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Targeting neuronal populations of the striatum

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The striatum is critically involved in motor and motivational functions. The dorsal striatum, caudate–putamen, is primarily implicated in motor control and the learning of habits and skills, whereas the ventral striatum, the nucleus accumbens, is essential for motivation and drug reinforcement. The GABA medium-sized spiny neurons (MSNs, about 95% of striatal neurons), which are targets of the cerebral cortex and the midbrain dopaminergic neurons, form two pathways. The dopamine D_1 receptor-positive (D_1R) striatonigral MSNs project to the medial globus pallidus and substantia nigra pars reticulata (direct pathway) and co-express D_1R and substance P, whereas dopamine D_2 receptor-positive (D_2R) striatopallidal MSNs project to the lateral globus pallidus (indirect pathway) and co-express D_2R, adenosine A_{2A} receptor (A_{2A}R) and enkephalin (Enk). The specific role of the two efferent pathways in motor and motivational control remained poorly understood until recently. Indeed, D_1R striatonigral and D_2R striatopallidal neurons, are intermingled and morphologically indistinguishable, and, hence, cannot be functionally dissociated with techniques such as chemical lesions or surgery. In view of the still debated respective functions of projection D_2R striatopallidal and D_1R striatonigral neurons and striatal interneurons, both in motor control and learning but also in more cognitive processes such as motivation, the present review sum up the development of new models and techniques (bacterial artificial chromosome transgenesis, optogenetic, viral transgenesis) allowing the selective targeting of these striatal neuronal populations in adult animal brain to understand their specific roles.

Keywords: striatum, medium-sized spiny neurons, interneurons, transgenesis, BAC

INTRODUCTION

The striatum represents the main input nucleus of the basal ganglia, a system of subcortical nuclei critically involved in motor control and motivational processes and altered in several conditions such as Parkinson's and Huntington's diseases or drug addiction and schizophrenia (Nestler, 2005; Graybiel, 2008; Kreitzer and Malenka, 2008). The projection neurons of the striatum are GABAergic (γ-aminobutyric acid) medium-sized spiny neurons (MSNs), which account for the large majority of striatal neurons (95% in rodents; Tepper et al., 2007). The MSNs are subdivided into two subpopulations that form two main efferent pathways. The striatonigral MSNs mainly project to the entopeduncular nucleus (EP or medial globus pallidus) and substantia nigra *pars reticulata* SNr (direct pathway) and express dopamine D_1 receptor (D_1R, *drd1a*), M4 muscarinic acetylcholine receptor (*chrm4*) and substance P neuropeptide (SP) whereas striatopallidal MSNs mostly project to the lateral globus pallidus (LGP; indirect pathway) and co-express dopamine D_2 receptor (D_2R, *drd2*), adenosine A_{2A} receptor (A_{2A}R) and enkephalin (Enk; Young and Bonner, 1986; Gerfen et al., 1990; Graybiel, 1990; Schiffmann and Vanderhaegen, 1993). Striatal interneurons, which account for a small proportion of striatal neurons (2–3% in rodent and possibly up to 20% in primates; Tepper and Bolam, 2004), are phenotypically diverse and consist of four different populations: one cholinergic and three GABAergic. The cholinergic interneurons are giant aspiny neurons (with a somatic diameter that can be in excess of 40 μm) expressing the choline acetyltransferase (ChAT; Kawaguchi et al., 1995). The three subtypes of GABAergic interneurons can be distinguished neurochemically: two express either the calcium binding protein parvalbumin or calretinin, and one co-expresses the peptides somatostatin and neuropeptideY (NPY) as well as the enzyme neuronal nitric oxide synthase (nNOS; Tepper and Bolam, 2004). Based on the origin of cortical glutamatergic and midbrain dopaminergic (DA) afferents, the striatum can be functionally divided into dorsal and ventral subregions. The dorsal striatum is thought to be involved mostly in motor behaviors, while ventral striatum (or nucleus accumbens, NAc) is crucial for motivational processes (Robbins and Everitt, 1996; Groenewegen, 2003; Nestler, 2005).

The striatal neurons are mosaically distributed through all the striatum (Bolam et al., 2000) and cannot be targeted with techniques such as electrolysis or surgery. Selective modulations of striatal subpopulation activity by drugs remains difficult since most of pharmacological agents have multiple targets widely distributed in the brain. Moreover, striatal MSNs are similar in number, in size, and shape. Because of these technical limitations, the deciphering of striatum cell-type specific functions remained unsatisfactory for decades. In this paper, we will review the different strategies developed to target intrinsic striatal neuronal subtypes, with an emphasis on the most recent state of the art genetic tools and the major functional processes illuminated in these models.

FIRST EXPERIMENTAL TOOLS TO TARGET STRIATAL SUBPOPULATIONS

Because striatal neuronal types differ one from each other notably according to their gene expression pattern, expression of a particular gene can lead to selective susceptibility to a given pharmacological agent. Then, initial approaches attempting to target neuronal population in the striatum consisted in using toxic compound with a relative specificity for a neuronal subtype in wildtype animals. For instance, stereotaxic injection of the aziridinium ion of ethylcholine (AF64A; Mantione et al., 1981) in the rat striatum led to a desired and expected decrease in ChAT activity but with a concomitant loss of glutamate decarboxylase (GAD) and tyrosine hydroxylase (TH) activities (Sandberg et al., 1984), suggesting a toxic effect against striatal cholinergic neurons as well as other neuronal populations. Intraventricular administration of an immunotoxin composed of a monoclonal antibody directed to the low-affinity rat nerve growth factor (NGF) receptor (192 IgG) chemically linked to the ribosome inactivating protein saporin (192 IgG-saporin; Wiley et al., 1991) was shown to destroy cholinergic neurons in the basal forebrain but not in the striatum, perhaps due to the low NGF receptor expression level in the latter cells (Book et al., 1992; Leanza et al., 1995). A similar immunotoxin-based strategy was developed to target striatal neurons expressing high levels of tachykinin (NK-1) receptor, ablating both NPY and ChAT expressing striatal interneurons (Saka et al., 2002).

In other experiments, neurotoxic non-selective agents were injected in target nuclei of the striatum efferent populations (i.e., the SNr or the LGP) to selectively kill the striatonigral or the striatopallidal neurons by axonal transport (Harrison et al., 1990; Hervé et al., 1993; Roberts et al., 1993). While this strategy can lead to modest reduction of striatopallidal or striatonigral neuron number (Roberts et al., 1993), it lacks striatum specificity since the neurotoxin can be axonally transported from the injection site to multiple areas.

To target a specific neuronal population, either to ablate these cells or to inactivate a specific gene within them, the genetic targeting is the most promising approach given the large number of available tools (inducible systems, specific recombination) and regulatory sequences. The first success to target a striatal cell population by classical additional transgenesis was obtained by Nestler and colleagues (Chen et al., 1998) on the striatonigral neurons using a 1.8-kb fragment of the NSE (neuronal specific enolase) promoter to express the tetracycline transactivator (tTA) of the tetracycline (Tet) system. The Tet system is based on two elements. On one hand, the transcriptional transactivator tTA (tetracycline transactivator) is under the control of a cell-specific promoter. On the other hand, the gene necessary to affect the target neuronal population is under the control of the TRE (tetracycline-responsive element) promoter that binds tTA and will be, therefore, regulated by the presence or absence of tetracycline (Gossen and Bujard, 2002). Despite the fact that NSE is not specifically expressed in striatonigral neurons, 1 the 12 lines obtained, the line A, expressed tTA in these neurons. The specificity of this cell expression pattern is due to the insertion site of the transgene in the genome. Using this transgenic line, Nestler and colleagues have demonstrated that overexpression of the transcription factor ΔFosB in striatonigral neurons increases the responsiveness of mice to the rewarding effect of cocaine (Kelz et al., 1999; Colby et al., 2003) as well as the rewarding effect and physical dependence to morphine (Zachariou et al., 2006).

The second striatal cell population successfully targeted by classical additional transgenesis were the cholinergic interneurons. Nakanishi and colleagues ablated them by immunotoxin-mediated cell targeting (IMTC) which allows a conditional and time controlled destruction of neurons (Kaneko et al., 2000). A transgene was made containing the alpha subunit of human interleukin-2 receptor (hIL-2Rα) under the control of the mouse 18.3 kb 5′-upstream genomic sequence containing the first and second exons of the mGluR2 gene. Out of the 14 transgenic lines obtained, two lines (IG16 and IG17) expressed hIL-2Rα specifically in striatal cholinergic interneurons (Kaneko et al., 2000). The ablation of these cholinergic interneurons was achieved by means of the stereotaxic injection of a monoclonal antibody against hIL-2Rα fused with a truncated *Pseudomonas* exotoxin. The study of these mice unraveled the concerted and adapted interactions between acetylcholine and dopamine transmissions in the basal ganglia during motor behavior (Kaneko et al., 2000) the cocaine-elicited behavior (Hikida et al., 2001) and procedural learning (Kitabatake et al., 2003).

KNOCK-IN STRATEGIES

The most accurate method to preserve all regulatory elements and sequences allowing the exact spatial–temporal expression of a gene is to insert a transgene into the native locus of the gene of interest by homologous recombination (Knock-in). Again, the first population targeted by this method were the striatonigral neurons. A transgene composed of a LoxP-NEO/STOP-LoxP cassette and the diphtheria toxin A-chain gene was inserted into the *drd1a* gene (Drago et al., 1998). After crossing these knock-in mice with EIIa/Cre mice, expressing Cre recombinase in the fertilized oocyte, the resulting pups express the diphtheria toxin A-chain under the control of *drd1a* promoter. Although most pups died in the first postnatal week, some mice survived to P19 and displayed bradykinesia, dystonia and reduced striatal volume. These mice did not express D_1R, substance P and dynorphin mRNAs in the striatum but had normal levels of D_2R and enkephalin mRNAs (Drago et al., 1998). To circumvent the short lifespan and potential developmental compensation of these mice, Drago and colleagues (Gantois et al., 2007) crossed the knock-in mice with calcium/calmodulin-dependent protein kinase II a/Cre mice (Casanova et al., 2001). The resulting mice express Cre in the forebrain starting from 1 to 2 weeks after birth. These mice are viable and displayed, among other, hindlimb dystonia and locomotor hyperactivity. An important limitation of these mice to decipher the role of the striatonigral neurons results from the destruction of other populations of neurons expressing *drd1a* in the forebrain (Weiner et al., 1991).

Palmiter's group targeted the expression of Cre recombinase in *drd1a* neurons by a knock-in strategy by insertion in the *drd1a* loci (Heusner et al., 2008). These *drd1a*-Cre knock-in mice have

been crossed with Gad1 floxed mice to selectively reduce GABA synthesis in striatonigral neurons (Heusner et al., 2008). The resulting mice showed altered motor skill learning in some behavioral test such as rotarod (Heusner et al., 2008). Using the same knock-in mice, this group inactivated the NR1 subunit of the glutamate NMDA receptor in *drd1a* neurons and demonstrated the impairment of amphetamine sensitization and conditioned place preference (CPP) in the mutant mice (Beutler et al., 2011). To decipher the role of these neurons in amphetamine-induced plastic phenomena and to bypass the fact that *drd1a* gene is not only expressed in striatonigral neurons they re-expressed NR1 selectively in the *drd1a* neurons of the NAc by Cre/lox viral strategy. NR1 re-expression rescued amphetamine sensitization, demonstrating that striatonigral neurons in the NAc are sufficient to retain this behavioral effect (Beutler et al., 2011).

An IMTC (see above) of striatal *drd2* neurons has been undertaken by a knock-in strategy in *drd2* loci (Sano et al., 2003). One week after intrastriatal immunotoxin treatment and ablation of *drd2* striatal neurons, mice had spontaneous hyperactivity (Sano et al., 2003). However, *drd2* is expressed not only in striatopallidal neurons but also in striatal cholinergic interneurons and in dopaminergic neurons of the SNc and VTA projecting to striatal regions (Weiner et al., 1991). In this model, the intrastriatal injection of the immunotoxin preserved the DA neurons and destroyed the striatopallidal neurons and cholinergic interneurons (Sano et al., 2003).

Knock-in parvalbumin (PV)-Cre mice were generated by insertion of an internal ribosome entry site (IRES)-*Cre* cassette 3′ to the translational stop codon of the *Pvalb* locus allowing a bicistronic translation (Hippenmeyer et al., 2005). Striatal Cre recombinase expression in these *Pvalb*-IRES-Cre mice was not described (Hippenmeyer et al., 2005), but preliminary results obtained with a LacZ reporter strain (Soriano, 1999) indicate a very low colocalization of PV immunostaining and LacZ expression (PFD et al., unpublished data). More recently, Madisen et al. (2009) generated a *Pvalb*-2A-Cre knock-in line with a *2A-Cre* sequence (mediating bicistronic translation in a mechanism different from the IRES) inserted 3′ to the PV stop codon. While *Pvalb*-2A-Cre mice, as compared to *Pvalb*-IRES-Cre mice, exhibited higher level of Cre recombination in brain areas such as cerebral cortex or thalamus, Cre recombination in the striatum was not investigated (Madisen et al., 2009).

While the knock-in strategy is the best strategy to mimic the exact expression of a gene, it has the potentially important limitation that the insertion of the transgene causes the loss of one of the two loci of the target gene. In fact, there are several reports on the effects of *drd1a* or *drd2* heterozygosity. For example, acoustic startle reactivity (Ralph-Williams et al., 2002) and dopamine-elicited late-phase LTP and facilitation of the LTD in the medial prefrontal cortex are significantly altered in *drd1a* +/− mice (Huang et al., 2004). In the case of *drd2*, locomotor activity is determined by gene dosage (Kelly et al., 1998) and non-selective attention is different in *drd2* +/− and *drd2* +/+ mice (Vallone et al., 2002).

BAC STRATEGIES

In late 1990s, the possibility to manipulate bacterial artificial chromosome (BAC) containing large fragments of genomic mouse DNA (100–250 kb) to obtain transgenic mice allowed to combine the advantages of classical transgenesis and Knock-in strategy (Heintz, 2001). Firstly, the use of large genomic DNA fragments increases the possibility to preserve the key regulatory elements of a gene to allow an accurate expression *in vivo*. Secondly, BAC constructs insert at random locations throughout the genome and the two loci of the gene of interest are then more likely to be preserved as compare to a knock-in approach. Thirdly, since the site of insertion of the transgene could maintain only part of the native expression profile of the targeted gene, it is possible to select transgenic lines that target specific cell subpopulations or selected brain nuclei.

To target the neuronal population of the striatum the two main transgenes used were fluorescent proteins (GFP or d Tomato) and Cre recombinase (Valjent et al., 2009; see **Table 1** for the different cell-type targeting tools). It is worth to mention that the fluorescent proteins are mainly used to target neuronal populations in adult mice to characterize them. Thus it is important that the regulatory sequence used in the BAC mimics the adult *in vivo* expression in the transgenic mice. In contrast, for Cre recombinase expression, transgenic mice are made for mating with floxed mice, and the resulting mice recapitulate the complete developmental profile (before and after birth) of the targeted gene that could be different from adult expression profile only.

MEDIUM SPINY NEURON TARGETING

BAC–EGFP for D_1R- and D_2R-MSN visualization

In 2003, the GENSAT project published transgenic BAC reporter mouse lines in which the enhanced green fluorescent protein (EGFP) gene is selectively expressed in a large variety of central nervous system cell-types including the D_1R or D_2R expressing cells (*drd1a*-EGFP and *drd2*-EGFP; Gong et al., 2003). Extensive characterization of *drd1a*-EGFP and *drd2*-EGFP lines (Bertran-Gonzalez et al., 2008; Matamales et al., 2009) revealed that EGFP expression patterns correlate with previous *in situ* hybridization or immunohistochemical studies (Hersch et al., 1995; Le Moine and Bloch, 1995) showing that D_1R and D_2R are expressed in distinct striatal neuronal populations. In the *drd1a*-EGFP strain, fluorescence was restricted to the striatonigral neuron population while both striatopallidal MSNs and cholinergic interneurons were EGFP-positive in the *drd2*-EGFP mice (Bertran-Gonzalez et al., 2008). Other reporter BAC strains were also generated to target the striatonigral neurons as *chrm4*-EGFP line (Gong et al., 2003) and the red fluorophore strain *drd1a*-tdTomato (Shuen et al., 2008).

These reporter mice represent a powerful tool for identifying each MSN-subtype in multiple experimental paradigms using fixed tissue preparations, fluorescence-activated cell sorting (FACS) or *ex vivo* electrophysiological recordings (Day et al., 2006, 2008; Lee et al., 2006; Lobo et al., 2006; Wang et al., 2006; Kreitzer and Malenka, 2007; Shen et al., 2007, 2008; Bertran-Gonzalez et al., 2008; Gertler et al., 2008; Matamales et al., 2009; Tecuapetla et al., 2009; Grueter et al., 2010). For instance, they allowed to show that DA depletion induces a loss of spines and glutamatergic synapses selectively on striatopallidal neurons (Day et al., 2006) and that striatopallidal neurons selectively express

Table 1 | Distinct strategies to target the subpopulations of the striatum.

	Classical transgenesis or knock-in	BAC	Other
D_1R striatonigral neurons	NSE-tTA (Chen et al., 1998) *drd1a*-tox176 (Drago et al., 1998) *drd1a*-Cre (Heusner et al., 2008)	*drd1a*-EGFP, *Chrm4*-EGFP (Gong et al., 2003) *drd1a*-tdTomato (Shuen et al., 2008) *drd1a*-Cre (YAC) (Mantamadiotis et al., 2002) *drd1a*-Cre (EY262 line) (Gong et al., 2007) *drd1a*-DARPP-32/Flag (Bateup et al., 2008) *drd1a*-EGFP-L10A (Heiman et al., 2008) *drd1a*-iRNA (mGluR5 knock-down) (Novak et al., 2010)	Volkensin toxin (Harrison et al., 1990) PPTA-tTA AAV (Hikida et al., 2010) pDyn-hM_4D HSV (Ferguson et al., 2011)
D_2R striatopallidal neurons	*drd2*-hIL-2Rα (Sano et al., 2003)	*drd2*-EGFP (Gong et al., 2003) *drd2*-Cre (ER44 line) (Gong et al., 2007) *drd2*-DARPP-32/Myc (Bateup et al., 2008) *drd2*-EGFP-L10a (Heiman et al., 2008) *adora2a*-Cre (Durieux et al., 2009)	OX7-saporin toxin (Roberts et al., 1993) PPE-tTA AAV (Hikida et al., 2010) pEnk-hM_4D HSV (Ferguson et al., 2011)
ChAT interneurons	mGluR2-hIL-2Rα (Kaneko et al., 2000)	*Chat*-EGFP (Gong et al., 2003) *Chat*-Cre (GM24 and GM60) (Gong et al., 2007)	AF64A toxin (Mantione et al., 1981) SP-PE35 toxin (Saka et al., 2002)
NPY-NO interneurons	n/a	*Npy*-Cre (DeFalco et al., 2001) *Npy*-Tau-Sapphire-GFP (Pinto et al., 2004; Roseberry et al., 2004) *Npy-hr*GFP (van den Pol et al., 2009) *Nos1*-EGFP, *Npy*-Cre (RH26-CRE and RH28-CRE) (http://www.gensat.org)	SP-PE35 toxin (Saka et al., 2002)
PV-interneurons	*Pvalb-IRES*-Cre (Hippenmeyer et al., 2005) *Pvalb-2A*-Cre (Madisen et al., 2009)	*Pvalb*-EGFP (Meyer et al., 2002) *Pvalb*-EGFP (Gong et al., 2003) *Pvalb*-Cre (Tanahira et al., 2009)	n/a
CR interneurons	n/a	n/a	n/a

n/a: not available.

an endocannabinoid-mediated long-term depression (eCB-LTD) that is disrupted in DA-depleted animals (Kreitzer and Malenka, 2007). Specific expression profiles of D_1R and D_2R MSNs were determined using FACS assays coupled to microarray analysis in *drd1a*-, *drd2*-, and *chrm4*-EGFP mice (Lobo et al., 2006), identifying multiple new genes preferentially expressed in each MSN-subtype.

Recent report pointed out major abnormalities in *drd2*-EGFP mice from GENSAT (Kramer et al., 2010). Alvarez and colleagues showed that, as compare to WT mice, *drd2*-EGFP mice exhibit a D_2R overexpression (i.e., increase in striatal D_2R binding and D_2R mRNA levels), enhanced electrophysiological responses to D_2R activation in midbrain dopaminergic neurons as well as impaired DA clearance after NAc stimulation or cocaine administration (Kramer et al., 2010). Behaviorally, *drd2*-EGFP mice display hyperactivity in novel environment, hypersensitivity to D_2R-like agonists and deficient acute and chronic response to cocaine (Kramer et al., 2010).

These results highlight the importance of extensive characterization and adequate controls when using transgenic mice in neuroscience research.

Cre BAC for manipulation of striatonigral and striatopallidal neurons or genes to shed light on their physiological roles

The first attempt to use a large mouse genomic fragment (140 kb) of *drd1a* gene has been undertaken with a yeast artificial chromosome (YAC) to unravel the role of cAMP responsive element binding protein (CREB) and cAMP responsive element modulatory protein (CREM) in striatal neurodegeneration (Mantamadiotis et al., 2002). The extensive characterization of these mice showed that the transgene has a spatiotemporal expression pattern that closely recapitulated the pattern of *drd1a* gene but is expressed in most dopaminoceptive neurons of the striatum and not only in the striatonigral neurons (Lemberger et al., 2007).

Having generated a large library of BAC–GFP mice, the GENSAT project then proceeded to generate a BAC-Cre mice library (Gong et al., 2007). In a first study, Greengard and colleagues used the *drd1a*-Cre (EY262 line) and *drd2*-Cre (ER44 line) mice to demonstrate that deletion of the histone methylase GLP/G9a led to de-repression of non-neuronal genes in each MSN populations. The study showed an altered locomotor response to pharmacological agents that target striatonigral and striatopallidal neurons in the conditional KO mice (Schaefer

et al., 2009). In a second study, they crossed the same lines of *drd1a*-Cre and *drd2*-Cre mice with dopamine- and cAMP-regulated phosphoprotein Mr 32 kDa (DARPP-32) floxed mice. Striatonigral DARPP-32-deleted mice show a decrease in basal and cocaine-induced locomotion and an abolition of L-DOPA-induced dyskinesia. Conversely, striatopallidal DARP32-deleted mice have increased locomotor activity and reduced haloperidol-induced catalepsy (Bateup et al., 2010). In this last study, they clearly showed that the deletions of DARPP-32 in striatonigral or striatopallidal neurons are specific. However, since DARPP-32 is only expressed in MSNs, these experiments do not rule out that, *drd2*-Cre mice, Cre recombinase is also expressed in cholinergic interneurons and/or DA neurons as shown for D_2-GFP (Matamales et al., 2009).

In parallel with the GENSAT BAC-Cre, other striatopallidal-Cre mice have been developed by choosing the *adora2a* gene (adenosine A_{2A} receptor) which is expressed in striatopallidal neurons but not in other striatal neuronal populations or in the presynaptic DA neurons (Durieux et al., 2009). The characterization of mice resulting from the crossing between *adora2a*-Cre mice and inducible diphtheria toxin receptor (iDTR) mice (Buch et al., 2005) after striatal diphtheria toxin injection have demonstrated that only striatopallidal neurons were targeted. The resulting mice show an increase in spontaneous locomotor activity after a whole striatum striatopallidal neuron ablation and an increase of amphetamine CPP after ventral striatum striatopallidal neuron ablation, thus demonstrating the *in vivo* roles of striatopallidal neurons in both locomotion and drug reinforcement inhibition (Durieux et al., 2009).

The most recent advances in this domain have been obtained by combining BAC-Cre expressing mice with optogenetics, allowing control of neuron-type activity with high temporal and spatial resolutions (Kravitz et al., 2010; Lobo et al., 2010). In this paradigm, two types of membrane light-sensitive proteins are used to respectively activate or silence neurons: the channelrhodopsin-2 (ChR2) cation channel and the halorhodopsin (NpHR) chloride pump respectively (Boyden et al., 2005; Zhang et al., 2007). Blue light induces a conformational change that opens ChR2 pore which rapidly and reversibly depolarizes the cell membrane and triggers action potentials (Boyden et al., 2005), while yellow light activates NpHR, which generates a chloride flow and causes rapid and reversible cell membrane hyperpolarization that prevents action potentials (Zhang et al., 2007).

Stereotactic injections of Cre-dependent ChR2 or NpHR adeno associated virus (AAV) in Cre lines lead to cell-type specific expressions of the light-sensitive proteins and optical stimulation is applied locally, through an optical fiber coupled to a diode laser, inserted in the brain area of interest (Sohal et al., 2009; Carter et al., 2010).

A first study using striatal BAC-Cre strains has demonstrated that bilateral excitation of dorsomedial striatal neurons of *drd1a*-Cre mice injected with AAV-DIO-ChR2-YFP (in which Cre-dependent expression of ChR2 is coupled to the yellow fluorescent protein, YFP) causes reduced freezing and increased locomotion (Kravitz et al., 2010). The same dorsomedial neuron excitation of *drd2*-Cre mice injected with AAV-DIO-ChR2-YFP increases freezing, and bradykinesia, and decreases locomotor initiation. In a mouse model of Parkinson's disease (injection of 6-hydroxydopamine in dorsomedial striatum), dorsomedial excitation of striatonigral neurons rescued these deficits (Kravitz et al., 2010). They showed that the AAV recombined mainly in striatonigral and striatopallidal neurons, respectively, by demonstrating the presence of YFP staining in the targets nuclei of the two neuronal populations (SNr and GP, respectively). However, they did not specify which *drd1a*-Cre (EY217, EY242, EY262, EY266, FK150, and FK164) and *drd2*-Cre (ER43 and ER44) GENSAT lines were used and this could be of importance because the Cre expression profiles are different in these lines. Yet, some striatal PV and NPY interneurons are also targeted in *drd1a*-Cre and ChAT interneurons are targeted in *drd2*-Cre mice (Kravitz et al., 2010). Finally, DA neurons are not targeted in *drd2*-Cre mice by these stereotaxic injections of AAV-DIO-ChR2-YFP in the dorsomedial striatum.

The second optogenetic study using also the *drd1a*-Cre and *drd2*-Cre GENSAT mice and AAV-DIO-ChR2-YFP, targets the NAc to unravel the role of direct and indirect pathways (Lobo et al., 2010). Illumination of NAc neurons in *drd2*-Cre mice injected with AAV-DIO-ChR2-YFP suppresses cocaine reward whereas the opposite effect is found by illumination of the neurons in the NAc of *drd1a*-Cre mice injected with AAV-DIO-ChR2-YFP (Lobo et al., 2010). These results mimic those obtained in Trkb (the brain-derived neurotrophic factor receptor) floxed mice with the same *drd2*-Cre and *drd1a*-Cre mice (Lobo et al., 2010).

It is important to conclude by noting that currently GENSAT offers six different *drd1a*-Cre lines and two *drd2*-Cre lines[1] with variable Cre recombinase expression profile that potentially allow to target different populations and subregions in the striatum. It is worth also to note that the Cre recombinase expression in the BAC-Cre mice is generally evaluated by crossing them with reporter strains as Rosa26 Lox-Stop-Lox EGFP; meaning that only the spatiotemporal profile of the Cre recombinase can be deduced and not its the level of expression.

Others transgene-BAC

drd1a and *drd2* BAC have been used to express other transgenes than eGFP and Cre recombinase. Indeed, to unravel the specific role of DARPP-32 in striatonigral and striatopallidal neurons Greengard and colleagues have generated two different BAC transgenic mice: one containing a bicistronic transgene composed of a C-terminal Flag-tagged DARPP-32 and a Venus fluorescent protein under the control of the *drd1a* promoter on a BAC and the other containing the bicistronic transgene composed of a C-terminal Myc-tagged DARPP-32 and enhanced cyan fluorescent protein (Bateup et al., 2008). Characterization of these two transgenic mice show that 36.7% of striatal cells expressed GFP mRNA in *drd1a*-DARPP-32/Flag mice and 41.4% in *drd2*-DARPP-32/Myc mice and that the expression is specific to striatonigral or striatopallidal neurons, respectively (Bateup et al., 2008). These transgenic mice have allowed to demonstrate that cocaine increases T-34 phosphorylation of DARPP-32 specifically in striatonigral neurons whereas haloperidol induces the

[1] http://www.gensat.org/cre.jsp

same type of phosphorylation selectively in striatopallidal neurons (Bateup et al., 2008).

Greengard, Heintz, and colleagues have also generated two other mouse lines with *drd1a* or *drd2* BAC that allow to purify polysomal mRNAs of specific neuronal populations to identify molecular changes in normal condition or after cocaine injection (Heiman et al., 2008). With this purpose, a fusion of EGFP to the N terminal of the large subunit ribosomal protein L10a was inserted under the control of the promoter of either *drd1a* or *drd2* in an appropriate BAC. The *drd1a*-EGFP-L10a line (CP73) showed expression of EGFP in the dorsal and ventral striatum, olfactory bulb, olfactory tubercle, and cortical layers five and six. The *drd2*-EGFP-L10a line (CP101) showed expression of EGFP in the dorsal and ventral striatum, olfactory tubercle, and hippocampus, as well as in the substantia nigra pars compacta and ventral tegmental area. Enkephalin immunostaining in the striatum colocalized with EGFP in *drd2*-EGFP-L10a mice but not in *drd1a*-EGFP-L10a. Polysomal mRNAs of isolated and homogenized striata were immunoprecipitated with magnetic beads coated with an antibody against EGFP on each of this two lines and translational profiling was determined by microarrays. Around 70 additional striatopallidal-enriched transcripts and more than 150 additional striatonigral-enriched transcripts with respect to the FACS-isolated MSN study (Lobo et al., 2006) were identified by this method (Heiman et al., 2008). The same analysis was performed after acute or chronic cocaine injection and hundreds of genes whose expression was increased or decreased in each cell-type were identified (Heiman et al., 2008).

Another study has used a *drd1a* BAC to express a short hairpin RNA to knock-down mGluR5 (Novak et al., 2010). GFP was introduced in tandem with two microRNAs against mGluR5 to track the expression of the sh-RNA. The GFP was expressed in 53% of striatal neurons that correspond to DARPP-32 neurons that do not express enkephalin, thus striatonigral neurons as expected (Novak et al., 2010). Moreover, the mRNA level of mGluR5 decreases significantly only in the striatum. These transgenic mice show impairment in the reinstatement of cocaine-seeking induced by a cocaine-paired stimulus due to a deficit in specific incentive learning processes (Novak et al., 2010).

TARGETING OF STRIATAL INTERNEURONS

GENSAT project generated choline acetyltransferase (ChAT)-EGFP (Gong et al., 2003) and ChAT-Cre (Gong et al., 2007) mice. Two *Chat* BAC-Cre transgenic lines (GM24 and GM60) showed brain Cre recombination that matched the expression of *Chat* in the adult, while the GM53 founder exhibited Cre recombination only in brainstem and spinal cord motor neurons (see text footnote 1). The GM24 strain was recently used in an optogenetic paradigm showing that ChAT interneurons in the NAc could be activated by cocaine and that silencing this drug-induced activity during cocaine exposure disrupted cocaine place preference conditioning (Witten et al., 2010).

Enhanced green fluorescent protein expression in parvalbumin (PV) interneurons of the striatum was initially obtained in a BAC line (*Pvalb*-EGFP; Meyer et al., 2002) with 96% of colocalization, calculated as the number of EGFP-positive cells stained with PV antibody. Using these *Pvalb*-EGFP mice, Freiman et al. (2006) found that activation of CB1 cannabinoid receptors led to presynaptic inhibition of PV-interneurons to MSN neurotransmission. *Pvalb*-EGFP mice were also developed by the GENSAT project (Gong et al., 2003) in which EGFP detection in the striatum seems to match the endogenous pattern of *Pvalb* expression but was not extensively characterized.

A *Pvalb*-Cre BAC strain, with a Cre recombinase DNA inserted into the *Pvalb* start codon, was generated by Tanahira et al. (2009), in which the Cre expression seems to match the PV expression in basal ganglia, including the caudate nucleus and the putamen (determined by Cre and PV double immunohistochemistry).

Npy-Cre BAC mice were generated by Friedman and colleagues at the Rockefeller University (DeFalco et al., 2001) to trace hypothalamic NPY neuron connections after stereotactic injection of a Cre-dependent GFP virus. Extensive characterization of Cre expression with a reporter strain was not performed in these mice and, notably, presence of Cre recombinase in the striatum remains elusive. Recently, the GENSAT project generated *Npy*-Cre mice (founders RH26-CRE and RH28-CRE) exhibiting a Cre expression pattern spatially similar to NPY (see text footnote 1) but Cre and NPY colocalization needs to be performed in these mice.

Friedman's laboratory also developed *Npy*-Tau-Sapphire-GFP BAC mice to investigate functions of NPY-positive neurons in the arcuate nucleus of the hypothalamus (Pinto et al., 2004; Roseberry et al., 2004). Unfortunately, the GFP expression in these mice appeared lower than revealed by histochemistry (van den Pol et al., 2009), and recent study showed morphological and electrophysiological pathological abnormalities in *Npy*-Tau-Sapphire-GFP interneurons compared with WT NPY interneurons that could be due to the overexpression of tau-coupled reporter constructs (Rancillac et al., 2010). Another group generated a BAC *Npy*-human codon corrected *Renilla* (hr)-GFP transgenic mice, with a GFP from a type of soft coral (sea pansy *Renilla*) that seems to be more stable and brighter than other green fluorescent protein (van den Pol et al., 2009). In these *Npy-hr*GFP mice, the caudate–putamen and the NAc contained scattered intensely GFP-positive cells (van den Pol et al., 2009) and colocalization of NPY and GFP in the striatum was confirmed by Partridge et al. (2009) that used these mice to specify properties of synaptic connections onto striatal NPY interneurons.

Finally, the GENSAT project also generated BAC lines in which GFP is under control of the neuronal nitric oxide synthase (nNOS) promoter (*Nos1*)[2]. However, none of the *Nos1*-EGFP lines generated show EGFP in striatal nNOS interneurons.

To date, to the best of our knowledge, no genetic tool has been developed to target selectively the striatal calretinin-positive interneurons.

VIRAL TARGETING

Recently, two papers have targeted striatonigral and striatopallidal neurons in mice and rats by using viral vectors (Hikida et al., 2010; Ferguson et al., 2011). To target mice striatonigral neurons, the −1525 to +543 residues of the mouse *PPTA* gene (Substance P) have been inserted in front of Flag-tagged tTA in

[2]http://www.gensat.org

an AAV vector whereas the −1834 to +148 residues of the mouse *Penk* gene (Enkephalin) have been used to target the striatopallidal neurons (Hikida et al., 2010). The AAVs have been injected in transgenic mice (TN) containing the TRE–GFP-tetanus toxin transgene allowing that cause the inhibition of neurotransmission in neurons expressing tTA when doxycycline is withdrawn. The TN mice bilaterally injected into 11 sites of the striatum with AAV-PPTA-tTA showed an exclusive GFP immunostaining in 74.2% of SP-positive cells whereas in TN mice injected with AAV-PPE-tTA 71.1% of ENK-containing neurons were immunostained by GFP, demonstrating a specific targeting of striatonigral and striatopallidal neurons, respectively (Hikida et al., 2010). Surprisingly, despite these percentage of targeted neurons targeted in either direct or indirect pathways whose neurotransmission was abolished, the resulting mice did not show a decrease or increase of spontaneous locomotor activity (Hikida et al., 2010) in contrast to the results obtained with BAC (Durieux et al., 2009; Bateup et al., 2010; Kravitz et al., 2010). TN mice bilaterally injected into four sites of the NAc with the transgenic AAV that results in the blockade of the direct pathway showed a decrease in cocaine-induced CPP (Hikida et al., 2010) in accordance with the results obtained in BAC mice using optogenetic approaches (Lobo et al., 2010). No difference in cocaine-induced CCP was found when the indirect pathway was inhibited (Hikida et al., 2010) in contrast with the results obtained with BAC technology (Durieux et al., 2009; Lobo et al., 2010). These differences could be explained by the different proportion of neurons that were targeted by the two approaches.

In rats, the availability of transgenic tools is much more limited than in mice. A very recent study reports the genetic targeting of both MSN populations. The −2609 to +52 residues of the rat *Penk* gene (Enkephalin) and the −1858 to +135 residues of the rat *Pdyn* gene (Dynorphin) were inserted in herpes simplex virus (HSV) in front of an hemagglutinin-tagged engineered GPCR (derived from the $G_{i/o}$-coupled human muscarinic M_4, hM_4D, activated by an inert ligand, CNO; Ferguson et al., 2011). Administration of CNO induced the activation of Kir3 channel in neurons expressing hM_4D, resulting in membrane hyperpolarization, and transient neuronal silencing. In rats infused into one site of the dorsal striatum with pEnk-hM_4D HSV, 90% of hemagglutinin cells were Enk-positive and 6% were SP-positive whereas with pDyn-hM_4D HSV 95% of hemagglutinin cells were substance P positive and 5% were Enk-positive (Ferguson et al., 2011). The percentage of Enk- or SP- positive neurons infected in the dorsal striatum by these HSV vectors was unknown. CNO treatment decreased the number of evoked action potentials in hM_4D-expressing neurons. In pEnk- hM_4D rats, CNO treatment facilitated the development of a robust amphetamine sensitization in contrast with pDyn- hM_4D rats (Ferguson et al., 2011).

CONCLUSION AND PERSPECTIVES

The recent advances in genetic targeting have allowed to address a lot of important physiological issues in the striatum which were unattainable in the past. However each of these approaches (Knock-in, BAC, Virus. . .) needs an extensive characterization of the resulting transgenic mice to avoid some overstated conclusions. The optogenetics by its flexibility and its time resolution will certainly allow to solve new important questions in striatal physiology. Moreover, a very recent advance in optogenetic tools (Ye et al., 2011) has demonstrated the possibility to control transcription of specific transgenes by light. This additional progress will allow not only to control the activity of a neuronal population but also to control the expression of a specific gene in this neuronal population. A next important step will be to export all these tools to other models than mouse, as rat and monkey, where more elaborated behavioral paradigms are possible.

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