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Subsets of spiny striosomal striatal neurons revealed in the Gad1-GFP BAC transgenic mouse

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ABSTRACT

Objective: To characterize GFP-expressing cells in the striatum of Cb6-Tg(Gad1-EGFP)G42Zjh/J mice, in which the Gad1 (also referred to as GAD67) promoter drives GFP expression (Gad1-GFP mouse).
Background: GFP-expressing cells of the GAD1-GFP mouse have been described to be a population of parvalbumin-positive basket interneurons residing in the cerebral cortex and the cerebellum. However, the cells in the dorsal striatum of these mice have not been characterized.
Methods: Using a combination of immunohistochemistry, electrophysiology, DiI labeling, and retrograde tracing, we investigated the phenotypes of GFP-expressing cells in the GAD1-GFP mice.
Results: A small number of striatal neurons express GFP in these mice. In the mature striatum, these cells are preferentially located in the lateral striatum with a strong expression in the lateral striatal streak. The GAD1-GFP positive neurons are distinct from the standard fast-spiking and low-threshold-spiking GAD-67 expressing striatal interneurons and appear to be a subset of medium spiny neurons. These neurons are generally colocalized with striosomal markers such as dynorphin, mu-opioid receptors, as well as CB1 and calretinin-immunopositive fibers. Striatal Gad1-GFP neurons can be separated into two groups based on the shape of the somata and patterns of action potential firing. Retrograde labeling indicated that a proportion of these cells are projection neurons.
Conclusions: The examination of GAD1-GFP cells in these mice revealed two subpopulations of ventral striosomal striatal medium spiny neurons, based on morphology, patch–matrix segregation and membrane properties.

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Introduction

The generation of Bacterial Artificial Chromosome (BAC) transgenic mice in which BACs containing specific promoters drive the expression of enhanced green fluorescent protein (GFP) has allowed the examination of morphology, protein expression, pharmacology and physiology of specific subsets of neurons. Since BAC clones have the ability to carry several hundred kilobases of DNA, they are able to include the entire transcription unit and all of the associated regulatory elements for a gene allowing for a high rate of success in reproducing the expression pattern of a gene of interest [1]. However, unexpected information about neuronal subtypes has also been revealed by these mice.

In this study, characterization of GFP-expressing cells in the striatum of transgenic mice in which a BAC containing the glutamic acid decarboxylase 1 (GAD1 also referred to as GAD67) promoter drives the expression of GFP [2] was performed. These transgenic mice were first described by Chattopadhyaya et al. [2] in which their G42 line selectively expresses GFP in the calcium-binding protein parvalbumin-expressing subclass of basket interneurons in the cortex and the cerebellum [2–4]. More recently, GAD1-GFP positive cells, including SNAP-25 or 5-HT immunoreactive neurons, were observed in the taste bud and horizontal cells of the retina, respectively [5,6].

The striatum is the primary input site of the Basal Ganglia, a subcortical brain region with crucial roles in action control and learning and memory. Cytoarchitecturally, the striatum appears to be a homogeneous structure composed mainly of one neuronal type, the medium spiny neuron (MSN). However, numerous Golgi, intracellular injection, and immunohistochemical studies have revealed a mosaic organization based on neuroanatomical markers and patterns of afferent, efferent, and intrinsic connections. There is general agreement that there are six morphologically distinct types of neurons that can be divided by cell body size (large vs medium) as well as the presence or absence of spines [7].

The most frequently and well-studied striatal neuronal type is the MSN that makes up ~95% of the total neuronal number and

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is the main projection neuron from the striatum [8]. The morphological features of this cell type are a medium-sized perikaryon and the possession of many dendritic spines [9,10]. The remaining cells of the striatum, the aspiny intrinsic neurons, are characterized by the size of their cell bodies and mainly aspiny or sparsely spiny dendrites [9–11]. These cells are immunoreactive for either choline acetyltransferase, or a combination of glutamate decarboxylse (GAD) coexpressed with either somatostatin, neuropeptide Y, or parvalbumin [12–17].

MSNs can be subdivided by their expression of either the D1 or D2 dopamine receptor as well as their output pathways; those that project to the substantia nigra pars reticulata (direct pathway) and to the globus pallidus (indirect pathway). Another division is the segregation of MSNs into striosome (aka patch) and matrix compartments. Striosomes have been identified as regions of high mu-opioid receptor (μOR) binding and immunoreactivity [18,19]. Striosomes are enriched in enkephalin- and substance P-like immunoreactivity as well as a calretinin-rich fiber plexus [20–22]. This is complementary to a matrix marked by immunoreactivity for acetylcholinesterase, and somatostatin- and calbindin-positive processes [13,14,18,23,24]. Dynorphin, a class of opioid peptide that exerts effects via the kappa opioid receptor, appears to be expressed slightly higher in ventral striatal areas and distinctly higher in striosomal neurons [21].

The patch/striosome and matrix also differ in their cortical innervation. The prelimbic cortex preferentially innervates striosomes [13], whereas other prefrontal, motor, and sensory cortical areas provide inputs to the striatal matrix [25,26]. The orbitofrontal cortex has been shown to project to both striosomes and matrix [27,28]. In addition, the two striatal compartments give rise to separate projection systems to the substantia nigra; striosomal neurons project to the pars compacta whereas matrix neurons project to the pars reticulata [13,14].

Our original intent was to use the GAD1-GFP to identify and study the parvalbumin-expressing GABAergic interneurons in the striatum. Upon investigation of these mice, however, we found that a small number of striatal neurons express GFP, were spiny, and were mainly localized to the ventral lateral region of the dorsal striatum and the lateral striatal streak. Further characterization of these cells has produced unexpected results.

Materials and methods

Mice

All procedures involving animals were in full compliance with the NIH guidelines for the Care and Use of Laboratory Animals, and approved by the Animal Care and Use Committee, Division of Intramural Clinical and Biological Research of NIAAA. GAD1-GFP mice were originally created by Z. Josh Huang in which the Bacterial Artificial Chromosome (BAC) clone 56I19 containing the mouse Gad1 gene (and 60 kb of upstream and downstream sequence) was modified by insertion of an enhanced green fluorescent protein (GFP) cDNA and phosphoglycerate kinase polyadenylation sequence in the first coding exon at the translation initiation site of the Gad1 gene [2]. We obtained the mice from the Jackson Laboratory (Bar Harbor, ME). In previous studies, these GAD1-GFP (line G42) mice were found to selectively express enhanced green fluorescent protein (GFP) in the calcium-binding protein parvalbumin (PV)-expressing subclass of basket interneurons (soma, dendrites, and axons) and also in putative presynaptic boutons in brain regions including cortex and the cerebellum [2–4]. Expression of GFP was not observed in other interneuron classes expressing markers such as somatostatin (SOM), cholecystokinin (CCK), calretinin (CR), and VIP. The expression of GFP is detected as early as embryonic day 14 (E14) with high expression levels throughout postnatal development [2,3].

Transgenic mice (Tg(GAD65_3e/gfp3.3)#15) expressing GFP under the GAD65 promoter on C6CBA background were generated as described [29] and maintained in house by homozygous breeding. Briefly, a genomic clone containing 5.5 kb upstream region and the first six exons of the mouse GAD65 gene was isolated from a mouse λ phase genomic library, and the GFP marker gene without its own translation start site was fused in frame to the first or third exon of the GAD65 gene. Transgenic mice were derived by standard pronuclear injection of CBA/C57BL6F2 fertilized eggs. The expression of GFP is almost exclusively in GABAergic neurons in many brain regions including the neocortex and hippocampus.

In our studies, genotyping for GFP in both mouse lines was either performed at P0–P2 by use of an ultraviolet light source and goggles fitted with a UV filter. For adult GAD1-GFP mice, genotyping for the GFP gene was performed by PCR using the following primers: forward 5′ AAGTTCATCTGCACCACCG; reverse 5′ TGCTCA GGTACTGGTTGTCG.

Immunohistochemistry

Mice were briefly anesthetized with isofluorane (Baxter) and perfused intracardially with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). Brains were then removed and immersion fixed overnight in 4% PFA. Brains were sectioned in PBS at a thickness of 40 μm on a Vibratome Series 1000 (Leica, Buffalo Grove, IL). Sections were blocked in 5% BSA, 0.2% triton X-100 in 1× PBS for 2 h. Sections were then incubated overnight at 4 °C in primary antibodies diluted in 0.2% Triton X-100 in PBS. Table 1 lists the concentrations and sources for the primary antibodies used. After several washes in 0.2% Triton X-100 in PBS, sections were incubated in secondary antibodies (Alexa 488, 568; Invitrogen, Carlsbad, CA) overnight at 4 °C. After several washes in PBS, sections were mounted and coverslipped in Fluoromount (Southernbiotech, Birmingham, AL).

Retrograde labeling

GAD1-GFP mice at least P60 were placed into a stereotaxic instrument (Kopf, Tujunga, CA) fitted with an isofluorane rebreather system (Euthanex, Palmer, PA). A 0.1% solution of the retrograde neuronal tract tracer Alexa Fluor 555-conjugated cholera toxin B subunit (Invitrogen, Eugene, OR) was made in PBS. A 50 nL syringe (Hamilton, Reno, NV) was used to pressure inject 300 nL of the tracer solution at a rate of 50 nL/min into the substantia nigra. The coordinates for targeting the substantia nigra were (in mm relative to Bregma) AP = −3.20, ML = +1.45, and DV = −5.0. Animals were sacrificed 10 days later and immunohistochemical methods were used to reveal retrogradely labeled neurons in the dorsal striatum.

Table 1
Primary antibody concentrations.

Antibody	Species	Source	Dilution
Calbindin	Rabbit	Millipore (Billerica, MA)	1:1000
Calretinin	Rabbit	Millipore	1:1000
Darpp-32	Rabbit	Cell Signaling (Davers, MA)	1:500
Dynorphin	Rabbit	EMD Chemicals (Gibbstown, NJ)	1:200
GFP	Chicken	Abcam (Cambridge, MA)	1:2000
μ-Opiod receptor	Rabbit	Millipore	1:500
Neuropeptide Y	Rabbit	Millipore	1:1000
Parvalbumin	Rabbit	Swant (Bellinzona, Switzerland)	1:1000
PanGAD	Rabbit	Sigma (St. Louis, MO)	1:200
Somatostatin	Rabbit	Zymed (San Francisco, CA)	1:1000