Differential regulation of motor control and response to dopaminergic drugs by D₁R and D₂R neurons in distinct dorsal striatum subregions

Pierre F Durieux, Serge N Schiffmann¹ and Alban de Kerchove d'Exaerde^{1,*}

Laboratory of Neurophysiology, School of Medicine, Université Libre de Bruxelles, ULB, Brussels, Belgium

The dorsal striatum is critically involved in a variety of motor behaviours, including regulation of motor activity, motor skill learning and motor response to psychostimulant and neuroleptic drugs, but contribution of D₂R-striatopallidal and D₁R-striatonigral neurons in the dorsomedial (DMS, associative) and dorsolateral (DLS, sensorimotor) striatum to distinct functions remains elusive. To delineate cell type-specific motor functions of the DMS or the DLS, we selectively ablated D₂R- and D₁R-expressing striatal neurons with spatial resolution. We found that associative striatum exerts a population-selective control over locomotion and reactivity to novelty, striatopallidal and striatonigral neurons inhibiting and stimulating exploration, respectively. Further, DMS-striatopallidal neurons are involved only in early motor learning whereas gradual motor skill acquisition depends on striatonigral neurons in the sensorimotor striatum. Finally, associative striatum D₂R neurons are required for the cataleptic effect of the typical neuroleptic drug haloperidol and for amphetamine motor response sensitization. Altogether, these data provide direct experimental evidence for cell-specific topographic functional organization of the dorsal striatum.

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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; Kreitzer and Malenka, 2008). The projection neurons of the striatum are GABAergic medium-sized spiny neurons (MSNs) subdivided into two sub-populations, the striatonigral and striatopallidal neurons (Gerfen *et al*, 1990;

Schiffmann *et al*, 2007) that form two main efferent pathways. The striatonigral MSNs (direct pathway) co-express dopamine D₁ receptor (D₁R) and substance P (SP), while striatopallidal MSNs (indirect pathway) co-express dopamine D₂ receptor (D₂R), adenosine A_{2A} receptor (A_{2A}R) and enkephalin (Enk) (Schiffmann and Vanderhaeghen, 1993). MSNs in the direct and indirect pathways are equal in number and shape, mosaically distributed throughout the striatum (Gerfen, 1992), and are not dissociable with techniques such as surgical or excitotoxic lesions.

The striatum can be functionally divided into dorsal and ventral subregions based on the origin of cortical glutamatergic and midbrain dopaminergic (DA) afferents. The dorsal striatum is thought to be involved mostly in motor behaviours, while ventral striatum is crucial for motivational processes (Robbins and Everitt, 1996; Groenewegen, 2003). The dorsal striatum is often subdivided into an external portion (the dorsolateral striatum (DLS) corresponding to the primate putamen, predominantly innervated by the sensorimotor cortex) and an internal part (the dorsomedial striatum (DMS) homologous to primate caudate nucleus, receiving projections from prefrontal and other association cortices) (Voorn *et al*, 2004; Graybiel, 2008). Cell-specific functions of the DMS or DLS in motor control and learning or in basal ganglia-related disorders (such as schizophrenia or drug addiction) remain poorly understood.

While the DMS seems more engaged during initial stages of motor skill learning, when the task is more dependent on attention and susceptible to interference (Jueptner and Weiller, 1998; Luft and Buitrago, 2005), the DLS seems required for progressive skill automatization and habit learning (Miyachi *et al*, 2002; Yin *et al*, 2004).

Treatment of schizophrenia-positive symptoms with typical antipsychotics, such as haloperidol, often induces a dramatic rigidity and locomotor immobility, called catalepsy (Lieberman *et al*, 2008). While striatal D₂R antagonism is a common characteristic of antipsychotic drugs (Karam *et al*, 2010), a cell type-specific involvement of dorsal striatum subregions in the haloperidol-induced catalepsy remains elusive.

Behavioural sensitization to psychostimulants provides a model of addictive behaviours such as those associated with craving and relapse (Robinson and Berridge, 1993; Hyman *et al*, 2006), but involvement of D₁R- and D₂R-neuron pathways in this process remains controversial (Mattingly *et al*, 1996; Chen *et al*, 2003; Karlsson *et al*, 2008).

To date, to the best of our knowledge, no *in-vivo* approach has unravelled respective functions of D₁R and D₂R MSNs in the DMS and DLS. We selectively ablated each class of neurons in adult mice using an inducible diphtheria toxin receptor (DTR)-mediated cell targeting strategy (Durieux *et al*, 2009) and delineated distinct roles of D₁R and D₂R MSNs in associative and sensorimotor striatum during

*Corresponding author. Laboratory of Neurophysiology, School of Medicine, Université Libre de Bruxelles, Campus Erasme, C.P. 601, Route de Lennik, 808, Brussels 1070, Belgium. Tel.: +32 2 555 41 20; Fax: +32 2 555 41 21; E-mail: adekerch@ulb.ac.be

¹These authors contributed equally to this work

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novelty- or drug-induced locomotor behaviours and motor skill learning.

Results

We bred lines of *Drd1a-EY262-cre*^{+/-} (Gong *et al*, 2007; founder EY262) or *Adora2a-cre*^{+/-} (Durieux *et al*, 2009) mice to inducible *DTR*^{+/+} (*iDTR*^{+/+}) (Buch *et al*, 2005) mice, leading to mice that selectively expressed the *DTR* in *D1R* or *D2R* MSNs (*Drd1a-EY262-cre*^{+/-} *iDTR*^{+/+} (*D1-DTR*⁺ mice) or *Adora2a-cre*^{+/-} *iDTR*^{+/+} (*A2A-DTR*⁺ mice), respectively); and control mice (*Drd1a-EY262-cre*^{-/-} *iDTR*^{+/+} (*D1-DTR*⁻ mice) or *Adora2a-cre*^{-/-} *iDTR*^{+/+} (*A2A-DTR*⁻ mice)). This approach allows inducible ablation of *D1R* or *D2R* neurons in striatum of adult mice with high spatial resolution, avoiding neuronal ablation in other brain areas or potential developmental adaptations (Drago *et al*, 1998; Gantois *et al*, 2007; Durieux *et al*, 2009). All animal procedures were approved by the Université Libre de Bruxelles School of Medicine Ethical Committee. Diphtheria toxin (DT) was stereotactically injected into the striatum to ablate the class of neurons throughout the entire striatum (full ablation) or to restrict the ablation to DMS (DMS ablation) or DLS (DLS ablation).

Selective ablation of *D1R*-striatonigral neurons

We first characterized full ablation of *D1R*-striatonigral neurons by DT injections in the entire striatum of *D1-DTR*⁺ mice (Figures 1 and 2). Bilateral full DT-injected *D1-DTR*⁺ mice showed specific loss of *D1R* and *SP* mRNAs (Figure 1A) as well as *D1R* binding (Figure 1B) with full preservation of *D2R*-striatopallidal neurons markers as *D2R*, *A2AR* and *Enk* mRNAs (Figure 1A) and *A2AR* binding (Figure 1B). The four sub-populations of striatal interneurons were spared in full *D1R* MSN ablated striatum (Figure 2). We also demonstrated that DT injections in the entire striatum of *D1-DTR*⁺ or *A2A-DTR*⁺ animals lead to a reduction of nearly 45% of dopamine- and cAMP-regulated phosphoprotein Mr 32 kDa (DARPP-32, a protein expressed in both MSN sub-populations)-positive cells in each model (Figure 3), confirming that striatal *D1R* and *D2R* neurons are approximately equal in number (Bertran-Gonzalez *et al*, 2008). Altogether, these results indicate that striatal DT injections in *D1-DTR*⁺ and, as previously described (Durieux *et al*, 2009), in *A2A-DTR*⁺ mice lead to selective elimination of *D1R*-striatonigral and *D2R*-striatopallidal neurons, respectively.

Motor activity and rotarod learning after *D1R*- or *D2R*-neuron removal in the entire striatum

We next compared the respective functions of *D1R* or *D2R* MSNs during locomotion and motor skill learning following full ablation of *D1*-positive or *D2*-positive MSNs (Figure 4) that was verified by quantitative *in-situ* hybridization after completion of behavioural observations (Figure 4A and B). As shown in *A2A-DTR*⁺ mice (Durieux *et al*, 2009), the reduction of striatopallidal *A2AR* binding and behavioural abnormalities appear 1 week after DT injections. For that reason, all the behavioural experiments were conducted after a minimum delay of 1 week post DT injections. Spontaneous locomotion was recorded in a videotracked open field and mice were trained in a motor skill learning task (accelerating rotarod). In this task, mice have to learn a novel sequence of

movements to maintain balance on a rotating rod in constant acceleration and receive several trials per day for consecutive days (Buitrago *et al*, 2004). While *A2A-DTR*⁺ mice exhibited persistent hyperactivity as previously described (Durieux *et al*, 2009) (Figure 4E, genotype: $F(1,12) = 208.55$, $P < 0.001$; time $\times$ genotype: $F(11,12) = 2.48$, $P = 0.007$), *D1-DTR*⁺ mice showed reduced locomotion (Figure 4C, genotype: $F(1,14) = 12.88$, $P = 0.003$; time $\times$ genotype: $F(11,14) = 2.60$, $P = 0.005$) that was still present 2 weeks after DT injections ($39 \pm 8.9\%$ of *D1-DTR*⁻ distance moved in 60 min, $P = 0.03$, data not shown). Full ablation of *D2*-positive MSN resulted in early impairments in the rotarod task, but progressive improvement of performance and finally performance equivalent to control level (Figure 4F, genotype: $F(1,12) = 16.45$, $P = 0.002$; trials $\times$ genotype: $F(33,12) = 3.13$, $P < 0.001$). In contrast, full DT-injected *D1-DTR*⁺ mice were unable to learn the task and displayed a permanent deficit (Figure 4D, genotype: $F(1,14) = 206.446$, $P < 0.001$; trials $\times$ genotype: $F(33,14) = 4.30$, $P < 0.001$).

In view of these cell type-specific deficits during the rotarod acquisition, we investigated impact of *D1R*- or *D2R*-MSN removal after extensive rotarod training. *D1-DTR* and *A2A-DTR* mice were overtrained on the rotarod for 10 days before receiving full DT injections (Figure 4G and H). One week after DT injections, these mice were retested on the rotarod. While *D1R* neuron-ablated mice displayed profound rotarod impairments (Figure 4G, genotype: $F(1,12) = 12.57$, $P = 0.004$; time $\times$ genotype: $F(53,12) = 18.82$, $P < 0.001$), mice lacking *D2R* neurons showed similar performances as compared with controls (Figure 4H, genotype: $F(1,12) = 0.55$, $P = 0.472$; time $\times$ genotype: $F(53,12) = 1.65$, $P = 0.003$). Thus, after extensive training, *D2R* MSNs from the entire striatum are not required for rotarod task execution while *D1R* neurons are still necessary for performance.

D1R and *D2R* MSNs in the DMS or DLS oppositely regulate locomotor activity and novelty exploration

To further investigate the roles of *D2R* and *D1R* MSNs in functionally distinct portions of the dorsal striatum, *A2A-DTR*⁺ and *D1-DTR*⁺ mice were bilaterally injected with DT in either the DMS or the DLS (Figures 5–10). The cell-type ablation was characterized (Figure 5) and its regional location measured and topographically represented after behavioural observations were completed (see respective figures). Elimination of *D1R*-striatonigral neurons in the DMS (Figure 6A and B) induced a reduction in ambulation (genotype: $F(1,38) = 14.096$, $P = 0.001$) that was not observed following the DLS lesion (Figure 6E and F, genotype: $F(1,49) = 1.38$, $P = 0.245$). In contrast, DMS *D2R*-striatopallidal neuron-ablated mice displayed hyperlocomotion over the trial (Figure 6D, genotype: $F(1,40) = 48.78$, $P < 0.001$; time: $F(11,40) = 3.01$, $P = 0.001$) while *D2R*-striatopallidal neuron loss in the DLS did not produce any locomotor activity increase (Figure 6H, genotype: $F(1,41) = 3.18$, $P = 0.082$). It is worth to note that the increased locomotor activity observed in DMS *D2R*-striatopallidal neuron ablated mice exhibited an incremental kinetics in contrast to the decremental locomotor activity observed in all other groups (Figure 6B, time: $F(11,38) = 24.1$, $P < 0.001$; Figure 6F, time: $F(11,49) = 16.85$, $P < 0.001$; Figure 6H, time: $F(11,41) = 9.11$, $P < 0.001$, see also histograms on each figure).

Figure 1 Characterization of D1R-striatonigral neuron ablation after full striatum DT injections in D1-DTR⁺ mice. **(A)** *In-situ* hybridization autoradiograms (coronal sections, level +1.2 mm relative to bregma) of D1R-striatonigral (D1R, SP) and D2R-striatopallidal (D2R, Enk and A_{2A}R) neuron mRNAs and respective levels in the striatum of D1-DTR⁻ and D1-DTR⁺ mice 2 weeks after bilateral full DT injections ($n = 6$ per group). **(B)** Autoradiograms (coronal sections, level +1.2 mm relative to bregma) of D1 and A_{2A} receptors and respective striatal binding levels of D1-DTR⁻ and D1-DTR⁺ mice 2 weeks after bilateral full striatum DT injections ($n = 5-6$ per group). Scale bars = 1 mm. Data are reported as mean $\pm$ s.e.m. *** $P < 0.001$.

In view of this impairment in novel environment habituation, we tested whether or not novel object exploration and recognition (Hughes, 2007) were also altered (Figure 7). Each ablated group was tested in a decoupled delayed spontaneous

object recognition task (McTighe *et al*, 2010; see Materials and methods) in which mice have to explore an object placed in the middle of an open field. DMS D2R neuron-ablated mice spent less time in the open field core in the absence of object

Figure 2 Preservation of striatal interneurons after ablation of striatonigral neurons in D₁-DTR⁺ mice. (A–D) Immunostaining and quantitative analysis of (A) choline acetyltransferase (ChAT), (B) parvalbumin (PV), (C) neuropeptide Y (NPY) and (D) calretinin (CR)-positive cells in full DT-injected striatum as compared with uninjected striatum of D₁-DTR⁺ mice (day 22 after unilateral full DT injections). Scale bars = 100 μ m. Columns represent the mean $\pm$ s.e.m. ($n = 7$).

(Figure 7G, $P = 0.0497$), but exhibited a dramatic increase in novel object exploration (Figure 7H, $P < 0.001$) and were unable to reduce their exploratory behaviour when an object was repeatedly presented (Figure 7E, I, M and Q, condition: $F(1,57) = 5.211$, $P = 0.026$; condition $\times$ group: $F(7,57) = 3.284$, $P = 0.005$; Figure 7I, group: $P = 0.027$). On the other hand, DMS D₁R neuron-ablated mice showed no difference in exploring the open field core without object (Figure 7C, $P = 0.899$), but displayed a reduced novel object exploration (Figure 7D, $P = 0.033$). These effects were specific of the associative striatum, since neither DLS D₂R neuron (Figure 7O, $P = 0.833$; Figure 7P, $P = 0.90$) nor DLS D₁R neuron (Figure 7K, $P = 0.386$; Figure 7L, $P = 0.912$) ablations altered the task.

Thus, DMS-specific ablations, but not the DLS lesions, demonstrate a cell type-specific modulation of locomotor activity and novel object exploration in which D₂R MSNs and D₁R MSNs inhibit and stimulate locomotion and novelty exploration, respectively.

DMS D₂R neurons and DLS D₁R neurons are required for early motor learning and progressive skill acquisition, respectively

Evaluation of involvement of DLS and DMS D₁R and D₂R neurons in motor skill learning by the accelerating rotarod (Figure 8) showed that DMS D₂R neuron-ablated mice (Figure 8D, genotype: $F(1,14) = 1.17$, $P = 0.297$;

trials $\times$ genotype: $F(25,14) = 2.87$, $P < 0.001$) were impaired during initial trials but gradually improved their performances to reach control levels from the second learning day, while D₂R-neuron elimination in the DLS did not affect the task (Figure 8H, genotype: $F(1,14) = 0.32$, $P = 0.58$; trials $\times$ genotype: $F(25,14) = 1.51$, $P = 0.06$). On the other hand, mice lacking D₁R-expressing neurons in the DLS (Figure 8F) showed rotarod impairments until 6 days of learning (genotype: $F(1,16) = 39.96$, $P < 0.001$; trials $\times$ genotype: $F(33,16) = 2.50$, $P < 0.001$), an effect totally absent in DMS D₁R neuron-ablated mice (Figure 8B, genotype: $F(1,14) = 0.16$, $P = 0.69$; trials $\times$ genotype: $F(25,14) = 1.19$, $P = 0.244$). Importantly, after extensive rotarod training, ablation of DMS D₂R neurons (Figure 8J, genotype: $F(1,14) = 0.285$, $P = 0.602$) or DLS D₁R neurons (Figure 8I, genotype: $F(1,18) = 0.308$, $P = 0.586$) did not induce performance deficit, indicating that rotarod learning impairments (see Figure 8D and F) are not due to task execution disruption.

Distinct contribution of DMS and DLS direct or indirect pathways in haloperidol-induced catalepsy and amphetamine sensitization

Treatment of schizophrenia-positive symptoms with typical neuroleptic drugs is often associated with motor side effects such as catalepsy (Lieberman *et al*, 2008). We then evaluated

Figure 3 Reduction of striatal DARPP-32-positive neurons in DT-injected $D_1\text{-DTR}^+$ or $A_{2A}\text{-DTR}^+$ mice. (A, B) DARPP-32 immunostaining 1 month after unilateral DT injection in the entire striatum showing a 44.5 ± 2.5 and $42.9 \pm 2.62\%$ reduction of DARPP-32-positive cells in the injected striatum as compared with the uninjected striatum of $D_1\text{-DTR}^+$ (A) (6870 ± 244 cells versus 3807 ± 40 cells, $n = 2$) and $A_{2A}\text{-DTR}^+$ (B) (6046 ± 1094 cells versus 3481.5 ± 783.5 cells, $n = 2$) mice, respectively. Scale bars = $200 \mu\text{m}$.

involvement of each neuronal population in motor responses to neuroleptic drugs (Figure 9). Investigation of these locomotor responses revealed that D_2R -striatopallidal neuron removal selectively in the associative striatum completely abolished haloperidol-induced immobility (Figure 9E, treatment: $F(1,12) = 0.127$, $P = 0.728$) and catalepsy (Figure 9F, $P < 0.001$). Neither ablation of D_2R neurons in the DLS (Figure 9K, treatment: $F(1,10) = 11.81$, $P = 0.006$; Figure 9L, $P = 0.17$) nor D_1R -neuron ablations in both dorsal striatum subregions (Figure 9B, treatment: $F(1,10) = 99.26$, $P < 0.001$; Figure 9C, $P = 0.32$; Figure 9H, treatment: $F(1,16) = 56.15$, $P < 0.001$; Figure 9I, $P = 0.59$) altered haloperidol responses, indicating that D_2R antagonism in striatopallidal neurons of the DMS is crucial for the motor effects of haloperidol.

Locomotor sensitization to psychostimulants is produced by repeated drug administration and is defined as an increase in the locomotor effect of the drug upon readministration. We evaluated involvement of each neuronal population in these motor responses to psychostimulant (Figure 10). Mice lacking D_1R neurons in the DMS (Figure 10B, genotype: $F(2,24) = 4.56$, $P = 0.043$), but not in the DLS (Figure 10H, genotype: $F(1,29) = 1.3$, $P = 0.26$) showed a reduced acute amphetamine-induced hyperlocomotion as compared with controls; but repeated amphetamine injections sensitized this locomotor response (Figure 10B, repeated injections: $F(2,24) = 13.47$, $P < 0.001$; repeated injections $\times$ genotype: $F(2,24) = 1.45$, $P = 0.246$; Figure 10C, $P = 0.003$). In contrast, ablation of DMS D_2R neurons totally disrupted amphetamine locomotor sensitization (Figure 10E, repeated injections $\times$ genotype: $F(2,24) = 4.345$, $P = 0.018$; Figure 10F, $P = 0.995$). On the other hand, DLS lesions of D_2R neurons induced an increase in amphetamine acute locomotor activation (Figure 10K, genotype: $F(1,21) = 5.926$, $P = 0.024$) but

sensitization was preserved in these mice (Figure 10K, repeated injections: $F(2,21) = 12.085$, $P < 0.001$; repeated injections $\times$ genotype: $F(2,21) = 0.482$, $P = 0.621$; Figure 10L, $P = 0.026$).

Discussion

While it is currently accepted that dorsal striatum plays an important role in locomotor behaviours and motor learning (Graybiel, 1991; Mink and Thach, 1993; Groenewegen, 2003), MSN-type and dorsal striatum subregion involvement in these behaviours remains difficult to decipher without cell-type targeting tools. In the present work, we produced selective and inducible D_2R and D_1R MSN ablation in $Drd1a\text{-cre}^{+/-}$ $iDTR^{+/-}$ and $Adora2a\text{-Cre}^{+/-}$ $iDTR^{+/-}$ mice with a spatial resolution allowing their functional dissection selectively in the DMS and DLS (see Table I for a synthetic view of the observed behaviours in the different ablation subtypes).

First, the selective loss of D_1R and SP in $D_1\text{-DTR}^+$, taken together with the previously described loss of D_2R , $A_{2A}R$ and Enk in $A_{2A}\text{-DTR}^+$ DT-injected mice (Durieux *et al*, 2009), confirms that expression of these receptors and neuropeptides is largely segregated in the two MSN populations (Gerfen and Young, 1988; Gerfen *et al*, 1990; Lobo *et al*, 2006; Bateup *et al*, 2008; Bertran-Gonzalez *et al*, 2008; Heiman *et al*, 2008).

Full D_2R -MSN ablation produced hyperlocomotion in contrast to the reduction of ambulation observed in D_1R neuron full ablated mice. These data provide direct experimental evidence for an opposite control of the two populations over motor activity in freely moving animals, showing that D_2R and D_1R MSNs inhibit and stimulate motor activity respectively. Moreover, this modulatory influence on

Figure 4 Motor activity and rotarod learning in D1R and D2R MSN full ablation mice. (A, B) *In-situ* hybridization for substance P (SP) (A) and enkephalin (Enk) (B) mRNA and respective quantitation, in rostral (bregma +1.2 mm) and caudal (bregma -0.1 mm) coronal brain sections of full striatum DT-injected D1-DTR^{-/-}/D1-DTR^{+/+} (A) and A_{2A}-DTR^{-/-}/A_{2A}-DTR^{+/+} (B) mice. Data are expressed as optical density values of the injected striatum in DTR^{+/+} as a percentage of the respective DTR^{-/-} mice. Scale bars represent 1 mm. (C, E) Locomotor activity over 60 min in an open field of D1-DTR^{-/-} (C) and A_{2A}-DTR^{-/-} (E) mice, and respective controls, 1 week after full DT injections. (D, F) Rotarod performance in D1-DTR^{-/-}/D1-DTR^{+/+} (D) and A_{2A}-DTR^{-/-}/A_{2A}-DTR^{+/+} (F) mice 1 week after full DT injections (*n* = 6–10 per group). (G, H) Ten days of rotarod training and three days of performance recall one week after full DT injections in D1-DTR^{-/-}/D1-DTR^{+/+} (G) and A_{2A}-DTR^{-/-}/A_{2A}-DTR^{+/+} (H) mice (*n* = 6–8 per group). Data are reported as mean ± s.e.m. Statistical comparisons were made between DTR^{+/+} and respective DTR^{-/-} control mice. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

locomotion was partially recapitulated in DMS, but not in DLS, restricted ablations, indicating that associative striatum area exerts an MSN population-dependent control over spontaneous locomotion. Consistently, recent works using optogenetics showed that stimulation of D1R or D2R neurons selectively in the DMS increases and reduces spontaneous ambulation, respectively (Kravitz *et al*, 2010), while cell-type stimulation in the ventral striatum had no effect on spontaneous locomotion (Lobo *et al*, 2010).

Mice with selective DMS neuron ablation show alterations in several behaviours related to novelty. In D1R neuron-ablated mice, a decrease in novelty-induced exploration of objects (novel object test) and environment (open field) was observed, indicating that the direct pathway in the associative cortico-striatal loop is necessary for novelty-induced exploration. In D2R neuron-ablated mice, the hyperlocomotion in the open field and increased exploration of a novel object when it is repeatedly presented, can be, at least in part, interpreted as a form of hyperexploration, that is, the reciprocal of the behaviours of DMS D1R neuron-ablated mice. In mice lacking DMS D2R neurons, hyperlocomotion/hyperexploration becomes evident after an initial inhibition (see progressive increase in locomotion in the open field test

and increased exploration from novel to repeated object in the novel object test), suggesting the existence in these mice of a higher sensitivity to the aversive effects of novelty. These results point to DMS as a crucial component of the circuits mediating novelty reactivity. Interestingly, it has been shown that novelty exposure preferentially induces immediate early gene expression in DMS and other brain regions of the executive prefrontocortico-striatal loop, in particular when novel objects are involved (Rinaldi *et al*, 2010). DMS neurons would select the access to the limited motor and cognitive resources of the conflicting sensory stimuli associated with the increased number of objects or environmental cues that become salient by virtue of their novelty (Redgrave and Gurney, 2006). Within the DMS, Go units and No-Go units mediated by D1 and D2 MSNs, respectively (Maia and Frank, 2011), are physiologically balanced. After selective cell ablations, the prevalence of Go or No-Go units would cause hypoexploration/hypolocomotion or hyperexploration/hyperlocomotion. In particular, in D2R neuron-ablated mice, a continuous translation of sensory stimuli to locomotion would lead to a state of continuous exploration/locomotion.

Rotarod motor skill learning experiments demonstrated that D1R MSNs are crucially required to acquire a motor

Figure 7 Object recognition task of DMS or DLS D₁R- and D₂R-MSN ablated mice. **(A)** Decoupled delayed spontaneous object recognition task, in which time spent in an open field core zone (dot line) is recorded without object, with novel object or repeated object. **(B, F, J, N)** Topographic representation of the lesioned areas in D₁-DTR⁺ (**B, J**) and A_{2A}-DTR⁺ (**F, N**) DT-injected mice into the DMS (**B, F**) or the DLS (**J, N**). Colours represent percent of superimposed lesioned areas. **(C-E, G-I, K-M, O-Q)** Time spent in the open field core in the absence of object (**C, G, K, O**), time spent in open field core in the presence of novel objects divided by the time spent in the open field core without object (**D, H, L, P**) and time spent in the open field core during the test phase divided by time spent in the open field core during the study phase in novel or repeated conditions (**F, I, M, Q**) of DMS D₁-DTR (C-E) and A_{2A}-DTR (G-I) or DLS D₁-DTR (K-M) and A_{2A}-DTR (O-Q) DT-injected mice. Data are reported as mean $\pm$ s.e.m. ($n = 6-10$ per group). Statistical comparisons were made as described in Materials and methods. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

task as their entire striatum removal disrupts permanently skill performance in naive animals. In contrast, mice lacking the D₂R MSNs showed task impairments only during the initial trials and displayed late performances similar to controls. After extensive rotarod training, D₁R neurons in the entire striatum are still required for task performance while D₂R MSNs can be ablated. These findings demonstrate that execution of a previously learned motor sequence is not dependent on the D₂R MSN pathway (Ashby *et al*, 2010). In a previous work (Gantois *et al*, 2007), ablation of D₁R neurons led to locomotor hyperactivity and normal rotarod performances. It is worth to note that ablation in Gantois *et al* model was achieved by DT expression at 1–2 weeks after birth in forebrain D₁R neurons, including the striatum, the cortex and the hippocampus. These differences in the neuronal population targeted and the age of ablation onset could explain the behavioural discordances between Gantois *et al* model and the present work.

DMS D₂R neuron-ablated mice showed early rotarod deficits, while a permanent rotarod performance deficit was found in DLS D₁R neuron-ablated mice. After extensive

rotarod training, DMS D₂R neurons or DLS D₁R neuron can be ablated without affecting task performances, indicating that rotarod defects observed in naive animals cannot be attributed to task execution disruption. These results suggest that in naive animals, when task performance is more susceptible to interference and more dependent on attention (Luft and Buitrago, 2005), D₁R and D₂R MSNs work in concert to promote acquisition of a new motor skill: activation of D₁R MSNs in the sensorimotor striatum is required for progressive automaticity of task performance, by development of correct motor strategies (Graybiel, 2008), while activation of D₂R MSNs in associative striatum inhibits competing exploratory activity. During later skill learning stage, attention to action is less required (Jueptner and Weiller, 1998) and DMS D₂R MSNs progressively disengage the process while DLS D₁R MSN pathway is still required for skill automatization. Recent work (Yin *et al*, 2009) showed a potentiation of synaptic strength in the DMS after early rotarod learning that returns back to naive animal level after extensive training. Our results are in accord with this dynamic recruitment of DMS during motor skill learning.

Figure 8 Rotarod performance after ablation of D1R and D2R MSNs in the DMS or DLS. (A, C, E, G) Topographic representation of the lesioned areas in D1-DTR⁺ (A, E) and A2A-DTR⁺ (C, G) DT-injected mice into the DMS (A, D) or the DLS (E, G). Colours represent percent of superimposed lesioned areas. (B, D, F, H) Rotarod performance of DMS D1-DTR⁺ (B) and A2A-DTR⁺ (D) and DLS D1-DTR⁺ (F) and A2A-DTR⁺ (H) DT-injected mice and respective controls. (I, J) Ten days of rotarod training and three days of performance recall one week after DT injections in DLS of D1-DTR (I) and DMS of A2A-DTR (J) mice; respective topographic representation of the lesioned areas can be found on Figure 5F and J. Data are reported as mean $\pm$ s.e.m. ($n = 7-9$ per group). Statistical comparisons were made between DTR⁺ and respective DTR⁻ control mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 9 Haloperidol-induced immobility and catalepsy in mice lacking D1R or D2R MSNs in the DMS or DLS. (A, B, D, E, G, H, J, K) Locomotor activity in a 60-min open field after saline or haloperidol (1.5 mg/kg) administration in DMS D1-DTR (A, B) and A2A-DTR (D, E) or DLS D1-DTR (G, H) and A2A-DTR (J, K) DT-injected mice. (C, F, I, L) Catalepsy score (latency to move) 30 min after haloperidol (1.5 mg/kg) administration in D1-DTR⁺ (C, I) and A2A-DTR⁺ (F, L) DT-injected mice into the DMS (C, F) or the DLS (I, L). Respective topographic representation of the lesioned areas can be found on Figure 5B, F, J and N. Data are reported as mean $\pm$ s.e.m. ($n = 6-11$ per group). Statistical comparisons were made between haloperidol and saline treatment (dot chart) or DTR⁺ and respective DTR⁻ control mice (histograms). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 10 Amphetamine locomotor sensitization after D₁R or D₂R MSN ablation in the DMS or DLS. (A, D, G, J) Topographic representation of the lesioned areas in D₁-DTR⁺ (A, G) and A_{2A}-DTR⁺ (D, J) DT-injected mice into the DMS (A, D) or the DLS (G, J). Colours represent percent of superimposed lesioned areas. (B, C, E, F, H, I, K, L) Locomotor activity in a 60-min open field after repeated d-amphetamine (3 mg/kg) administration in DMS D₁-DTR (B, C) and A_{2A}-DTR (E, F) or DLS D₁-DTR (H, I) and A_{2A}-DTR (K, L) DT-injected mice. Histograms represent ambulation following first and last d-amphetamine administration. Data are reported as mean $\pm$ s.e.m. ($n=10-17$ per group). Statistical comparisons were made between DTR⁺ and respective DTR⁻ control mice (dot chart) or between first and last amphetamine injection (histograms). * $P < 0.05$, ** $P < 0.01$.

Table 1 Summary of the observed behaviours in each ablation model

	DMS ablation		DLS ablation		Entire striatum ablation	
	D1 neurons	D2 neurons	D1 neurons	D2 neurons	D1 neurons	D2 neurons
<i>Open field</i>						
Total locomotion	↔	↑	↔	↔	↔	↔
Habituation	↔	Inverted	↔	↔	↔	Absent
<i>Rotarod</i>						
Learning curve	↔	Initial ↓	↓	↔	↓	Initial ↓
Performance after learning	ND	↔	↔	ND	↓	↔
<i>Novel object</i>						
Exploration of novel object	↓	↑	↔	↔	ND	ND
Exploration of old object	↔	↑	↔	↔	ND	ND
Object memory	↔	↔	↔	↔	ND	ND
<i>Haloperidol-induced catalepsy</i>	↔	↓	↔	↔	ND	ND
<i>Amphetamine-induced locomotion</i>						
Acute effect	↓	↔	↔	↑	ND	ND
Sensitization	↔	↓	↔	↔	ND	ND

ND, not determined.

The authors further found a DLS increase in long-lasting potentiation only after rotarod overtraining that was more important in D₂R than in D₁R MSNs (Yin *et al*, 2009), a result which is in contrast to the present results, as D₂R MSN elimination in the DLS did not disrupt rotarod learning, and full ablation of D₂R MSNs interfered neither with late rotarod trials nor with the recall of a rotarod task previously learned through extensive training. On the other hand, DLS D₁R MSN ablation disrupts rotarod performances at both initial and late

motor skill learning stages. This view is supported by a recent *in-vivo* electrophysiological study showing that task-related ensemble activity in the DLS can emerge early during procedural learning (Thorn *et al*, 2010).

Neuroleptic-induced catalepsy represents a dramatic side effect of schizophrenia-positive symptom treatment (Lieberman *et al*, 2008). While most of neuroleptic drugs are D₂R antagonists (Karam *et al*, 2010), identification of a restricted neuronal population involved in their motor effects

remained elusive because of the wide D₂R distribution (Beaulieu and Gainetdinov, 2011). Using genetic models, previous works have suggested that striatopallidal neurons mediate the cataleptic effect of haloperidol (Bertran-Gonzalez *et al*, 2008; Bateup *et al*, 2010). The complete insensitivity of DMS D₂R neuron-ablated mice to haloperidol, strongly indicates that, in normal subject, a selective activation of striatopallidal neurons in the associative striatum is critical for the haloperidol hypolocomotor effect.

Repeated drug of abuse administration can lead to a gradual increase in behavioural responsiveness, such as locomotor activation in rodents, called behavioural sensitization (Robinson and Berridge, 1993). While striatum DA transmission is crucial for psychostimulant locomotor effects (Swerdlow *et al*, 1986; Nestler, 2005), a selective involvement of distinct dorsal striatum area neuronal subtypes in these processes remained elusive. Acute amphetamine locomotor response was increased in mice lacking DLS striatopallidal neurons while DMS D₂R neuron-ablated mice displayed a deficit in amphetamine response sensitization, suggesting that sensorimotor and associative striatum indirect pathways are normally involved in regulation of acute amphetamine locomotor response and its sensitization respectively. In contrast, dorsal striatum D₁R-neuron ablations did not affect amphetamine sensitization, but DMS direct pathway neuron removal reduces acute locomotor response to amphetamine. The interpretation of amphetamine acute effects is not straightforward due to the effects of region-selective neuron ablations on spontaneous locomotion. On the other hand, the lack of amphetamine sensitization in DMS D₂R neuron-ablated mice but its maintenance in DLS D₂R neuron-ablated mice as well as DMS or DLS D₁R neuron-ablated mice highlights the relevance of the former neuronal population for amphetamine sensitization. Experiments with receptor antagonists and knockout mice indicate an involvement of both D₁R and D₂R in addictive drug sensitization (Vanderschuren and Kalivas 2000; Karlsson *et al*, 2008; Harrison and Nobrega, 2009). These approaches do not allow a precise identification of striatal target cell populations that may include multiple dorsal as well as ventral D₁R- or D₂R-expressing striatal sub-populations. Much evidence points to the ventral tegmental area and nucleus accumbens as main striatal regions contributing to the development of psychostimulant sensitization (Steketee and Kalivas 2011), although an involvement of caudate-putamen, especially its medial portion, has been proposed (see, e.g. Conversi *et al*, 2008). While present results are compatible with a main role of ventral striatal circuits, they strongly point to a necessary contribution of DMS D₂R neurons in amphetamine sensitization.

Recently, other groups have targeted striatonigral and striatopallidal neurons in mice (Hikida *et al*, 2010) or rats (Ferguson *et al*, 2011). These authors developed inducible approaches with viral transgenesis to inhibit neurotransmission by cell-type expression of a tetanus toxin (Hikida *et al*, 2010) or to silence neuronal activity by selective expression of a Gi/o-coupled receptor in Enk- or SP-expressing neurons and pharmacological agent injections (Ferguson *et al*, 2011). Surprisingly, the resulting animals did not show a decrease or increase of spontaneous locomotor activity (Hikida *et al*, 2010; Ferguson *et al*, 2011), in contrast to the results obtained in other models (Durieux *et al*, 2009;

Bateup *et al*, 2010; Kravitz *et al*, 2010), while acute locomotor response (Hikida *et al*, 2010) or sensitization (Ferguson *et al*, 2011) to amphetamine was altered. These differences could be explained by the different proportion of neurons targeted in the different models (see Durieux *et al*, 2011 for an extended discussion) and/or by the ability of each approach to effectively strongly and durably inhibit or stimulate neuronal activity.

Taken together, these results provide direct *in-vivo* experimental evidence for dissociation between neuronal subtypes and striatal subregions in the regulation of novelty- or drug-induced motor responses and motor learning.

Materials and methods

Animal care

All procedures were performed according to the Institutional Animal Care Committee guidelines and were approved by the Local Ethical Committee.

Animal breeding

C57BL/6 Adora2a-Cre^{+/−} 2M strain mice were crossed with C57BL/6 iDTR^{+/+} mice (Durieux *et al*, 2009) resulting in 50% double heterozygous Adora2a-Cre^{+/−} iDTR^{+/−} (A_{2A}-DTR⁺ mice) and 50% Adora2a-Cre^{−/−} iDTR^{+/−} (A_{2A}-DTR[−] mice), which were used as controls. Outbred Drd1a-cre^{+/−} EY262 strain (<http://www.gensat.org>; Gong *et al*, 2007) mice were mated with C57BL/6 iDTR^{+/+} mice resulting in 50% of Drd1a-cre^{+/−} iDTR^{+/−} (D₁-DTR⁺ mice) and 50% of Drd1a-cre^{−/−} iDTR^{+/−} (D₁-DTR[−]) control mice. For the two models, all experiments were then performed on animals with the same strain background. Experiments were conducted on male mice, but to increase the group size in some experiments (Figure 6), females were added in a counterbalanced way between double transgenic and control groups without gender effect (Figure 6B, $F(1,12)=0.364$, $P=0.557$; Figure 6F, $F(1,14)=1.128$, $P=0.306$).

Stereotaxic injections

Adora2a-Cre^{+/−} iDTR^{+/−}, Drd1a-cre^{+/−} iDTR^{+/−} and respective control mice were deeply anaesthetized at the age of 16–20 weeks, and placed in a stereotaxic apparatus. DT (Sigma-Aldrich) was diluted in 0.01 M PBS (pH 7.4) to a concentration of 100 pg/μl and was stereotaxically injected with a blunt needle in both sides of the striatum over 10 min. For the full striatum ablation, 1 μl of DT was injected at two sites with the coordinates (adapted from atlas of Paxinos and Franklin, 2001, with *bregma* and *dura* as references): anterior +1.2 mm, lateral ±1.5 mm, ventral +3 mm, and anterior +0.5 mm, lateral ±1.8 mm, ventral +3 mm.

For the DLS and DMS ablation, 0.2 μl of DT was injected at two sites with the coordinates:

DLS: anterior +1.2 mm, lateral ±2.6 mm, ventral +1.6 mm; anterior +0.1 mm, lateral ±3.2 mm, ventral +1.6 mm;

DMS: anterior +1.2 mm, lateral ±1.1 mm, ventral +1.9 mm; anterior +0.1 mm, lateral ±1.3 mm, ventral +1.9 mm.

Locomotor activity and object recognition tasks

Locomotor activity was assessed by videotracking (Ethovision, Noldus). Mice were placed for 60 min in open field locomotor activity boxes (20 cm × 40 cm) under non-stressful conditions (15 lux illumination) and horizontal ambulation was recorded.

Object recognition task was adapted from McTighe *et al* (2010). Decoupled delayed spontaneous object recognition was conducted in a 40 × 40 cm open field coupled to a videotracking system (Ethovision, Noldus). The objects used in the experiment differed in shape and colour, had a 5–6 cm diameter and were placed in the centre of the open field. During each trial, the mouse explored the open field for 20 min and the time spent in a central zone (10 cm in diameter) was recorded. At the end of the trial, the mouse was removed from the open field and placed in its home cage. The experiment started with a trial without object in the open field, followed by sessions consisting of two phases: a study and a test trial. During the study trial, a novel object was placed in the centre of the open field. One hour after the study, the test phase could take

one of two forms: the novel condition or the repeated condition. In the novel condition, a novel object is placed in the apparatus and in the repeated condition, the mouse explores a new identical copy of the object used in the study trial. Mice were tested in novel and repeated conditions in a counterbalanced order. Which object of the pair was viewed in the study trial was also counterbalanced and both objects were viewed an equal number of times in the study phase in novel and repeated trials.

Haloperidol and amphetamine challenges

Mice were placed in open field locomotor activity boxes (20 cm × 40 cm) under non-stressful conditions (15 lux illumination) and horizontal ambulation was recorded.

For haloperidol-induced hypolocomotion, locomotor activity was first assessed for 60 min following saline (NaCl 0.9%) intraperitoneal (i.p.) injection and mice were placed in their home cage at the end of the challenge. Twenty-four hours later, locomotor activity was recorded during 60 min after haloperidol (JANSSEN-CILAG, 1.5 mg/kg) i.p. administration.

For haloperidol-induced catalepsy, mice received an injection of haloperidol (1.5 mg/kg, i.p.) in their home cage. After a delay of 30 min, mice forepaws were placed on a bar (0.2 cm in diameter) 4 cm above the tabletop. The latency to remove both forepaws from the bar was recorded with a maximum cutoff time of 180 s.

For amphetamine locomotor response trials, mice were first placed in the open field for 1 h (habituation) and then received an i.p. d-amphetamine injection (Certa, Waregem, Belgium; 3 mg/kg). Horizontal distance was recorded for 60 min. Mice received three trials with a 48-h interval between each trial.

Accelerated rotarod task

The rotarod apparatus (mouse rotarod, Ugo Basile) consisted of a plastic roller with small grooves running along its turning axis (Bearzatto *et al*, 2005). One week after injections, mice were tested for six to eight consecutive daily sessions (Figures 4D, F, 8B, D, F and H). To increase the number of early learning trials, mice received six trials on the first session and then four trials per day for 5–7 days. On the first day, mice were habituated by placement on the rotarod at a constant speed (4 r.p.m.) for 60 s. During each trial, animals were placed on the rod rotating at a constant speed (4 r.p.m.), then the rod started to accelerate continuously from 4 to 40 r.p.m. over 300 s. The latency to fall off the rotarod was recorded. Animals that stayed on the rod for 300 s were removed from the rotarod and recorded as 300 s. Mice that clung to the rod for two complete revolutions were removed from the rod and time was recorded. Between each trial, mice were placed in their home cage for a 15–20-min interval. For the recall paradigm (Figures 4G, H, 8I and J), mice received 10 rotarod sessions before full DT injections. One week after DT injections, mice were retested on the rotarod for three consecutive daily sessions.

Immunohistochemistry

Interneuron immunostainings (Figure 2) were performed as previously described (Durieux *et al*, 2009). Striatal coronal 30 µm free-floating sections were incubated overnight at 4°C with a rabbit primary antibody: anti-NPY (1/6000) (a gift of Pr Pelletier), anti-calretinin (1/3000) (Chemicon), anti-ChAT (1/1000) (Chemicon) or anti-parvalbumin (1/1000) (Chemicon). The sections were then incubated with donkey anti-rabbit biotinylated antibody (1/500, Jackson ImmunoResearch) and visualized with the ABC-diaminobenzidine reaction.

DARPP-32 immunohistochemistry (Figure 3) was performed as previously described (Bertran-Gonzalez *et al*, 2008). Striatal coronal 30 µm free-floating sections were incubated overnight at 4°C with a mouse primary antibody anti-DARPP-32 (1/2000) (a gift of Dr Jean-Antoine Girault) and were then incubated with donkey anti-mouse IgG Dylight 549 conjugated (1/200) (Jackson ImmunoResearch). Slides were examined under an AxioImager Z1 (Zeiss, Oberkochen, Germany) equipped with a ×5 objective and band-pass filter sets nr 15 for red fluorochromes, using AxioVision software (Zeiss). Entire striatum images were obtained by juxtaposition of successive acquired images (1388 × 1040 pixels; 1.29 µm/pixel; fixed exposure) and DARPP-32-positive cells were counted with cell counter software (ImageJ).

In-situ hybridization histochemistry and in-vitro receptor autoradiography

Striatal 18 µm coronal sections were cut from fresh-frozen brain and mounted onto glass slides. *In-situ* hybridization was performed as described previously (Schiffmann and Vanderhaeghen, 1993; Dasselsette *et al*, 2001; Blum *et al*, 2004). After the hybridization procedure, sections were exposed to Kodak Biomax MR film for 1–2 week(s) depending on the marker studied and digitalized images were generated from the autoradiograms. For D₁R and A_{2A}R binding, [3H]SCH23390 and [3H]CGS21680 (Perkin-Elmer), respectively, were used as previously described (Blum *et al*, 2002). After the binding procedure, slides were exposed to a 3H storage phosphor screen (Amersham) for 2–3 days and autoradiographic signals were detected using a Cyclone storage phosphor scanner (Packard Instruments, Meridian, CT). For quantitation of *in-situ* hybridization and binding (ImageJ software), averaged optical density (OD) was measured in different areas of interest and background level was subtracted to obtain corrected values. In the bilateral full ablation models, a small reduction of the entire striatum area was found in A_{2A}-DTR⁺ and D₁-DTR⁺ mice as compared with respective controls (about 10% of the DTR area) and average OD measured in the striatum of these animals was corrected to account for this slight shrinkage. Two to three sections were used for each animal to calculate the mean OD. As Drd1a-cre EY262 mice show some level of cre-recombinase expression in cerebral cortical regions surrounding the striatum (<http://www.gensat.org>), we controlled for cortical integrity in DT-injected D₁-DTR mice by performing *in-situ* hybridization for the cortical marker cholecystokinin (Schiffmann and Vanderhaeghen, 1991) at the level of the two injection sites. Animals demonstrating reductions in cortical cholecystokinin mRNA levels (superior to two times the standard deviation of the control mean) were excluded from further analysis.

Statistical analysis

Rotarod experiments were analysed by a two-way ANOVA with genotype as between factor and trials as within factor. Open field locomotor behaviours (Figures 4C, E, 6B, D, F, H, 9A, B, D, E, F, G, H, J and K) were analysed by a two-way ANOVA with genotype as between factor and 5 min time bins as within factor. Amphetamine sensitization tests were analysed by a two-way ANOVA with genotype as between factor and amphetamine injection as within factor. Depending on the two-way ANOVA results, trial-by-trial, bin-by-bin or injection-by-injection one-way ANOVA was performed (see *P*-values on respective figures). Object recognition test in novel or repeated condition was analysed by a two-way ANOVA with groups as between factor and condition as within factor, followed by Bonferroni *post hoc* comparisons.

All other comparisons were subjected to one-way ANOVA analysis.

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Author contributions: PFD, SNS and AdKdE conceived and designed the experiments; PFD performed the experiments; PFD, SNS and AdKdE analysed the data and wrote the paper.

Conflicts of interest

The authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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