Distinct patterns of dyskinetic and dystonic features following D1 or D2 receptor stimulation in a mouse model of parkinsonism

Laura Andreoli *, Morteza Abbaszadeh 1, Xiao Cao 1, Maria Angela Cenci *

Basal Ganglia Pathophysiology Unit, Department of Experimental Medical Science, Lund University, BMC, 221 84 Lund, Sweden

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ABSTRACT

L-DOPA-induced dyskinesia (LID) is a significant complication of dopamine replacement therapy in Parkinson’s disease (PD), and the specific role of different dopamine receptors in this disorder is poorly understood. We set out to compare patterns of dyskinetic behaviours induced by the systemic administration of L-DOPA and D1 or D2 receptor (D1R, D2R) agonists in mice with unilateral 6-hydroxydopamine lesions. Mice were divided in four groups to receive increasing doses of L-DOPA, a D1R agonist (SKF38393), a D2/3 agonist (quinpirole), or a selective D2R agonist (sumanirole). Axial, limb and orofacial abnormal involuntary movements (AIMs) were rated using a well-established method, while dystonic features were quantified in different body segments using a new rating scale. Measures of abnormal limb and trunk posturing were extracted from high-speed videos using a software for markerless pose estimation (DeepLabCut). While L-DOPA induced the full spectrum of dyskinesias already described in this mouse model, SKF38393 induced mostly orofacial and limb AIMs. By contrast, both of the D2-class agonists (quinpirole, sumanirole) induced predominantly axial AIMs. Dystonia ratings revealed that these agonists elicited marked dystonic features in trunk/neck, forelimbs, and hindlimbs, which were overall more severe in sumanirole-treated mice. Accordingly, sumanirole induced pronounced axial bending and hindlimb divergence in the automated video analysis. In animals treated with SKF38393, the only appreciable dystonic-like reaction consisted in sustained tail dorsiflexion and stiffness. We next compared the effects of D1R or D2R selective antagonists in L-DOPA-treated mice, where only the D2R antagonist had a significant effect on dystonic features. Taken together these results indicate that the dystonic components of LID are predominantly mediated by the D2R.

1. Introduction

Poverty and slowness of movement (hypokinesia and bradykinesia) are cardinal motor features of Parkinson’s disease (PD). These and other features, such as rigidity, are caused by putaminal dopamine (DA) depletion (Brooks, 2006; Meder et al., 2019) and improved by dopaminergic medications, the most effective being L-DOPA (Cenci et al., 2011). Long term treatment with L-DOPA, however, leads to the development of abnormal involuntary movements (dyskinesia) in a majority of PD patients (Cenci et al., 2020; Manson et al., 2012; Muller and Russ, 2006). There is consensus that L-DOPA-induced dyskinesia (LID) develops as a consequence of large intermittent fluctuations in brain DA levels causing an abnormal stimulation of post-synaptic DA receptors, particularly those expressed in striatal neurons (Cenci, 2014; Kordower et al., 2013; Olanow et al., 2020). Indeed, a large number of studies have linked LID to the stimulation of supersensitive D1 receptors (D1Rs) and the consequent aberrant activation of D1R-mediated signaling in striatal neurons forming the direct pathway (Alcacer et al., 2012; Aubert et al., 2005; Darmopil et al., 2009; Santini et al., 2007; Sebastianutto et al., 2020; St-Hilaire et al., 2005; Westin et al., 2007). Nevertheless, dyskinesias have also been reported after administration of selective D2 receptor (D2R) agonists to parkinsonian rodents and monkeys (Bhide et al., 2015; Gomez-Mancilla and Bedard, 1992; Sebastianutto et al., 2020). In most of these studies, however, animals had been previously exposed to L-DOPA, which might condition the response to subsequent dopaminergic treatments through a phenomenon called “priming” and mediated by D1Rs (Di Chiara et al., 1992; Morelli, 1997). In the treatment of PD, agonists of D2-class DA receptors (commonly referred to as DA agonists) do not induce much dyskinesia when used as a monotherapy in early-stage PD, although they increase...
the risk of both dyskinesia and postural abnormalities when combined with L-DOPA at later disease stages (Ameghino et al., 2018; Stocchi et al., 2016; Stowe et al., 2011). These findings point to the clinical importance of investigating the specific contribution of D2Rs to the pathophysiology of LID and other motor complications.

In order to elucidate the dyskinetic action of D2R stimulation, we set out to compare patterns of abnormal involuntary movements (AIMs) and open-field motions induced by selective agonists of D2R vs D1R in drug-naive DA-denervated rodents. Studies were performed in mice sustaining unilateral 6-OHDA lesions of the medial forebrain bundle (MFB), an animal model of parkinsonism that is now widely used for pathophysiological investigations (Alcacer et al., 2017; Girasole et al., 2018; Parker et al., 2018; Ryan et al., 2018; Sebastianutto et al., 2020; Shen et al., 2020). Upon observing pronounced differences between D1 and D2 agonists on the liability to induce dystonia, we developed a new rating scale to specifically quantify dystonic features in different parts of the body, including trunk, hindlimbs, forelimbs, and tail. The scale was partly validated through correlation with geometric measures of mouse postures, and then used to study L-DOPA-treated mice receiving challenge injections with D1R- or D2R agonists.

Our results show that D1Rs and D2Rs mediate distinct patterns of dyskinetic and dystonic movements, possibly reflecting their preferential recruitment of different neuronal circuits.

2. Materials and methods

2.1. Animals

The study was performed on C57BL/6 J mice (Charles River/SCAN-BUR Research A/S; Denmark), approximately 12 weeks old at the beginning of the experiments. Mice were housed under a 12-h light/dark cycle with ad libitum access to water and food. All procedures were approved by the Malmö-Lund Ethical Committee on Animal Research.

2.2. Experimental design

In a first experiment, mice with unilateral 6-OHDA lesion were allocated to four groups (n = 7/8 per group) to receive treatment with incremental doses of one of the following compounds: (1) the partial D1R agonist SKF38393 (3/6 mg/kg); (2) the D2/3R agonist quinpirole (QUIN) (0.5/1 mg/kg); (3) the selective D2R agonist sumanirole (SUM) (2/4 mg/kg); (4) L-DOPA (3/6 mg/kg). Each dose was administered for five consecutive days (day 1 to day 5) with a drug free period of two days (Day 6 and 7) before moving to the higher dose (see design in Supplemental Fig. S1A).

Dyskinesia and dystonia rating sessions were carried out twice in each treatment period, at day 1 and 5. After completing this schedule, mice in group (4) received challenge injections of L-DOPA (6 mg/kg) combined with the selective D1R agonist SCH23390 (0.05 mg/kg) or the selective D2R antagonist L741626 (4 mg/kg), while mice in group (3) received challenge injections of SUM (4 mg/kg) combined with L741626 (4 mg/kg) according to a blinded cross-over test design (see design in Supplemental Fig. S1A).

In a second experiment, 20 additional mice were rendered parkinsonian according to the same procedure and recorded with a high-speed camera on the fifth day of the following treatments: vehicle (n = 5), L-DOPA 6 mg/kg (n = 5), SKF38393 3 mg/kg (n = 5) and SUM 4 mg/kg (n = 5) (see design in Supplemental Fig. S1B).

2.3. Compounds and treatments

Dopaminergic treatments were administered at doses previously used in rodents and reported to produce robust behavioural effects through the indicated target (see Table 1 for doses and main references). QUIN (–) quinpirole hydrochloride, Tocris Bioscience, SUM (sumanirole maleate, Tocris Bioscience), SKF38393 (Sigma-Aldrich AB), the

| Table 1 | Drugs and administration procedures. |
|---|---|
| Drug | Receptor target | Supplier | Doses (mg/kg) | Admin. route | Main references |
| L-DOPA | DAR | Sigma Aldrich | 3 and 6 | i.p. | Alcacer et al., 2017 |
| SKF38393 | D1/5 | Sigma Aldrich | 3 and 6 | i.p. | Sebastianutto et al., 2016 |
| Quinpirole | D2/3 | Tocris Bioscience | 0.5 and 1 | i.p. | Lanza et al., 2018 |
| Sumanirole | D2 | Tocris Bioscience | 2 and 4 | i.p. | Raddi et al., 2018 |
| SCH23390 | D1/5 | Tocris Bioscience | 0.05 | i.p. | Luque-Rojas et al., 2013 |
| L741626 | D2 | Tocris Bioscience | 4 | i.p. | Weber et al., 2010 |

Compounds were administered at doses reported to produce robust behavioural effects through the indicated target (DAR, all DA receptors).

D1R antagonist SCH23390 (SCH23390 hydrochloride, Tocris Bioscience) and the D2R antagonist L741626 (Tocris Bioscience) were given at doses devoid of intrinsic motor depressant effects (see references in Table 1). All compounds but L741626 were dissolved in physiological saline solution (9 g/l NaCl) in a sonicating bath for approximately 10 min. L741626 was first dissolved with some drops of 10% acetic acid and then diluted to the desired concentration in saline solution; the pH was adjusted to 5.5–6.0 by adding some drops of 1 M sodium hydroxide (NaOH). L-DOPA (Sigma-Aldrich AB) was dissolved in physiological saline together with the peripheral DOPA decarboxylase inhibitor benserazide-HCl (12 mg/kg, Sigma-Aldrich AB). All drugs used in the study, were freshly prepared on the same administration day and given intraperitoneally (i.p.). In the experiments including vehicle treated controls, vehicle consisted of saline solution (video tracking study) or saline solution containing acetic acid and NaOH (study with D2R antagonist). For all compounds, the injection volume was 10 μl/kg.

2.4. DA-denervating lesions and their assessment

Chronic striatal DA denervation was produced through unilateral injection of 6-OHDA in the MFB according to a well-established method (Francardo et al., 2011). The toxin 6-OHDA hydrochloride (Sigma-Aldrich) was dissolved at a fixed concentration of 3.5 μg/μl in 0.02% ice-cold ascorbate/saline and used within 2 h. Briefly, mice were anesthetized with a mixture of 1.5–2% isoflurane in air (Isovaet, Apo-ketsbolaget) and placed in a stereotaxic frame with a mouse adaptor (Kopf instruments). A local analgesic treatment was administered in the area of the skin incision via subcutaneous (s.c.) injection of the long-acting anesthetic Marcain (bupivacaine, 2.5 mg/ml, AstraZeneca; s.c.; 10 μl/10 g body weight).

After the skull was exposed, the toxin solution was injected at the following coordinates: antero-posterior (AP): –0.7, medio-lateral (ML): –1.2, dorso-ventral (DV): –4.7 (tooth bar: –4.0). A total of 1 μl toxin solution was injected at a rate of 0.2 μl/min using a glass capillary attached to a 10 μl Hamilton syringe. The capillary was left in place for 2 min before and 4 min after the injection. Sham lesions were carried out by injecting 1 μl of 0.02% ascorbic acid-saline solution at the same MFB coordinates. After the surgery, the wound was closed with tissue glue (Histoacryl®, B. Braun OEM Division).
To prevent dehydration, mice received a s.c. injection of sterile glucose-ringer acetate solution (0.5 ml) immediately after the surgery. During the first 3 postoperative weeks, mice were housed in a warming cabinet at a constant temperature of 30 °C. They were daily monitored for body weight and health status, and moreover received integrative diet with high-protein gel (DietGel®boost, ClearH20).

Approximately three weeks after surgery, forelimb use asymmetry was evaluated using a test of spontaneous limb use during vertical exploratory behaviour (cylinder test), as previously described (Francardo et al., 2011). Briefly, mice were placed individually in a glass cylinder (10 cm diameter, 14 cm high) flanked by mirrors and video recorded for 5 min. Video recordings were analysed offline to count the number of wall contacts executed independently with the right and the left forepaw to support the mouse body against the cylinder wall. The performance of the paw contralateral to the lesion (left paw) was expressed as a percentage of the total number of wall contacts recorded in a session. Only animals exhibiting ≤30% contralateral forelimb use were included in the study (Fig. S2A). This threshold value ensured selection of animals with a nearly complete DA denervation of motor striatal regions (Lundblad et al., 2004; Francardo et al., 2011).

Striatal DA denervation was moreover verified post-mortem using tyrosine hydroxylase (TH) immunohistochemistry (see Supplemental methods). All animals included in the study had >85% overall depletion of TH optical density in the striatum on the side ipsilateral to the lesion, and no differences were found among animals allocated to different treatment groups (Fig. S2B,C).

2.5. Ratings of abnormal involuntary movements (AIMs)

Treatment-induced dyskinesia was assessed using a well-established rating scale that does not discriminate between dystonic and hyperkinetic (choreiform-like) features (Francardo et al., 2011; Lundblad et al., 2004). The scale considers three subtypes of purposeless and abnormal movement sequences (axial, limb, orofacial AIMs) based exclusively on their topographic distribution. Axial AIMs consist of twisting-bending movements of the neck and upper body towards the side contralateral to the lesion, limb AIMs are jerky or fluttering movements of the contralateral forelimb, while orofacial AIMs involve rapid contractions of jaw and facial muscles, and are often accompanied by tongue protrusion towards the side contralateral to the lesion (Francardo et al., 2011; Lundblad et al., 2004). Mice were placed in individual transparent cages starting from 20 min before drug administration, and then observed for 1 min every 20 min during a total of 180 min after drug injection). No signs of dyskinesia were detected during the 20 min habituation period prior to drug treatment. Each AIM subtype was rated on a severity scale from 0 to 4 based on the duration and persistence of the dyskinetic features (0 = no dyskinesia; 1 = occasional signs of dyskinesia present for less than 50% of the observation time; 2 = frequent signs of dyskinesia present for more than 50% of the observation time; 3 = dyskinesia present during the entire observation period but easily interruptible by external stimuli; 4 = continuous dyskinesia not interrupted by external stimuli).

2.6. Ratings of dystonic features

During each AIMs rating session, an action camera (GoPro Hero 4, GoPro Inc., USA) was placed in front of the test cage and mice were video recorded for 1 min every 20 min. Videos were analysed offline. Dystonic features were defined as a pattern of slow movements and abnormal postures that visibly required sustained muscular activity. Dystonic features were defined as a pattern of slow movements and video recorded for 1 min every 20 min. Videos were analysed offline.

Each dystonic sequence turned out to last for 2 s. We set out to assign severity scores to different body segments using a time-based grading scale analogous to that used to rate AIMs, where score 1 indicates that a given item is present for less than 50% of the observation time; score 2 indicates that the item is present for more than 50% of this time, and score 3 indicates that the item is present throughout the monitoring period. In order to provide a more interval-like rating system, we however anchored these gross definitions to actual number of seconds and also introduced half-point scores (0.5, 1.5, 2.5). We could thus establish precise time-based severity scores according to the following criteria: 0 = no signs of dystonia; 0.5 = single or occasional dystonic events occurring for less than 10 s; 1 = signs of dystonia present for 11 to 20 s; 1.5 = signs of dystonia present for 21 to 30 s; 2 = signs of dystonia present for 31 to 40 s; 2.5 = signs of dystonia present for 41 to 50 s; 3 = signs of dystonia present for 51 to 60 s). The rating method was validated by two independent experimenters, both blinded to the drug treatments. After its establishment and validation, the rating method was applied in a systematically blinded fashion in the rest of the study (cf. Figs. 4, 5, 8). A detailed description of the rated dystonic features is provided in the Results section.

2.7. Video tracking analysis

Animals were placed in an open-field arena defined by a circular enclosure of 33 cm diameter placed on the top of a transparent glass table. After a 10-min acclimatization, animals were filmed from below using a high-speed camera (GS3-U3-2356C-F, FLIR Grasshopper®3, 140 frames/s) while freely moving in the arena. Videos were acquired in 2 min periods, every 20 min, after drug or vehicle administration. Videos were preprocessed in order to standardize for variability in background and orientation of the arena between different recording session. The software package DeepLabCut (Mathis et al., 2018) was used to dynamically track different parts of the mouse body using virtual markers. The DeepLabCut network was trained on 95% of the dataset and tested on 5% of the dataset. With a 0.5 likelihood cutoff, the estimated error on the trained dataset was 1.26 pixels (<0.77 mm; 1 pixel = 0.61 ± 0.03 mm) while the error on the tested dataset was 2.03 pixels (<1.24 mm). For each of the markers, DeepLabCut provided three columns of data: X coordinates, Y coordinates and detection likelihood. Markers with a likelihood below 0.5 were excluded and replaced with the marker position in the previous frame (the average lengths of the excluded segments were below 0.15 s for each body part, see Supplementary Fig. S3). Jittering fluctuations were smoothed using Savitzky-Golay filter. The centroid of the animal was calculated by averaging the X and Y coordinates of 6 body parts, that is, nose tip (N), left and right forelimbs (LF and RF), left and right hindlimbs (LH and RH), and base of tail (T) through the 2 min recording periods. Patterns of open-field motions were examined by computing distance travelled and speed of movement with reference to the mouse body centroid. The distance travelled was calculated as the sum of distances (in pixels) that the animal’s centroid moved between two consecutive frames along the 2 min video. Distance in pixels was then converted into centimeter unit according to the following ratio: 1 pixel = 0.061 cm. Movement speed was calculated by dividing the total distance travelled between two consecutive frames by the frame duration, and then averaged by the total recording period (2 min recordings at 20, 40, 60 min post injection).

2.8. Automated measurement of axial bending angle

The axial bending angle was defined as the angle generated from the intersection between two vectors extending from the mid-point between hindlimbs (midHL), to the nose-tip and the tail base, respectively.

Knowing X and Y coordinates for each of the four body parts (N(xN, yN), LH(xLH,yLH), RH(xRH,yRH), T(xT,yT)), and the mid-point between hindlimbs (midHL), .Head and .Tail vectors were calculated as follow:

\[
\text{midHL} = \frac{\left( xRH - xLH \right) \hat{r} + \left( yRH - yLH \right) \hat{s}}{2}
\]

\[
\text{Head} = \frac{\left( xN - xmidHL \right) \hat{r} + \left( yN - ymidHL \right) \hat{s}}{2}
\]
\[ \text{Tail} = (xT - x\text{midHL}, yT - y\text{midHL}) \]

Next, we calculated the cosine of the angle between the \text{Head} and \text{Tail} vectors:

\[
\cos(\theta) = \frac{\text{Head} \cdot \text{Tail}}{|\text{Head}||\text{Tail}|}
\]

where \text{Head} and \text{Tail} is the dot product of the two vectors and |\text{Head}| and |\text{Tail}| are their Euclidean distances. The axial bending angle was calculated through the length of the 2-min videos acquired at 20, 40, and 60 min post vehicle or drug injection.

### 2.9. Measurement of hindlimb divergence

A measure of hindlimb divergence was obtained by calculating the angle at the intersection between two vectors traced across the longitudinal axes of the hindpaws. Because DeepLabCut does not allow for changing the zooming factor during the marking of body parts, it was necessary to examine the hindpaws at high magnification on frozen video frames so that vectors could be traced with precision. Thus, 50 frames were randomly selected from the 2 min recording periods acquired at 20, 40, and 60 min post drug injection (corresponding to 150 frames analysed per animal) using an ad-hoc developed algorithm based on Python (Python Software Foundation, Python Language Reference, version 3.9.5. Available at [http://www.python.org](http://www.python.org)).

Frames showing grooming, rearing and resting were excluded and replaced with new randomly selected frames to account for a total number of 50 frames per recording period. Each frame was first saved in JPEG format and then opened with the free software FIJI ([Schindelin et al., 2012](http://rsbweb.nih.gov/ij/)). In FIJI, XY coordinates referring to left and right paw placement in space were extracted using the following criteria: four marks were made using the “multipoint” tool in a chronological order, where marks 1 and 2 always referred to right paw, heel and middle toe respectively, while marks 3 and 4 always referred to the left paw, heel and middle toe respectively. Next, XY coordinates were used to extract vectors referring to the right (R) and left (L) paw longitudinal axis:

\[
\begin{align*}
R &= (x_2 - x_1, y_2 - y_1) \\
L &= (x_4 - x_3, y_4 - y_3)
\end{align*}
\]

The angle between the two vectors was calculated using the dot product formula:

\[
\cos(\theta) = \frac{R \cdot L}{|R||L|}
\]

where |R| and |L| are the magnitude of the vectors. The final value was then converted from radians to degrees.

### 2.10. Statistical analyses

Statistical analyses were performed using Prism 9 (GraphPad software). Scores recorded during a course of chronic drug treatment (AIMs, dystonia) were analysed using repeated measures ANOVA. This test provides valuable information on the interaction between time points and treatment effects which is not readily available in non-parametric tests. Post hoc analyses were carried out using Bonferroni’s multiple comparison test, and data were presented as group ± SEM. When compared between groups on single sessions or time points, AIMs and dystonia ratings were analysed using non-parametric statistics (Kruskal-Wallis and post hoc Mann-Whitney test). Relations between variables were examined using the Pearson’s r. All data analysed using non-parametric statistics are presented as box plot and median, with whiskers annotating minimum and maximum values. The level of significance was set at \( p < 0.05 \).

### 3. Results

#### 3.1. Different patterns of dyskinesia upon stimulation of D1 or D2 receptors

Axial, limb, and orofacial (ALO) AIMs were compared between animals treated with standard doses of L-DOPA or with subtype-selective DA receptor agonists, namely, SKF38393 (D1/5), QUIN (D2/3), or SUM (D2). Each agonist was used at relatively high doses, yet presumably devoid of significant off-target effects based on the available literature ([Anzalone et al., 2012](http://www.ncbi.nlm.nih.gov/pubmed/22853912); [Collins et al., 2007](http://www.ncbi.nlm.nih.gov/pubmed/17105272); [Gangarosa et al., 2013](http://www.ncbi.nlm.nih.gov/pubmed/23904006); [Iderberg et al., 2013](http://www.ncbi.nlm.nih.gov/pubmed/23338390); [Lanza et al., 2018](http://www.ncbi.nlm.nih.gov/pubmed/29715172); [Luque-Rojas et al., 2013](http://www.ncbi.nlm.nih.gov/pubmed/24399004); [Raddi et al., 2018](http://www.ncbi.nlm.nih.gov/pubmed/29370020); [Weber et al., 2010](http://www.ncbi.nlm.nih.gov/pubmed/20700210)).

Dyskinetic behaviours were induced by all the compounds tested although with differences in duration and overall severity (Fig. 1A–D). In order to facilitate further comparisons between treatments in a way that prescinded from pharmacodynamic aspects, we focussed our analyses on AIMs scores recorded at 20–60 min following drug administration, which is the interval corresponding to peak AIM severity for all the tested compounds (see grey-shaded areas in Fig. 1A–D).

Peak ALO AIMs were significantly more severe in mice treated with L-DOPA compared to DA receptor agonists, but did not differ significantly between the groups treated with SKF38393, QUIN, or SUM (Fig. 1E). However, conspicuous differences between treatments were uncovered upon analyzing the individual AIM subtypes (Fig. 1F–H) and their relative representation (Fig. 1I). Specifically, axial AIMs were mildest in mice treated with SKF38393 when considering both the absolute scores (Fig. 1F; \( p < 0.001 \) vs L-DOPA, \( p < 0.05 \) vs QUIN) and their percentage of total AIMs (Fig. 1I, \( p < 0.01 \) vs L-DOPA and \( p < 0.001 \) vs QUIN and SUM). Orofacial and limb AIMs were, however, mildest in mice treated with D2-class agonists (Fig. 1G; \( p < 0.001 \) vs L-DOPA for both QUIN and SUM; Fig. 1H, \( p < 0.01 \) vs L-DOPA for both QUIN and SUM). Thus, in QUIN- and SUM-treated mice, over 70% of the recorded ALO AIMs were accounted for by the axial subtype (Fig. 1I; \( p < 0.01 \) for QUIN and SUM vs both L-DOPA and SKF38393).

Taken together, these results show that dyskinetic behaviours can develop upon pharmacological stimulation of both D1R and D2R, but that the two receptor classes preferentially mediate different types of AIMs. In particular, D2-class agonists induced prominent axial dystonia but very mild orofacial and forelimb AIMs, whereas the inverse pattern was seen after treatment with the D1-class agonist SKF38393. Compared to orofacial and forelimb AIMs, the axial subtype has a predominantly dystonic character. This raised the question, whether D2-class agonists have a high liability to induce dystonic forms of dystonia also in other body segments.

#### 3.2. A new rating scale to assess dystonic features in dyskinetic mice

To enable a further characterization of dyskinetic behaviours induced by D1R or D2R stimulation, we developed a rating scale specifically focused on dystonic features, i.e. slow movements and abnormal postures visibly maintained by active muscular contraction ([Albanese et al., 2013](http://www.ncbi.nlm.nih.gov/pubmed/23461454)). In dyskinetic mice, such features were detected in all body segments accessible to visual inspection (Table 2, Fig. 2). We developed a rating scale assigning dystonia severity scores to the following body segments: trunk/neck (tr/ne), forelimb (FL) hindlimb (HL), and tail. The limbs contralateral and ipsilateral to the 6-OHDA lesion were given separate ratings, referred to as contralateral and ipsilateral forelimb (cFL, iFL), or contralateral and ipsilateral hindlimb (cHL and iHL). The scale was applied to videos that had been recorded simultaneously with the on-line AIM ratings, and each of the indicated body segments received severity grades from 0 to 3 based on the proportion of observation time during which well-defined dystonic feature were detectable. All the features included in our scale are presented in Table 2, which reports both an easily recognizable dystonic trait for each body part (“criterion”) and describes the corresponding movement/
Fig. 1. Dyskinesia profiles induced by different dopaminergic treatments.

A–D. Time course of the summed axial, limb and orofacial AIM scores during the 180 min test sessions. A. L-DOPA (n = 8) - Dose: F(1.0, 7.0) 10.38, p < 0.05; Time: F(3.2, 22.6) 57.53, p < 0.001; Interaction: F(2.3, 16.3) 4.60, p < 0.05. B. SF38393 (n = 8) - Dose: F(1.0, 7.0) 1.68, p = 0.236; Time: F(2.3, 16.1) 16.82, p < 0.001; Interaction: F(2.5, 17.8) 1.69, p = 0.208. C. Quinpirole (QUIN, n = 7) - Dose: F(1.0, 6.0) 5.19, p = 0.062; Time: F(1.8, 11.1) 38.58, p < 0.001; Interaction: F(2.6, 15.9) 0.98, p = 0.415. D. Sumanirole (SUM, n = 7) - Dose: F(1.0, 6.0) 81.52, p < 0.001; Time: F(3.3, 20.0) 25.51, p < 0.001; Interaction: F(2.3, 14.0) 12.48, p < 0.001. *p < 0.05, ***p < 0.001 vs the lower dose of the same compound. E–H. Box and whiskers diagrams show AIM scores in the time window of peak drug effect (20–60 min, cf. grey shaded area in A–D) upon treatment with L-DOPA (black), SKF38393 (red), QUIN (green) and SUM (blue). E. Sum of axial, limb and orofacial scores (total ALO); the separate scores are reported in F–H. *p < 0.05, **p < 0.01 and ***p < 0.001 in the indicated comparisons (see horizontal lines). I. Axial, limb and orofacial AIM scores are here represented as a percentage of the total ALO AIM scores after each treatment – AIM subtype: F(1.3, 34.8) 50.28, p < 0.001; Treatment: F(3.0, 27.0) 1.23, p = 0.31; Interaction: F(6.0, 54.0) 53.84, p < 0.001. **p < 0.01 vs L-DOPA and ###p < 0.001 vs SKF38393.

Fig. 2. Video frames illustrating the dystonic features reported in Table 2.
3.3. Different patterns of dystonic features upon stimulation of D1 or D2 receptors

Similarly to the AIMS, the sum of dystonia scores (“tot. Dystonia”) reached peak values between 20 and 60 min post injection for all the treatments tested (Fig. 3A-D). We therefore carried out further group comparisons on the scores collected during this time window (Fig. 3E-K).

Dystonia was overall more severe after treatment with QUIN, or SUM than after SKF38393 administration (Fig. 3E; p < 0.05 for SKF vs QUIN, p < 0.001 vs SUM).

Important differences in the topographical distribution of dystonic features induced by D1R or D2R stimulation were unveiled when examining the individual subtypes. In particular, the two D2R-agonists QUIN and SUM induced significantly more dystonia than did the D1 agonist SKF38393 in the trunk-neck region (Fig. 3E), the forelimb ipsilateral to the lesion (Fig. 3H) and both hindlimbs (Fig. 3I, J). Dystonic features expressed on/towards the side contralateral to the lesion (i.e., tr/ne, cFL and cHL) had similar severity in mice treated with the D2R agonists or L-DOPA (Fig. 3F,G, I), while dystonic features in the ipsilateral limbs (iFL and iHL) were mainly induced by SUM (Fig. 3H, J; p < 0.05 for SUM vs L-DOPA).

The only dystonic-like reaction that became prominent after treatment with SKF38393 consisted in a sustained stiffness and dorsiflexion of the tail (Fig. 3K; p < 0.05 for SKF vs QUIN, p < 0.01 vs L-DOPA, p < 0.001 vs SUM). A similar phenomenon, defined as Straub tail, has been previously reported after the administration of morphine, apomorphine, or D1R agonists to intact mice (Babovic et al., 2013; Bilbey et al., 1960; Zarrindast et al., 1993).

Taken together, these results reveal that the selective pharmacological stimulation of D2Rs has a significantly higher liability to induce dystonic forms of dyskinesia in all parts of the mouse body (except for the Straub tail response). Importantly, the selective D2R agonist SUM had the most generalized dystonic effects, being the only treatment to induce abnormal postures also in the hindlimb ipsilateral to the lesion.

3.4. Dystonia scores correlate with geometric measures of altered postures

We next set out to define geometric correlates of altered limb and axial postures that could provide an independent verification of the observed movement patterns.

To this end, we prepared new groups of 6-OHDA-lesioned mice, and randomly divided them into four groups to receive treatment with vehicle, L-DOPA, SKF38393, or SUM (we focused this analysis on SUM, a more selective D2 agonist than QUIN). Mice were video recorded with a high frame-rate camera placed below the test arena, which provided an accurate visualization of mouse body axis, nose tip, hindpaws, and tail base in all the recorded frames. These parts of the mouse body were therefore used as references points for a video tracking analysis employing specifically designed macroinstructions.

To obtain a geometrical correlate of abnormal trunk/neck posturing, we traced two longitudinal vectors that extended from the midpoint between hindpaws to nose tip and tail base, respectively (Fig. 4A). The angle generated by the intersection of these two vectors (“axial bending angle”) was monitored over 2 min recording bins every 20 min using the open-source software DeepLabCut (Mathis et al., 2018). During the time window corresponding to peak drug effect (20–60 min post injection), the average values of the axial bending angle were markedly lower after the administration of L-DOPA or SUM compared to both SKF38393 and vehicle (Fig. 4B; p < 0.01 for L-DOPA and SUM vs the two other groups), indicating a more pronounced degree of spine bending after the former treatments. Accordingly, tr/ne dystonia ratings from the same videoclips revealed that dystonic features involving axial muscles were markedly more severe in L-DOPA- and SUM-treated animals (Fig. 4C; p < 0.01 for L-DOPA and SUM vs the other two groups), validating the reliability of our manual rating system, there was a strong linear inverse correlation between axial bending angles and tr/ne dystonia scores (Fig. 4D; r = −0.956, p < 0.001).

In a second analysis, we traced longitudinal vectors across the hindpaws, and then calculated the angle at the intersection between these vectors, referred to as the “hindpaw angle” (Fig. 5A). At the peak of drug effect (20–60 min post injection) this angle was significantly wider after treatment with SUM and L-DOPA compared to vehicle and SKF38393 (Fig. 5B; **p < 0.01 for L-DOPA vs SKF, and vehicle, ***p < 0.01 for SUM vs SKF, and vehicle). In addition, there was a trend for the angle to be larger in SUM compared to the L-DOPA treated animals (p = 0.056). Since the hindpaw angle reflects a divergence between hindlimbs during free ambulation, we asked whether larger values were related to the severity of dystonic features affecting the hindlimbs. We therefore compared the four treatment groups on the total HL dystonia
mice treated with SKF3839 did not differ from vehicle-treated animals (Fig. 4C; p vs vehicle and SKF3839 vs SUM, p

significantly elevated in both groups compared to vehicle-treated controls (Fig. 6C; p < 0.001 for SKF3839 vs vehicle and SUM, p < 0.001 for each antagonist vs vehicle). Nevertheless, the profile of efficacy on individual AIM components was different between the two antagonist compounds. Thus, axial AIMs were not significantly affected by SCH23390 (Fig. 7C) but significantly reduced by L741626 (Fig. 7D; p < 0.01 vs vehicle). Limb and orofacial AIMs were significantly reduced by both antagonist treatments, although SCH23390 had a more pronounced effect (cf.

Fig. 3. Dystonic features induced by different dopaminergic treatments.

A–D. Time course of the sum of dystonia scores during the 180 min test session. A. L-DOPA (n = 8) - Dose: F[1,0, 7,0] = 5.55, p = 0.056; Time: F[20, 144] = 22.93, p < 0.001; Interaction: F[2,0, 18,0] = 2.48, p = 0.097. B. SKF38393 (n = 8) - Dose: F[1,0, 7,0] = 2.04, p = 0.196; Time: F[3,0, 27,0] = 32.63, p < 0.001; Interaction: F[3,1, 27,0] = 1.52, p = 0.234. C. QUIN (n = 7) - Dose: F[1,0, 6,0] = 4.42, p = 0.080; Time: F[1,0, 9,0] = 53.39, p < 0.001; Interaction: F[1,0, 6,0] = 1.05, p = 0.380. D. SUM (n = 7) - Dose: F[1,0, 6,0] = 40.71, p < 0.001; Time: F[2,1, 16,0] = 40.22, p < 0.001; Interaction: F[2,1, 12,0] = 14.08, p < 0.001; ***p < 0.001 vs lower dose of the same compound. E–K. Box and whiskers diagrams show dystonia scores at the peak of drug effect (20–60 min, cf. grey shaded area in A–D) after treatment with L-DOPA (black), SKF38393 (red), QUIN (green) and SUM (blue). E. Sum of dystonia scores. F–K. Scores for individual body regions. *p < 0.05, **p < 0.01 and ***p < 0.001 in the indicated comparisons (see horizontal lines).

score (cHL + iHL at 20–60 min post vehicle/drug injection) on the same videoclips used for the vectorial analysis. The most severe cases of HL dystonia occurred in the SUM-treated group (Fig. 5C; p < 0.01 for SUM vs vehicle and SKF38393, p > 0.05 for SUM vs L-DOPA). In contrast, mice treated with SKF3839 did not differ from vehicle-treated animals (Fig. 4C; p < 0.01 for SKF vs both SUM and L-DOPA). Accordingly, there was a significant positive correlation between hindpaw angle values and total HL dystonia scores across the treatment groups (Fig. 5D; r = 0.854, p < 0.001).

3.5. Patterns of open field-motions

On the same videos used for vector analyses, we examined the animals’ ambulation in the open-field arena by tracking body centroid coordinates (Fig. 6A, B). The different treatments resulted in markedly different patterns of motions. At the peak of drug effect, SKF38393-treated mice were able to move across the entire extent of the arena whereas sumanirole-treated animals remained confined to one quadrant (Fig. 6B). The different treatments resulted in markedly different patterns of motions. At the peak of drug effect, SKF38393-treated mice were able to move across the entire extent of the arena whereas sumanirole-treated animals remained confined to one quadrant (Fig. 6B).

3.6. Effect of D1R and D2R antagonist on dyskinesia and dystonia induced by L-DOPA

We next asked whether hyperkinetic and dystonic forms of LID would respond differently to the inhibition of D1- or D2Rs. To this end, L-DOPA-treated dyskinetic mice received challenge injections of the selective D1R antagonist SCH23390 or the D2R antagonist L741626, used at doses devoid of general motor depressant effects. On the day of testing, mice were coadministered with L-DOPA (6 mg/kg) and the antagonist or its vehicle, and were assessed using AIMs and dystonia rating scales in a blinded manner.

Both receptor antagonists were effective in blunting L-DOPA-induced AIMs, although with some differences in the time window of maximal efficacy (Fig. 7A; p < 0.05 for SCH23390 vs vehicle at 40–60 min; cf. Fig. 7F, p < 0.05 for L741626 vs vehicle at 60–80 min post L-DOPA injection). When comparing the two antagonists at 20–60 min post L-DOPA injection (i.e., the same time windows considered in Fig. 1A and Fig. 2A), we found that SCH23390 and L741626 had equal efficacy in reducing the severity of ALO AIMs, corresponding to a 27–28% reduction in total AIM scores (cf. Fig. 7B, G; p < 0.01 for each antagonist vs vehicle). Nevertheless, the profile of efficacy on individual AIM components was different between the two antagonist compounds. Thus, axial AIMs were not significantly affected by SCH23390 (Fig. 7C) but significantly reduced by L741626 (Fig. 7D; p < 0.01 vs vehicle). Limb and orofacial AIMs were significantly reduced by both antagonist treatments, although SCH23390 had a more pronounced effect (cf,
To further evaluate the role of D2Rs in the genesis of AIMs, we examined the effects of L741626 in mice treated with the highly selective D2R agonist SUM (as reported above, approx. 75% of SUM-induced AIMs consist of axial dyskinesia, see Fig. 1 I). Coadministration of L741626 with SUM resulted in a drastic general reduction in dyskinesia severity. Thus, L741626 was quite effective in all phases of the dyskinesia time curve (Fig. 7 K; $p < 0.01$ vs vehicle in all time points) and on all AIM subtypes (Fig. 7 M – O).

Next, we examined the two receptor antagonists on L-DOPA-induced dystonia ratings (the tail scores were omitted from this analysis because they were clearly detectable in two mice only). While SCH23390 did not have any significant impact (Fig. 8 A–G), L741626 proved to be overall effective both in the time-curve analysis (Fig. 8 H; $p < 0.001$ for treatment effect) and on the total dystonia scores at the peak of the curve (20–60 min) (Fig. 8 I, $p < 0.01$ for L-DOPA vs vehicle). Significant effects of L741626 were moreover detected on the analysis of individual dystonic features. Thus, Tr/ne and cHL (which are the most dystonic body segments in L-DOPA-treated mice) displayed lower levels of dystonia when the D2R-antagonist L741626 was coadministered with L-DOPA (Fig. 8 J, M; $p < 0.05$ and $p < 0.01$ for antagonist vs vehicle, respectively).

As a comparison, we examined the effects of L741626 on SUM-induced dystonia ratings. The D2R antagonist proved to completely suppress SUM-induced dystonia (Fig. 8 O–P), being quite effective in each body segment, i.e. trunk-neck (Fig. 8 Q) and the four limbs (Fig. 8 R–U; $p < 0.05$ vs vehicle).

4. Discussion

Since the introduction of L-DOPA as a treatment for PD, the role of D1 and D2Rs in the beneficial or untoward effects of this treatment has been the object of extensive investigation. Animal models of PD have been used to examine the modulatory effects of D1R and D2R agonists on different aspects of motor behaviour. While it is clear that both D1 and D2R stimulation can ameliorate parkinsonian motor features in different PD models (Gomez-Mancilla and Bedard, 1992; Sebastianutto et al., 2020), the specific contribution of these receptor classes to the development and expression of dyskinesias is still a matter of debate.

Clinical studies have indicated that long-acting D2/3 agonists are basically devoid of dyskinesiogenic effects if given de novo (Constantinescu, 2008; Rascol et al., 2006). In contrast, short-acting D2R agonists were reported to produce as much as dyskinesia as L-DOPA in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys (Gomez-Mancilla and Bedard, 1992; Sebastianutto et al., 2020), the specific contribution of these receptor classes to the development and expression of dyskinesias is still a matter of debate. Clinical studies have indicated that long-acting D2/3 agonists are basically devoid of dyskinesiogenic effects if given de novo (Constantinescu, 2008; Rascol et al., 2006). In contrast, short-acting D2R agonists were reported to produce as much as dyskinesia as L-DOPA in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys (Gomez-Mancilla and Bedard, 1992; Luquin et al., 1992b). Using the same animal model, one study indicated that D2R stimulation can only cause dystonic movements but not choreoathetosis (Boyce et al., 1990b), although this notion was not confirmed by other studies (Luquin et al., 1992b). Discrepancies between pharmacological data obtained from MPTP-lesioned non-human primates (NHP) may depend on varying levels of DA denervation and/or different previous drug exposure in the animal model. In particular, it is not uncommon for MPTP-lesioned NHPs to receive some form of L-DOPA treatment in order to alleviate the motor disability caused by severe lesions, although this supporting therapy is rarely specified in the published articles.

When considering rodent PD models, most of the available
information regarding the effects of DAR ligands has been obtained from unilaterally 6-OHDA-lesioned rats. Upon testing clinically used D2/3 agonists in this model, some studies have detected conspicuous AIMs (Bagetta et al., 2012; Shin et al., 2014), whereas others have reported very mild or no AIMs, at least when these compounds were given de novo (Carta et al., 2008; Lundblad et al., 2002). What is clear is that D2/3 agonists can induce the expression of dyskinetic behaviours when administered to animals already primed with L-DOPA (Bhide et al., 2015; Sebastianutto et al., 2020; Shin et al., 2014). The extent to which these dyskinesias share the same features as those induced by L-DOPA has not yet been clarified. Moreover, to the best of our knowledge, the dyskinetic effects of different DAR ligands have not been examined in the mouse, which currently represents the preferred species for advanced studies of circuit dysfunctions in LID (reviewed in (Cenci et al., 2018)).

This is the first study presenting a detailed analysis of abnormal movements and postures that fulfilled a phenomenological definition of dystonia. All the items included in this new rating scale featured an abnormal posturing of the affected body part, apparently maintained by sustained muscular contractions, in animals that were motorically active. Thanks to this scale, we could show that treatment with SKF38393 had a negligible dystonic effect, whereas treatment with QUIN and SUM induced overt dystonia. Notably, SUM was the only treatment eliciting dystonic features in all of the body segments examined, including the forelimb and hindlimb ipsilateral to the lesion. SUM-induced dystonias and dyskinesias were completely suppressed upon coadministering the highly selective D2R antagonist L741626. Moreover, the same compound significantly attenuated axial and limb dystonias in mice treated with L-DOPA, whereas the D1-class receptor antagonist SCH23390 was without any effect on the same features.

In mice treated with SKF38393, the only appreciable dystonic-like feature consisted in a sustained dorsiflexion of the tail, which was kept erected in a nearly vertical position. The latter behaviour closely resembles the so-called Straub tail, originally described in mice treated with morphine and found to depend on sustained activity of the sacrococcygeus dorsalis muscle (Bilbey et al., 1960). The Straub phenomenon has been variably interpreted as reflecting a transient spastic reaction (Belozertseva et al., 2016) or a component of complex dystonic phenotypes (Teper et al., 2007). The significance of this phenomenon in our SKF38393-treated mice remains uncertain, particularly because the same treatment induced negligible dystonic features at the level of trunk or limb muscles. Moreover, the Straub phenomenon has been reported to occur in intact mice receiving treatment with high doses of...
apomorphine or SKF38393 (Zarrindast et al., 1993). Yet, intact mice treated with dopaminergic agents do not develop dyskinesia (our unpublished observations). Despite these reservations, the Straub tail was scored using the same principles as frank dystonic features. The significance of this phenomenon, however, needs to be clarified by further investigation.

The question could be asked why the partial D1R agonist SKF38393 was chosen over other compounds with full D1R agonist activity for the comparisons made in this study. The reason is that SKF38393 mimics the molecular effects of therapeutic L-DOPA dosages as well as the time course of L-DOPA-induced AIMS in this animal model (Sebastianutto et al., 2020). In addition to having a much faster pharmacodynamic profile, full D1R agonists are prone to induce desensitization when administered repeatedly (Asin and Wirtshafter, 1993; Conroy et al., 2015). The low dystonic activity of SKF38393 in this study cannot be ascribed to a potentially low level of D1R stimulation, because this treatment had a potent motor stimulant effect, as demonstrated by the high values measured from SKF38393-treated mice in terms of distance travelled and movement speed in the open field.

The term dystonia is used in various clinical contexts to indicate repetitive twisting movements and/or abnormal postures caused by sustained muscle contractions. Dystonic features are typically initiated or worsened by voluntary action, and they are associated with overflow of muscle activation to nearby or distant muscular groups (Albanese et al., 2011; Balint et al., 2018). Although dystonia is often attributed to imbalances in basal ganglia pathways (Balint et al., 2018; Ribot et al., 2019), the clinical heterogeneity of this movement disorder most likely reflects distinct underlying network dysfunctions that are poorly understood.

PD patients may experience dystonia both off medications (e.g. early morning foot dystonia) and on medications, as a component of LID (Calabresi and Standaert, 2019; Poewe et al., 1988). After chorea/choreoathetosis, dystonia is the second most common form of LID. L-DOPA-induced dystonia can be either peak-of-dose, beginning/end of dose, or “off” dystonia. Peak-dose dystonia may be associated with choreic movements, making the abnormal movements more severe and with more interference with the activities of daily living (Fahn, 2000). Detecting dystonic features during peak-dose LID is generally difficult (Fahn, 2000; Hagell and Widner, 1999) and most dyskinesia rating scales do not attempt to distinguish between hyperkinetic-choreic and dystonic components. Part of the difficulty may arise from the fact that, when dystonia occurs concomitantly with rapid and abrupt movements, these are easier to recognize and rate. Another difficulty depends on dystonia being a dynamic condition, often changing in severity depending on the voluntary activity of the involved body area (Albanese et al., 2011).

Considering these difficulties, it is not surprising that most studies using animal models of PD-LID have neglected the presence of dystonia. Still, it is important to acknowledge that LID rating scales discriminating between dystonic and choreatic movements are available for NHP models of PD. These scales have proven to be especially effective when applied to Old-World Monkeys, where an overall less motor activity induced by L-DOPA leads to an easier independent detection of choreatic vs dystonic components (Boyce et al., 1990a; Fox et al., 2012; Johnston...
and Fox, 2015). On the other hand, current AIM scales developed for rodents do not define the dyskinetic movements as being hyperkinetic or dystonic, and just recognize them as being abnormal and purposeless. However, as previously noted (Alcacer et al., 2017; Lee et al., 2000), axial AIMs conform to the definition of slow twisting movements leading to sustained abnormal postures. To our knowledge, the only scale specifically rating dystonic movements in a rodent model of LID is the one developed by Steece-Collier and collaborators (Stece-Collier et al., 2003). Upon examining unilaterally 6-OHDA-lesioned rats treated with L-DOPA, this group described and quantified dystonic movements and
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postures involving neck, trunk, and the limbs contralateral to the lesion. Although Steece-Collier and collaborators provided a fundamental proof-of-principle that dystonia is a component of LID also in parkinsonian rodents, their rating scale differs from ours in several respects and could not be applied *tout court* to the mice in this study. First, the scale was produced by observing rats, a larger animal model where it is possible to reliably discriminate between neck and upper trunk dystonia, something that proved to be difficult in the mouse. Second, the scale was tailored to the effects of L-DOPA. By testing a larger number of dopaminergic agents, we were here able to detect additional features that were not reported by Steece-Collier and collaborators. In particular, our scale also considers the limbs ipsilateral to the lesion, which exhibited markedly abnormal postures in mice treated with SUM. Moreover, thanks to the inclusion of a D1R agonist in the study, we were able to recognize overtly abnormal postures of the tail (Straub tail behaviour).

Fig. 8. Dystonic features are improved by the pharmacological antagonism of D2R but not D1R. A–G. Dystonia ratings in mice coadministered with L-DOPA and the D1R antagonist SCH23390 (SCH, 0.05 mg/kg) or its vehicle. H–N. Effects of the D2R antagonist L741626 (L74, 4 mg/kg) on L-DOPA-induced dystonia scores. O–U. For a comparison, L74 (4 mg/kg) was also tested in mice treated with sumanireole (SUM), a highly selective D2R agonist. A, H and O show the time course of dystonic features (sum of dystonia scores from different body segments) during the 180 min test session. B–G, I–N, P–U. Box and whiskers diagrams show dystonia scores at the peak of drug effect (20–60 min, cf. grey shaded area in A, H, O) after treatment with SCH + L-DOPA vs vehicle + L-DOPA (B–G), L74 + L-DOPA vs vehicle + L-DOPA (I–N), and L74 + SUM vs vehicle + SUM (P–U). Statistical analyses: A. vehicle + L-DOPA vs SCH + L-DOPA (n = 9) – Treatment: F(1, 8) = 0.76, p = 0.40; Time: F(2.4, 19.8) = 30.68, p < 0.001; Interaction: F(2.9, 23.4) = 1.05, p = 0.38. H. vehicle + L-DOPA vs L74 + L-DOPA (n = 9) – Treatment: F(1, 8) = 27.5, p < 0.001; Time: F(2.2, 17.8) = 1.65, p = 0.21. O. vehicle + SUM vs L74 + SUM (n = 7) – Treatment: F(1, 6, 6.0) = 28.44, p < 0.01; Time: F(1.6, 10.6) = 17.35, p < 0.001; Interaction: F(2.6, 16.0) = 12.62, p < 0.001. *p < 0.05, **p < 0.01 vs antagonist + agonist. *p < 0.05 and **p < 0.01. The antagonists were tested in a systematically blinded manner.
Another important difference between the previous dystonia scale and ours lies in method used to grade severity. Rather than attributing scores to descriptive movement categories, we opted to anchor our severity scores to the time during which a pre-defined dystonic feature was visible, and the same scoring method could therefore be applied to all body segments. The ‘time yardstick’ made it possible to use not only full scores (from 1 to 3) but also half-point scores according to well-defined criteria, thus rendering this scale more interval-like than ordinal-like. We believe that our time-based severity scale should be easy to use in a consistent manner also by other laboratories.

In addition to providing a new dystonia scale for unilateral mouse models of PD-LID, this work contributes novel information on the differential role of D1- and D2Rs in the generation of dystonic features. In particular, our results establish a causal link between D2R stimulation and dystonic postures involving axial and hindlimb muscles in a parkinsonian setting. Altered D2R-mediated signaling has been implicated in several types of dystonia, including familial or sporadic forms of primary dystonia (Black et al., 2014; Todd and Perlmutter, 1998; van der Weijden et al., 2020) and acquired dystonic syndromes, such as those developing upon treatment with antipsychotic drugs (Miller and Jankovic, 1990; Rupniak et al., 1986). Taken together, the available clinical findings would suggest that both hypo- and hyperfunction of D2R signaling can generate dystonic features. This is indeed the case for PD, where dystonia can occur either in a virtual absence of putaminal DAR stimulation (e.g., off-period dystonia) or upon treatment with L-DOPA and DAR agonists.

The here-demonstrated link between D2R stimulation and dystonic features is in keeping with several reports of reversible postural abnormalities in PD patients treated with D2/3 agonists such as pergolide, pramipexole, and ropinirole (Ameghino et al., 2018; Cannas et al., 2009; Pandey and Jain, 2016; Stocchi et al., 2016). Interestingly, reversibly pleurothotonus (a form of axial dystonia) has also been reported after treatment with the adenosine A2a receptor antagonist idarabine (Yasuda, 2018), whose action mimics that of D2R agonists on indirect pathway striatal neurons (Fredholm and Svenningsson, 2020).

An intriguing question is why the most severe dystonic features induced by D2 agonists affected the trunk and the hindlimbs, which showed the most conspicuous deviations from their normal positional range as well as high time-based scores. In PD too, “on-medication” dystonias are most prominent in the legs and lower trunk, whereas hyperkinetic-choreatic features predominate in the upper part of the body (Fahn, 2000; Luquin et al., 1992a). Although an explanation for this topographic pattern is currently lacking, it is relevant to mention that dystonia has been hypothesized to stem from the excessive function of a postural control system coordinated by the indirect pathway of the basal ganglia (Blood, 2008; Ribot et al., 2019). Axial and hindlimbs muscles are key effectors of this postural control system, as they implement anticipatory postural adjustments to maintain balance during ongoing movements. Given the bilaterality of SUM-induced limb dystonias, it is here relevant to mention that axial and proximal limb muscles are subjected to a high degree of bilateral control at both spinal and supraspinal levels (Galea et al., 2010; Goetz et al., 2015).

4.1. Concluding remarks

Dyskinesia and dystonia are core manifestations of basal ganglia dysfunction in PD and other neurodegenerative disorders. Despite conspicuous differences in their clinical presentation, both movement disorders are currently attributed to an imbalanced activation of direct vs indirect striatal output pathway, ultimately releasing thalamo-cortical neurons that promote movement (Cenci et al., 2018; Wichmann, 2018). A refinement of these pathophysiological notions is critically dependent on our capacity to discern and quantify specific movement abnormalities in different body regions and DA-dependent states. It is particularly important that this research is pursued in experimental models that allow for combining advanced behavioural analyses with molecular and systems-level investigations. We believe that the new observations and methods of behavioural analysis presented in this study will greatly facilitate further efforts in this direction, paving the way for an improved understanding of dyskinesia and dystonia in PD.

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References

Albanese, A., et al., 2011. EFNS guidelines on diagnosis and treatment of primary dystonias. Eur. J. Neurol. 18, 5–18.
Albanese, A., et al., 2013. Phenomenology and classification of dystonia: a consensus update. Mov. Disord. 28, 863–873.
Alcacer, C., et al., 2012. Gabaβα(β1) mutation allows parsing the role of DαM-dependent and extracellular signal-regulated kinase-dependent signaling in L-3,4-dihydroxyphenylalanine-induced dyskinesia. J. Neurosci. 32, 5900–5910.
Alcacer, C., et al., 2017. Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson’s disease therapy. J. Clin. Invest. 127, 720–734.
Ameghino, L., et al., 2018. Postural disorders and antiparkinsonian treatments in Parkinson disease: an exploratory case-control study. Clin. Neuropharmacol. 41, 123–128.
Anzalone, A., et al., 2012. Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. J. Neurosci. 32, 9023–9034.
Asin, K.E., Wirthsafter, D., 1993. Effects of repeated dopamine D1 receptor stimulation on rotation and c-fos expression. Eur. J. Pharmacol. 235, 167–168.
Aubert, L., et al., 2005. Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann. Neurol. 57, 17–26.
Babovic, D., et al., 2013. Behavioural and anatomical characterization of mutant mice with targeted deletion of D1 dopamine receptor-expressing cells in response to acute morphine. J. Pharmacol. Sci. 121, 39–47.
Bagetta, V., et al., 2012. Rebalance of striatal NMDA/AMPA receptor ratio underlies the reduced emergence of dyskinesia during D2-like dopamine agonist treatment in experimental Parkinson’s disease. J. Neurosci. 32, 17921–17931.
Balint, B., et al., 2018. Dystonia. Nat. Rev. Dis. Prim. 4, 25.
Belozerseva, I.V., et al., 2016. Morphine-induced Straub tail reaction in mice treated with serotonergic compounds. Eur. J. Pharmacol. 791, 1–7.
Blide, N., et al., 2015. Effects of the beta-adrenergic receptor antagonist propranolol on dyskinesia and L-DOPA-induced striatal DA efflux in the hemi-parkinsonian rat. J. Neurochem. 134, 222–232.
Billery, D.L., et al., 1960. The anatomical basis of the straub phenomenon. Br. J. Pharmacol. Chemother. 15, 540–543.
Black, K.J., et al., 2014. Spatial reorganization of putaminal dopamine D2-like receptors in cranial and hand dystonia. PLoS One 9, e88121.
Bloom, A.J., 2008. New hypotheses about postural control support the notion that all dystonias are manifestations of excessive brain postural function. Bioi Hypotheses 1, 14–25.
Boyd, S., et al., 1990a. Induction of chorea and dystonia in parkinsonian primates. Mov. Disord. 5, 3–7.
Boyd, S., et al., 1990b. Differential effects of D1 and D2 agonists in MPTP-treated primates: functional implications for Parkinson’s disease. Neurology. 40, 927–933.
Brooks, D.J., 2006. Imaging the role of dopamine in health and disease Parkinson’s disease as a lesion model. Wien. Klin. Wochenschr. 119, 570–572.
Calabresi, P., Standaert, D.G., 2019. Dystonia and levodopa-induced dyskinesias in Parkinson’s disease: is there a connection? Neurobiol. Dis. 132, 104579.
Cenci, M.A., et al., 2011. Current options and future possibilities for the treatment of dystonia and dystonic postures involving axial and hindlimb muscles in a parkinsonian setting. Neurology. 40, 927–933.
Collins, G.T., et al., 2007. Yawning and hyperventilation in rats: effects of dopamine D3 and D2 agonists and antagonists. Psychopharmacology 193, 159–168.
Conroy, J.L., et al., 2015. Identification of G protein-biased agonists that fail to recruit and activate extracellular signal-regulated kinase-dependent signaling in L-3,4-dihydroxyphenylalanine-induced dyskinesia. J. Neurosci. 35, 232.
D2R agonists and antagonists. Psychopharmacology 193, 159–168.
Dopamine D2 agonists and antagonists. Psychopharmacology 193, 159–168.
Constantinescu, R., 2008. Update on the use of pramipexole in the treatment of Parkinson’s disease. Neuropsychiatr. Dis. Treat. 4, 337–352.

Darmopil, S., et al., 2009. Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. Biol. Psychiatry 66, 603–613.

Di Chian, G., et al., 1992. Priming as a model of behavioural sensitization. Dev. Pharmacol. Ther. 18, 223–227.

Fahn, S., 2000. The spectrum of levodopa-induced dyskinesias. Ann. Neurol. 47, S2–9; discussion S9–11.

Fox, S.H., et al., 2012. A critique of available scales and presentation of the Non-Human Primate Dyskinesia Rating Scale. Mov. Disord. 27, 1373–1378.

Francardo, V., et al., 2011. Impact of the lesion procedure on the profiles of motor impairment and molecular responsiveness to L-DOPA in the 6-hydroxydopamine mouse model of Parkinson’s disease. Neurobiol. Dis. 42, 327–340.

Fredholm, B.B., et al., 2020. Why target brain adenosine receptors? A historical perspective. Parkinsonism Relat. Disord. 80 (Suppl. 1), S3–S6.

Galea, M.P., et al., 2010. Bilateral postynaptic actions of pyramidal tract and reticulospinal neurons on feline erector spinae motoneurons. J. Neurosci. 30, 858–869.

Gangarossa, G., et al., 2013. Combinatorial topography and cell-type specific regulation of the ERK pathway by dopaminergic agonists in the mouse striatum. Brain Struct. Function. 218, 405–419.

Girasole, A.E., et al., 2018. A subpopulation of striatal neurons mediates levodopa-induced dyskinesia. Neuron 97 (787–795), e6.

Goetz, C., et al., 2015. Distinct limb and trunk premotor circuits establish laterality in the human motor control system. Ann. Neurol. 77, 221–235.

Kordower, J.H., et al., 2013. Disease duration and the integrity of the nigrostriatal system in Parkinson’s disease. Brain 136, 2419–2431.

Lanza, K., et al., 2018. Behavioral and cellular dopamine D1 and D3 receptor-mediated synergy: implications for L-DOPA-induced dyskinesia. Neuropharmacology 138, 204–214.

Lee, R.K., et al., 2000. Embryonic dormancy phenomenon in obstructed healthy mouse fallopian tubes. J. Assist. Reprod. Genet. 17, 540–545.

Li, et al., 2010. Yawning and locomotor behavior induced by dopamine receptor agonists in mice and rats. Behav Pharmacol 21. https://doi.org/10.1097/FBP.0b013e32833a5c68.

Lundblad, M., et al., 2002. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson’s disease. Eur. J. Neurosci. 15, 120–132.

Lundblad, M., et al., 2004. A model of L-DOPA-induced dyskinesia in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of nigrostriatal function. Neurobiol. Dis. 16, 110–123.

Luque-Rojas, M.J., et al., 2013. Hyperactivity induced by the dopamine D2/D3 receptor agonist quinpirole is attenuated by inhibitors of endocannabinoid degradation in mice. Int. J. Neuropsychopharmacol. 16, 661–676.

Luquin, M., et al., 1992a. Identification of sulpholipid I by thin-layer chromatography in Mycobacterium tuberculosis. Res. Microbiol. 143, 225–227.

Luquin, M.R., et al., 1992b. Selective D2 receptor stimulation induces dyskinesia in parkinsonian monkeys. Ann. Neurol. 31, 551–561.

Mathis, A., et al., 2018. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat. Neurosci. 21, 1281–1289.

Meder, D., et al., 2019. The role of dopamine in the brain - lessons learned from Parkinson’s disease. Neuroimage. 190, 79–93.

Miller, L.G., Jankovic, J., 1990. Sulpiride-induced tardive dyskinesia. Mov. Disord. 5, 83–84.

Morelli, M., 1997. Dopamine/glutamate interaction as studied by combining turning behaviour and c-Fos expression. Neurosci. Biobehav. Rev. 21, 505–509.

Muller, T., Rans, H., 2006. Levodopa, motor fluctuations and dyskinesia in Parkinson’s disease. Expert. Opin. Pharmacother. 7, 1715–1730.

Olanow, C.W., et al., 2020. Continuous dopaminergic stimulation as a treatment for Parkinson’s disease: current status and future opportunities. Mov. Disord. 35, 1731–1744.

Pandey, S., Jain, S., 2016. Pramipexole-associated fixed limb dystonia in Parkinson’s disease. Parkinsonism Relat. Disord. 31, 159–160.

Parker, J.G., et al., 2018. Diabetic neural ensemble dynamics in parkinsonian and dyskinetic states. Nature 557, 177–182.

Poewe, W.H., et al., 1988. Dyskinesia in Parkinson’s disease: clinical and pharmacological features. Ann. Neurol. 23, 73–78.

Radd, D., et al., 2018. Differential regulation of striatal motor behavior and related cellular responses by dopamine D2L and D2S isoforms. Proc. Natl. Acad. Sci. U. S. A. 115, 198–203.

Rascov, O., et al., 2006. Development of dyskinesias in a 5-year trial of ropinirole and L-dopa. Mov. Disord. 21, 1844–1850.

Ribot, B., et al., 2019. Dystonia and dopamine: from phenomenology to pathophysiology. Prog. Neurobiol. 182, 101678.

Rupniak, N.M., et al., 1986. Acute dystonia induced by neuroleptic drugs. Psychopharmacology 88, 403–419.

Ryan, M.B., et al., 2018. Abrupt striatal activity in parkinsonism and levodopa-induced dyskinesia. Cell Rep. 23, 3438–3446 e6.

Santini, E., et al., 2007. Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J. Neurosci. 27, 6995–7005.

Schindelin, J., et al., 2012. Fiji: an open-source platform for biological image analysis. Nat. Methods 9, 676–682.

Sebastianutto, et al., 2016. Validation of an improved scale for rating L-DOPA-induced dyskinesia in the mouse and effects of specific dopamine receptor antagonists. Neurobiology of Disease 96, 156–170. https://doi.org/10.1016/j.nbd.2016.09.001.

Sebastianutto, L., et al., 2020. D1-mGlu5 heteromers mediate noncanonical dopamine signaling in Parkinson’s disease. J. Clin. Invest. 130, 1168–1184.

Shen, W., et al., 2020. Striatal Kir2.1+ channel inhibition mediates the antidysecretic effects of amantadine. J. Clin. Invest. 130, 2593–2601.

Shin, E., et al., 2014. The anti-dyskinetic effect of dopamine receptor blockade is enhanced in parkinsonian rats following dopamine neuron transplantation. Neurobiol. Dis. 62, 233–240.

Steece-Collier, K., et al., 2003. Embryonic mesencephalic grafts increase levodopa-induced forelimb hyperkinesia in parkinsonian rats. Mov. Disord. 18, 1442–1454.

St-Hilaire, M., et al., 2005. Denervation and repeated L-DOPA induce complex regulatory changes in neurochemical phenotypes of striatal neurons: implication of a dopamine D1-dependent mechanism. Neurobiol. Dis. 20, 456–460.

Todd, R.D., 1998. Mutational and biochemical analysis of dopamine D2 receptor and progressive chorea and dystonia phenotype. Mov. Disord. 13, 739–745.

Vander Weijden, M.C.M., et al., 2020. A gain-of-function variant in dopamine D2 receptor inhibits L-DOPA-induced dyskinesia and histone activation. Biol. Psychiatry 66, 603–613.

Ward, H., et al., 2006. Development of dyskinesias in a 5-year trial of ropinirole and L-dopa. Mov. Disord. 21, 1844–1850.

Weber, M., et al., 2010. The effects of the dopamine D2 agonist sumanirole on prepulse inhibition in rats. Eur. Neuropsychopharmacol. 20, 421–425.

Westin, J.E., et al., 2007. Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol. Psychiatry 62, 800–810.

Wichmann, T., 2018. Pathophysiological basis of movement disorders. Prog. Neurog. Surg. 33, 13–24.

Yasuda, T., 2018. Reversible intradreyline-induced pleurothostomus in a patient with Parkinson’s disease: a case report and literature review. eNeurologicalSci. 13, 5–7.

Zinnadost, M.R., et al., 1993. Involvement of dopaminergic receptor subtypes in straitil body parts in mice. Gen. Pharmacol. 24, 127–130.