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ABLATION OF FAST-SPIKING INTERNEURONS IN THE DORSAL STRIATUM, RECAPITULATING ABNORMALITIES SEEN POST-MORTEM IN TOURETTE SYNDROME, PRODUCES ANXIETY AND ELEVATED GROOMING

M. XU, [a†] **L. LI** [a] **AND C. PITTENGER** [a,b,c,d*]

[a] *Department of Psychiatry, Yale University, New Haven, CT, United States*

[b] *Department of Psychology, Yale University, New Haven, CT, United States*

[c] *Child Study Center, Yale University, New Haven, CT, United States*

[d] *Interdepartmental Neuroscience Program, Yale University, New Haven, CT, United States*

Abstract—Tic disorders, including Tourette syndrome (TS), are thought to involve pathology of cortico-basal ganglia loops, but their pathology is not well understood. Post-mortem studies have shown a reduced number of several populations of striatal interneurons, including the parvalbumin-expressing fast-spiking interneurons (FSIs), in individuals with severe, refractory TS. We tested the causal role of this interneuronal deficit by recapitulating it in an otherwise normal adult mouse using a combination transgenic-viral cell ablation approach. FSIs were reduced bilaterally by ∼40%, paralleling the deficit found post-mortem. This did not produce spontaneous stereotypies or tic-like movements, but there was increased stereotypic grooming after acute stress in two validated paradigms. Stereotypy after amphetamine, in contrast, was not elevated. FSI ablation also led to increased anxiety-like behavior in the elevated plus maze, but not to alterations in motor learning on the rotorod or to alterations in prepulse inhibition, a measure of sensorimotor gating. These findings indicate that a striatal FSI deficit can produce stress-triggered repetitive movements and anxiety. These repetitive movements may recapitulate aspects of the pathophysiology of tic disorders.

Key words: basal ganglia, fast-spiking interneurons, parvalbumin, Tourette syndrome, tics.

INTRODUCTION

Tic disorders affect 5% of the population and produce significant morbidity (Du et al., 2010). Gilles de la Tourette syndrome (TS), characterized by both vocal and motor tics, represents part of this continuum. Tics are commonly comorbid with other forms of neuropsychiatric pathology, including obsessive–compulsive disorder (OCD) and attention deficit disorder (ADHD); indeed, 90% of patients diagnosed with TS have at least one additional diagnosis (Du et al., 2010; Hirschtritt et al., 2015). Existing pharmacotherapies are of limited efficacy (Bloch, 2008). Tics fluctuate over time and are exacerbated by stress (Leckman, 2002; Du et al., 2010; Kurlan, 2010) and by acute psychostimulant challenge (Denys et al., 2013). Individuals with TS often have deficits in procedural learning (Marsh et al., 2004), sensorimotor gating (Swerdlow et al., 2001; Castellan Baldan et al., 2014), and fine motor control (Bloch et al., 2006), though these are not part of contemporary diagnostic criteria.

Convergent data implicate abnormalities of the basal ganglia-thalamo-cortical circuitry in TS (Leckman et al., 2010; Williams et al., 2013b), although it is increasingly clear that dysfunction in other brain circuitries is also involved (Leckman et al., 2010; Neuner et al., 2013; Williams et al., 2013a). The striatum is the main input nucleus of the basal ganglia. Its principal cells, the medium spiny neurons (MSNs), receive glutamatergic input from the cortex and thalamus and dopaminergic modulation from the substantia nigra. MSNs comprise > 90% of the neurons in the rodent striatum; their activity is modulated by several different populations of interneuron.

Parvalbumin-expressing fast-spiking interneurons (FSIs) constitute about 1% of striatal neurons. PV interneurons integrate glutamatergic inputs from the cortex and form strong GABAergic synapses on the somata of nearby MSNs, forming a potent feedforward inhibitory circuit (Mallet et al., 2005; Tepper et al., 2010). A single FSI can exert powerful inhibitory control on the activity of a large number of nearby MSNs (Koos and Tepper, 1999). This feed-forward inhibition is thought to have an important role in orchestrating striatal information processing (Berke, 2011). FSIs coordinate MSN firing in the theta-band range in certain behavioral states (Berke et al., 2004) and fire in concert during action selection (Gage et al., 2010).

*Correspondence to: C. Pittenger, 34 Park Street, W315, New Haven, CT 06519, United States. Tel: +1-203-974-7675.
E-mail address: Christopher.pittenger@yale.edu (C. Pittenger).
† Current address: Brown University, Providence, RI, United States.

Abbreviations: ANOVA, analysis of variance; CINs, cholinergic interneurons; CS, conditioned stimulus; eGFP, enhanced green fluorescent protein; FSIs, fast-spiking interneurons; MSNs, medium spiny neurons; OCD, obsessive–compulsive disorder; sDTR, simian diphtheria toxin receptor; TS, Tourette syndrome; US, unconditioned stimulus.

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Post-mortem examination of brains from individuals with a history of severe TS has revealed abnormalities in striatal FSIs (Kalanithi et al., 2005; Kataoka et al., 2010). However, such observations cannot elucidate the causal role of this deficit: whether it is causal, compensatory, epiphenomenal, or a consequence of years of treatment. Several lines of evidence suggest a casual role. Transient pharmacological inhibition of FSIs produces movement abnormalities (Gittis et al., 2011), but it is unclear what would happen with more chronic disruption of FSI activity, which is implied by the absence of these cells in post-mortem material. Reduced FSIs are also seen in the dt^{sz} dystonic hamster, a spontaneously occurring mutant with movement abnormalities (Gernert et al., 2000), and in the SAPAP3 knockout mouse, which exhibits elevated, repetitive grooming (Burguiere et al., 2013); but these deficits occur in the context of widespread abnormalities, some of which may be developmental, and thus their causal role in the development of abnormal behaviors is no more clear than it is patients.

We sought to more directly address the question of whether chronic disruption of striatal FSIs, in an otherwise normal brain, is sufficient to produce TS-relevant phenomenology. We have recently described a strategy for the targeted ablation of defined interneuronal populations (Xu et al., 2015a). This permits the temporally, spatially, and cell type-specific recapitulation of the neuropathological changes seen post-mortem in TS in an otherwise normal adult brain. Here we applied this strategy to striatal FSIs and examined the consequences of their ablation.

Importantly, demonstration of a causal connection between a FSI deficit and behavioral pathology does not require recapitulation of all aspects of TS phenomenology. It is increasingly recognized that many neuropsychiatric diagnoses do not represent unitary natural kinds (Insel et al., 2010) and are causally heterogeneous, and that modeling trandiagnostic endophenotypes is a more realistic prospect than modeling diagnostic entities in all of their complexity (Nestler and Hyman, 2010). Striatal FSI deficiency has been associated with severe TS (Kalanithi et al., 2005; Kataoka et al., 2010), but it is not known whether this finding is specific to severe, treatment-refractory disease, to TS or tic disorders more generally, or to a range of behavioral pathology that extends beyond the boundaries of current diagnostic entities. We therefore tested a range of behaviors that capture different aspects of the symptomatology of TS and related disorders.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with the NIH Guide for the Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee. Mice were housed in a temperature- and climate-controlled facility on a 12-h light/dark schedule.

Vector constructs and AAV virus production

We engineered a recombinant adeno-associated virus that expresses enhanced green fluorescent protein (eGFP) (Heim et al., 1995) in infected cells in the absence of *Cre* recombinase but expresses the simian diphtheria toxin receptor (sDTR; Buch et al., 2005) in *Cre*-expressing cells, rendering them sensitive to ablation after systemic administration of diphtheria toxin (Xu et al., 2015a). EGFP and sDTR, in the antisense orientation and tagged with a FLAG epitope for ready immunodetection, are contained within a modified FLEX cassette, with eGFP between the lox p and lox 2722 sites at the 5′ end of the FLEX cassette. We have previously used the elongation factor 1a promoter to drive expression from the eGFP/sDTR cassette (Xu et al., 2015a); however, in pilot experiments we found sDTR expression from this promoter to be low in virus-infected striatal FSIs (data not shown), and we therefore replaced it with the more powerful CAG promoter (Miyazaki et al., 1989). To prevent excessive and potentially toxic eGFP expression in virus-infected *Cre*-negative cells, we introduced a stem-loop structure sequence (TACTGCTATACTAATAG GTATAGCAGTA) into the 5′ end of the eGFP mRNA, which reduces eGFP translation without affecting the sDTR mRNA in *Cre*-positive cells. The resulting vector is termed iDTR A46 (Fig. 1A).

A negative control construct, C46, is identical to A46 except for multiple point mutations in the 3′ Lox sites, as in Xu et al. (2015a). This prevents *Cre*-mediated inversion, such that infected cells always express eGFP (and not sDTR), irrespective of whether they express *Cre* (Fig. 1A).

These constructs were packaged into AAV (rh10 serotype) by the Salk Institute viral vector core (vectorcore.salk.edu), with a titer of 1×10^{13} genomic copies per mL by real-time PCR. rh10 provides superior spread in the striatum after a single injection without significant anterograde or retrograde infection (Cearley and Wolfe, 2006; Xu et al., 2015a).

In vivo viral infusion and virus validation

Adult male hemizygous PV-*Cre* transgenic mice were produced in our vivarium by crossing heterozygous male Pvalbm1(*Cre*)Arbr mice (www.jax.org: 008069) with female wild-type C57BL/6 breeders (Jackson Laboratories, Bar Harbor, ME, USA). Stereotaxic surgery was performed at age 3–4 months following standard procedures. PV-*Cre* mice were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg, Bayer, Whippany, NJ, USA) and ketamine (100 mg/kg, Merial, Duluth, GA, USA). A 2-μL Hamilton syringe attached to a micropump (UltramicroPump II; World Precision Instruments, Sarasota, FL, USA) was lowered through a skull burr hole into the striatum at the coordinates: AP +0.8 mm; ML ±2.3 mm; DV −3.5 mm relative to the bregma (Paxinos and Franklin, 2004). 0.5 μL of virus was infused bilaterally at a flow rate of 0.1 μL/min. The Hamilton syringe was left in place for 10 min after completion of the infusion, to eliminate back-flow, and then slowly withdrawn. This process was repeated on the other side. The scalp was then sutured; after recovery, mice were returned to their home cage for a minimum of 2 weeks post-surgery.

For validation experiments (Fig. 1A–C), mice received A46 virus on one side and C46 contralaterally; for