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# Dopaminergic and cholinergic modulation of striatal tyrosine hydroxylase interneurons

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ABSTRACT

The recent electrophysiological characterization of TH-expressing GABAergic interneurons (THINs) in the neostriatum revealed an unexpected degree of diversity of interneurons in this brain area (Ibáñez-Sandoval et al., 2010, Unal et al., 2011, 2015). Despite being relatively few in number, THINs may play a significant role in transmitting and distributing extra- and intrastriatal neuromodulatory signals in the striatal circuitry. Here we investigated the dopaminergic and cholinergic regulation of THINs *in vitro*. We found that the dominant effect of dopamine was a dramatic enhancement of the ability of THINs to generate long-lasting depolarizing plateau potentials (PPs). Interestingly, the same effect could also be elicited by amphetamine-induced release of endogenous dopamine suggesting that THINs may exhibit similar responses to changes in extracellular dopamine concentration *in vivo*. The enhancement of PPs in THINs is perhaps the most pronounced effect of dopamine on the intrinsic excitability of neostriatal neurons described to date. Further, we demonstrate that all subtypes of THINSs tested also express nicotinic cholinergic receptors. All THIS responded, albeit differentially, with depolarization, PPs and spiking to brief application of nicotinic agonists. Powerful modulation of the nonlinear integrative properties of THINs by dopamine and the direct depolarization of these neurons by acetylcholine may play important roles in mediating the effects of these neuromodulators in the neostriatum with potentially important implications for understanding the mechanisms of neuropsychiatric disorders affecting the basal ganglia.



## 1. Introduction

Until recently, the neostriatum has been thought to contain only a few types of GABAergic interneurons in comparison to the large diversity of GABAergic cell types in the neocortex or the hippocampus (Freund and Buzsáki, 1996; Tepper et al., 2010; DeFelipe et al., 2013). This picture changed significantly with the introduction of transgenic reporter mouse lines that revealed the existence of 5 new electrophysiologically distinct cell types, more than doubling the number of interneuron classes recognized in this brain area (Ibáñez-Sandoval et al., 2010; Ibáñez-Sandoval et al., 2011; Unal et al., 2011). In addition to a neuropeptide Y (NPY) expressing neuron described in a NPY-GFP line (Ibáñez-Sandoval et al., 2011), the newly discovered interneurons include 4 additional types of GABAergic neurons that were termed TH-interneurons (THINs) reflecting their initial identification in a TH-EGFP strain (Ibáñez-Sandoval et al., 2010; Unal et al., 2011). The function of THINs remains unclear. Their small population size and connectivity place some important constraints on the possible function of these neurons. On particularly interesting possibility is that these neurons may distribute intra- and extrastriatal neuromodulatory signals to projection neurons.

In the neostriatum, dopamine (DA) and acetylcholine (ACh) are 2 major neuromodulators that exert pronounced effects on most functions of the basal ganglia. Here we investigated how these neuromodulators or control the intrinsic electrophysiological properties of THINs. Since preliminary experiments indicated that the most salient effect of these modulators was the triggering and enhancement of a semi-stable depolarizing state we characterized in more detail this important dynamic feature of THINs.

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## 2. Materials and methods

### 2.1. Subjects

We used transgenic mice Tg (Th-EGFP) DJ76Gsat/Mmmc (GENSAT; Gong et al., 2003), obtained from the Mutant Mouse Regional Resource Center at UCLA and bred in our colony at Rutgers for all experiments. Hemizygous progeny were mated to wild type FVB or Swiss Webster mice each generation thereafter. All offspring were genotyped from tail samples and only those expressing the EGFP transgene were used in these experiments. Henceforth these mice are referred to as EGFP-TH mice.

All procedures were performed with the approval of the Rutgers University Institutional Animal Care and Use Committee and in accordance with the NIH *Guide to the Care and Use of Laboratory Animals* and all efforts were made to minimize the number of mice used and any possible discomfort.

### 2.2. Preparation of brain slices

Experiments were performed on brain slices obtained from adult EGFP-TH mice older than one month of age. Mice were deeply anesthetized with 150 mg/kg ketamine and 30 mg/kg xylazine i.p. and transcardially perfused with ice-cold, modified Ringer's solution containing (in mM) 248 sucrose, 2.5 KCl, 7 MgCl2, 23 $NaHCO_3$, 1.2 $NaH_2PO_4$, 7 glucose, 1 ascorbate, 3 pyruvate, and bubbled with 95% $O_2$ and 5% $CO_2$ (pH 7.3). The brain was quickly removed into a beaker containing ice-cold oxygenated Ringer's and trimmed to a block containing the striatum. Coronal or para-horizontal sections (250–300 μm) were cut in the same medium using a Vibratome 3000 and immediately transferred to normal Ringer's solution containing (in mM) 124 NaCl, 2.5 KCl, 1.2 $NaH_2PO_4$, 26 $NaHCO_3$, 1.3 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 1 ascorbate, 3 pyruvate, and 0.4 myo-inositol that was heated to 34 °C and continuously bubbled with 95% $O_2$ and 5% $CO_2$ (pH 7.3) for 1 h prior to recording and then maintained at room temperature until use. In some experiments, we substituted in an equimolar manner: choline Cl for NaCl, choline bicarbonate for $NaHCO_3$ and sucrose for $CaCl_2$. Slices were transferred to the recording chamber and submerged in continuously flowing oxygenated buffer (2–4 ml/min) which was heated with an inline heater (Warner Instruments) to approximately 33 °C.

### 2.3. Fluorescence and DIC imaging and recording

Slices were initially visualized under epifluorescence illumination with a high sensitivity digital frame transfer camera (Cooke SensiCam) mounted on an Olympus BX50-WI epifluorescence microscope and a 40× long working distance water immersion lens. Once an EGFP-TH neuron was identified, visualization was switched to infrared differential interference contrast (IR-DIC) microscopy for the actual patching of the neuron, usually performed under current clamp.

Micropipettes for whole cell recording were constructed from 1.2 mm o.d. and 0.94 mm i.d. borosilicate pipettes (Harvard apparatus) on a Narishige PP-83 vertical puller. The standard internal solution for whole cell current clamp recording was (in mM): 130 K gluconate, 10 NaCl, 2 $MgCl_2$, 10 HEPES, 3 $Na_2ATP$, 0.3 GTP, 1 EGTA plus 0.1–0.3% biocytin (pH 7.3–7.4). These pipettes typically exhibited a DC impedance of 4–6 MΩ measured in the recording chamber.

Current clamp recordings were made with a Neurodata IR-283 current clamp amplifier and voltage and/or current clamp recordings were made with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) whose output was filtered online with a second order Bessel filter at 1 kHz and digitized at 20-40 KHz with either a CED Micro 1401 Mk II and a PC running Signal v. 4 (Cambridge Electronic Design, Cambridge UK) or an ITC-16 and a Mac running Axograph. Acquired data was stored on a PC or Mac for offline analysis.

At the completion of the experiments slices containing biocytin-injected neurons were fixed by immersion in 4% paraformaldehyde-0.5% glutaraldehyde for 30 min at room temperature or microwaved to 60 °C for 12 s and stored overnight at 4 °C.

### 2.4. Pharmacology

Drugs were applied in the perfusion medium or locally via a micropipette using a Picospritzer (General Valve, Fairfield, NJ), at 20 psi/30–100 ms, at 0.1 Hz. Cobalt chloride, nimodipine, flufenamic acid, SCH-23390 hydrochloride, SKF-38393 hydrochloride, dopamine, choline bicarbonate and amphetamine were purchased from Sigma–Aldrich and tetrodotoxin (TTX), carbamylcholine chloride (carbachol), mecamylamine hydrochloride (MEC), Dihydro-β-erythroidine hydrobromide (DHβE) and methyllycaconitine citrate (MLA) were purchased from Tocris. Cobalt chloride was prepared as an equimolar substitute for sucrose in modified Ringer's solution. Nimodipine and flufenamic acid were dissolved in dimethyl sulfoxide. All other drugs were dissolved freshly in Ringer's solution.

### 2.5. Extracellular stimulation

Stimulating electrodes consisted of concentric bipolar electrodes of 25 μm at the tip, and 1 kΩ DC resistance were used (FHC, Bowdoinham, ME). Electrodes were placed onto the surface of the slice within 200–500 μm of the recorded cells. Stimuli consisted of single square wave pulses (typically 0.01–1 mA, 200 μs duration at 0.1–0.5 Hz) and was generated by a Winston A-65 timer and SC-100 constant current stimulus isolation unit (eg., Lee and Tepper, 2007; Ibánez-Sandoval et al., 2010).

### 2.6. Biocytin histochemistry

Slices containing biocytin-filled neurons were transferred into 4% paraformaldehyde overnight. In some cases, the thick sections were resectioned on a Vibratome at 100 μm. Sections were washed for 3 × 10 min in 0.1 M phosphate buffer (PB) followed by 10% methanol and 3% $H_2O_2$ for 15 min, and incubated with avidin-biotin-peroxidase complex (Vector Laboratories; 1:200) and 0.1% Triton X-100 overnight at 4 °C. After washing 6 × 10 min in 0.1 M PB the sections were reacted with 3,-3′-diaminobenzidine (DAB; 0.025%) and $H_2O_2$ (0.0008%) in PB. In some cases nickel intensification (Adams, 1981) was used (2.5 mM nickel ammonium sulfate and 7 mM ammonium chloride in the DAB and $H_2O_2$ incubation). The sections were then postfixed in osmium tetroxide (0.1% in PB) for 30 min, dehydrated through a graded series of ethanol, followed by propylene oxide, and infiltrated overnight with a mixture of propylene oxide and epoxy resin (Durcupan; Fluka Chemie, Buchs, Switzerland). The sections were then transferred to fresh resin mixture for several hours and flat embedded between glass slides and coverslips and polymerized at 60 °C for 24 h.

### 2.7. Statistical analysis

Most numerical values are reported as the mean ± SEM. Data were analyzed by using ANOVA with Prism (GraphPad Software), followed with Tukey's post hoc tests to multiple comparisons means. Differences were considered to be significant at $p < 0.05$.

## 3. Results

### 3.1. Electrophysiological and anatomical properties of striatal TH interneurons

*Ex vivo* whole-cell recordings were obtained in current clamp mode from fluorescent neurons in the striatum of adult EGFP-TH mice. In agreement with previously reported data (Ibáñez-Sandoval et al., 2010), several distinct subtypes of THINs could be characterized on the basis of their intrinsic electrophysiological properties (input resistance, $R_{in}$, resting membrane potential (RMP), action potential duration at half-amplitude ($AP_{50\%}$), their ability or inability to maintain firing throughout modest depolarizing current injections and the presence of a low-threshold spike (LTS). These were: Type I ($R_{in}$ = 554 ± 48 MΩ, RMP = −63 ± 2 mV and $AP_{50\%}$ = 1 ± 0.08 ms; n = 45/68 [66%]), Type II ($R_{in}$ = 379 ± 79 MΩ, RMP = −75 ± 2 mV and $AP_{50\%}$ = 0.45 + 0.03 ms; n = 8/68 [12%]), and Type IV ($R_{in}$ = 486 ± 67 MΩ, RMP = −75 ± 1 mV, $AP_{50\%}$ = 0.61 + 0.06 ms and LTS; n = 15/68 [22%]). Type III striatal THINs that made up only about 5% of the total population in our original study (Ibáñez-Sandoval et al., 2010) were not encountered in the present study, while the remaining subtypes were present in roughly the same proportions as reported previously. Representative examples of responses to current injection and the resulting current–voltage curves for Type I, II and IV THINs are shown in Fig. 1.

Striatal THINs frequently (46%, 21/45 of Type I, and 25%, 2/8 of Type II) exhibited an intrinsic PP in response to strong depolarizing current pulses delivered at rest as previously described (Ibáñez-Sandoval et al., 2010). PPs ranged in duration from 15 ms to over 5 s. A typical example of a PP from a Type I striatal THIN is shown in Fig. 1.

During whole cell recordings, all neurons were filled with biocytin for subsequent anatomical study. Eleven neurons were chosen for further anatomical analysis. These neurons exhibited morphologies consistent with our previous reports (Ibáñez-Sandoval et al., 2010; Tepper et al., 2010). Striatal THIN somata were medium sized (14.5 ± 1 μm × 10 ± 0.5 μm) and emitted from 2 to 5 thick primary dendrites that branched modestly, forming a simple dendritic arborization rarely exceeding 300 microns in diameter. The dendrites of many Type I neurons were sparsely invested with spine-like spines processes. The most characteristic feature of the THINs was their axon arborization. The axon