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Modulation of the basal ganglia dopaminergic system in a transgenic mouse exhibiting dystonia-like features

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Abstract

Dystonia is a movement disorder characterized by involuntary excessive muscle activity and abnormal postures. There are data supporting the hypothesis that basal ganglia dysfunction, and specifically dopaminergic system dysfunction, plays a role in dystonia. In the present study, we used hyperkinetic transgenic mice generated as a model of DYT1 dystonia and compared the basal ganglia dopaminergic system between transgenic mice exhibiting hyperkinesia (affected) transgenic mice not showing movement abnormalities (unaffected), and non-transgenic littermates A decrease in the density of striatal D2 binding sites, measured by [3H]raclopride binding, and D2 mRNA expression in substantia nigra pars compacta (SNpc) was revealed in affected an unaffected transgenic mice when compared with non-transgenic. No difference in D1 receptor binding and DAT binding, measured by [3H]SCH23390 and [3H]WIN35428 binding, respectively, was found in striatum of transgenic animals. In SNpc, increased levels of DAT binding sites were observed in affected and unaffected animals compared to non-transgenic, whereas no change in DAT mRNA expression was found. Our results show selective neurochemical changes in the basal ganglia dopaminergic system, suggesting a possible involvement in the pathophysiology of dystonialike motor hyperactivity.

Keywords

Hyperkinesia; DYT1 dystonia; Dopamine transporter; D2 dopamine receptor; Basal ganglia; Movement disorder

Introduction

Dystonia is a movement disorder characterized by involuntary excessive muscle contractions, which frequently cause twisting, repetitive movements and abnormal postures (Fahn et al. 1998). The crucial role of the basal ganglia in hyperkinetic movement disorders is being increasingly recognized. Evidence for a role of the basal ganglia motor circuit in the pathogenesis of dystonia is provided from clinicopathological studies in patients with dystonia (Marsden et al. 1985; Lee and Marsden 1994) and from patients with Parkinson's disease or other forms of parkinsonism in which dystonia may occur as a presenting symptom or as a consequence of medical therapy (Schneider et al. 2009). While most

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dystonias are non-genetic in origin, an important subset of them is inherited (Bressman 2007). Dystonia linked to a mutation of the DYT1 gene, also known as primary generalized torsion dystonia, is one of the most severe forms of the inherited dystonias. It is inherited in an autosomal dominant mode with a reduced penetrance of 30–40% (Fahn 1991; Bressman 2000). Most cases of DYT1 dystonia are caused by a three-base pair (GAG) deletion in the coding region of the DYT1 gene on chromosome 9q34, resulting in loss of a glutamic acid residue (DE) near the carboxyl terminus of a protein termed torsinA (Ozelius et al. 1997, 1998). Genetic and biochemical studies indicate that dysfunction of the dopamine (DA) system in the basal ganglia may underlie the clinical features of human DYT1 dystonia (Todd and Perlmutter 1998; Augood et al. 2002, 2004; Carbon et al. 2009). Postmortem examination revealed a moderate reduction of DA levels in the caudate and rostral putamen of a dystonia patient with the DYT1 genotype (Furukawa et al. 2000). Additionally, modifications in the turnover of DA, expressed as the ratio of DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) to DA (Augood et al. 2004), as well as a moderate reduction in D1 and D2 DA receptor binding have been reported in the postmortem striatum of DYT1 human brains (Augood et al. 2002). Furthermore, PET imaging studies revealed reduced striatal D2 receptor binding in manifesting and non-manifesting carriers of DYT1 dystonia mutation (Asanuma et al. 2005; Carbon et al. 2009).

Several genetic mouse models for dystonia have been produced and have provided valuable information about DA transmission. Sharma et al. (2005) developed mice overexpressing mutant torsinA which showed unaffected striatal DA levels or binding densities of presynaptic transporter sites and D1 and D2 receptors. In these transgenic animals DA metabolites were also found either unchanged or increased, while amphetamine-induced DA release was reduced (Balcioglu et al. 2007; Zhao et al. 2008). In knock-in and knock-down DYT1 animals, tissue levels of DA were not altered but there was a slight reduction of DA metabolites (Dang et al. 2005, 2006). Shashidharan et al. (2005) generated a transgenic mouse model of human DE-torsinA, using a neuron-specific enolase promoter. These transgenic animals showed a hyperkinetic phenotype and altered striatal dopamine levels. They also exhibited this abnormal phenotype even in the absence of detectable amounts of transgene expression in subsequent generations and therefore, the abnormal behavior cannot be attributed to the overexpression of ΔE-torsinA. Nonetheless owing to the dystonic-like abnormal movements, neurochemical and electrophysiological similarities to human dystonia exhibited by this mouse model (Chiken et al. 2008), we were interested in further examining the involvement of the basal ganglia dopaminergic system in the pathophysiology of dystonia like motor hyperactivity using these transgenic mice. For this purpose, we examined protein and mRNA levels of the dopamine transporter (DAT), D1 and D2 DA receptors in the striatum and substantia nigra pars compacta, by in situ hybridization and quantitative autoradiography, using transgenic mice exhibiting a hyperkinetic phenotype (affected), transgenic mice exhibiting normal movement phenotype (unaffected) and non-transgenic littermates.

Methods

Experimental Animals

In the present study, male transgenic mice 8 weeks of age were generated (Shashidharan et al. 2005) early in 2006. On behavioral testing, after genotype analysis, approximately 30–40% of the transgenic mice developed hyperkinetic and rapid bidirectional circling movements, as well as abnormal involuntary movements with dystonic appearing self-clasping of limbs and head-shaking. The rest of the transgenic mice did not exhibit such behavioural abnormalities. We used five transgenic mice exhibiting a hyperkinetic-like phenotype (affected), five transgenic mice not exhibiting a hyperkinetic-like phenotype

(unaffected) and five age-matched non-transgenic littermates. The experimental protocols were approved by the Animal Care and Use Committees of the Mount Sinai School of Medicine and the University of Patras. All experimentation was carried out in accordance to the National Institutes of Health Guide for Care and Use of Laboratory Animals and the European Communities Council Directive (86/609/ EEC). In the present study efforts were made to minimize animal suffering and to reduce the number of animals used.

Autoradiography

All studies were performed on coded samples by experimenters blinded to genotype. Brains were rapidly frozen in liquid isopentane. Sections (15μm) containing striatum or substantia nigra were mounted onto gelatine-chromalum coated glass slides, kept at −80°C and thawed 1 hour prior to use.

D2-like receptor binding

The D2 receptor binding was performed according to the method of Tarazi et al. (1998) using [^{3}H]raclopride (PerkinElmer Life Sciences, Belgium), which labels D2 and D3 receptors. Sections were preincubated for 1 h at room temperature (RT) in buffer containing 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, pH 7.4 and then incubated for 1 h at RT in fresh buffer containing 1.8 nM [^{3}H]raclopride (60.1 Ci/mmol). Non-specific binding was determined in the presence of 1 M cis-flupenthixol (Sigma-Aldrich, Greece). Sections were washed 2x5 min in ice-cold buffer, briefly dipped in ice-cold distilled water and dried under a stream of cold air.

D1-like receptor binding

The D1 receptor binding was performed according to the method of Tarazi et al. (1998) using [3H]SCH23390 (Perkin–Elmer Life Sciences, Belgium), which labels D1 and D5 receptors. Sections were preincubated at RT for 1 h in buffer containing 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, pH 7.4 and then incubated for 1 h at RT in fresh buffer in the presence of 2.5 nM [3H]SCH23390 (85 Ci/mmol) and 40 nM ketanserin (Tocris, UK) to block the 5-HT2 serotonin binding sites. Sections were washed 2 9 5 min in ice-cold buffer, briefly dipped in ice-cold distilled water and dried under a stream of cold air. Nonspecific binding was determined with 1 lM cis-flupenthixol (Sigma–Aldrich, Greece).

DAT binding

DAT binding was assayed according to the method of Dickinson et al. (1999) using [^{3}H]WIN35428 (PerkinElmer Life Sciences, Belgium) as radioligand. Sections were preincubated for 30 min in 20 mM sodium phosphate buffer pH 7.4 at 4°C and incubated for 90 min in fresh buffer containing 0.32 M sucrose, pH 7.4 at 4°C in the presence of 5 nM [^{3}H]WIN35428 (87 Ci/mmol). The sections were then washed for 2x1 min in 20 mM sodium phosphate buffer pH 7.4 at 4 °C, briefly dipped in ice-cold distilled water and dried under a stream of cold air. Non-specific binding was determined in the presence of 30 μM benztropine (Sigma Aldrich, Greece).

The labelled sections were apposed to [^{3}H]sensitive hyperfilm (Amersham, UK) for 8–22 weeks. Tritium microscales (Amersham, England), calibrated as nCi/mg tissue equivalent, had been exposed along with the tissue samples and were used as standards. The films were developed in Kodak D19 developer.

In situ hybridization histochemistry

Frozen, coronal sections were cut in a cryostat, thaw-mounted on poly-L-lysine-coated slides and allowed to air dry at RT. Sections were then fixed for 5 min by immersion in 4% paraformaldehyde in DEPC-treated phosphate-buffered saline (PBS; 0.1 M, pH 7.4), rinsed in PBS and dehydrated in graded ethanol. The sections were stored at 4 °C in sterile jars containing 95% ethanol (DEPC-treated water added).

The oligodeoxyribonucleotide probes (Institute of Research and Technology, Greece) were complementary to the mRNAs encoding mouse D2 and DAT. The sequence for D2 was: 5′ ggc agg gtt ggc aat gat aca ctc att ctg gtc tgt 3′, corresponding to the D2 mRNA sequence 623-658 (Chen, et al., 1991). The sequence for DAT was: 5′ tac ccc agg aag gag aag aca acg aag cca gag gag aag c 3′, corresponding to the DAT mRNA sequence 1147-1186 (Delis, et al., 2004). An NCBI BLAST network search confirmed the specificity of all probes.

Each probe was diluted to a concentration of 3pmol/ml and was labeled with ^{35}S-ATP (Hartmann Analytic GmbH, Germany) at the 3′ end using the 3′ terminal transferase enzyme (Roche, Germany) to a specific activity of 2×10^7 cpm/pmol. Unincorporated nucleotides were removed using chromatography Sephadex G-50 columns (BioRad, Greece).

Hybridization was performed in a solution containing 50% formamide (v/v), 4× SSC (1× SSC: 0.15 M sodium chloride, 0.015M sodium citrate), 10% dextran sulfate (w/v) and 10 mM dithiothreitol (DTT), with 1:100 labeled probe (final concentration of the labeled probe 6×10^{-5} pmol/μl). Tissue sections on slides were covered with 100 μl of hybridization solution, immediately covered with a strip of parafilm and incubated overnight (18 h) in a humid chamber at 42 °C. Nonspecific signal was determined by addition of 100-fold excess of unlabeled probe to the hybridization solution on some slides. Following hybridization, the parafilm was removed and sections were first rinsed in 1× SSC at RT, washed in 1× SSC for 20 min at 60 °C, dipped in 1× SSC at RT, washed in 0.1× SSC for 3 min at RT and finally dehydrated in 70%, 95% and 100% ethanol and allowed to air-dry at RT.

For each mRNA, sections from transgenic and wild-type mice were exposed to a BioMax MR film (Kodak). Exposure times varied depending on the mRNA and on the labelling of the probe (ranging from 3 weeks to 4 months). After exposure, the films were developed in Kodak GBX developer.

Quantification

Autoradiograms were scanned with an Epson 4990 scanner. Optical densities and bound radioactivity were measured with the MCID 7.0 software (Imaging Research Inc, St. Catharines, ON, Canada). In autoradiography and in situ hybridization experiments the results are expressed in fmol/mg protein and in relative optical density (ROD) values, respectively (mean ± standard error of the mean).

The anatomical structures were defined according to stereotaxic coordinates.(Franklin KBJ, 1997) The striatum was divided in rostral (interaural: 5.34–4.42 mm), intermediate (interaural: 4.42–3.70 mm) and caudal (interaural: 3.70–2.98 mm) parts. The rostral and intermediate striatal sections were divided in quadrants (dorsolateral, dorsomedial, ventrolateral, ventromedial). Eight to fifteen sections per animal were measured for each brain region. Measurements were taken for total as well as nonspecific binding signal from each animal. The specific signal was determined by subtracting non specific from total signal.

Statistics

The statistics software program SPSS was employed throughout and the appropriate statistical test was chosen by the type of data. For multiple comparisons of D2 binding, DAT binding, D2 mRNA levels and DAT mRNA levels in SNpc, one-way analysis of variance (ANOVA) was used. For multiple comparisons of D1 binding, D2 binding, DAT binding, D2 mRNA levels and DAT mRNA levels in striatum, a two-way ANOVA, with group and striatal subregions as factors, was used. ANOVAs were followed when allowed by post hoc *t* tests corrected for multiple comparisons by the method of Bonferroni. A probability level of 5% ($p<0.05$) was considered statistically significant.

Results

Measurements were initially taken from right and left striatum and SNpc separately. Since no significant differences were observed in binding levels or mRNA expression of D2 receptors and DAT, right and left striatal and nigral measurements were averaged in all analyses.

D2 dopamine receptors in striatum

The levels of D2 receptor binding sites were measured in the striatum of affected and unaffected transgenic mice and non-transgenic littermates using [3H]raclopride. Densitometric measurements were taken from dorsolateral, dorsomedial, ventrolateral and ventromedial quadrants of striatum. Analyses of variance revealed a significant decrease of [3H]raclopride binding throughout the whole striatum (total) of transgenic animals that exhibited a hyperkinetic-like phenotype (affected) compared to non-transgenic animals (effect of group; $F2,45 = 10.814$, $p = 0.001$, post hoc; Table 1). The 12% decrease in raclopride in the dorsolateral part of the striatum was statistically significant ($F2,13 = 5.978$; $p = 0.017$), while only a trend for decrease was observed in the other quadrants of striatum. There was also a trend for decrease in raclopride binding in the dorsolateral part of striatum of unaffected transgenic animals compared to non-transgenic ones (Fig. 1; Table 1). When comparing the affected with the unaffected transgenic group, a significant decrease of 9% in raclopride binding was found in the striatum when measured as a whole (effect of group; $F2,45 = 10.814$, $p = 0.002$, post hoc; Fig. 1).

To further study the D2 DA receptor expression in the striatum of transgenic mice, D2 mRNA was measured using in situ hybridization. There was no significant difference in the expression of D2 DA receptor mRNA in the striatum of both affected and unaffected transgenic animals compared to non-transgenic mice. No difference in D2 DA mRNA expression was also observed between affected and unaffected transgenic mice (Fig. 2; Table 1).

D2 dopamine receptors in substantia nigra pars compacta

The levels of D2 DA receptor binding were measured in SNpc of affected and unaffected transgenic mice and non-transgenic mice. A reduction in [3H]raclopride binding in SNpc was observed between affected transgenic animals and non-transgenic mice, although the decrease was not statistically significant. A similar trend for decrease in raclopride binding in SNpc was found between affected and unaffected transgenic mice (Fig. 1; Table 1).

However, as seen in Fig. 2 and Table 1, there was a noteworthy alteration in the expression of D2 receptor mRNA in SNpc of transgenic mice ($F2,10 = 5.494$, $p = 0.032$). Compared to non-transgenic mice, D2 receptor mRNA was significantly decreased in affected animals by 24% ($p = 0.013$, post hoc) and in unaffected transgenic animals by 19% ($p = 0.034$, post

hoc). No significant change was observed between affected and unaffected transgenic animals.

Dopamine transporter in striatum

The levels of [3H]WIN35428 binding were measured in the striatum of affected and unaffected transgenic mice and non-transgenic mice. Densitometric measurements were taken from dorsolateral, dorsomedial, ventrolateral and ventromedial quadrants of striatum. No change was revealed in the density of DA transporter sites in striatum of transgenic mice (Table 3).

Dopamine transporter in substantia nigra pars compacta

The levels of [3H]WIN35428 binding were measured in SNpc of affected and unaffected transgenic mice and non-transgenic mice. A small, but significant, increase of 8% in [3H]WIN binding was observed in SNpc in both affected and unaffected transgenic animals in comparison with non-transgenic littermates ($F2,13 = 4.755$, $p = 0.029$, $p = 0.018$ post hoc tests, respectively; Fig. 3a; Table 3). A similar, but not significant, increase was observed in the expression of DAT mRNA in SNpc between transgenic (affected and unaffected) and non-transgenic littermates (Fig. 3B; Table 3).

Discussion

Using hyperkinetic transgenic mice generated as a model of dystonia by Shashidharan et al. (2005), we observed selective changes in the dopaminergic system in basal ganglia. The present data revealed a decrease in the mRNA of DA D2 receptors in SNpc of both transgenic miceexhibiting hyperkinesia and dystonic-like abnormal movements (affected) and not exhibiting these abnormal movements (unaffected) compared to non-transgenic ones. A decrease in the density of D2 DA receptors was also observed in the striatum between affected transgenic and non-transgenic mice. It is noteworthy that D2 binding in affected transgenic animals showed a trend for decrease compared to unaffected in all quadrants of the striatum and this decrease was significant when measured in the striatum as a whole. In addition, we found no change in the density of presynaptic DA uptake sites in the striatum, while an increase in the density of DAT in SNpc was observed between both affected and unaffected transgenic mice, and non-transgenic mice. Neurochemical analysis carried out by Shashidharan et al. (2005) on the same colony of transgenic mice, showed decreased striatal DOPAC/DA ratios in both affected and unaffected transgenic mice compared to non-transgenic mice, suggesting an overall decrease in DA turnover. Taken together, these findings suggest that functional abnormalities of the nigrostriatal pathway may be important to the pathophysiology of dystonia-like motor hyperactivity.

It is well known that D2 DA receptors are located on striato-pallidal GABAergic projection neurons and corticostriatal glutamatergic fibers (Gerfen 1992; Robertson et al. 1992; Hsu et al. 1995; Delgado et al. 2000). Only a few striatal interneurons appear to express D2 receptors (Centonze et al. 2003). In addition to postsynaptic localization of D2 receptors in the striatum, D2 receptors are localized presynaptically on both the somatodendritic andterminal regions of nigrostriatal dopaminergic neurons. Thus, dopamine release is under the inhibitory control ofD2 dopamine receptors (Benoit-Marand et al. 2001).

Postmortem biochemical studies in patients with genetically confirmed DYT1 dystonia demonstrated findings consistent with a trend towards a decrease in D2 receptor binding in striatum (Augood et al. 2002, 2004). Imaging studies have revealed similar reductions in striatal D2 receptor binding in manifesting and non-manifesting DYT1 mutation human carriers (Asanuma et al. 2005; Carbon et al. 2009). In DYT1 transgenic mice the density of

striatal D2 DA receptors was not altered (Balcioglu et al. 2007). Similar to neuroimaging and postmortem neurochemical studies of human DYT1 dystonia, our results revealed a statistically significant decrease in D2-binding sites in the dorsal striatum of affected mice and a trend towards a reduction in striatal D2 binding sites of unaffected mice. The presence of a lower reduction in D2 receptor binding in unaffected transgenic mice compared to affected transgenic mice raises the possibility that the degree to which striatal D2 DA receptors are reduced may be necessary for symptoms to become manifest.

While one could argue that the present changes in D2-like receptor binding seem to be too small to be of great importance in mediating motor abnormalities in DYT1 dystonia, the fact that the raclopride autoradiographic approach does not differentiate between presynaptic and postsynaptic location of dopamine D2 receptors, may lead to a marked underestimation of changes confined to one receptor location. Furthermore, a decrease of mRNA expression of D2 receptors in SNpc was observed between transgenic mice with motor abnormalities and control. This decrease may lead to a decrease in D2 autoreceptors and the latter could explain the decreased raclopride binding in striatum.

The possible decrease in presynaptic D2 autoreceptor sites could lead to an increase in presynaptic release of DA in striatum (Benoit-Marand et al. 2001). The increased levels of DA would further lead to an increased inhibitory input to GPi and GPe from the GABAergic striatal neurons through ‘‘direct’’ and ‘‘indirect’’ pathways (Gerfen 1992, 2000), resulting in the motor hyperactivity seen in the transgenic mice studied. The above explanation of the present data is substantiated by recent results on the same transgenic model, showing decreased electrophysiological activity of the GPi and GPe (Chiken et al. 2008). Another explanation for the increased inhibitory striatopallidal input may be an enhanced GABAergic activity in GABAergic projection neurons of transgenic mice. Indeed, in a transgenic mouse model expressing mutant torsinA, it has been recently shown that GABaergic signaling is increased in these neurons. Furthermore, in these transgenic mice, D2 receptor activation by quinpirole, a D2 receptor agonist, failed to inhibit GABAergic currents (Sciamanna et al. 2009). Taking into account the above findings, the observed reduction of D2 receptors in the striatum of transgenic mice in our study may underlie the impairment of D2 receptor-dependent control of striatal GABA transmission.

With respect to the finding that significant changes of D2 receptor density in striatum seemed to be restricted to the dorsolateral part of this region, it may be important to note that several animal studies showed that the mediolateral organization of striatum is functionally relevant. The lateral striatum and the anatomically related sensorimotor cortical regions have been suggested to mediate motor functions (DeLong, 1990, Hallett, 1993).

Studies in a small number of clinically affected patients with DYT1 dystonia have shown no change in striatal DA uptake sites (Naumann, et al., 1998). In mouse models of DYT1 dystonia no change has been observed in the density of striatal presynaptic DA uptake sites, as well (Balcioglu et al. 2007). It is well known that DAT is localized to axonal as well as dendritic plasma membranes of nigrostriatal dopaminergic neurons (Nirenberg et al.1996). This localization supports a role for DAT in reuptake of DA into dendrites and axon terminals and indicates the potential sites of DAT-mediated DA release (Cheramyet al. 1981). In the present study, we found that DAT sites in striatum were not altered similar to the above reports, while in SNpc they were significantly increased in both affected and unaffected transgenic animals. Thus, our results indicate that DAT sites were increased in the dendrites of nigral neurons, suggesting that the density of DAT sites may be regulated differently in the different compartments of the nigrostriatal dopaminergic neurons in this transgenic mouse.

Furthermore, studies using in vitro slice techniques, synaptosomes, and in vivo microdialysis showed that presynaptic D2-like autoreceptors can modulate DAT function (Cass and Gerhardt, 1994, Meiergerd, et al., 1993, Parsons, et al., 1993). In our study, the decreased levels of D2 receptors may modulate DAT function, which did not appear to result in altered transporter expression because no differences in the density of striatal WIN binding sites between transgenic animals and control were observed, but may result in an altered DAT activity as indicated by the decreased striatal DA turnover (Shashidharan, et al., 2005).

Overall, our results showed decreased levels of D2 protein in striatum and D2 mRNA expression in SNpc in transgenic mice compared to non-transgenic mice. In addition, DAT binding showed an increase in the SNpc but no change was observed in the striatum. The identification of selective dopaminergic abnormalities in these genetically altered mice characterized by motor hyperactivity is important since this defect may contribute to the clinical symptoms of dystonia-like movement disorders.

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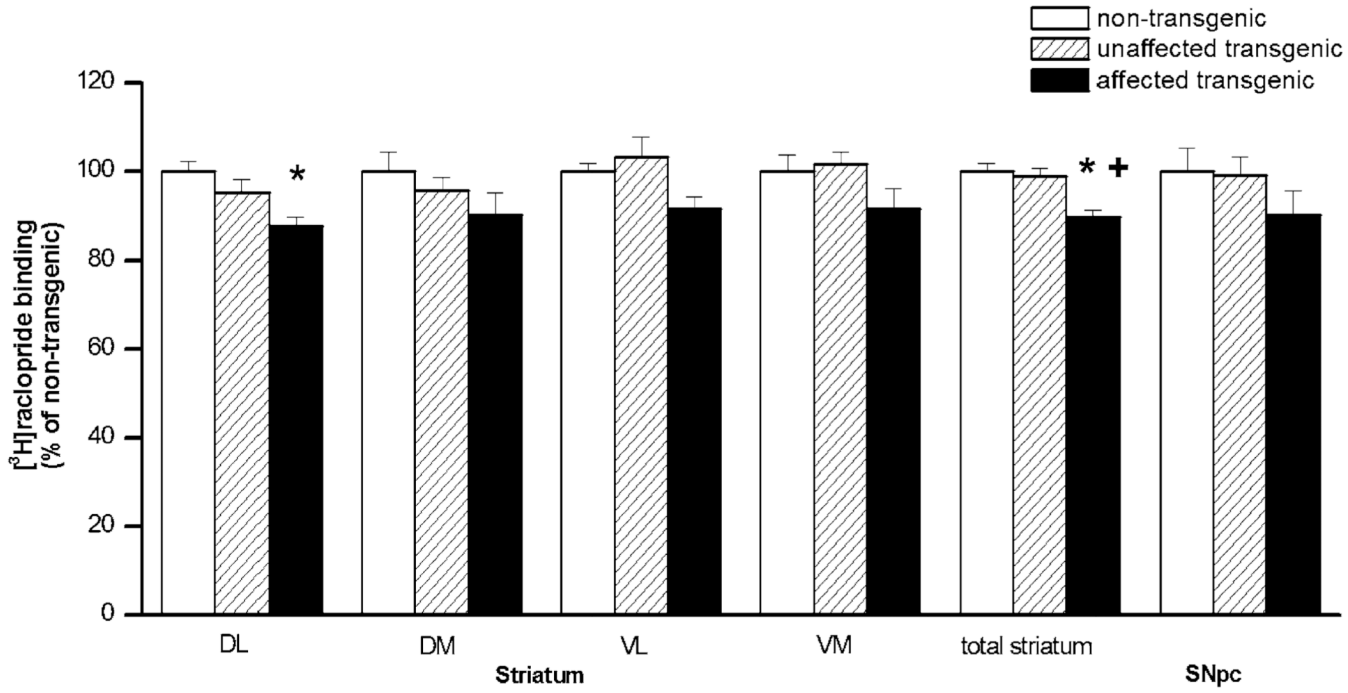

Figure 1.

D2 dopamine receptor binding. [3H]raclopride binding levels in the striatum and SNpc of non-transgenic, unaffected transgenic and affected transgenic mice. Results are mean ± SEM of specific [3H]raclopride binding (fmol/ mg tissue) expressed as the percentage of non-transgenic animals. DL dorsolateral, DM dorsomedial, VL ventrolateral, VM ventromedial, SNpc substantia nigra pars compacta, *affected compared to nontransgenic,$p<0.05$; $^{+}$affected compared to unaffected, $p<0.05$.

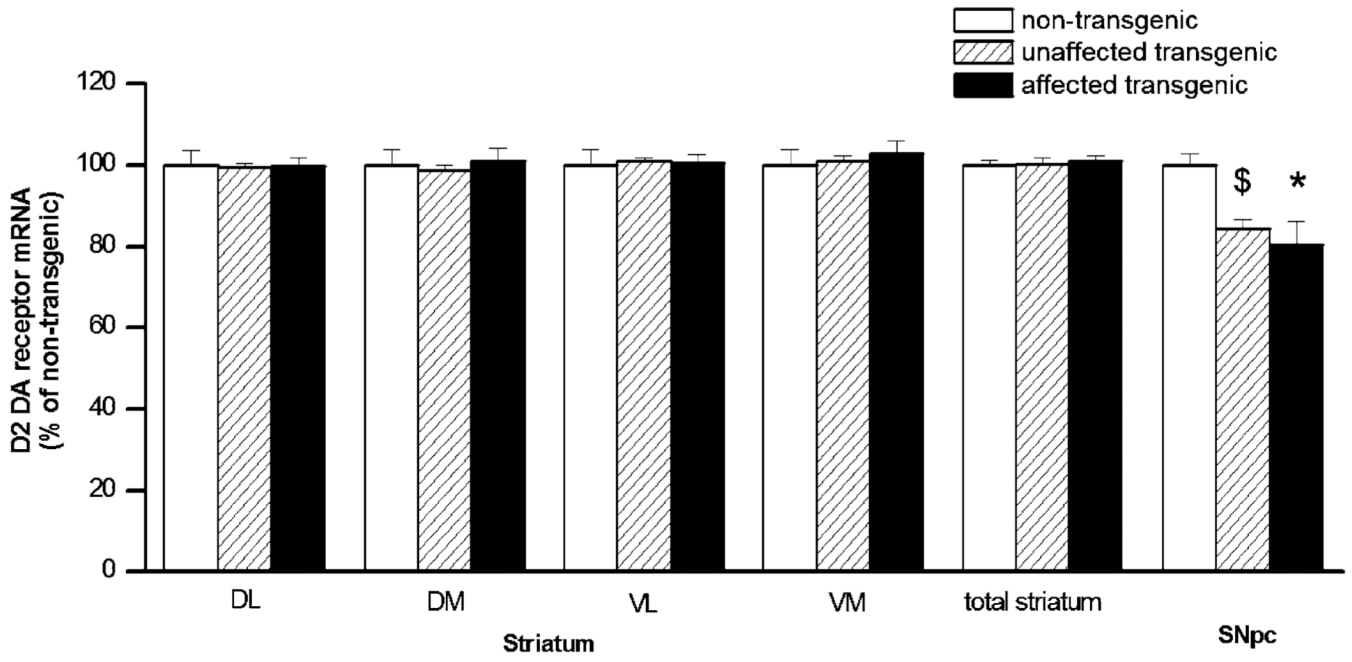

Figure 2.

D2 dopamine receptor mRNA expression in the striatum and SNpc of nontransgenic, unaffected transgenic and affected transgenic mice. Results are mean ± SEM of relative optical density (ROD) expressed as the percentage of non-transgenic animals. DL dorsolateral, DM dorsomedial, VL ventrolateral, VM ventromedial, SNpc substantia nigra pars compacta, *affected compared to nontransgenic, $p<0.05$; $^{\$}$unaffected compared to nontransgenic, $p<0.05$.

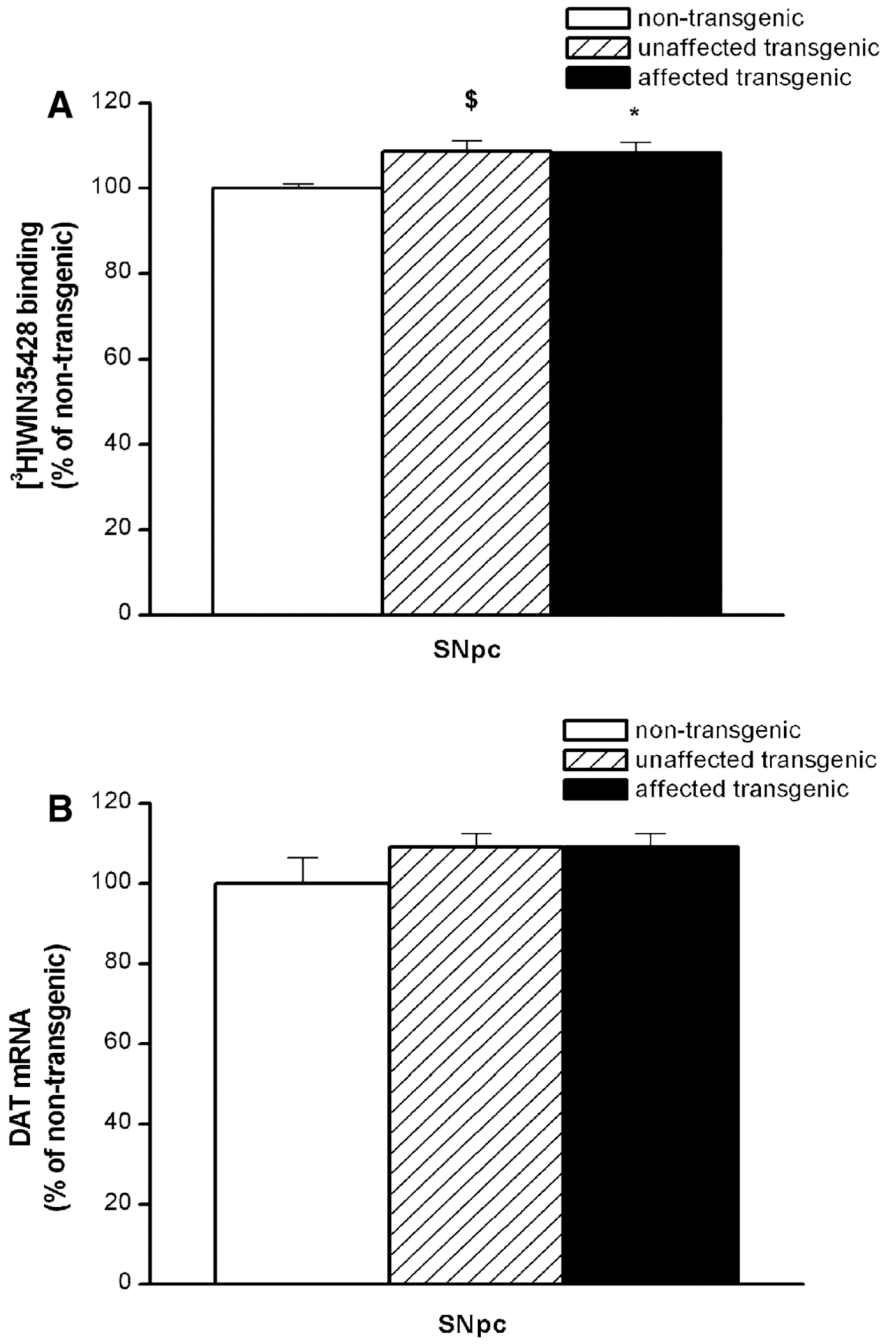

Figure 3.

Dopamine transporter (DAT) binding and mRNA expression. **A)** [3H]WIN35428 binding levels and **B)** DAT mRNA expressio levels in SNpc of non-transgenic, unaffected transgenic and affecte transgenic mice. Results are mean ± SEM of specific [3H]WIN3542 binding (fmol/mg tissue) or relative optical density (ROD) expresse as the percentage of non-transgenic animals. DL dorsolateral, D dorsomedial, VL ventrolateral, VM ventromedial, SNpc substanti nigra pars compacta, *affected compared to non-transgenic, $p<0.05$; $^{\$}$unaffected compared to non-transgenic, $p<0.05$.

Table 1

D2 binding sites ([^{3}H]raclopride) and D2 receptor mRNA expression levels in striatum and SNpc of non-transgenic mice, unaffected transgenic and affected transgenic mice

Brain region	D2 receptor binding			D2 receptor mRNA		
Striatum	**Non-transgenic**	**Unaffected**	**Affected**	**Non-transgenic**	**Unaffected**	**Affected**
Dorsolateral	175.36 ± 3.96	167.10 ± 5.14	153.80 ± 3.70*	0.303 ± 0.010	0.302 ± 0.002	0.302 ± 0.007
Dorsomedial	140.45 ± 6.10	134.39 ± 4.20	126.82 ± 6.52	0.283 ± 0.011	0.279 ± 0.004	0.286 ± 0.009
Ventrolateral	173.01 ± 3.10	178.30 ± 8.20	158.71 ± 4.75	0.298 ± 0.011	0.301 ± 0.025	0.300 ± 0.059
Ventromedial	134.64 ± 4.86	136.82 ± 3.63	123.31 ± 6.49	0.272 ± 0.011	0.275 ± 0.004	0.280 ± 0.009
Total striatum	156.13 ± 2.93	154.40 ± 2.62	140.31 ± 2.54^{*+}	0.289 ± 0.004	0.290 ± 0.004	0.292 ± 0.004
SNpc	132.60 ± 6.78	131.31 ± 5.30	119.65 ± 6.59	0.486 ± 0.014	0.410 ± 0.012$^{\$}$	0.391 ± 0.029*

Data are mean ± SEM expressed in fmol/mg tissue for receptor binding or ROD for in situ hybridization (n = 5/group)

*Affected compared to non-transgenic, $p < 0.05$;

$^{+}$affected compared to unaffected, $p < 0.05$;

$^{\$}$unaffected compared to non-transgenic, $p < 0.05$

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Table 3

DAT binding sites ([^{3}H]WIN35428) and DAT mRNA expression levels in striatum and SNpc of non-transgenic mice, unaffected transgenic and affected transgenic mice

Brain region	DAT binding			DAT mRNA		
Striatum	**Non-transgenic**	**Unaffected**	**Affected**	**Non-transgenic**	**Unaffected**	**Affected**
Dorsolateral	338.42 ± 13.34	331.65 ± 25.98	350.38 ± 15.00			
Dorsomedial	274.11 ± 16.32	233.79 ± 15.29	234.23 ± 17.80			
Ventrolateral	431.35 ± 13.31	439.27 ± 33.73	451.48 ± 22.59			
Ventromedial	335.56 ± 22.19	315.98 ± 16.62	303.50 ± 13.65			
Total striatum	349.52 ± 18.41	334.15 ± 24.52	341.10 ± 23.67			
SNpc	134.35 ± 1.59	145.92 ± 3.75$^{\$}$	145.50 ± 3.54*	0.51 ± 0.03	0.56 ± 0.02	0.56 ± 0.02

Data are mean ± SEL expressed in fmol/mg tissue for receptor binding or ROD for in situ hybridization (n = 5/group)

*Affected compared to non-transgenic, $p < 0.05$;

$^{\$}$unaffected compared to non-transgenic, $p < 0.05$