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Human COMT over-expression confers a heightened susceptibility to dyskinesia in mice

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Abstract

Catechol-*O*-methyltransferase (COMT) degrades dopamine and its precursor L-DOPA and plays a critical role in regulating synaptic dopamine actions. We investigated the effects of heightened levels of COMT on dopamine-regulated motor behaviors and molecular alterations in a mouse model of dyskinesia. Transgenic mice overexpressing human COMT (TG) and their wildtype (WT) littermates received unilateral 6-OHDA lesions in the dorsal striatum and were treated chronically with L-DOPA for two weeks. L-DOPA-induced dyskinesia was exacerbated in TG mice without altering L-DOPA motor efficacy as determined by contralateral rotations or motor coordination. Inductions of FosB and phospho-acetylated histone 3 (molecular correlates of dyskinesia) were potentiated in the lesioned striatum of TG mice compared with their WT littermates. The TG mice had lower basal levels of dopamine in the striatum. In mice with lesions, L-DOPA induces a greater increase in the dopamine metabolite 3-methoxytyramine in the lesioned striatum of dyskinetic TG mice than in WT mice. The levels of serotonin and its metabolite were similar in TG and WT mice. Our results demonstrate that human COMT overexpression confers a heightened susceptibility to L-DOPA-induced dyskinesia and alters molecular and neurochemical responses in the lesioned striatum of mice.

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Keywords

L-DOPA; LID; abnormal involuntary movements; *TXNRD2*; *COMT*; *ARVCF*; 22q11.2; dopamine; striatum

Introduction

Chronic use of the dopamine (DA) precursor L-3,4-dihydroxyphenylalanine (L-DOPA) for effective noninvasive treatment of Parkinson's disease (LeWitt 2015) induces abnormal involuntary motor movements known as L-DOPA-induced dyskinesia (LID). In mice, these abnormal involuntary movements appear in the limbs as hyperkinetic and jerky stepping movements of the forelimbs or small circular movements of the forelimb to and from the snout. There are also axial (lateral flexion of the neck or torsional movements) and orolingual (bursts of masticatory movements from facial, jaw, and tongue muscles) areas (Pavón et al., 2006; Cenci and Lundblad, 2007). LID has been associated with pulsatile stimulation of dopamine receptors (Bastide et al., 2015)—mainly the sensitized dopamine D1 receptors (Darmopil et al., 2009), which increase cell-surface expression and traffic (Porras et al., 2012; Berthet et al., 2009). This activation leads to aberrant expression of L-DOPA-induced molecular markers such as FosB and phospho-acetylated histone3 (pAcH3) (Ruiz-DeDiego et al., 2015; Solís et al., 2015a, b).

The enzymes that regulate DA concentrations may play a role in the development of LID (Marin and Obeso, 2010; Müller, 2015). Catechol-*O*-methyltransferase (COMT) is encoded within the 22q11.2 chromosomal segment. It has been linked to LID. COMT catalyzes the *O*-methylation of catecholamines including DA and plays an important role in regulating the synaptic actions of DA and its precursor L-DOPA (Müller, 2015). The role of COMT in dopamine (DA) metabolism in the striatum is less important than in other areas such as the frontal cortex and the hypothalamus (Gogos et al., 1998). However, previous studies have demonstrated that L-DOPA treatment increases the expression and activity of COMT in the striatum (Zhao et al., 2001). Clinical observations suggest that the impact of COMT in regulating LID is underscored by the fact that COMT inhibitors can reduce LID by enhancing the L-DOPA delivery to the brain and prolonging DA action in the striatum (Müller, 2015). However, these studies were not conclusive (Stocchi et al., 2010; Nyholm et al., 2011; Muhlack et al., 2014), possibly because of the lower efficiency of COMT inhibitors in the brain than in the periphery.

To establish the impact of COMT in LID, we used bacterial artificial chromosome (BAC)-transgenic mice (TG) that overexpress a 190-kb human chromosomal segment of 22q11.2 including the genes *TXNRD2*, *COMT*, and *ARVCF*. In this BAC-transgenic mouse, COMT enzymatic activity in the striatum, prefrontal cortex, and hippocampus is 2-fold greater compared with their wild-type littermates (Suzuki et al., 2009). We used a LID model as a reliable and robust method to induce abnormal limb movements (Pavón et al., 2006; Solís et al., 2017) and measured markers of cellular responses of striatal neurons FosB and histone 3 (H3) activation (Hiroi and Graybiel, 1996; Hiroi et al., 2002; Ruiz-DeDiego et al., 2015; Solís et al., 2015a, b) and levels of DA, serotonin (5-HT), and their metabolites.

Materials and methods

Animals

This study was carried out using congenic transgenic mice that carry a BAC comprising a 190-kb region of human chromosome 22q11.2 (BAC467.8) containing *TXNRD2*, *COMT*, and *ARVCF*. These mice were backcrossed into the C57BL/6J line for more than ten generations (Suzuki et al., 2009). We used the offspring of BAC-transgenic (TG) and wild-type (WT) breeder pairs. Genotypes were determined by polymerase chain reactions of tail-tip DNA. Mice aged 5–7 months were used for all experiments. The animals were housed and maintained in accordance with the guidelines of the European Union Council Directive (86/609/European Economic Community). The protocol was approved by the Consejo Superior de Investigaciones Científicas ethics committee.

6-OHDA lesions and treatment

Mice were anesthetized with isoflurane and placed in a stereotaxic apparatus (Kopf Instruments, CA, USA). As previously described (Solís et al., 2017), the animals received unilateral injections of either $2 \times 2 \mu\text{L}$ of 6-hydroxydopamine hydrobromide (6-OHDA) (20 mmol/L containing .02% ascorbic acid; Sigma-Aldrich, Spain) or saline solution (0.9% NaCl) into the dorsal striatum at the following coordinates: (mm from bregma/dura) anteroposterior, +0.65; lateral, -2.0; and dorsoventral, -4.0 and -3.5. Desipramine (20 mg/kg, i.p.; Sigma-Aldrich, Spain) was injected 30 min before the intrastriatal injections of 6-OHDA to avoid destroying noradrenergic neurons. Two to three weeks after the lesion procedures, mice were treated daily with benserazide (10 mg/kg, i.p.; Sigma-Aldrich, Spain) and L-DOPA (20 mg/kg, i.p.; Sigma-Aldrich, Spain) for 2 wk. Benserazide and L-DOPA were freshly prepared before use and injected in a volume of 10 mL/kg.

Behavioral assessment

To evaluate LID, animals were placed in clear-glass cylinders and were rated by a trained observer. Dyskinesia was evaluated for 4 min 2–3 times per week 40 min after L-DOPA was injected. Previous studies demonstrated that the incidence and intensity of abnormal involuntary movements are maximal 30 and 60 min following L-DOPA administration (Pavón et al., 2006; Solís et al., 2015a). Three subtypes (axial, limb, and orolingual) of dyskinetic symptoms were given scores ranging from 0 (not present) to 4 (severe) (Suárez et al., 2014; Ruiz-deDiego et al., 2015). The total score represents the sum of these three dyskinetic subtypes (ALO score). To evaluate the time course of LID, on day 16 of treatment, we evaluated LID for 1 min, every 30 min for a period of 180 min immediately after L-DOPA was injected.

Motor coordination was assessed using the rotarod test following an accelerating protocol with increasing speed from 4 to 40 rpm over a 5-min period as previously described (Ruiz-deDiego et al., 2015; Solís et al., 2017). The latency to fall off the rod was measured before 6-OHDA lesions (prelesion), before treatment with L-DOPA (pre-L-DOPA; day 0), and during the chronic L-DOPA treatment on day 14 (post-L-DOPA). This latter measure was carried out 90 min after the L-DOPA, to avoid the peak of dyskinetic symptoms.

Rotational behaviors were measured to evaluate behavioral sensitization. All animals were videotaped with a vertically mounted video camera for 15 min starting 5 min after L-DOPA injection on days 3, 6, 9, 12, and 14. Contralateral turns were analyzed in Viewer2 (Biobserve GmbH, Bonn, Germany). All experiments were performed by investigators blinded to the treatment conditions and mouse genotypes.

Immunohistochemistry

The mice were deeply anesthetized with pentobarbital and perfused transcardially with cold saline followed by a solution of 4% formaldehyde in phosphate-buffered saline 1 h after the last L-DOPA injection. Immunostaining was performed on free-floating coronal brain sections as described previously (Solís et al., 2015a) with the following rabbit antibodies: tyrosine hydroxylase (TH, 1:1000; Chemicon, Temecula, California), FosB (1:7500; Santa Cruz Biotechnology, Dallas, Texas), and phospho-(Ser10)-acetyl (Lys14)-histone 3 (pACh3; 1:1500; Upstate Biotechnology, Inc., Lake Placid, New York).

The extent of the dopaminergic lesions was quantified using Stereo Investigator (MBF Bioscience, Williston, Vermont) by depicting the borders of the dorsal striatal areas with complete loss of TH-immunoreactive fibers under 4× objective in 5-7 serial rostrocaudal sections per animal. The lesioned areas were quantified as the percentage volume of the dorsal striatum without TH immunostaining (Supplementary Fig. 1). Quantification of FosB and pACh3-positive cells was carried out using ImageJ. The numbers of immunolabeled cells were determined for all animals in each group using two serial rostrocaudal sections per animal from the lesioned dorsolateral striatum for a total of six images per animal. The digital images were obtained under a Leica microscope using a 40× objective. The data are presented as the number of stained nuclei per mm² in the lesioned striatum.

Neurochemical procedures

A separate cohort of mice was sacrificed by decapitation 60 min after the last injection of L-DOPA (dyskinetic group) or saline (sham-operated and Parkinsonian groups). The brains were removed rapidly, and the dorsal striata were dissected.

Separate tissue samples were taken from the left and right dorsal striata and were frozen at -80°C for analyses of neurotransmitter contents. The levels of monoamine neurotransmitters and metabolites were determined by high performance liquid chromatography (HPLC) using electrochemical detection (Solís et al., 2016). Briefly, levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were measured with an ESA Coulochem III detector according to Mena et al. (1984) with minor modifications. Samples were sonicated in 8 volumes (w/v) of 0.4 N perchloric acid with 0.5 mM Na₂S₂O₅ and 2% EDTA, and then centrifuged for 10 min. DA levels were determined from 20 µL of the supernatant. The chromatographic conditions were as follows: column ACE 5 C18, 150 × 4.6 mm (UK); mobile phase, 109.3 mM citrate buffer/1.1 mM acetate buffer (pH 3.55) with 10% methanol, 1 mM EDTA, and 5 mM sodium 1-heptanesulfonate at a flow rate of 1 mL/min. Peaks of DA, 5-HT, and their metabolites were identified by their retention time, and their amounts

were calculated against a calibrated external standard solution (0.6 μ M). The investigator performing HPLC measurements was blinded to the sample identities.

Statistical analysis

Data were analyzed by repeated measures analyses of variance (ANOVAs) with levels of significance adjusted by Bonferroni corrections or by unpaired *t* tests. Statistical analyses were performed with SPSS 23.0 for Windows. The data are expressed as the means $\pm$ standard errors (SEM). The minimal significance level was set at 5%.

Results

Overexpression of COMT increases LID

To assess the role of COMT overexpression in the development of LID, WT and TG mice were depleted of DA in the striatum in one hemisphere and treated daily with L-DOPA (20 mg/kg, i.p.) for 2 wk. As expected, this treatment induced dyskinetic behaviors in lesioned mice. TG mice displayed more severe axial, limb, and orolingual dyskinetic behaviors than their WT littermates (Fig. 1A–C). The total dyskinetic scores were also greater in TG mice than in WT controls (Fig. 1D).

LID persisted for at least 180 min after L-DOPA was injected, but the greatest difference between TG and WT mice occurred during the first hour (Fig. 2A). LID was present but very mild before a 30-min time point (data not shown) and peaked at 30 and 60 min, at which times the phenotypic differences between TG and WT mice were most pronounced. However, the overall effect during the entire 180 min was still robust; the sum of all observation periods over the entire 180-min period shows that TG mice displayed enhanced dyskinesia compared with that in WT mice (Fig. 2B).

We measured motor coordination using the rotarod test (Solís et al., 2017). Basal motor coordination was indistinguishable between the TG and WT animals (Fig. 2C, prelesion). 6-OHDA lesions decreased the latencies to fall from the rotarod equally in the two genotypes (Fig. 2C, pre-L-DOPA). L-DOPA treatment restored motor coordination equally in WT and TG mice (Fig. 2C, post-L-DOPA).

The repeated administration of L-DOPA results in behavioral sensitization that can be measured by the development of contralateral rotations (Pavón et al. 2006). We assessed this behavior for 15 min beginning 5 min after L-DOPA was injected. Contralateral rotations increased gradually over several days, reaching a plateau at approximately day 9 of treatment. We found no differences in L-DOPA-induced contralateral rotations between the TG and WT mice (Fig. 2D).

Overexpression of COMT potentiates L-DOPA-induced FosB and pACh3 expression in the dorsal striatum

LID has been attributed to enhancement of dopamine receptor 1 (D1R) signaling (Darmopil et al. 2009; Santini et al. 2009). We measured D1R-dependent molecular responses, FosB and pACh3 expression, in DA-depleted striata. Immunostaining for TH confirmed that DA

denervation was similar in TG and WT mice (Fig. 3A, B). However, TG mice had significantly greater densities of FosB⁺ (Fig. 3C) and pACh3⁺ (Fig. 3D) cells than WT mice.

Overexpression of COMT alters basal levels of DA and its metabolite but not 5-HT or its metabolite in mouse striata

As one of the three genes in the 190-kb segment (*COMT*) encodes for an enzyme that degrades DA and its metabolites, we next examined striatal monoamine levels. We used HPLC to analyze the levels of DA and its metabolites DOPAC and 3-MT in WT and TG mice that were sham-operated, 6-OHDA-lesioned (Parkinsonian [Park] group), or 6-OHDA lesioned and treated with L-DOPA (dyskinetic [dysk] group). We confirmed that TG mice in this cohort also displayed more severe dyskinesia than their WT littermates (see Supplementary Fig. 2).

The levels of DA in striata were significantly lower in sham-operated TG mice than in their WT counterparts (Fig 4, sham). 6-OHDA lesions induced severe decreases in DA levels in the ipsilateral (I) striata compared with levels in the contralateral (C) striata in TG and WT mice (Fig 4, Park). Chronic L-DOPA treatment did not alter DA levels in the striata ipsilateral to lesions in dyskinetic WT or TG mice, although it increased DA levels in contralateral striata in TG mice (Fig. 4, dysk).

Regarding DA metabolites, DOPAC levels were not significantly different between TG and WT sham-operated mice (Fig. 4, DOPAC, Sham). 6-OHDA lesions decreased DOPAC levels equally in the ipsilateral striata of the two genotypes (Fig. 4, DOPAC, Park). Similarly, chronic treatment with L-DOPA increased DOPAC levels equally in the two genotypes (Fig. 4, DOPAC, dysk). Levels of 3-MT were significantly elevated in the striata of sham-operated TG mice compared with the levels in WT mice. 6-OHDA lesions reduced the levels 3-MT in the striata ipsilateral to 6-OHDA lesions equally in TG and WT mice (Fig. 4, 3-MT, Park). L-DOPA increased the levels of 3-MT to a greater extent in the striata ipsilateral to lesions in TG mice than in WT mice (Fig. 4, 3-MT, dysk).

We next assessed the levels of 5-HT and its metabolite 5-HIAA. There were no differences in the levels of 5-HT or 5-HIAA between WT and TG mice under any condition. We found that the striatal 5-HT levels were similar in sham-operated TG and WT mice (Fig. 5, 5-HT, sham). 5-HT levels were augmented equally in the lesioned (DA-depleted) striata of TG and WT mice (Fig. 5, 5-HT, Park). L-DOPA treatment in 6-OHDA-lesioned mice normalized this effect equally in the two genotypes (Fig. 5, 5-HT, dysk). 5-HIAA striatal levels mirrored 5-HT levels in the sham and Park groups; 5-HIAA levels remained elevated in the striata ipsilateral to lesions in both WT and TG mice (Fig. 5, 5-HIAA).

Discussion

Our findings show that the overexpression of COMT included in a 190-kb segment of 22q11.2 in mice confers a heightened susceptibility to abnormal axial, limb, and orolingual movements. We also observed potentiated expressions of FosB and pACh3 and DA metabolism in DA-depleted striata concomitant with lower basal DA levels in mice without 6-OHDA lesions. In contrast, the overexpression of COMT had no effect on the therapeutic

actions of L-DOPA in mice as measured by contralateral rotations and motor coordination and by levels of 5-HT and its metabolite.

The constitutive transgenic mouse is ideal for testing our hypothesis that COMT overexpression confers altered susceptibility to dyskinesia. However, the BAC transgene construct could potentially have disrupted or interacted with endogenous murine genes. As such, the observed phenotypes might reflect these secondary effects rather than the effects from overexpressing genes that are encoded in the BAC.

Although the TG mice express TXNRD2, COMT, and ARVCF, it is not clear which of these contributed to the observed phenotypes. The existing evidence suggests that COMT and DA contribute to dyskinesia (de Lau et al., 2012). We previously demonstrated that this TG mouse has 2-fold higher levels of COMT enzymatic activity in the brain including in the striatum (Suzuki et al., 2009). Our data also show that basal levels of DA—an enzymatic target of COMT but not 5-HT—are significantly reduced in the striata of TG mice. Thus, we surmise that COMT contributed to the observed phenotypes.

There is an interesting dissociation between the motor effects induced by L-DOPA. Although the transgenic mice used here demonstrate more severe LID, the basal locomotor activity is not altered (Suzuki et al., 2009) nor were the therapeutic behavioral effects on motor coordination and rotational behavior. TG mice exhibit elevated basal levels of COMT and reduced levels of DA. Thus, L-DOPA treatment likely induces a shorter duration of dopaminergic stimulation in these mice. This condition is known to induce severe dyskinesia (Papathanou et al., 2011; Mulas et al., 2016). On the other hand, continuous L-DOPA administration reduces LID in Parkinson's patients (Timpka et al., 2016) and prolongs the half-life of L-DOPA via COMT inhibition to decrease dyskinesia (Espinoza et al., 2012; Müller, 2015). Thus, elevated COMT levels might cause repeated and short stimulation by L-DOPA thereby potentiating the dyskinetic phenotype.

Our interpretations on the lack of phenotypes in contralateral turns and rotarod tests should be considered with caution because the results might have been due to the timing of the testing. Contralateral turns were measured for 15 min starting 5 min after L-DOPA. Mice were examined for motor coordination on the rotarod before and 3 weeks after 6-OHDA lesions and again 90 min after the last injection on day 14. In contrast, dyskinesia was measured 40 minutes after L-DOPA injection or for 180 min on day 16 of L-DOPA treatment. A significant genotype effect on dyskinetic responses was seen 30 and 60 min after L-DOPA on day 16, but not thereafter. The time points for rotational and motor coordination assessments were chosen to avoid the secondary effects of dyskinesia, which non-selectively interferes with their detection. The data show that the therapeutic effects of L-DOPA (i.e., restoration of motor coordination and contralateral turns) were equally maintained in both WT and TG mice while inducing more severe dyskinesia in TG mice than in WT mice.

Although the post-synaptic mechanisms for potentiated dyskinetic responses in TG mice remain unclear, LID is associated with aberrant expression of protein kinases, transcription factors, and epigenetic modifications (Pavón et al., 2006; Westin et al., 2007; Santini et al.,

2009). In particular, the expression of the transcription factor FosB is enhanced in the denervated striatum upon chronic L-DOPA treatment in a D1R-dependent manner (Darmopil et al., 2009; Murer and Moratalla, 2011; Porras et al., 2012; Berther et al., 2009; Ruiz-DeDiego et al., 2015). H3 activation also contributes to the L-DOPA-induced accumulation of FosB (Feyder et al., 2016), which occurs in the direct striatal pathway (Pavón et al., 2006, Darmopil et al., 2009, Suarez et al., 2016; Solís et al., 2017). Upon repeated injection of L-DOPA, pronounced and continuous LID develops due to the pulsatile stimulation of DA receptors (Bastide et al., 2015). This results from the sensitization (Darmopil et al., 2009) and increased cell-surface expression and trafficking (Porras et al., 2012, Berther et al., 2009) of D1Rs consequently causes aberrant expression of FosB and pAcH3 (Ruiz-DeDiego et al., 2015; Solís et al., 2015a, b). A future challenge is to definitively determine that FosB and pAcH3 in the dorsal striatum functionally contribute to heightened LID intensity in TG mice.

COMT catalyzes DA to produce 3-MT (Männistö and Kaakkola, 1999). In accordance with this, we observed lower levels of DA and higher levels of 3-MT in the striata of TG mice than in their WT counterparts. However, these differences disappeared after the 6-OHDA lesions. In dyskinetic animals, L-DOPA treatment does not increase DA levels in the lesioned striatum (Del-Bel et al., 2014; Solís et al., 2016). Interestingly, dyskinetic TG animals had higher DOPAC and 3-MT levels in the lesioned striata than dyskinetic WT mice. Previous studies suggested a modulatory effect of striatal 3-MT on dyskinesia due to activation of trace-amine associated receptor 1 (TAAR1) (Rajput et al., 2004; Sotnikova et al., 2010; Espinoza et al., 2012). Therefore, the increase in LID seen in the TG mice might be due to the increase in 3-MT.

TH staining was completely absent (TH-negative) in ~50% of the entire dorsal striatum in WT and TG mice after 6-OHDA lesions. We applied a stringent criterion to judge the absence of TH-positive fibers; an area where weakly or very weakly stained fibers were present was regarded as a TH-positive area. Our HPLC analysis showed ~90% reduction in DA levels in the dorsal striatum. The explanation for this apparent discrepancy is that either dopaminergic cells with weakly TH-labeled fibers were not sufficient to produce DA or that residual TH-positive fibers included dying cells.

Since the serotonergic system is also implicated in the development of LID (Carta et al., 2007), we evaluated whether 5-HT and its metabolite 5-HIAA were modified in TG mice. The 5-HT and 5-HIAA levels were indistinguishable between TG and WT mice, and 5-HT and 5-HIAA levels were increased in both WT and TG mice with DA-depleted striata. Interestingly, L-DOPA treatment restored 5-HT levels to normal in both TG and WT mice. Thus, 5-HT might not be involved in the potentiated dyskinetic responses in TG mice, although we do not rule out the possibility that it—along with its pre- and post-synaptic components (e.g., 5-HT transporters and receptors)—might exert an inhibitory effect on dyskinesia.

Human studies have identified a low-activity allele of COMT with a methionine at position 108 as well as a high-activity allele with valine at the same position (Männistö and Kaakkola, 1999; Tunbridge, 2010). The role of this COMT polymorphism in dyskinesia in

L-DOPA-treated Parkinson's disease patients is not clear. One study found that the high-activity COMT allele was associated with an increased risk of developing LID (de Lau et al., 2012), but others failed to find any significant association (Bialecka et al., 2008; Contin et al., 2005; Watanabe et al., 2003). On the other hand, patients with higher erythrocyte COMT activity displayed more dyskinesia (Reilly et al., 1980; Rivera-Calimlim and Reilly, 1984).

Our results are consistent with the idea that prolonging the half-life of L-DOPA thorough COMT inhibition can decrease dyskinesia (Espinoza et al., 2012; Müller, 2015). This work using genetically modified mice strengthens and consolidates clinical studies using COMT inhibitors. To the best of our knowledge, this is the first study to show that over-expressing COMT increases LID.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BAC	bacterial artificial chromosome
COMT	catechol- <i>O</i> -methyltransferase

DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
D1R	dopamine receptor 1
H3	histone 3
HPLC	high performance liquid chromatography
L-DOPA	L-3,4-dihydroxyphenylalanine
LID	L-DOPA-induced dyskinesia
pAcH3	phospho-acetyl-histone 3
TG	transgenic mice that carry a BAC comprising a 190-kb region of human chromosome 22q11.2 (BAC467.8)
TH	tyrosine hydroxylase
WT	wild type
3-MT	3-methoxytyramine
5-HT	serotonin
5-HIAA	5-hydroxyindoleacetic acid
6-OHDA	6-hydroxydopamine hydrobromide

Highlights

Overexpression of a 190kb chromosomal segment including the human COMT increases dyskinesia.

This chromosomal segment contains *TXNRD2*, *COMT*, *ARVCF*.

Potentiated expression of FosB and pACh3 is correlated with the degree of dyskinesia.

The dopamine metabolites DOPAC and 3-MT are correlated with the degree of dyskinesia.

Serotonin or its metabolite 5HIAA is not correlated with the degree of dyskinesia.

Fig. 1.

Dyskinetic responses to L-DOPA are potentiated in COMT-overexpressing mice. Scores for axial (A), limb (B), orolingual (C), and the sum total (ALO) (D) dyskinetic behaviors were evaluated for 4 min, 40 min after L-DOPA injections in mice. A repeated two-way ANOVA indicated significant main effects for axial ([A] genotype, $F_{1,17} = 13.21$, $P = 0.0021$; day, $F_{4,68} = 34.59$, $P < 0.0001$; interaction, $F_{4,68} = 0.63$, not significant [n.s.]), limb ([B] genotype, $F_{1,17} = 10.00$, $P = 0.0057$; day, $F_{4,68} = 37.65$, $P < 0.0001$; interaction, $F_{4,68} = 0.60$, n.s.), orolingual ([C] genotype, $F_{1,17} = 8.73$, $P = 0.0089$; day, $F_{4,68} = 22.46$, $P < 0.0001$; interaction, $F_{4,68} = 0.44$, n.s.), and total ([D] genotype, $F_{1,17} = 13.62$, $P = 0.0018$; day, $F_{4,68} = 78.53$, $P < 0.0001$; interaction, $F_{4,68} = 0.38$, n.s.) dyskinesia. Data are expressed as the means $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ vs. WT as determined by Bonferroni post hoc test; $n = 9$ –10/group.

Fig. 2.

Overexpression of COMT enhances dyskinesia in mice but not motor coordination or rotational behaviors. (A) Time course of dyskinetic (axial, limb, and orolingual [ALO]) behaviors on day 16, evaluated for 1 min once every 30 min during a 180-min period following L-DOPA treatment. A repeated two-way ANOVA indicated significant main effects (genotype, $F_{1,17} = 5.88$, $P = 0.0268$; time, $F_{5,85} = 191.63$, $P < 0.0001$; interaction, $F_{5,85} = 1.45$, $P = 0.215$), and a genotype effect at each time point, as determined by Bonferroni's tests. * $P < 0.05$ vs. WT. (B) Sum of all scores over the 180-min period ($t[17] = 2.425$) * $P = 0.0268$ vs. WT. (C) Motor coordination and balance assessed on the rotarod before 6-OHDA lesions (prelesion), 3 weeks after lesions (pre-L-DOPA), and 90 min after L-DOPA was injected on day 14 of the chronic treatment (post-L-DOPA). A two-way ANOVA indicates a significant group effect only (genotype, $F_{1,17} = 0.02$, not significant [n.s.]; group, $F_{2,34} = 60.70$, $P < 0.0001$; interaction, $F_{2,34} = 0.06$, n.s.). *** $P < 0.001$ vs. TG pre-L-DOPA, ### $P < 0.001$ vs. WT pre-L-DOPA as determined by Bonferroni post hoc tests. (D) Contralateral turns were measured for a period of 15 min, 5 min after L-DOPA was injected during chronic treatment. A repeated two-way ANOVA indicates a significant effect of day only (genotype, $F_{1,17} = 0.46$, n.s.; day, $F_{4,68} = 18.12$, $P < 0.0001$; interaction, $F_{4,68} = 1.10$, n.s.). Data are expressed as the means $\pm$ SEM. $n = 9$ –10/group.

Fig. 3.

Induction of FosB and pACh3 in striata of COMT-overexpressing mice. (A) Immunostaining for TH, FosB, and pACh3. Photomicrographs of adjacent coronal striatal sections of the lesioned striata at low and high (40x) magnification from WT and TG mice. Scale bar = 100 μ m for low-magnification and 50 μ m for high-magnification images. The continuous outline represents the dorsal striatum and the dashed outline represents the completely denervated striatum in the low magnification TH pictures. (B) The extent of striatal lesions was assessed by quantifying the percentage of striatal volume that did not stain for TH ($t[17] = 0.53$, n.s.). The densities of FosB-positive (C) ($t[17] = 2.38$, $P = 0.0292$) and pACh3-positive (D) ($t[17] = 2.80$, $P = 0.0123$) cells in the lesioned striata. Data are expressed as the means $\pm$ SEM.

* $P < 0.05$ vs. WT mice; $n = 9$ –10/group.

Fig. 4.

Levels of DA and its metabolites in lesioned mice after L-DOPA treatment. Levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxytyramine (3-MT) in the striata contralateral (C) and ipsilateral (I) to 6-OHDA lesions in sham-operated, lesioned and saline-administered (Park), and lesioned and L-DOPA-administered (dysk) mice. Mice were sacrificed 60 min after the last saline or L-DOPA injection, and levels were measured by HPLC. A three-way ANOVA with significant *P* levels adjusted by Bonferroni corrections showed genotype $\times$ condition interactions for DA ($F_{2,29} = 10.838$, $P = 0.001$) and DOPAC ($F_{2,28} = 6.326$, $P = 0.005$). For 3-MT levels, significant effects were found according to genotype ($F_{1,29} = 21.205$, $P < 0.001$), condition ($F_{2,29} = 11.01$, $P < 0.001$), and hemisphere ($F_{1,29} = 71.61$, $P < 0.001$) without interactions (genotype $\times$ condition, $F_{2,29} = 0.659$, $P = 0.525$; genotype $\times$ hemisphere, $F_{1,29} = 0.026$, $P = 0.873$; genotype $\times$ condition $\times$ hemisphere, $F_{2,29} = 0.588$, $P = 0.562$). * $P < 0.05$ and ** $P < 0.01$ vs. WT ipsilateral striatum. The data are shown as the means $\pm$ SEM. $n = 5-7$ /group.

Fig. 5.

Levels of serotonin and its metabolite in lesioned mice after L-DOPA treatment. Levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the striata contralateral (C) and ipsilateral (I) to 6-OHDA lesions in sham-operated, lesioned and saline-administered (Park), and lesioned and L-DOPA-administered (dysk) mice. Mice were sacrificed 60 min after the last saline or L-DOPA injection, and levels were measured by HPLC. A three-way ANOVA with significant *P* levels adjusted by Bonferroni corrections showed significant differences for effects of condition ($F_{2,29} = 8.215$, $P = 0.001$) and a condition $\times$ hemisphere interaction ($F_{2,29} = 12.61$, $P < 0.001$) for 5-HT; no other effect was significant. For 5-HIAA, no main or interaction effect involving genotype was significant (genotype, $F_{1,28} = 0.018$, not significant [n.s.]; genotype $\times$ condition, $F_{2,28} = 0.283$, n.s.; genotype $\times$ hemisphere, $F_{1,28} = 0.415$, n.s.; genotype $\times$ condition $\times$ hemisphere, $F_{2,28} = 0.543$, n.s.). * $P < 0.05$ and ** $P < 0.01$ vs. contralateral striatum. Data are presented as the means $\pm$ SEM. $n = 5-7$ /group.