4E1, F1) (df = 30, t = 3.39, p = 0.0009, SNc vs SNr, unpaired t test). Local ejection of PNU-282987 in STN had an opposite profile, increasing sEPSC frequency in SNr GABAergic neurons to 123 ± 7% of baseline (n = 14, p = 0.006; Fig. 4D2, F2), but to 160 ± 8% of baseline (n = 10, p < 0.0001; Fig. 4E2) in SNc DA neurons (df = 22, t = 6.1, p < 0.0001, SNc vs SNr; Fig. 4F2). These data suggest that α4β2 neurons preferentially stimulate downstream SNr GABAergic neurons, whereas α7 neurons preferentially stimulate SNc dopaminergic neurons.

Using the same puffing procedure, we applied a fluororescent dye, Dil, into the STN for 5 s, and it spread spherically, to a radius of ~50 μm (~1/10 of STN). We applied the agonists at their EC50, which may not maximally activate nAChRs.

Figure 4. α4β2 and α7 neurons differentially modulate SN neurons. A1, A2, BDA (red), an anterograde tracer, injected in STN, was transported to the SN. Green neurons were TH-positive (A1).

B1, A stimulating electrode (Stim) and a recording electrode (Rec) were placed in STN and SNc or SNr, respectively. B2, B3, In the presence of 10 μM bicuculline, electrical stimulation in STN evoked CNQX-sensitive EPSCs in both SNc (B2) and SNr neurons (B3). C, Either RJR-2403 (RJR, 50 μM) or PNU-282987 (PNU, 20 μM) was locally applied (5 s) to alternatively activate α4β2 or α7 nAChRs in STN. Meanwhile, sEPSCs were recorded from either an SNr neuron or an SNc neuron. Either RJR-2403 (D1, E1, F1) or PNU-282987 (D2, E2, F2) in STN increased sEPSC frequency in SNr neurons. PNU-282987 (D2, E2, F2), but not RJR-2403 (D1, E1, F2) in STN, increased sEPSC frequency in SNc neurons. B1, C, The rectangles in SNc and SNr indicate the area containing the recorded neurons.
nAChRs modulate firing rates of STN neuron subsets in vivo

We performed in vivo single-unit recordings to test the expectation that STN neurons containing nAChRs would respond to local ACh application in vivo. During single-unit recordings, ACh (0.5 mM) was delivered by iontophoresis (30 nA for 10 s) (Fig. 5A, B). We observed that ACh stimulated 21% (17 of 81) of neurons (Fig. 5B).

To address receptor subtypes mediating in vivo ACh responses, we tested the effects of ACh iontophoresis on the firing rate of STN neurons in brain slices. In this set of experiments, we used α4-YFP mice in which α4-YFP neurons were visibly fluorescent (Fig. 5C1). We consistently observed that ACh stimulated α4-YFP neurons (Fig. 5C2, top), but not other neurons (Fig. 5C2, bottom). Atropine perfusion (0.5 mM, 5 min) altered ACh responses in neither α4-YFP (before atropine: 186 ± 30% of pre-
Figure 6. Chronic nicotine upregulates α4β2 nAChRs in STN. α4-YFP was visualized with GFP antibody staining (A, B). More α4-YFP-positive neurons were found in STN of chronic nicotine-treated mice (B) than chronic vehicle-treated mice (A). A, B. Insets, 60× images. C. The number of neurons per STN section was greater in chronic nicotine-treated mice. D1, D2. The integrated YFP density in STN neurons was higher in chronic nicotine-treated mice. E. STN neurons in chronic nicotine-treated (14 d) mice (Nicotine) had larger α4β2 nAChR currents (df = 43, t = 2.77, p = 0.009, unpaired t test; K-S statistics, 0.45, p < 0.05) but similar α7 nAChR currents, compared with those in chronic vehicle-treated mice (Vehicle). F. The 14 d nicotine treatment increased the proportion of α4β2 neurons (black stack) but decreased that of neurons not responding to ACh (dark gray stack) (df = 2, χ² = 10.7, p < 0.01). G. The 3 μM ACh-induced currents in α4β2 neurons of 14 d nicotine-treated (right) mice, but not in those of 14 d vehicle-treated (left) mice. H. The 14 d nicotine treatment increased spontaneous firing rate in α4β2 neurons (df = 29, t = 3.39, p = 0.02, unpaired t test), but not in α7 neurons (df = 31, t = 1.1, p > 0.05). M, N. In chronic vehicle- and nicotine-treated mice, ACh iontophoresis in vivo stimulated 21% (17 of 81) and 44% (38 of 86), respectively, of STN neurons (df = 1, χ² = 10.1, p < 0.01). Spontaneous firing rate was higher in STN neurons stimulated by ACh iontophoresis in chronic nicotine-treated mice than those in chronic vehicle-treated mice (df = 53, u = 196, p < 0.01, Mann–Whitney test). Numbers of neurons are indicated in each box. *p < 0.05; **p < 0.01.

ACh, n = 6; after atropine: 238 ± 48% of pre-ACh, n = 5; t = 1.89, p = 0.12) nor non-α4β2 (before atropine: 108 ± 6% of pre-ACh, n = 11; after atropine: 97 ± 6% of pre-ACh, n = 5; t = 1.52, p = 0.15) neurons. These data suggest that iontophoresis delivered ACh at low concentrations, which could activate α4β2 nAChRs, but not the less sensitive α7 nAChRs.

If STN neurons interact, ACh-stimulated α4β2 neurons, then, could excite nearby STN neurons in vivo. To test this possibility, we recorded pairs of neurons 50–100 μm apart (Fig. 5D1). In each neuron pair, we injected 10 ms 50–80 pA currents to stimulate a single spike in the current-clamped neuron and recorded signals from the voltage-clamped neuron (VTh = −50 mV) (Fig. 5D2). The time-locked current in the voltage-clamped neuron initiating within 0.1–0.5 ms after the peak of spike in the current-clamped neuron indicates monosynaptic connections (Berry and Pentreath, 1976). We ran 10 trials at 15 s intervals in each pair, switched recording modes in two neurons, and ran 10 trials again. In 78 pairs of STN neurons, we elicited no time-locked postsynaptic current (Fig. 5D3). These data suggest that STN neurons may not form synapses with nearby neurons (<100 μm).

These results indicate that we could use ACh stimulation as an approach to identify α4β2 neurons in STN in vivo. Indeed, our in vivo and in vitro recordings revealed a similar percentage (20%) of α4β2 neurons in STN.

Chronic nicotine modifies nicotinic responses in STN neurons

Previous studies show that chronic nicotine selectively upregulates α4β2, but not α7 nAChRs, in midbrain GABAergic neurons (Nashmi et al., 2007; Xiao et al., 2009a). If upregulation also occurs in STN, this could enhance the activity in α4β2 neurons. To address this issue, we infused vehicle or nicotine into α4-GFP mice with osmotic minipumps for 7 or 14 d and counted fluorescent neurons and measured fluorescent intensity in STN. Chronic nicotine increased the number of fluorescent neurons in STN (Fig. 6A–C) and the average fluorescent intensity of each neuron (Fig. 6D1, D2).

We also compared nicotinic currents in STN neurons between chronic vehicle- and nicotine-treated mice (Fig. 6E; Table 2). We detected larger α4β2 currents, but similar α7 currents, in STN neurons from 14 d nicotine-treated mice, than in those from 14 d vehicle-treated mice (Fig. 6E; Table 2). Because 300 μM ACh activates the majority of α4β2 nAChRs, and >50% of α7 nAChRs (Chavez-Noriega et al., 1997; Gerzanich et al., 1997), the evoked responses likely reflect the number of nAChRs.

Interestingly, both 7 and 14 nicotine-treated mice had increased proportions of α4β2 neurons, accompanied by decreased proportion of non-nAChR neurons (Table 3; Fig. 6F). For interpretation of this phenomenon, we propose that some neurons contain α4β2 nAChRs that produce little or no agonist-induced current, either because they are intracellular, otherwise inactive, or simply too sparse on the membrane. Chronic nicotine increases the plasma membrane population of these receptors so that nicotinic currents can be detected electrophysiologically.

After 3 weeks withdrawal, the percentage of α4β2 neurons was still significantly higher in nicotine-treated mice than that in vehicle-treated mice (Table 3). However, ACh-induced current size in chronic nicotine-treated mice became the same as that in chronic vehicle-treated mice (Table 2). The recovery in current

Table 2. Chronic nicotine effects on ACh currents in STN neurons

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
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<td>α4β2</td>
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<tr>
<td>α4β2</td>
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<td>46 ± 7</td>
<td>23</td>
<td>73 ± 7</td>
<td>0.009</td>
</tr>
<tr>
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<td>19</td>
<td>94 ± 19</td>
<td>21</td>
<td>82 ± 15</td>
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<tr>
<td>α4β2</td>
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<td>15</td>
<td>43 ± 11</td>
<td>0.55</td>
</tr>
<tr>
<td>α7</td>
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<td>93 ± 19</td>
<td>9</td>
<td>88 ± 22</td>
<td>0.86</td>
</tr>
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</table>
size of α4β2 nAChRs after nicotine withdrawal supports the notion that nicotine upregulation of nAChRs was reversible.

An extensive electrophysiological literature, mostly with nAChRs expressed in clonal cell lines, suggests that α4β2 nAChRs exist in two forms (Buisson and Bertrand, 2001; López-Hernández et al., 2004; Moroni et al., 2006; Son et al., 2009). These are termed high-sensitivity (EC50 ~ 1 μM) and low-sensitivity (EC50 ~ 90 μM) forms. The high-sensitivity form is capable of being activated by the ambient ACh and can be upregulated by chronic exposure to nicotine (Son et al., 2009; Henderson et al., 2014). Within the technical limitations of puff application in the slice preparation, we studied this point for STN neurons. We chose to study the responses to 3 μM ACh, which activates some high-sensitivity but essentially no low-sensitivity α4β2 nAChRs. In such cases, an increased proportion likely reflects enhanced sensitivity (rather than simple number) of upregulated α4β2 nAChRs. We applied 3 μM ACh to STN neurons from both 14 d nicotine- and vehicle-treated mice that responded to 300 μM ACh with slow currents (Fig. 6G). We detected 3 μM ACh-evoked current in only 2 of 6 neurons of vehicle-treated mice (3.7 ± 2.5% of 300 μM ACh-induced currents, n = 6), but in all of 6 neurons from nicotine-treated mice (13.4 ± 0.6% of 300 μM ACh-induced currents, n = 6) (p = 0.01, chronic nicotine vs chronic vehicle, Mann–Whitney Rank Sum Test). The data are consistent with the notion that chronic nicotine upregulates high-sensitivity α4β2 nAChRs and can explain that spontaneous firing rate of α4β2 neurons was significantly higher in 14 d nicotine-treated mice than in 14 d vehicle-treated mice (Fig. 6H), suggesting that α4β2 nAChRs on these neurons have an enhanced response to ambient ACh.

To test whether chronic nicotine upregulation of α4β2 nAChRs in STN neurons modifies neural circuits and neuronal activity in vivo, we conducted in vivo single-unit recordings from either chronic vehicle- or nicotine-treated mice. We observed that chronic nicotine treatment increased the proportion of neurons being stimulated by ACh iontophoresis (chronic vehicle: 21%, 17 of 81; chronic nicotine: 44%, 38 of 86; χ² = 10.1, p = 0.0011) (Fig. 6HJ) and that spontaneous firing rates in ACh-stimulated neurons were higher in chronic nicotine-treated mice than in vehicle-treated mice (Fig. 6HJ).

Synaptic inputs to STN neurons are subject to regulation by dopamine (Shen and Johnson, 2000; Heida et al., 2008; Marani et al., 2008). Chronic exposure to nicotine, by affecting dopamine levels (Rahman et al., 2004; Lester et al., 2009; Zhang et al., 2012; Koranda et al., 2014), could affect synaptic inputs to STN neurons. To test this possibility, we analyzed spontaneous post-synaptic currents in STN neurons in chronic vehicle- and nicotine-treated mice. The sIPSC and sEPSC frequency in α4β2 and α7 nAChs did not significantly differ between chronic vehicle-treated mice (α4β2 neurons: sIPSCs, 1.6 ± 0.3 Hz, n = 19; sEPSCs, 2.8 ± 0.5 Hz, n = 19; α7 neurons: sIPSCs, 8.0 ± 2.0 Hz, n = 19; sEPSCs, 1.6 ± 0.2 Hz, n = 19) and nicotine-treated mice (α4β2 neurons: sIPSCs, 1.9 ± 0.5 Hz, n = 10; sEPSCs, 3.4 ± 0.8 Hz, n = 10; α7 neurons: sIPSCs, 11.2 ± 2.8 Hz, n = 15; sEPSCs, 2.4 ± 0.3 Hz, n = 15). Moreover, the ratios of sIPSC frequency to sEPSC frequency in both α4β2 and α7 neurons were similar in chronic vehicle- and nicotine-treated mice (α4β2 neurons, chronic vehicle: 0.71 ± 0.11, n = 19; chronic nicotine: 0.75 ± 0.19, n = 10, p = 0.84, Mann–Whitney Rank Sum Test) (α7 neurons, chronic vehicle: 8.2 ± 3.1, n = 19; chronic nicotine: 7.6 ± 3.1, n = 15, p = 0.59, Mann–Whitney Rank Sum Test). These data suggest that chronic nicotine may not alter the excitatory and inhibitory synaptic inputs impinging on two populations of STN neurons.

**Discussion**

α4β2 and α7 nAChRs regulate separate microcircuits in STN

We provide electrophysiological and pharmacological evidence showing two distinct microcircuits operating within the STN and diverging into SNr and SNC. One, enhanced by the activation of α7 nAChRs, preferentially forms glutamatergic synapses onto SNC dopaminergic neurons. The other one, augmented by the activation of α4β2 nAChRs, preferentially targets SNr GABAergic inhibitory neurons. Selective stimulation of the two microcircuits may stimulate and inhibit, respectively, SNC dopaminergic neurons.

Previous studies show that a large proportion of SN neurons contain both α4β2 and α7 nAChRs (Wooltorton et al., 2003; Xiao et al., 2009a), but in the STN, α4β2 and α7 nAChRs separately exist in two distinct neuron populations (Figs. 2 and 7). STN neurons containing α4β2 nAChRs displayed slower kinetics than STN neurons containing α7 nAChRs in both deactivation and desensitization (Figs. 1 and 2). Thus, the activation of α4β2 and α7 nAChRs excited STN neurons on the time scales of >1 s and <1 s, respectively (Fig. 1). Our data also show that α4β2 nAChRs were only partially desensitized during agonist application at moderate concentrations (e.g., 300 μM) for several seconds (Fig. 1C), leading to maintained membrane depolarization and maintained elevation of neuronal firing rates. Given that α4β2 nAChRs ex-
hibit higher sensitivity to ACh, we suggest that, in STN, α4β2 neurons might be among the major targets of PPTg stimulation, which boosts therapeutic effects of STN stimulation in late-phase PD (Stefani et al., 2007).

Beta oscillations, comprised of synchronized firings, occur in the STN in both PD patients and parkinsonian animals (Brown et al., 2001; Magill et al., 2004; Priori et al., 2004; Brown and Williams, 2005; Weinberger et al., 2006). High-frequency optogenetic stimulation in cortical glutamatergic inputs to STN, but not in STN neurons themselves, disrupts beta oscillations and improves motor symptoms in parkinsonian animals (Gradinaru et al., 2009). It is likely that glutamatergic inputs vary among neurons, so that stimulation of glutamatergic inputs would cause differential excitation among neurons and disrupt the oscillation. Our data showing that α4β2 neurons receive more glutamatergic inputs than α7 neurons (Fig. 3 B, D, E) provide cellular and synaptic evidence for this point.

In STN neurons, we also observed >2-fold less GABAergic inputs in α4β2 than in α7 neurons (Fig. 3 B, D, E). As GABAergic terminals synapsing onto STN neurons originate in the globus pallidus (Loucif et al., 2005; Benarroch, 2008; Marani et al., 2008), these data suggest that α7 neurons receive more GABAergic inputs than α4β2 neurons (Fig. 7).

We found that α4β2 neurons receive more glutamatergic inputs and less GABAergic inputs compared with α7 neurons (Figs. 3 and 7). Electrical stimuli in STN increased firing rate in more α4β2 neurons than α7 neurons (79% vs 42%) while inhibiting firing rate in more α7 neurons than α4β2 neurons (58% vs 21%) (Fig. 3H2). This suggests that local electrical stimulation may differentially alter neuronal activity in STN.

α4β2 and α7 neurons were disparate, not only in synaptic inputs, but also in their major synaptic targets. Stimulating α4β2 neurons caused a greater enhancement in glutamatergic inputs to SNr GABAergic neurons, but stimulating α7 nAChRs caused a greater enhancement in glutamatergic input to SNc DA neurons (Fig. 4). Because SNr GABAergic neurons inhibit SNc DA neurons (Tepper et al., 1995), selectively enhancing the activity of α4β2 neurons in STN may indirectly inhibit SNc DA neurons. These data extended the previous finding that electrically stimulating STN neurons induces a complex postsynaptic potential waveform in SNc DA neurons, with an STN-SNc monosynaptic glutamatergic EPSP and an STN-SNr-SNc polysynaptic GABAergic IPSP (Iribe et al., 1999).

Considering our data shown in Figures 1, 2, 3, and 4 with those in Figure 5D2, showing no synaptic interaction between STN neurons, we propose that, in the STN, there are at least two rather divergent microcircuits involving α4β2 or α7 neurons (Fig. 7). As local electrical stimulation lacks selectivity toward synaptic projections from different nuclei, further investigations are warranted to use cell type-specific manipulations to characterize the differential inputs from motor cortex/PPTg and from the globus pallidus/SNr, which is essential to fully address the circuit patterns.

Some nAChRs containing α4 and β2 subunits govern voluntary movement, via activation of these nAChRs on SNc DA neurons and on DA terminals in the striatum (Labarca et al., 2001; Tapper et al., 2004; Nashmi et al., 2007; Drenan et al., 2008, 2010; Xiao et al., 2009a; Quik and Wonacott, 2011; Drenan and Lester, 2012). Our data provide an additional pathway: a subpopulation of STN neurons can be stimulated by nicotinic agonists selective for α4β2* nAChRs and may consequently excite SNr GABAergic neurons, which could increase inhibitory tone to SNc DA neurons. This pathway might be important in fine-tuning the activity of SNc DA neurons.

**Chronic nicotine modifies α4β2 nAChRs in STN neurons**

Similar to previous studies in other brain regions (Nashmi et al., 2007; Lester et al., 2009; Son et al., 2009; Xiao et al., 2009a; Srinivasan et al., 2011; Henderson et al., 2014), chronic nicotine up-regulated both the number and sensitivity of α4β2* nAChRs in STN (Fig. 6D1, D2, E; Table 2). Interestingly, chronic nicotine increased the percentage of α4β2-containing neurons; thus, there were functional nAChRs in neurons not previously responding to ACh (Fig. 6F, H2; Table 3). These results can be explained by previous findings show that many α4β2 nAChRs reside in intracellular organelles and that nicotine acts as a pharmacological chaperone to facilitate receptor assembly, so that additional receptors appear both in organelles and on the plasma membrane (Lester et al., 2009; Miwa et al., 2011; Richards et al., 2011; Srinivasan et al., 2011, 2012; Henderson et al., 2014). This upregulation may especially occur in somatodendritic regions, which give the largest signals in whole-cell recordings.

The upregulation of α4β2* nAChRs by chronic nicotine enhanced responses to basal levels of ACh and increased firing rate in α4β2 neurons, but not in α7 neurons (Fig. 6F, H2). Because chronic nicotine changed synaptic inputs neither in α4β2 neurons nor in α7 neurons, the increased firing in α4β2 neurons by chronic nicotine might be primarily mediated by the upregulated nAChRs in the somata or dendrites of these neurons. According to the neural circuit diagram in Figure 7, the contrast in chronic nicotine effects on α4β2 and α7 neurons could exaggerate glutamatergic inputs to SNr GABAergic neurons over those to SNc DA neurons. The effect is demonstrated by our observation that, compared with vehicle-treated mice, chronic nicotine-treated mice had higher sEPSC frequency in SNr GABAergic neurons (vehicle: 5.0 ± 0.6 Hz, n = 18; nicotine: 8.3 ± 1.1 Hz, n = 13, p = 0.006), but similar sEPSC frequency in SNc DA neurons (vehicle: 1.2 ± 0.3 Hz, n = 10; nicotine: 1.5 ± 0.4, n = 8; p = 0.24). Together with the fact that chronic nicotine upregulates α4β2* nAChRs in SNr GABAergic neurons, but not SNc DA neurons (Nashmi et al., 2007; Xiao et al., 2009a), we propose that chronic nicotine enhances the activity of SNr GABAergic neurons through upregulating α4β2* nAChRs in both their somata and presynaptic STN glutamatergic neurons. This consequently could lead to a suppression and protection of SNc DA neurons (Nashmi et al., 2007; Lester et al., 2009; Xiao et al., 2009a) because GABAergic inhibition protects neurons from excitotoxicity (Luo et al., 2002).

That chronic nicotine suppresses firing in SNc DA neurons is consistent with decreased dopamine release in the striatum of chronic nicotine-treated animals, observed in previous in vivo microdialysis and in vivo cyclic voltammetry studies (Rahman et al., 2004; Zhang et al., 2012; Koranda et al., 2014). In addition, in chronic nicotine-treated animals, even when DA neuron firing frequency is directly controlled by stimulating the SN, impulse-evoked striatal dopamine release is almost as low as in β2 knock-out mice (Koranda et al., 2014). It was suggested that unknown adaptive mechanisms associated with chronically desensitized nAChRs, or with these low striatal DA levels, participate in the apparent neuroprotective effects of chronic exposure to tobacco products (Koranda et al., 2014).

The upregulation of α4β2* nAChRs in STN leads to a selective enhancement of the activity in α4β2 neurons (Fig. 6H1, H2). This consequently could decrease the probability of synchronized firing, which is associated with increased beta oscillations in local field potentials, motor symptoms, and L-dopa-induced dyskinesia in PD (Kreiss et al., 1997; Lozano et al., 2000; Levy et al., 2001, 2002; Weinberger et al., 2006). This could be a possible circuit mechanism underlying the phenomenon that chronic nicotine
and avoid the disadvantages of nicotine: lack of selectivity for nAChR subtypes and rapid metabolism.

References


recording in Parkinson’s disease patients with dyskinesias induced by apomorphine. Ann Neurol 47 [Suppl]:S141–S146.


