Behavioural Brain Research 226 (2012) 281–292

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Unilateral nigrostriatal 6-hydroxydopamine lesions in mice II: Predicting L-DOPA-induced dyskinesia

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ARTICLE INFO

Article history:
Received 28 July 2011
Received in revised form 8 September 2011
Accepted 13 September 2011
Available online xxx

Keywords:
L-3,4-dihydroxyphenylalanine
Dyskinesia
6-Hydroxydopamine
TH
5-HT
FosB

ABSTRACT

In the 6-hydroxydopamine (6-OHDA) lesioned rodent the location of the lesion produces significantly different behavioural phenotypes, responses to the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) and neuropathology. Lesion extent is commonly determined by a series of motor tests, but whether any of these tests have a relationship to the development and predictability of dyskinesia is unknown. We used mice with 6-OHDA lesions of the striatum, medial forebrain bundle and substantia nigra to examine the relationship between a range of tests used to determine motor function in the absence of L-DOPA: rotarod, cylinder, corridor, the balance beam, locomotor activity, psycho-stimulant and spontaneous rotational behaviour. The mice were subsequently treated with L-DOPA in progressively increasing doses and the development of L-DOPA-induced dyskinesia assessed. Most of these tests predict dopamine depletion but only rotarod, spontaneous rotations, apomorphine-induced rotations and locomotor activities were significantly correlated with the development of dyskinesia at 6 mg/kg and 25 mg/kg L-DOPA. The losses of dopaminergic neurons and serotonergic density in the ventral and dorsal striatum were dependent upon lesion type and were also correlated with L-DOPA-induced dyskinesia. The expression of FosB/ΔFosB was differentially affected in the striatum and nucleus accumbens regions in dyskinetic mice according to lesion type.

1. Introduction

Chronic treatment of the movement disorder Parkinson's disease (PD) with the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) is necessitated by the prolonged course of the disease and the lack of equally effective alternative pharmaceutics. Unfortunately, this long time course of treatment increases the risk of developing L-DOPA-induced dyskinesia (LID) [1]. These abnormal involuntary movements (AIMs) can be choreic and/or dystonic in nature and can be highly debilitating, limiting treatment options when severe. If we are to have a better understanding of the development of these abnormal movements and ultimately avoid, limit or treat them, development of good animal models of LID are invaluable. The classic rodent model of PD described by Ungerstedt [2] recapitulates the dopaminergic nigrostriatal depletion through the unilateral administration of 6-hydroxydopamine (6-OHDA). Now in widespread use, this rat model has been used to assess the role of the nigrostriatal pathway in motor function, and most importantly to screen compounds for potential anti-parkinsonian efficacy, with contralateral rotations being indicative of dopamine agonist activity [3–5]. However, the role of this model was revolutionised by the description of AIMs of the forelimb, body axis and orofacial region following chronic L-DOPA administration [6,7]. These behaviours were found to develop in addition to the more commonly recognised rotational responses and are reminiscent of the LID observed in patients.

Forebrain dopaminergic depletion can be initiated by injection of 6-OHDA at any point along the trajectory of the nigrostriatal pathway: into the originating nucleus in the substantia nigra (SN), along the axonal projection (the medial forebrain bundle; MFB), and into the terminal regions (most prominently, the striatum). These targets have advantages and disadvantages in practical terms (e.g., the terminal regions are more diffuse in the striatum, requiring multiple injections to produce a large lesion) but also have consequences for the behavioural repertoire produced in terms of motor deficits and response to L-DOPA [8–17]. AIMs following lesions at each of these intervals along the nigrostriatal tract have now been characterised in rats [6,7,18–20]. However, there has been increasing demand for mouse models of PD and LID, to take advantage of the development in transgenic technologies. 6-OHDA lesion protocols in mice can be extremely variable, as

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doi:10.1016/j.bbr.2011.09.025

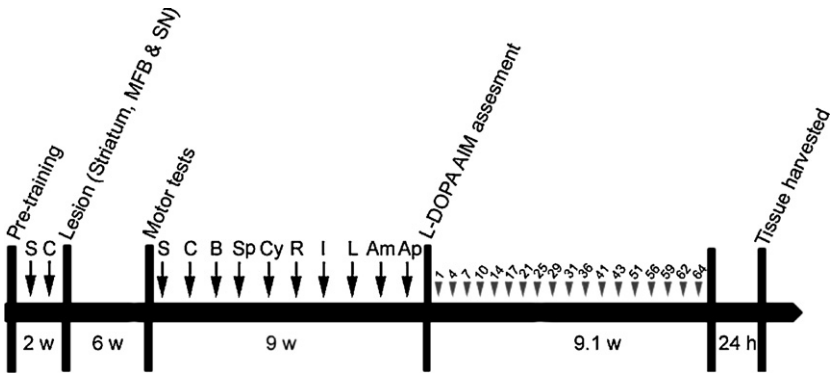

Fig. 1. Graphical representation of the experimental design. Motor tests were carried out consecutively: staircase (S), corridor (C), balance beam (B), spontaneous rotation (Sp), cylinder (Cy), rotarod (R), inverted cage lid (I), locomotor activity (L) amphetamine rotation (Am) and apomorphine rotation sessions (Ap). Grey arrow heads indicate the days in which dyskinesia was scored. In addition to days times are also indicated in hours (h) and weeks (w).

can the behavioural methods employed to assess lesion extent [14,15,17,21–24]. Indeed, in a concurrent study we systematically assessed an extensive list of simple motor tests in each of the lesion models [17]. Furthermore, despite apparently consistent dopaminergic lesions to the striatum and MFB in mice with equivocal drug administration regimes, there remains a high variation in the expression of AIMs. The reason(s) for this variability is unknown and unpredictable, but may relate at least in part to relative variability in stereotaxic placement of lesions in the smaller mouse brain. Therefore this study sets out firstly to examine dyskinesia expression in mice of three separate groups with lesions targeted at the SN, at the striatum, or at the MFB in response to different doses of L-DOPA, and then to determine whether motor deficits identified in simple test of motor function can predict susceptibility to L-DOPA-induced AIMs. We also explored the relationship between LID and behavioural parameters in comparison to the magnitude of dopamine cell loss in the ventral midbrain, dopaminergic fibre and seratonergic depletion in the striatum and changes in striatal immediate early gene expression in response to chronic L-DOPA treatment.

2. Methods

2.1. Experimental design

The sequence of experiments carried out is outlined in Fig. 1. Firstly, all mice were subject to staircase and corridor tests to obtain a pre-lesion baseline. Ninety adult male mice received 6-OHDA unilateral lesions of the nigrostriatal pathway, targeted at the SN ($n = 30$), MFB ($n = 30$), and striatum ($n = 30$). Unlesioned mice were also used as controls for behavioural and histochemical analysis ($n = 10$). Six weeks following lesion surgery, dopamine depletion was assessed in a series of motor tests (see below and Fig. 1). 20 mice from each lesion group were then selected for subsequent L-DOPA administration based on the following criteria: amphetamine turns of over 5/min, a greater than 65% right forelimb preference on the cylinder test and latency to fall on the accelerating rotarod of 75 s or less. Half of each lesion group ($n = 10$ per group) were treated with L-DOPA, with the others ($n = 10$) serving as lesion-only controls. L-DOPA was administered daily, starting at 2 mg/kg, during which mice were assessed for AIMs every 3–4 days, on 18 separate scoring sessions. Once AIMs had reached a stable plateau, the dose was increased, a process that was then repeated over a further 3 phases (6, 12, 25 mg/kg). 24 h after the final L-DOPA injection animals were terminally anaesthetised and transcardially perfuse-fixated in 1.5% paraformaldehyde. Brains were removed for histology and immunohistochemical analysis performed for tyrosine hydroxylase (TH), serotonin (5-HT) and FosB/ΔFosB.

2.2. Animals

C57/Bl6 mice (Charles River), weighing 20–25 g at the time of surgery were housed in standard cages, under a 12/12 h light/dark cycle. While being tested on motor tasks motivated by sugar pellets, mice were placed on a food-restriction regime to maintain 85% of their free-feeding bodyweight. At all other times, food and water were available *ad libitum* in the home cage. In particular, mice were on *ad libitum* feeding prior to surgery and during all drug administration periods. All procedures were conducted in accordance with personal, project and facility licences issued under the Animals (Scientific Procedures) Act 1986 and subject to Local Ethics Review.

2.3. 6-OHDA lesions

General anaesthesia was induced with 1–2% isoflurane in oxygen. Mice were then placed in a stereotaxic frame with incisor bar set at the level of the interaural line, and maintained under 1.5–2.0% isoflurane in a 2:1 oxygen/nitrous oxide mix. The skull was exposed and coordinates located on the skull relative to bregma. 6-OHDA hydrobromide (Sigma, Poole, UK) was injected in a single deposit in the MFB or SN, or in 2 deposits in the striatum at the following coordinates: striatum: (i) AP = +1.0, $L = -2.1$, DV = −2.9; (ii) AP = +0.3, $L = -2.3$, DV = −2.9, MFB: AP = −1.2, $L = -1.2$, DV = −4.75; SN: AP = −3.0, ML = −1.2, DV = −4.5. 6-OHDA was injected at a concentration of 6 μg/μl (free base weight, in 0.2 mg/ml ascorbic acid 0.9% sterile saline) through a 30-gauge stainless steel cannula, connected via fine polyethylene tubing to a 10 μl Hamilton syringe mounted in a Harvard microdrive pump. 6-OHDA was infused at 1 μl/min flow rate and infusion volumes were adjusted to 1 μl into the MFB, 1.5 μl at each site in the striatum and 1.5 μl into the SN. Following surgery, animals were administered 0.5 ml sterile glucose/saline (4% glucose s.c.) and analgesia provided in the form of soluble paracetamol (1 mg/ml in the drinking water).

2.4. Behavioural tests

2.4.1. Cylinder

Forelimb asymmetry was assessed, as described by Schallert et al. [26] by placing the mouse in a glass cylinder (H 14 cm, D 5 cm), illustrated in Smith and Heuer [25] and noting the forelimb preference for weight bearing touches onto the wall. Two mirrors were placed directly behind the cylinder so that a 360° view was seen by the observer. Animals that failed to reach 20 touches were removed from the cylinder after 10 min. Simultaneous ipsilateral and contralateral forelimb weight-bearing touches were excluded from the count. The session was videotaped and scored by detailed freeze frame analysis at a later date by an observer blind to the lesion condition of the animals.

2.4.2. Inverted grid

Animals were placed at the centre of the metal grid: dimensions 30 cm × 30 cm. The grid was then elevated 40 cm over the workbench and rotated 180°, by the investigator so that the mouse was grasping the underside. The latency to release the first paw and latency to fall was recorded.

2.4.3. Raised beam

The beam test assessed the motor coordination and dexterity between the right and left side, and was adapted to be used in mice [8,25]. Over the two days immediately prior to the test day all mice were trained to use the beam (beam dimensions: L 80 cm, W 0.5–1.5 cm, H 34–54 cm; house dimensions: L 11 cm, W 11 cm, H 10 cm) by placing them at the start and within the house to allow exploration. Each mouse was then allowed three attempts to traverse the beam after being placed at the base, where foot slips of the left side were counted live and the right side by video analysis. For each mouse the time taken to traverse the beam and the total number of hindlimb and forelimb foot slips were counted. The two quickest times and foot slip counts were averaged to give the final score.

2.4.4. Locomotor activity

Horizontal locomotor activity was assessed using Perspex activity cages (Med Associates, dimensions: L 42 cm, W 26 cm, D 19 cm) over a 2 h period in the light phase, run consistently from 11 am to 1 pm daily in a randomised fashion, where mice were habituated to the cages 1 h prior to recording. Automated Med Associates (St Albans, Vermont, USA) hardware and MED-PC IV software were used to record

the number of breaks for each of the three infrared beams, subsequently totalling the three for each animal to give an overall count.

2.4.5. Rotarod

All mice were subject to a pre-training protocol, as described in [27] and in detailed in [17]. On the test day mice were placed on a 5 cm division of the rotating beam (Jones and Roberts, Ugo Basile, Italy) for 5 min rotating in an accelerating protocol of 3–44 rpm over five min [27]. Time taken to fall was automatically logged by the mouse tripping a time switch upon landing on the base of the equipment. Mice performed the test three times with intervals of 60 min; the two highest scores averaged.

2.4.6. Rotation

Mice were placed in a cylinder (*H* 14 cm, *D* 11.5 cm) and behaviour recorded for 5 min. Full ipsilateral and contralateral rotations for spontaneous, apomorphine and amphetamine mediated conditions were counted manually. Mice were habituated to the same cylinder for 5 min prior to the administration of apomorphine (0.05 mg/kg s.c. 0.9% saline) or methamphetamine (2.5 mg/kg i.p. in 0.9% saline). Mice were recorded using an overhead camera and full rotations made in the ipsilateral and contralateral direction counted to determine net turning capacity. Mice were recorded over a 20 min window of peak rotation, either immediately after injection (amphetamine) or beginning 20 min post injection (apomorphine) [25,28]. Drug challenges were carried out 2 weeks apart and animals were not given repeated injections of apomorphine prior to the test day and no stereotyped behaviour was observed during the rotation period. Spontaneous rotations were assessed before amphetamine and apomorphine-induced rotation in the same apparatus to avoid conditioning effects.

2.4.7. Corridor

Initially designed for rats, the corridor test assesses lateralized sensory-motor integration and proprioception [11]. This test has previously been adapted for mice, dimensions: 60 cm long, 4 cm wide and 15 cm high [14]. Two days prior to testing, mice were habituated to the apparatus by the placement of sugar pellets randomly along the floor where mice were free to explore for 20 min. Immediately prior to testing, mice were acclimatised in the identical parallel corridor. For the test session, ten pairs of adjacent 1 cm pots containing six 20 mg sugar pellets were equally spaced at 5 cm intervals along the length of the corridor. The number of successful retrievals made on ipsilateral and contralateral sides, as defined by published protocols [14], was counted until a total of 20 retrievals were recorded or 5 min had been spent in the corridor.Staircase

The independent assessment of skilled forelimb use by paw reaching was achieved through the use of the staircase test [9,29]. The apparatus is composed of two Plexiglas staircases, one on either side of a central platform, such that the animal can only reach the staircase nearest to the right and left forelimbs. This staircase is enclosed within a container adjoined to a start chamber, and the rat enters via hinged lid, illustrated in Smith and Heuer [25]. Each step contains two sugar pellets (20 mg) and the numbers of pellets retrieved over a 30 min session are evaluated with respect to each paw. Animals were trained prior to lesioning to establish baseline performance [17].

2.5. L-DOPA administration and behavioural assessment

2.5.1. Administration

L-DOPA methyl ester HCl and the peripheral DOPA decarboxylase inhibitor, benserazide HCl (Sigma) were dissolved in 0.9% saline immediately before use. The drugs were injected at the volume of 10 ml/kg in a single daily subcutaneous (s.c) injection. Daily treatment with L-DOPA and benserazide continued for 64 days in four phases of incremental doses, in a modification of the Lundblad [16] regime:

- Phase 1 (days 1–13): 2 mg/kg of L-DOPA with 2 mg/kg of benserazide;
- phase 2 (days 14–40): 6 mg/kg of L-DOPA with 6 mg/kg of benzerazide;
- phase 3 (days 41–55): 12 mg/kg of L-DOPA and 12 mg/kg of benzerazide;
- phase 4 (days 56–64): 25 mg/kg of L-DOPA with 12 mg/kg of benzerazide.

Phase 2a refers to the days 14–24 of daily L-DOPA injections at 6 mg/kg and phase 2b refers to the days 25–40 of injections at the same dose, in order to monitor priming and AIM plateau responses respectively. Phase 4a refers to the days 56–59 where all three-lesion models received 25 mg/kg of L-DOPA before treatment was continued for mice with SN lesions, for a further 5 days, to reach a stable AIM plateau for that dose. The locomotor activity of lesioned mice was recorded in clear Perspex activity cages simultaneous with AIM scoring and rotational bias was assessed on intermittent days at the end of phase 2 and phase 4a. Mice were taken for histology (see below) 24 h after the final injection of L-DOPA.

2.5.2. Abnormal involuntary movements

AIMs were scored every 3–4 days of L-DOPA administration by placing mice in individual Perspex cages to habituate 20 min prior to L-DOPA injection and were scored once every 20 min for 3 h. The AIMs scoring criteria was based on [6], as follows:

Amplitude score; orolingual; 1 = repetitive chewing of the jaw, 2 = with tongue protrusions, axial; 1 = consistent deviation of head and neck to up to 30°, 2 = deviation of the head and neck to between 30° and 60°, 3 = deviation of head, neck and upper trunk of 60–90°, 4 = consistent deviation to 90° with loss of balance; limb 1 = small oscillatory movements of paw and distal forelimb, 2 = low amplitude circular movements of the forelimb, 3 = extension of the forelimb including displacement of the shoulder, 4 = maximal amplitude movements of limb and shoulder.

Duration score; 0 = absent, 1 = present less than half the time, 2 = present for over half the time, 3 = present for the whole time but interruptible by an external stimulus, 4 = present throughout but uninterruptible by external stimuli.

Duration and amplitude scores are the sum of all orolingual, axial, forelimb and hindlimb AIMs at all time points in the respective category. These are then multiplied together to give a total AIMs score. Mice were classified as dyskinetic if they consistently displayed all categories of AIMs, between severity scores 3–4, and AIMs of sufficient duration within the 1 min monitoring period, as defined in [18]. Animals were defined as non-dyskinetic if they exhibited either no or occasional AIMs of minimal severity [30].

2.6. Immunohistochemistry

At the end of behavioural testing rats were terminally anaesthetised with sodium pentobarbital and perfused transcardially with 25 ml phosphate buffered 0.9% saline (PBS) followed by 100 ml of 1.5% paraformaldehyde in phosphate buffer. Brains were removed and post-fixed in 1.5% paraformaldehyde for a further 24 h before placing them 20% sucrose. Coronal sections were then cut, 40 μm thick, on a freezing sledge microtome and stored in PBS with 0.01% azide at 4 °C until use. Sections were rinsed in PBS before endogenous peroxidases were quenched in 10% methanol and 3% hydrogen peroxide for 10 min. After rinsing, the sections were incubated in 5% normal horse serum in 0.25% Triton X-100 in PBS. Tissue sections were then incubated in primary antibody (all rabbit polyclonal) for 16 h at room temperature at concentrations of anti-TH (1:1000; Chemicon International, CA, USA), anti-5-HT (1:15,000; Immunostar Inc, Hudson, WI, USA) and anti-FosB/ΔFosB (1:500; Santa Cruz, CA, USA). All protocols were carried out as reported previously in the rat [31]. After washing in PBS, sections were incubated in biotinylated secondary antibody (anti-rabbit antibody raised in horse (1:200 for all stains)). Staining of the tissue-bound antibody was visualised using a standard peroxidase-based method (Vectastain Elite, ABC kit, Vector Laboratories, Burlingame CA, USA) and the chromogen, 3,3′-diaminobenzadine (Sigma). Tissue sections were mounted, dehydrated and coverslipped.

2.7. Quantification and statistics

Behavioural measures in corridor, grip strength, rotarod, balance beam, apomorphine rotation, amphetamine rotation, spontaneous rotation, staircase, activity and cylinder tests are expressed as contralateral performance as a percentage of the ipsilateral (unaffected side). TH-positive cell bodies in the VTA and SNc were counted under a Leica RB/DME light microscope at the level of the medial terminal nucleus of the accessory nucleus of the optic tract interception, as described by Ref. [32]. We assessed the density of TH-immunopositive fibres and 5-HT in the dorsal and ventral striatum from images of every 4th section taken with Leica DFC420 camera and Leica application software (v3.6) on ImageJ freeware (Version 1.42, National Institutes of Health, USA). Density was normalised to density levels of the corpus callosum. TH positive cell bodies within the striatum were counted in all sections from a 1 in 5 series through the mouse brain and adjusted with the Abercrombie correction factor to calculate cell numbers in whole striatum [33]. The SPSS (v16) statistical package was used to undertake 2-way analyses of variance for comparisons between surgical groups and doses of L-DOPA, and Pearson's correlations between behavioural and histological measures. Further partial correlations were used to determine the extent of AIM-behaviour correlations controlling for TH-cell loss in the SNc. This statistical analysis factors out the relationship with dopamine depletion, permitting behavioural correlations with additional factors.

3. Results

3.1. 6-OHDA-induced deficits in a battery of motor tests

Motor tests carried out prior to L-DOPA treatment indicated no significant differences between L-DOPA-treated and untreated lesion subgroups. As shown in Table 1, the 6-OHDA lesions in all three surgical groups produced significant deficits in comparison to the unlesioned control animals on several of the behavioural tests, including the rotarod ($F_{3,69} = 28.76$, $p < 0.001$), inverted cage lid ($F_{3,69} = 2.91$, $p < 0.05$), time on balance beam ($F_{3,69} = 2.92$, $p < 0.05$), foot slips on beam ($F_{3,69} = 5.34$, $p < 0.01$), cylinder ($F_{3,69} = 4.51$, $p < 0.01$), corridor ($F_{3,69} = 3.04$, $p < 0.01$), staircase ($F_{3,69} = 3.31$, $p < 0.05$) and basal locomotor activity ($F_{3,69} = 3.83$,

Table 1
Lesion-induced deficits on behavioural hand test scores in mice with striatum, MFB and SN 6-OHDA lesions.[a]

Hand test/lesion type	Intact control	Striatum	MFB	SN
Rotarod	131.65 ± 6.08	66.75 ± 5.95***	45.82 ± 2.89***	77.15 ± 6.97***
Inverted grid test	215.26 ± 30.85	117.47 ± 11.09*	155.84 ± 17.91	153.09 ± 24.60
Amphetamine rot (total)	26.72 ± 6.84	81.61 ± 6.60***	87.55 ± 11.88***	72.34 ± 11.47***
Apomorphine rot (total)	2.70 ± 0.39	29.41 ± 4.44***	59.65 ± 9.43***	33.10 ± 6.16***
Spontaneous rot (total)	8.20 ± 0.78	12.90 ± 1.45	22.35 ± 2.31***	8.70 ± 0.76
% Balance beam	68.62 ± 8.13	73.52 ± 3.07	73.9 ± 3.22*	80.21 ± 3.59
% Cylinder	57.82 ± 2.12	77.18 ± 3.43*	73.41 ± 3.21*	66.37 ± 3.91
% Corridor	36.69 ± 6.04	18.69 ± 3.42*	16.81 ± 3.07*	27.17 ± 4.41
% Staircase	49.85 ± 2.33	49.61 ± 0.69	52.98 ± 0.76*	46.14 ± 2.41
Beam time	13.59 ± 2.57	16.79 ± 1.42	20.90 ± 1.16	17.27 ± 1.68
Activity (perseverative)	663.4 ± 27.4	631.7 ± 47.0	469.5 ± 25.7	710.5 ± 80.9
Activity (non-perseverative)	1320.7 ± 76.1	1382.6 ± 147.6	1137.7 ± 71.4	1429.4 ± 191.0

[a] Groups averages are expressed as means ± SEM; $n = 10$ control group, $n = 20$ per lesion group.
* $p < 0.05$.
*** $p < 0.001$.

$p < 0.01$). Rotational behaviours also demonstrated a clear lesion effect in total amphetamine ($F_{3,69} = 4.80$, $p < 0.01$), total apomorphine ($F_{3,69} = 9.18$, $p < 0.001$) and total spontaneous ($F_{3,69} = 16.33$, $p < 0.001$) rotation tests. However, when individual lesion groups were evaluated the effects were less pronounced. Specific deficits according to lesion type were noted by pair wise comparisons as indicated in Table 1, and described in more detail in the accompanying paper [17].

3.2. L-DOPA induced dyskinesia, activity and rotation

It is well established that in the absence of a lesion, intact mice do not develop AIMs following chronic L-DOPA treatment, and consequently, the analysis of L-DOPA-induced dyskinesia was only undertaken in lesioned animals, comparing the three surgical lesion targets. In general, AIMs in all lesioned mice chronically treated with L-DOPA increased over the course of L-DOPA administration ($F_{4,108} = 15.42$, $p < 0.001$). Moreover there was a difference between the lesion groups in the L-DOPA dose at which AIMs were exhibited and the magnitude of the AIMs that were expressed (group, $F_{2,27} = 14.15$, $p < 0.001$; group × phase, $F_{8,108} = 7.71$, $p < 0.001$). As shown in Fig. 2A and B the lowest dose of L-DOPA, 2 mg/kg, induced low level AIMs only in the mice with MFB lesions but failed to evoke a measurable response in the SN and striatal lesion groups. The MFB lesion group continued to express AIMs at progressively higher levels as the L-DOPA dose was increased in phase 2 (6 mg/kg), phase 3 (12 mg/kg) and phase 4 (25 mg/kg). By contrast, the SN and striatal groups did not display significant AIMs until phase 3 (12 mg/kg), but the severity of abnormal movements then increased further at phase 4 (25 mg/kg). AIMs in the MFB group were consistently greater than those in the SN and striatal groups at all phases (Fig. 2B). At 25 mg/kg 77% mice developed AIMs above threshold levels, however, the number within each group varied depending upon lesion type (striatum: 60%; MFB: 100%; SN: 70%).

Horizontal locomotor activity was consistently increased at doses of 6–25 mg/kg in comparison to activity at 2 mg/kg (Fig. 2C and D; $F_{4,108} = 5.19$, $p < 0.001$), with no significant overall effect of lesion type, although there was a significant group × phase interaction (Fig. 2C and D; $F_{8,108} = 3.71$, $p < 0.001$). The percentage of animals of the total cohort exhibiting turning behaviour gradually increased over the 4 phases (Fig. 2E). This was accompanied by a dose-dependent increase in total net rotations ($F_{1,28} = 4.21$, $p < 0.05$) where rotation was assessed quantitatively during the plateau phases 2 (6 mg/kg) and 4 (25 mg/kg). However, upon *post hoc* analysis, this was only significant in the striatal group (Fig. 2F; $p < 0.05$).

3.3. Immunohistochemistry.

TH immunohistochemistry indicated a marked loss of TH cells in the SNc on the side ipsilateral to the lesion in all three groups compared to the intact controls in which the distribution of cells remained symmetrical between the two sides (Fig. 3A–H). Quantification of TH-positive cell numbers in the ipsilateral and contralateral SNc indicated approximately 80% loss of SNc dopamine neurons in both the striatum and MFB lesion groups and a somewhat lower depletion of 60–70% in the SN lesion group (Fig. 3M; $F_{3,69} = 14.80$, $p < 0.001$). In the VTA dopaminergic cell loss was less uniform with a 50% loss typically seen in MFB lesion mice and a 20–40% loss in the SN and striatal lesion groups (Fig. 3N; $F_{3,69} = 11.63$, $p < 0.001$).

At the level of the forebrain, TH immunohistochemistry indicated a marked loss of TH fibre density in the dorsal and ventral striatum on the right side, ipsilateral to the lesions (Fig. 3). Quantification by densitometry of TH staining indicated 60–85% loss of staining in the three lesion groups in the dorsal striatum (Fig. 3O; $F_{3,69} = 13.05$, $p < 0.001$) and somewhat lower 55–75% loss of staining in the ventral striatum (Fig. 3P; $F_{3,69} = 13.05$, $p < 0.001$). Interestingly, the differences in terminal staining between groups did not exactly match the loss of cells in the midbrain: lesions targeted on the SN produced the least loss of cells throughout the nigra and lesions targeted on the striatum produced the least loss of terminal staining in the striatum (compare Fig. 3M and N), whereas lesions targeted at the MFB produced the most consistent retrograde and anterograde loss of cells and terminals in all areas. L-DOPA treated and non-L-DOPA treated groups were well matched with no differences in mean TH positive cell loss between groups.

There were small differences in the density of 5-HT staining in the lesioned striatum compared to the intact side (Fig. 3I–L), with the largest hemispheric reduction in 5-HT in the MFB group, although this was only observed in some animals of the group. 5-HT loss was assessed in terminal regions and was evident in both the dorsal (Fig. 3Q; $F_{3,69} = 3.14$, $p < 0.05$) and ventral striatum (Fig. 3R; $F_{3,69} = 3.15$, $p < 0.05$), although this was also highly variable within groups.

L-DOPA mediated increases in the expression of FosB/ΔFosB are seen in the striatum and NAcc regions in all lesion groups (Fig. 4A–H) with a denser and more widespread staining the in MFB lesion mice. The most significant increase in FosB/ΔFosB levels was found within the dorsal striatum where there was a significant effect of L-DOPA treatment (Fig. 4I; $F_{2,67} = 23.43$, $p < 0.001$) compared to lesion only mice and the intact control group. In this region there was also an effect of lesion type ($F_{3,69} = 21.04$, $p < 0.001$). When assessed in the NAcc core the number of FosB/ΔFosB positive cells

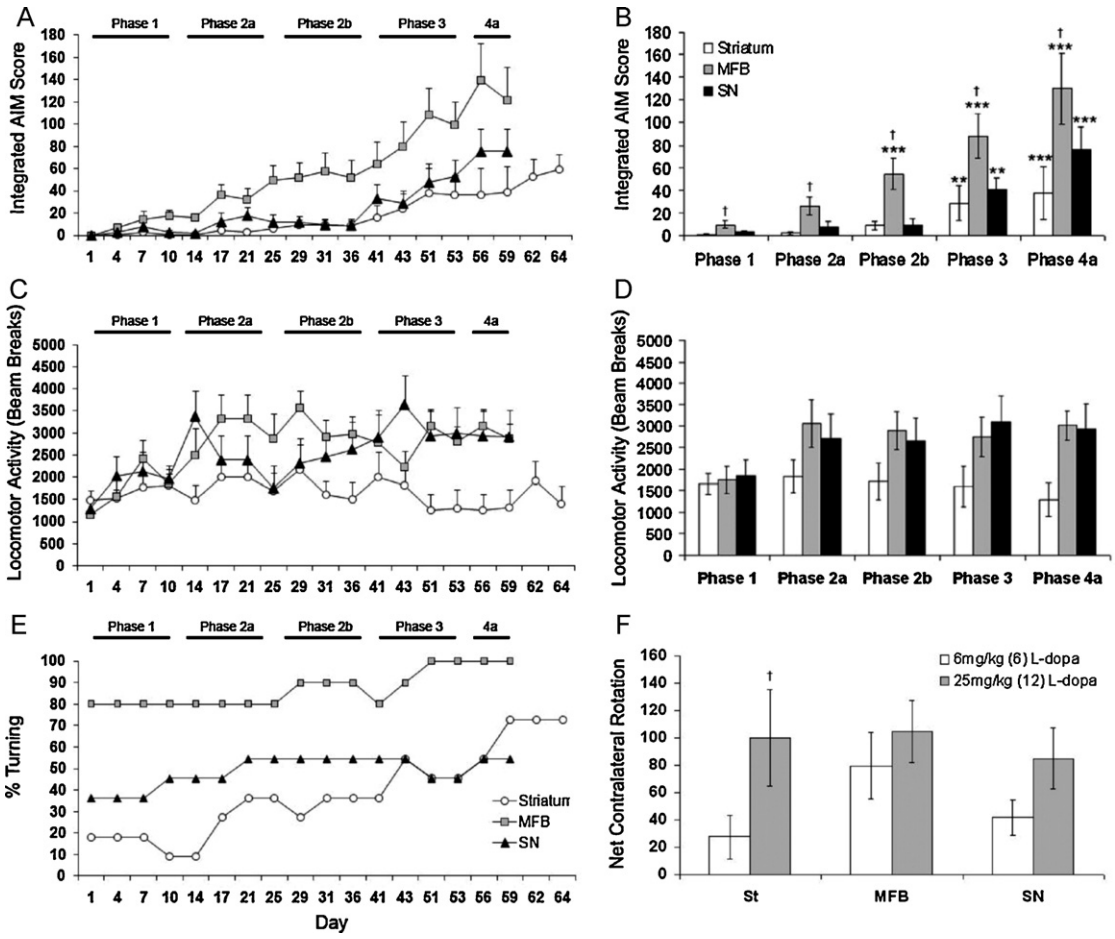

Fig. 2. AIMs induced by L-DOPA administration, in mice with striatal ($n = 10$), nigral ($n = 10$) or MFB ($n = 10$) 6-OHDA lesions scored on the Cenci and Lundblad rating scale each day (A) and averaged for each phase (B), where phase 1 = 2(2) mg/kg (days 1–13), phase 2 = 6(6) mg/kg (days 14–40), phase 3 = 12(12) mg/kg (days 41–55) and phase 4 = 25(12) mg/kg (days 56–59); brackets indicate dose of benzerazide used. Simultaneous horizontal locomotor recorded scored each day (C) and mean activity are shown (D). Also displayed is the % of the cohort eliciting turning behaviour in response to phasic L-DOPA treatment each scored day (E) and net controlateral rotations for each lesion type scored at 6 mg/kg and 25 mg/kg. *Post hoc* comparisons from a 2-way repeated measures ANOVA have shown AIM scores at phases 1–4 are indicated as $p < 0.01^{**}$ and $p < 0.001^{***}$ and compared to the MFB group at each phase as $p < 0.05^{\dagger}$ Significance found between rotational bias between 6 mg/kg and 12 mg/kg are annotated as $p < 0.05^{\dagger}$.

was only significantly increased in the striatal lesion L-DOPA group (Fig. 4J; $p < 0.05$) although a similar trend was evident for all groups. Within the NAcc shell region L-DOPA treatment increased the number of FosB/ΔFosB positive cells (Fig. 4K $F_{2,67} = 18.69$, $p < 0.001$) from specific increases in striatal and MFB lesion mice, with no overall difference between lesion type.

3.4. Histological correlates of L-DOPA induced AIMs

As shown in Table 2, there was a correlation between cell loss within the SNc with AIMs score at 25 mg/kg ($r = -0.533$, $p < 0.01$) and the VTA with AIMs score at 6 mg/kg ($r = -0.596$, $p < 0.01$), although there was a trend between cell loss in both regions and AIMs at both doses. When looking more closely at individual lesion groups, AIMs were correlated significantly in the MFB lesion group with respect to cell counts within the SNc ($r = -0.577$, $p < 0.05$) and the SN lesion group with respect to the VTA ($r = -0.577$, $p < 0.05$). Table 2 also shows that FosB/ΔFosB cell counts within the striatum, NAcc core and NAcc shell in relation to development of AIMs in the three different unilateral lesioned mouse models. At phase 2 (6 mg/kg) AIMs and FosB/ΔFosB cell counts are only positively correlated in the NAcc shell region ($r = 0.583$, $p < 0.05$). At 25 mg/kg FosB/ΔFosB is highly correlative to dyskinesia in the striatum ($r = 0.754$, $p < 0.01$) and NAcc core ($r = 0.525$, $p < 0.01$) in all mice. FosB/ΔFosB levels do not correlate significantly with AIMs present from 2 or 12 mg/kg of L-DOPA although a trend can be noted in the latter dose. In addition a positive correlation was noted between L-DOPA induced rotation at 25 mg/kg and FosB/ΔFosB positive cells in the NAcc shell in MFB lesion mice ($r = 0.664$, $p < 0.05$). There was also a weak overall positive correlation with ventral 5-HT density and AIMs at 6 mg/kg ($r = 0.325$, $p < 0.05$) and dorsal density at 25 mg/kg ($r = 0.343$, $p < 0.05$). In addition, L-DOPA induced AIMs were moderately correlated to 5-HT density only in the MFB group at 6 mg/kg, within the ventral striatum (Fig. 5: $r = 0.616$, $p < 0.05$) and dorsal striatum (Fig. 5: $r = 0.618$, $p < 0.05$). However, this trend was lost at high dose L-DOPA and was not found in mice receiving SN or striatal lesions.

3.5. Correlating dyskinesia to behavioural test scores

The severity of AIMs at the end of phases 2 and 4 (overall and for each lesion group) were correlated with motor test scores of lesion extent, factoring in the SNc cell loss (Table 3) of partial correlation with SNc depletion as co-variate). Contralateral rotation in response to L-DOPA correlated with AIMs for all lesioned mice at 6 mg/kg ($r = 0.422$, $p < 0.01$) and 25 mg/kg ($r = 0.445$, $p < 0.01$). When considering all lesioned animals and the full range of AIMs, a different array of motor deficit tests correlate to AIMs induced by 25 mg/kg of L-DOPA than by 6 mg/kg. At 6 mg/kg AIMs expressed by all mice are only correlated with latency to fall on

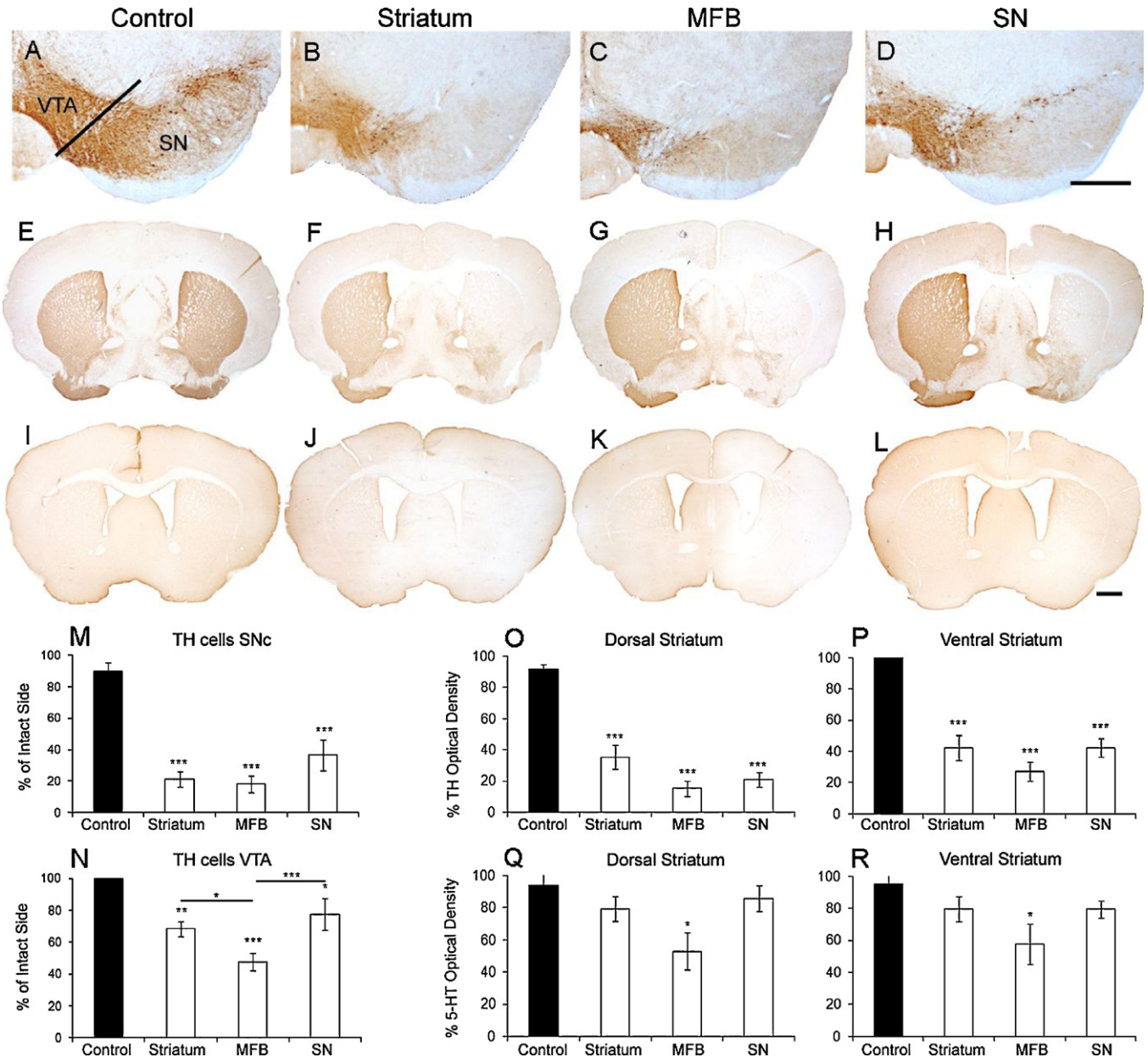

Fig. 3. Photomicrographs of the SN in mice, immunostained with TH, in an unlesioned control mouse (A) and mice with a 6-OHDA lesion of the striatum (B), MFB (C) and SN (D) (Scale bar 400 μm). Photomicrographs are also shown for whole forebrain sections immuno stained for TH (E–H) and 5-HT (I–L) on (scale bar 2 mm). Percentage TH cell loss from the SNc and VTA is shown for the 6-OHDA lesioned, L-DOPA-treated lesion groups ($n = 10$ for each) compared to the unlesioned side (M and N). Optical density was used to measure relative TH and 5-HT expression in the dorsal striatum and ventral striatum for each lesion type (O–R). Significant deviations from intact control animals and between lesion groups are indicated by $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$.

the rotarod ($r = -0.508$, $p < 0.01$), total apomorphine driven turns ($r = 0.305$, $p < 0.05$) and total spontaneous turns ($r = 0.541$, $p < 0.01$). In contrast, AIMs driven by 25 mg/kg were correlated to perseverative locomotor activity ($r = -0.420$, $p < 0.05$) and non-perseverative locomotor activity ($r = -0.460$, $p < 0.05$) but were no longer correlated to tests at the lower dose (Table 3).

When groups were split according to lesion type (Table 3) the most consistent correlations were on performance on the rotarod correlating with AIMs at 6 mg/kg (striatum: $r = -0.558$, $p < 0.05$, MFB: $r = -0.763$, $p < 0.05$; SN: $r = -0.784$, $p < 0.01$) with a general trend in all groups for correlation to apomorphine and amphetamine rotation. Interestingly, scores on the rotarod remained correlated with AIMs elicited by 2 mg/kg ($r = -0.498$, $p < 0.01$) but not 12 mg/kg or 25 mg/kg. At 25 mg/kg correlations of AIMs with perseverative locomotor activity and non-perseverative locomotor activity were noted in MFB and SN lesion groups ($p < 0.01$). Furthermore strong correlations between AIMs with total apomorphine rotations ($p < 0.001$) and rotarod ($p < 0.05$) were only seen in the MFB lesion group.

3.6. TH positive neurones in the denervated striatum

TH positive cells were identified in the striatum of all lesioned mice (Fig. 6A–C). They appear medium sized, displayed as apparent aspiny projections (Fig. 6G) and were darker in appearance in L-DOPA treated groups (Fig. 6D–F). The emergence of TH positive cells was also increased significantly by L-DOPA treatment compared to lesion only controls (Fig. 6G–I). The distribution of these cells was ubiquitous across the lesioned striatum with little observed difference when counts in the dorsal and ventral

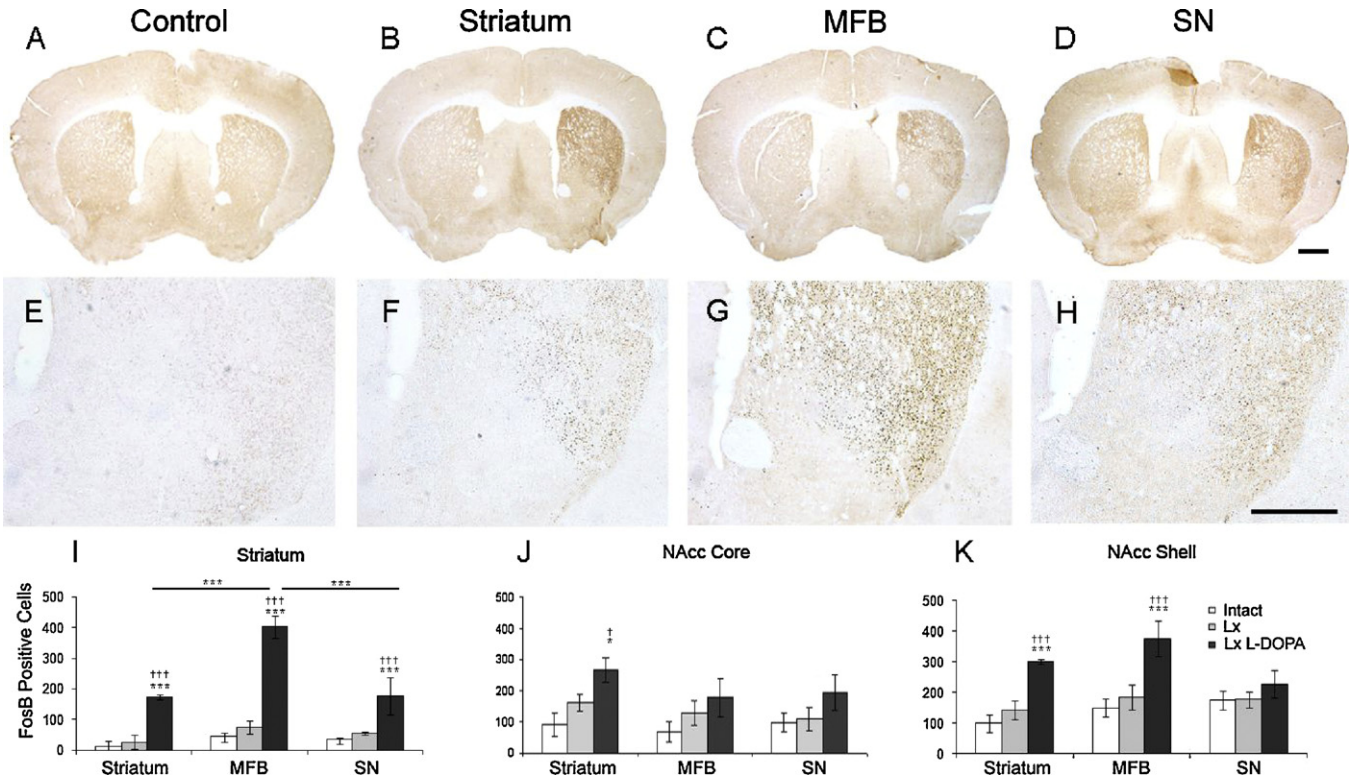

Fig. 4. Photomicrographs of sections immunostained for FosB/ΔFosB on mice with a 6-OHDA lesion of the striatum ($n = 10$), MFB ($n = 10$) and SN ($n = 10$) compared to unlesioned controls (scale bar 2 mm; A–D) and at 5× magnification FosB/ΔFosB (E–H), where scale bar shown as 500 μm. The number of FosB/ΔFosB positive cells within the whole striatum, NAcc core and NAcc shell and were quantified (I–K). Significant differences from intact control animals and between L-DOPA treated groups are indicated by $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$.

Table 2
Correlations between average integrated AIMs under 6 mg/kg and 25 mg/kg L-DOPA with TH cell counts in the midbrain, FosB/ΔFosB cell counts within striatal subdivisions, and with 5-HT density within cortex, dorsal and ventral striatum.[a,b,c]

AIMs	TH cell counts		FosB/ΔFosB cell counts			5-HT density		
	SNc	VTA	Dorsal Str.	NAc core	NAc shell	Cortex	Ventral Str.	Dorsal Str.
L-DOPA 6 mg/kg								
Overall	−0.310	−0.521**	0.217	0.199	0.316*	0.263	0.325*	0.245
Striatum	0.128	0.102	0.216	0.390	−0.230	0.226	0.051	0.193
MFB	−0.544	−0.550	0.317	0.164	0.583*	0.433	0.616*	0.618*
SN	−0.379	−0.544	0.272	−0.052	0.138	0.980	−0.054	0.168
L-DOPA 25 mg/kg								
Overall	−0.533**	−0.267	0.754**	0.525**	0.298	0.054	0.127	0.343*
Striatum	−0.527	−0.55	0.852**	0.469	0.195	0.450	−0.020	0.042
MFB	−0.577*	−0.452	0.357	0.733*	0.483	0.131	0.432	0.415
SN	−0.548	−0.577*	0.713*	0.138	0.418	−0.100	−0.020	0.188

[a] Values indicate correlation (Pearson's parametric) coefficients.
[b] $n = 10$ per lesion group.
[c] Abbreviations: NAc, nucleus accumbens; SNc, substantia nigra pars compacta; Str., striatum; VTA, ventral tegmental area.
* $p < 0.05$.
** $p < 0.01$.

striatum were considered independently. There was a significant increase of TH cell occurrence by L-DOPA treatment in the dorsal ($F_{1,59} = 14.68$, $p < 0.001$), ventral ($F_{1,59} = 8.11$, $p < 0.01$) and whole striatum ($F_{1,59} = 12.19$, $p < 0.001$), with no differences found between lesion type. *Post hoc* analysis has further shown that significant changes were seen in the whole striatum in MFB and SN lesion groups (both $p < 0.001$) but was not seen in the striatal lesion mice.

The number of cells in the striatum expressing TH in the whole striatum correlates with both % cell loss within the SNc in L-DOPA treated mice receiving MFB or SN lesions but not non-L-DOPA treated groups ($r = -0.690$ and $r = -0.811$ respectively $p < 0.01$). AIMs present at 25 mg/kg at phase 4 were also correlated to TH positive cells in the striatum in all lesioned animals irrespective of cell loss in the SNc ($r = 0.539$, $p < 0.01$). In addition, AIM development was only correlated to TH cells in the L-DOPA treated MFB group ($r = 0.642$, $p < 0.01$), with cell loss in the SNc taken into account.

4. Discussion

This is the first study to carry out a comprehensive longitudinal analysis comparing the dyskinetic responses of mice lesioned at the 3 main targets along the nigrostriatal tract, through a range of L-DOPA doses. All lesioned animals displayed deficits on some motor tests before the initiation of L-DOPA treatment, and these deficits

Table 3
Correlations between average AIMs at peak responses to 6 mg/kg and 25 mg/kg with L-DOPA induced behaviours and tests of motor function.[a,b,c].

Hand test/AIMs	(6 mg/kg L-DOPA)				(25 mg/kg L-DOPA)			
	Overall	Striatum	MFB	SN	Overall	Striatum	MFB	SN
L-DOPA rot (6 mg/kg)	0.422**	0.882**	−0.200	−0.492	–	–	–	–
L-DOPA rot (25 mg/kg)	–	–	–	–	0.445**	0.692*	0.045	0.041
Rotarod	−0.508**	−0.558*	−0.763*	−0.784**	−0.285	−0.440	−0.620*	−0.517
Cagelid	0.001	−0.317	−0.201	−0.519	−0.135	0.122	−0.507	−0.445
Amphetamine rot (total)	0.140	0.454	0.308	0.339	−0.320	0.194	−0.337	−0.110
Apomorphine rot (total)	0.305*	0.389	0.415	0.684*	0.304	−0.268	0.896**	0.552
Spontaneous rot (total)	0.541**	0.039	0.284	0.110	0.350	0.524	0.505	0.208
Balance beam	0.066	0.003	0.197	0.309	0.105	−0.183	0.431	0.026
Cylinder	0.034	0.309	−0.431	−0.707*	0.139	0.567	−0.497	−0.439
Corridor	−0.182	−0.229	−0.400	−0.367	−0.198	−0.268	−0.324	−0.147
Staircase	0.156	0.170	−0.267	−0.092	0.154	−0.214	−0.395	−0.357
Beam time	0.194	0.437	0.114	0.092	0.105	0.026	−0.183	0.431
Activity (perseverative)	−0.298	0.301	−0.310	−0.586	−0.420*	−0.260	−0.772**	−0.682*
Activity (non-preseverative)	−0.214	−0.319	−0.241	−0.546	−0.460**	−0.182	−0.699*	0.695*

[a] Correlation co-effects are indicated for the total L-DOPA treated cohort and for each lesion type.
[b] Controlled for confounding correlations between AIMs and % of cells remaining in the SNc.
[c] $n = 10$ per lesion group.
* $p < 0.05$.
** $p < 0.01$.

can be correlated both to dyskinesia expression and to cell loss in the SNc. Importantly, several of the behavioural tests remained predictive of subsequent development of LID, even once allowance is made (by partial correlation analysis) for the underlying variability between animals in the extent of loss of midbrain dopamine neurons. Consequently, mice with a susceptibility to express severe AIMs following chronic L-DOPA treatment can be preselected in future dyskinesia experiments. A detailed discussion on the use of known and novel behavioural mouse tests and their relationship to dopamine depletion is detailed in [17]. Herein we discuss importance of the degree of depletion in the VTA and SNc, 5-HT density reduction and FosB/ΔFosB positive cell upregulation in relation to LID, in each of the models.

4.1. Histological characterisation of the lesions

As expected based on previous reports and as experienced in the rat the greatest losses of TH positive neurons in the SNc were in the striatal and MFB groups with a more moderate depletion in the SN group. Importantly, clear differences in the lesion extent of non-nigral dopamine cell losses and 5-HT depletion characterised the different lesion types. VTA dopamine cell loss was the most depleted in the MFB lesion group, with a higher degree of sparing caused by striatal and SN lesion surgery. Indeed we report similar striatal lesion extents to Francardo et al. [13] with a dose twice as high, indicating the difficulty of depleting the whole striatum with a 2×1.5 μl volume. VTA dopamine loss was the greatest in the MFB group, with greater sparing when either the cell bodies or terminals were the target for the neurotoxin.

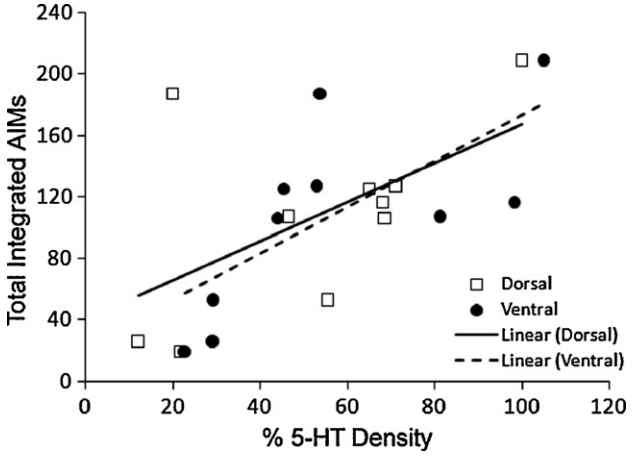

Fig. 5. Scatter plots of total integrated AIMs (6 mg/kg) and percentage 5-HT density of the dorsal and ventral striatum, in mice reciveing MFB lesions.

Importantly, clear differences in the lesion extent of non-nigral dopamine cell losses and 5-HT depletion characterised the different lesion types. Reductions in striatal 5-HT density were likely the result of 5-HT fibre losses and only evident in the MFB lesion group, although were highly variable between animals. This contrasts with the only other study to examine 5-HT changes in mice following 6-OHDA lesion and L-DOPA treatment in which no differences in whole 5-HT levels as measured by HPLC were observed [13]. This may be due to procedural differences, the total quantity of 6-OHDA infused was less and administration through a fine glass capillary as opposed to our metal cannula. These two factors may have produced less non-specific damage, leading to a more selective dopaminergic lesion. Equally, MFB lesions were large in this present study and comparable to levels found in the rat, where 5-HT fibre bundles have also been partially damaged, contributing to LID [34]. Depending on the primary outcome measures the effects on serotonin may not be a disadvantage so long as they are considered; we find an inverse relationship between total integrated AIM score and 5-HT density when using cell loss in the SNc as a covariate. This therefore may contribute to the aberrant pulsatile dopamine release that is a major factor in the development of LID [35]. Clearly it is therefore important that determination of 5-HT effects is made post mortem and taken into account in the analysis [36].

Elevated FosB/ΔFosB expression was induced in the striatum and NAcc regions in all lesioned mice. High FosB/ΔFosB expression in the NAcc in MFB lesion groups is mediated by the lack of TH fibre innervation of this area, a pattern well established in the striatum [13,30,37–39]. Although other studies have not looked specifically in the NAcc, striatal FosB/ΔFosB expression has previously been found to be highest in MFB lesioned mice compared to striatal lesion groups [13], also consistent with our findings. We show for the first time that dyskinetic mice with SN lesions have a large increase in FosB/ΔFosB expression in the dorsal striatum, which is comparable to the striatal lesion group, owing to sparing of TH fibres in the ventral striatum. It has been found that psychostimulant induced sensitisation can induce increases of FosB/ΔFosB in the NAcc shell [40], comparable to levels shown here, indicating the likely role of L-DOPA in supersensitisation specifically in this region. Although

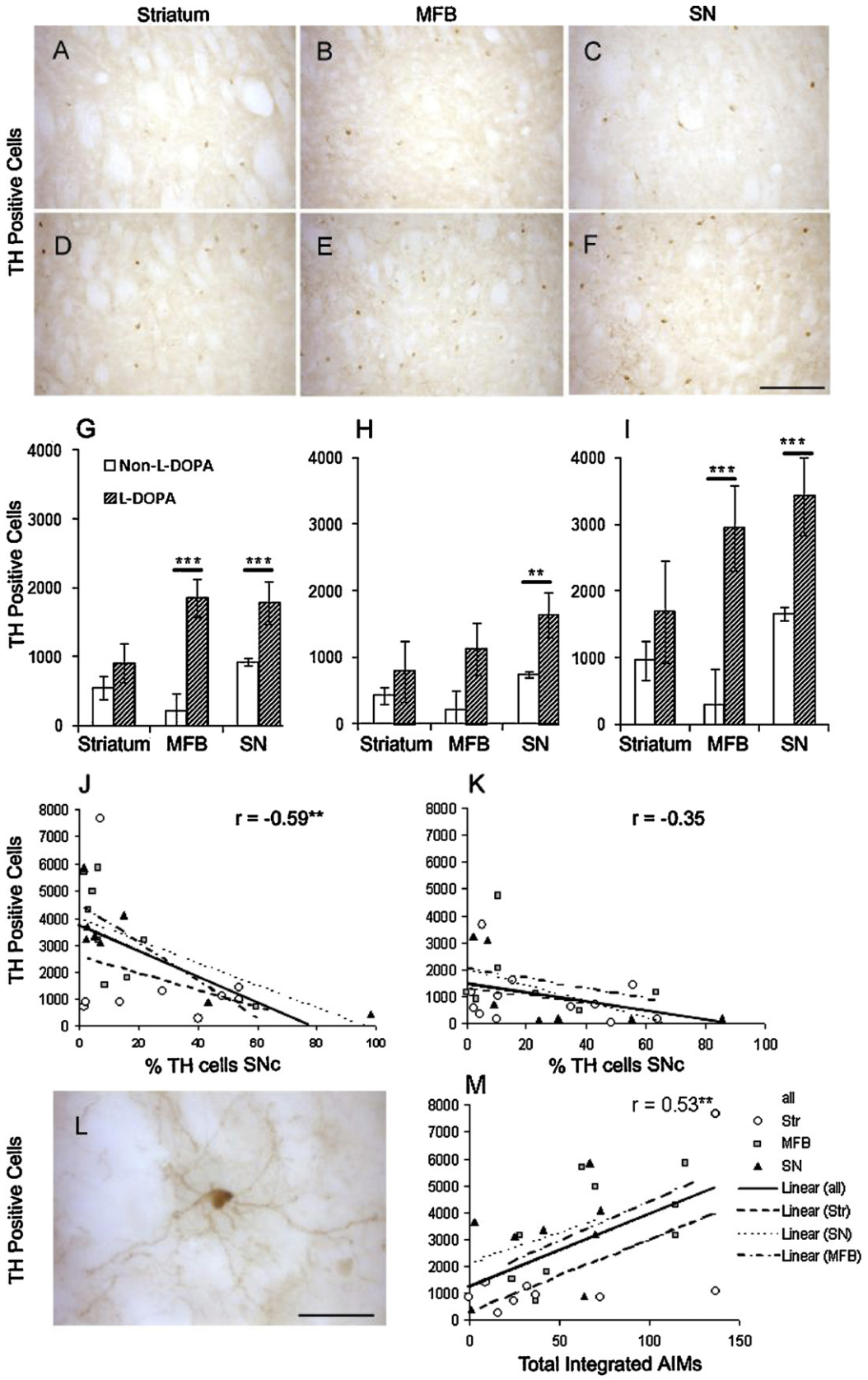

Fig. 6. Photomicrographs of TH immunopositive cells in the striatum of lesion only controls (A, striatum; B, MFB; C, SN) and L-DOPA treated groups (D, striatum; E, MFB; F, SN); scale bar is 200 μm. For each lesion only and L-DOPA treated group $n = 10$. A high magnifaction image is shown in (scale bar 50 μm; L). TH immunopositive cells were quantified in L-DOPA and non-L-DOPA treated groups in the dorsal (G), ventral (H) and whole (I) striatal areas. TH immunopositive cells were correlated with % cell loss in the SNc of L-DOPA treated (J) and Non-L-DOPA treated lesioned mice (K) and total integrated AIMs (25 mg/kg; M). Specific values and lesion trends are indicated for the striatal lesion group by circle outlines and dashed lines, the MFB group by filled circles and a dotted lines and SN group by filled triangles and dashed-dotted lines. Significant whole correlation coefficents and increases in TH cell numbers in L-DOPA treated mice are labelled as $p < 0.01^{**}$ and $p < 0.001^{***}$.

the diverse severity of AIMs enabled robust correlations to histological measures, coefficients were considered mild-moderate. This is likely because of the exclusion of partial lesioned and unlesioned mice.

4.2. Lesion effects on L-DOPA dyskinesia

In previous studies, the threshold doses of L-DOPA at which AIMs in mice have first been triggered were 6 mg/kg (MFB) and 18–20 mg/kg (striatal) [13,16,41]. In our study those with MFB lesions started to show dyskinesia at the same doses [16,41], while striatal or SN lesion mice developed AIMs at the lower dose of 12 mg/kg. This is a direct reflection of the degree of cell loss in the two models and is also observed in the magnitude of the behavioural response, L-DOPA induced AIMs being significantly lower in severity following striatal and SN lesions compared to the MFB group even at the highest doses.

The dyskinetic status of lesioned animals is in part due to dose. Raising the dose of L-DOPA increases AIM severity and also recruits previously non-dyskinetic members of the cohort (defined as having an integrated AIM score of less than 20 on the Cenci and Lundblad rating scale). In rats with striatal and MFB lesions approximately 20–35% of the cohorts continue to be 'non-dyskinetic' following chronic treatment with the moderate dose of 9–11 mg/kg [20]. Previous studies of LID have also reported low levels of non-dyskinetic mice and similarly in this study we found only 7% of MFB lesion mice were non-dyskinetic at 6 mg/kg. Conversely, 40% of striatal lesion mice were previously deemed dyskinetic at 6 mg/kg [13], while few mice from our cohort had significant AIMs at that dose. The lack of mice that could be scored below the dyskinetic threshold may in part relate to kinetic differences in basal movements, with mice having faster exploratory and grooming behaviours, which may be scored as AIMs by some investigators. In addition, the dose-dependent progressive development of AIMs to L-DOPA illustrates that the impact of apomorphine was likely minimal and crucially priming was still permitted.

These differences in dyskinetic profiles and dose responsiveness both between lesion types and between different studies are likely to be related to the cell loss observed in each cohort. Nigral dopamine cell loss in our study was equivalent in the striatal and MFB groups, more extensive than previous striatal lesion experiments using the same strain [16,41]. Importantly despite this similar nigrostriatal depletion the severity of AIMs in the SN and striatal group never reached that of the MFB lesioned group. With significant 5-HT loss in PD [36] the extent of this is of particular relevance to the study of L-DOPA mediated behaviours. As the site of dopamine conversion [42], 5-HT terminal losses might be expected to decrease dopamine synthesis and thereby reduce the amount of dopamine released and indeed there was a correlation in the MFB group between the level of AIMs and higher levels of 5-HT terminals. This does not explain, however, the reduced severity of AIMs in the SN and striatal groups which had undamaged serotonergic systems. Whereas dopaminergic losses in the SNc are comparable, the striatal and SN lesion groups in this study showed a greater sparing of the VTA. This strikingly similar pathology mirrors more closely the comparable behavioural responses to L-DOPA of the SN and striatal groups. This may implicate the VTA in having a role in the development of AIMs. The sparing of dopaminergic terminals in this region may be sufficient to offer buffering of dopamine levels and thereby limit dyskinesia severity. Strong L-DOPA driven net rotation and correspondingly high horizontal locomotor activity seen in the MFB group is may be attributed to the extensive VTA damage and hence supersensitisation in the NAcc region, in the same manner as amphetamine and apomorphine mediated turning traits [43]. The 5-HT changes in PD models and their relation to dyskinesia are debated, with studies variously finding no change or sprouting of terminals [34,44–47], warranting further investigation.

4.3. Behavioural prediction of susceptibility to dyskinesia

The extent of AIMs correlates with dopamine depletion in the SNc at 6 and 12 mg/kg irrespective of lesion type. We also find that a selection of motor tests can be correlated to AIM severity at 6 mg/kg after 40 days and 25 mg/kg after 64 days, even when controlling for SNc depletion. Consequently, tests correlating to AIMs may offer useful predictors of dyskinesia even before L-DOPA is given. However, the predictability of the behavioural test is dependent both on L-DOPA dose and on lesion type. The rotarod scores prior to L-DOPA dosing are correlated to AIMs at 2 mg/kg and 6 mg/kg but not at higher doses, indicating both the sensitivity of the test to distinguish potential dyskinetic mice and a ceiling effect from maximal AIM development. Further we find that at higher doses the correlative nature of basal horizontal activity measures and apomorphine rotations with AIMs reflects uncontrolled for lesion differences since this is exclusively found in MFB lesion animals. Nevertheless these tests can be used to predict dyskinesia in specific future experiments using high L-DOPA doses with this specific lesion type. Therefore we suggest that these tests can be used alongside other behavioural measures both to determine dopamine cell losses and to predict the mice with the highest potential to become dyskinetic after priming with L-DOPA. These data suggest that risk factors for LID development go beyond dopamine cell loss in the SNc and may reflect the interplay of other neurotransmitter systems, differences in plasticity and other maladaptive physiological changes. The predictive nature of apomorphine is interesting as it has a similar pharmacological action on dopamine receptors to L-DOPA, however, caution must be taken when using apomorphine to predict LID as this drug has the potential to supersensitise receptors [48].

4.4. TH expression in striatal neurons

It has been previously found that TH immunopostive cells can be found within the striatum of humans with PD, 6-OHDA lesioned rodents and MPTP treated primates [13,49–54]. The numbers of such cells have been found to peak 3–7 days after a 6-OHDA lesion, decrease from day 7 [50], and increase following L-DOPA treatment [13]. Previous studies suggest that the presence of TH immunoreactivity is not a marker for neurogenesis or induction of a specific dopaminergic phenotype in these cells reflects a phenotypic change of GABAergic cells that retain GABA as their primary neurotransmitter [52]. Here, we report that although small in number TH-positive cells were detected ubiquitously in the dopamine-depleted striatum of MFB lesion mice and that further L-DOPA treatment causes an up-regulation of these cells which were darker in appearance.

TH cells residing within the striatum of non-L-DOPA treated mice only mildly correlated with % cell loss within the SNc and this is likely because of the very low number of cells detectable in these lesion only groups, while cell loss was highly correlated with TH cells in L-DOPA treated mice, comparable to previous findings [13]. In a previous mouse study, L-DOPA was started 1 week after the striatal lesion and given over a shorter duration [50]. However, TH cell levels and changes were similar to those described here, despite a delay of 4 months between lesioning and the start of L-DOPA treatment in the present study. Our longitudinal study therefore shows two interesting findings; firstly that although TH cell numbers have been reported to decrease from 7 days post lesion those cells still maintain the ability to express TH when prompted by L-DOPA treatment despite a long delay, and secondly high doses and prolonged L-DOPA treatment does not cause a further increase in TH cell numbers.

5. Summary

We find for the first time that behavioural measures can be used to predict LID, distinctly to dopamine cell loss correlates. Combining both measures would provide a more appropriate selection procedure to study LID. In addition, AIM expression and locomotor responses caused by L-DOPA are differently expressed in mice lesioned to different regions along the nigrostriatal pathway, caused by differences in VTA dopamine losses and 5-HT concentrations in the striatum. We find new longitudinal L-DOPA driven responses in mice lesioned to the SN, which had distinct histological and behavioural characteristics compared to MFB and striatal lesion mice. Furthermore, FosB/ΔFosB upregulation and the emergence of TH positive cells are dependent on lesion type and striatal region.

Conflict of interest

SBD receives royalties from Campden Instruments on sales of the staircase paw reaching apparatus. The authors declare no other conflicts of interest.

Acknowledgements

The study was funded by the UK Biotechnology & Biological Sciences and Medical Research Councils, the EU Framework 7 NeuroStemCell and Replaces Collaborative Programmes, and the Parkinson's Disease Society.

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