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Continuous cerebroventricular administration of dopamine: A new treatment for severe dyskinesia in Parkinson's disease?

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ABSTRACT

In Parkinson's disease (PD) depletion of dopamine in the nigro-striatal pathway is a main pathological hallmark that requires continuous and focal restoration. Current predominant treatment with intermittent oral administration of its precursor, Levodopa (L-dopa), remains the gold standard but pharmacological drawbacks trigger motor fluctuations and dyskinesia. Continuous intracerebroventricular (i.c.v.) administration of dopamine previously failed as a therapy because of an inability to resolve the accelerated dopamine oxidation and tachyphylaxia. We aim to overcome prior challenges by demonstrating treatment feasibility and efficacy of continuous i.c.v. of dopamine close to the striatum. Dopamine prepared either anaerobically (A-dopamine) or aerobically (O-dopamine) in the presence or absence of a conservator (sodium metabisulfite, SMBS) was assessed upon acute MPTP and chronic 6-OHDA lesioning and compared to peripheral L-dopa treatment. A-dopamine restored motor function and induced a dose dependent increase of nigro-striatal tyrosine hydroxylase positive neurons in mice after 7 days of MPTP insult that was not evident with either O-dopamine or L-dopa. In the 6-OHDA rat model, continuous circadian i.c.v. injection of A-dopamine over 30 days also improved motor activity without occurrence of tachyphylaxia. This safety profile was highly favorable as A-dopamine did not induce dyskinesia or behavioral sensitization as observed with peripheral L-dopa treatment. Indicative of a new therapeutic strategy for patients suffering from L-dopa related complications with dyskinesia, continuous i.c.v. of A-dopamine has greater efficacy in mediating motor impairment over a large therapeutic index without inducing dyskinesia and tachyphylaxia.

1. Introduction

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder worldwide. The loss of dopamine through denervation in the striatum as a result of progressive neuronal degeneration in the substantia nigra pars compacta (SNpc), is the primary neurotransmitter marker of the disease (De Lau and Breteler, 2006). Since dopamine does not cross the digestive mucosa or the blood brain barrier, its lipophilic precursor L-dopa has been employed and remains the pivotal oral medication (Chaudhuri and Schapira, 2009). However, after persistent use over several years, many pharmacokinetic drawbacks contribute to the occurrence of motor fluctuations and dyskinesia (Fahn and Parkinson study group, 2005). Indeed L-dopa has a short half-life, limittped and variable reabsorption through the digestive and blood brain barriers and potentially harmful peripheral distribution. Moreover, L-dopa requires the aromatic L-amino acid decarboxylase for the synthesis of dopamine, which declines in the striatum with disease progression (Ciesielska et al., 2015).

Under normal conditions, dopaminergic neurons of the SNpc generate a short phasic discharge firing pattern. The frequency and duration of this pattern embeds them in the tonic low-frequency background range and maintains the striatal dopamine concentration at a relatively constant level (Paladini and Roeper, 2014). However, in the dopamine-depleted state relevant to PD, intermittent oral doses of L-dopa can

Abbreviations: DA, dopamine; i.c.v, intra-cerebro-ventricular; L-dopa, levodopa; MPTP, 1 méthyl-4-phenyl-1,2,3,6 terahydropyridine; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SMBS, sodium metabisulfite; SN*pc*, substantia nigra *pars compacta*.

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induce discontinuous stimulation of striatal dopamine receptors that in turn contribute to dysfunctional dopaminergic pathways. Thus, continuous dopamine administration is considered more physiologically appropriate by preventing oscillations in neurotransmitter concentration (Olanow et al., 2006; Gershanik and Jenner, 2012). De Yebenes et al. (1987) previously demonstrated that intracerebroventricular (i.c.v.) administered dopamine with an anti-oxidant adjuvant (sodium metabisulfite; SMBS) transiently improved motor handicap and increased dopamine in rat brains with unilateral neurotoxin 6-hydroxydopamine (6-OHDA)-induced damage as well as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated monkeys. The clinical feasibility of this administrative route has been supported by two PD patient case reports of dopamine infusion to the frontal ventricle, whereby a reduction in motor handicap was observed (Venna et al., 1984; Horne et al., 1989). However, both preclinical and clinical reports also highlight two overriding problems that prevented further development; (i) occurrence of tachyphylaxia and (ii) oxidation of dopamine causing enhanced dopamine metabolism and oxidative stress.

Dopamine oxidation can be limited by preparing, storing and administering dopamine in very low oxygen conditions. In addition, greater advances in programmable pumps now minimize tachyphylaxia by allowing administration of a lower effective dopamine dose in accordance with the circadian cycle. The purpose of this study is to demonstrate that continuous circadian i.c.v. administration of dopamine close to the striatum is feasible, efficient and safe in mouse and rat models of PD, supporting clinical development of this strategy to be revisited in PD patients with L-dopa related complications with dyskinesia.

2. Material and methods

2.1. LUHMES cells

Lund human mesencephalic (LUHMES) cells (gift from Pr. Marcel Leist; CAAT, University of Konstanz, Germany) were grown for 2 days in differentiation medium (advanced DMEM/F12, 1 × N2 supplement, 2 mM L-glutamine, 1 mM cAMP, 1 μg/ml tetracycline and 2 ng/ml recombinant GDNF) before seeding in 6 or 24 well plates and grown for a further 3 days before treatment. See supplementary material for details.

After 5 days of differentiation, LUHMES cells were treated with 1-methyl-4-phenylpyridinium (MPP+; 5 μM) for 24 h (h) before exposure to dopamine or L-3,4-dihydroxyphenylalanine (L-dopa) (Sigma Aldrich, St Louis, MO, USA) for a further 24 h. Viability was measured on 10,000 cells by flow cytometry (CANTO II) using propidium iodide (0.5 μM) and analysed with DIVA software (BD Biosciences, Le pont de Claix, France).

2.2. Rodent neurotoxic models

All experiments were carried out in accordance with the recommendations for the care and use of laboratory animals (FELASA) as well as European Directive 86/609-2010/63/UE guidelines for animal experimentation. Protocols were approved by an Ethical Committee (Nord-Pas-de-Calais; CEEA75) to induce MPTP neurotoxicity on 5 month old C57Bl/6 J mice (Ethical permit number: CEEA102012) or 6-OHDA neurotoxicity in 5 month old Wistar rats (Ethical permit number: CEEA 262011 and CEEA2016020911207601). Animals were group-housed (10 mice or 5 rats per cage) and a habituation period of 7 days after transportation was respected before any experimental manipulation was carried out. All surgery was performed under anesthesia and all efforts were made to minimize suffering.

Experimental procedures for obtaining the MPTP mice and 6-OHDA rat models have been previously described (Laloux et al., 2012). Briefly, mice received four intraperitoneal injections (with 2 h intervals) of either saline solution only or 20 mg/kg MPTP (Sigma Aldrich, St Louis, MO, USA). The rats received one cerebral unilateral injection of vehicle or 8 μg 6-OHDA (Sigma Aldrich, St Louis, MO, USA) through stereotaxic surgery to the right medial forebrain bundle.

2.3. Treatment parameters for rodent models

L-dopa methyl ester hydrochloride (Sigma Aldrich, St Louis, MO, USA) was extemporaneously prepared in saline with 12 mg/kg Benserazide, independent of L-dopa dose (Cenci and Lundblad, 2007). During the treatment regime L-dopa was administered intraperitoneally (i.p.) twice a day at doses previously reported for mice and rats (Espadas et al., 2012; Fornai et al., 2000). Anaerobia-dopamine (A-dopamine, Patent #WO2015173258 A1) was prepared by dissolving in saline (0.9% NaCl, pH 7.4) before the osmotic pump was filled and connected to a brain infusion cannula under an atmosphere that contained 5% hydrogen, 5% carbon dioxide and 90% nitrogen (BACTRON anaerobic/environmental chamber). Before stereotaxic surgery, osmotic pumps were primed under anaerobia for over 4 h at 37 °C. The stability of the A-dopamine solution in osmotic pump at 37 °C was checked for up to 30 days using an HPLC assay for dopamine (data not shown).

Treatment over 7 days began one week post MPTP or saline injections in mice. Mice were divided into 13 experimental groups; Saline only, MPTP only, MPTP + A-dopamine (3 to 5 different doses), MPTP + O-dopamine (3 different doses), and MPTP + L-dopa (3 different doses). A- or O-dopamine was administered continuously by i.c.v. (1 μl/h; 24 h/24 h) after surgical cannula-pump (ALZET 2001) implantation in the right lateral ventricle (see supplemental material for details). L-dopa was administered by i.p. twice a day over the same treatment period.

Chronic dopamine treatments (30 days) on rats began 3 weeks after unilateral 6-OHDA or saline cerebral injection. Only 6-OHDA rats displaying >5 turns/min in the Apomorphine-induced rotation test were used in the study (75% of the rats). Rats were divided into 6 experimental groups; saline only, 6-OHDA only, 6-OHDA + A-dopamine (3 different doses) and 6-OHDA + L-dopa. After surgical cannula-pump (programmable IPRECIO® SMP200 pumps) implantation on the 6-OHDA lesion side (see supplemental material for details) A-dopamine was administered to the right lateral ventricle by i.c.v. at a rate of 3 μl/h for 16 h out of 24 h (Zeitgeber time 13 to 5). Rats were housed in a 12 h light/12 h dark cycle and the implanted pump was set to deliver dopamine over 16 h, predominantly during the active (dark) phase of the rat while it is awake. However this also included part of the resting (light) phase to allow behavioral assessments under treatment (see Supplemental image 1 for the time delivery settings). L-dopa was administered by i.p. twice a day over the same treatment period.

2.4. Behavioral assessment

2.4.1. Actimetry

Spontaneous motor activity in mice and rats was recorded by an actimeter (Panlab, Barcelona, Spain) over 10 min (Laloux et al., 2012). This apparatus and the associated Actitrack software allowed distance travelled, speed and rearing behavior to be measured based on infrared beams obstructions.

2.4.2. Drug-induced rotation test

To assess rotational asymmetry, contralateral rotations over 10 min were counted 30 min after rats were subcutaneously (s.c.) injected with apomorphine (APOKINON®; 0.5 mg/kg). Only nigro-striatal-lesioned animals performing >5 turns/min were included in the experimental cohorts as these have >80% depletion of striatal dopamine terminals (Francardo et al., 2011).

2.4.3. Cylinder test

Rats performed the cylinder test (Schallert et al., 2000) to evaluate spontaneous forelimb lateralization. The number of vertical forepaw explorations on the cylinder wall using the right or left paw was assessed during 3 min.

2.4.4. Stepping test

The Stepping test (Olsson et al., 1995) is designed to monitor forelimb akinesia. Rats were gently dragged by the hindquarters across a bench over a distance of 0.90 m in 5 s. Supportive leg adjustments by the rat were counted over 3 consecutive trials.

2.4.5. Treatments-induced dyskinesia assessment

The principles and classifications of dyskinesia have been standardized and well-described for the rat (Cenci and Lundblad, 2007). Following the injection of L-dopa or during treatment, dyskinesia analysis consisted of scoring rat motor behavior for 1 min every 20 min over 3 h. Based on duration and persistence of the dyskinetic behavior during the 1-min observation period, abnormal involuntary movements (AIM) were divided into locomotive, axial, forelimbs and orolingual subtypes. For each subtype, a score from 0 to 4 was given to culminate into an overall dyskinesia score for each rat.

2.5. Nigro-striatal tyrosine hydroxylase staining and analysis

After treatment and behavioral analysis, mice and rats were sacrificed and 4% paraformaldehyde perfused brains were microscopically sectioned by cryostat for tyrosine hydroxylase immunochemistry (1:1000; Chemicon International, CA, USA). SN and striatal terminal THr + neurons were counted by stereological analysis software (Explora Nova, La Rochelle, France) (See supplemental material for details).

2.6. Striatum analysis by HPLC

Striatal samples were homogenized (0.1 M perchloric acid, 2.6 mM sodium disulfite, 0.7 mM EDTA, 25 ng/ml 3,4-dihydroxybenzilamine) before supernatants were separated by HPLC using a Chromsystems column for Dopamine, DOPAC and 5-cysteinyl-dopamine or an Uptisphere column for HVA. Glutathione status (*i.e.* GSSG/GSH) was also determined chromatographically after derivatization with orthophthalaldehyde was accomplished using isocratic elution on a Symmetry Shield C18 column (Waters SAS, France) (See supplemental material for details).

2.7. Statistical analysis

All data were expressed as mean ± SEM, mostly as a percentage from the saline or vehicle control group. For all parameters, a one-way ANOVA was used to assess group effect, followed by LSD Fisher *post hoc* test. If data did not follow a Gaussian distribution, a Kruskal-Wallis variance analysis was performed, followed by Mann-Whitney *post hoc* test. The threshold for statistical significance was set to $p < 0.05$. All statistical analyses were performed with IBM SPSS Statistics (Version 20).

3. Results

3.1. Dose-related effects of dopamine on dopaminergic neurons in vitro

In order to eliminate the possibility of dopamine being systematically deleterious, we demonstrated *in vitro* that low to moderate dopamine dose (≤3 μM) prevented MPP+ induced neurotoxicity whereas L-dopa was substantially less efficient (Fig. 1). Conversely, high doses of either dopamine or L-dopa (10- to 100-fold higher) had moderate to no neuroprotective effect and a very high dose of dopamine (1000-fold higher) increased cell death. The optimal window of neuroprotection for both dopamine and L-dopa was determined to be 0.05–0.2 μM.

3.2. Deleterious effect of dopamine oxidation avoided by anaerobic preparation of dopamine in vivo

Continuous i.c.v. administration of 240 μg/day O-dopamine induced dopamine oxidation even in the presence of SMBS. This was visually detectable *via* a brown to black discoloration of the brain's ventricular walls. Despite an improvement in motor activity compared to sham treated mice (Fig. 2A), addition of O-dopamine lowered the count of tyrosine hydroxylase positive (TH-ir+) neurons in SNpc (Fig. 2B). A reduction in the administered O-dopamine dose (120 μg/day) negated the changes to TH-ir + neuron counts, but strong discoloration of the ventricular walls was still observed in >65% of the mice.

In order to avoid the use of SMBS, due to its poor anti-oxidant properties with additional detrimental effects (Nair et al., 2003), an A-dopamine preparation protocol was developed to maintain the neurotransmitter in an anaerobic environment throughout the filling and pump priming procedures. Similar to O-dopamine with SMBS, high A-dopamine dose (240 μg/day) without SMBS improved motor impairment but was still deleterious to SNpc TH-ir + neurons (Fig. 2B). However, A-dopamine at 120 μg/day without SMBS improved motor activity without neuropathology to the nigro-striatal pathways

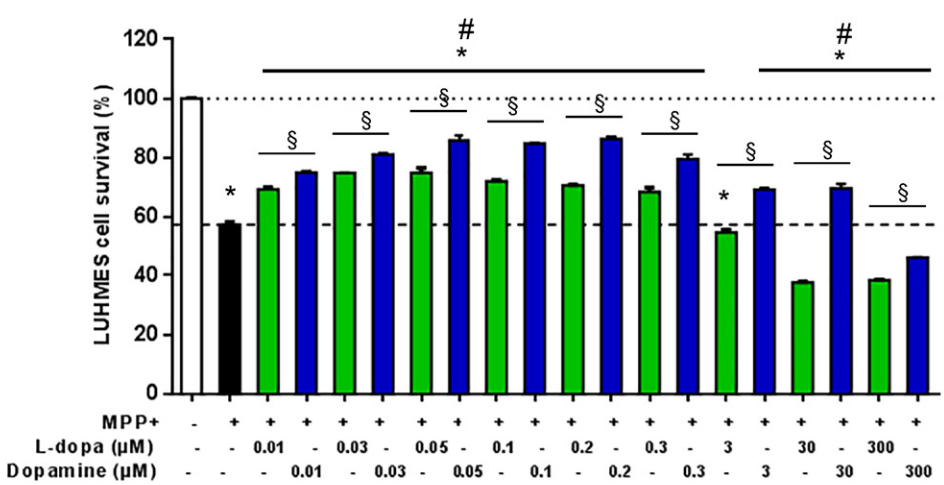

Fig. 1. Dose-related effects of dopamine on LUHMES dopaminergic neurons. Overall LUHMES viability was greater after A-dopamine compared to L-dopa treatment (0.01–300 μM). Significant differences * *vs.* saline condition, # *vs.* MPP+ condition, § between the dopamine and L-dopa conditions, $p < 0.05$ (Mann-Whitney comparison test).

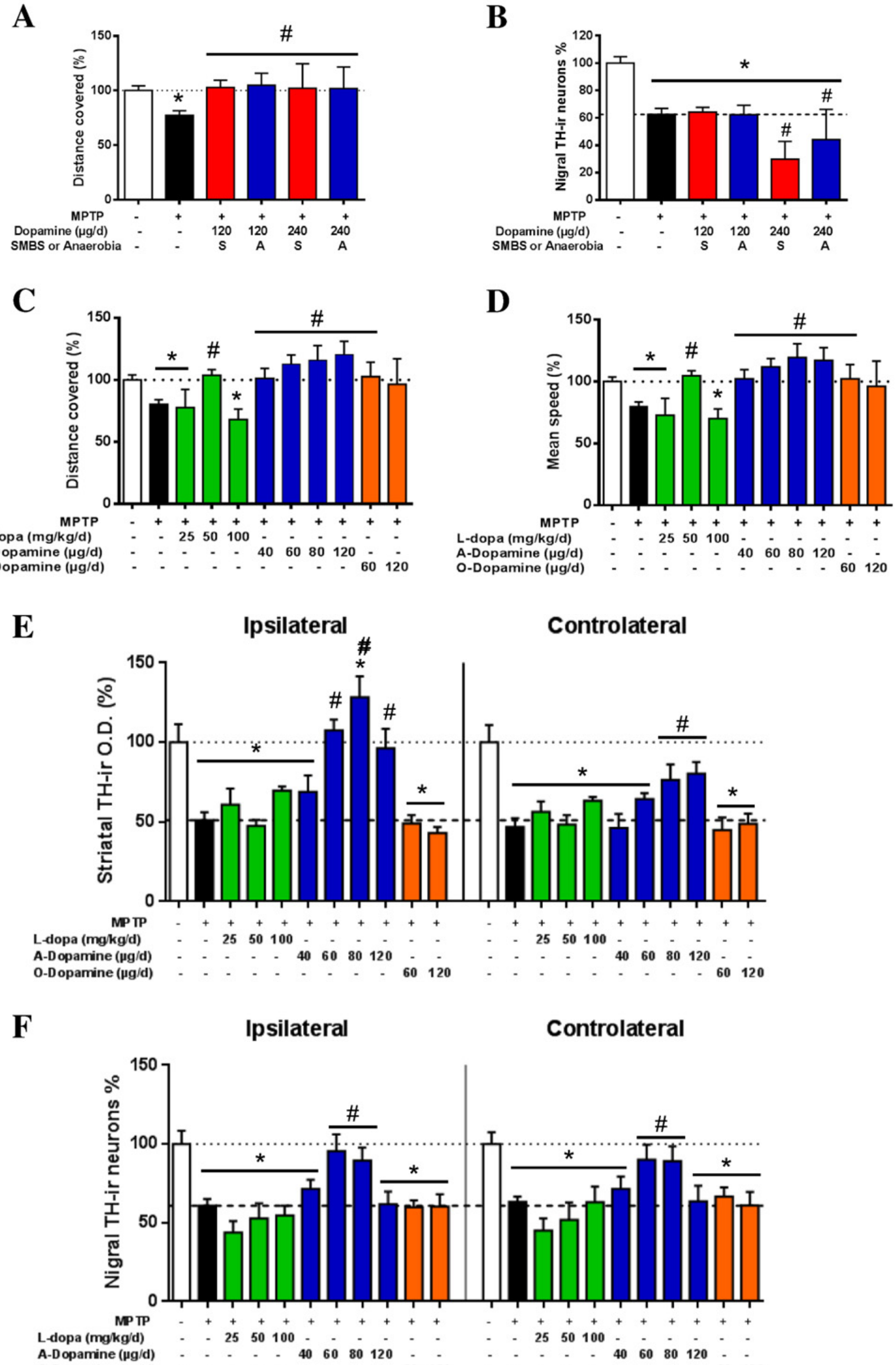

Fig. 2. Neuroprotection of nigro-striatal pathway and functional recovery induced by cerebral A-dopamine infusion in MPTP mice. Distance covered in actimetry arena (**A**) and TH-ir + neuronal count in SNpc (**B**) was measured in MPTP mice after 7 days of dopamine treatment, either supplemented with SMBS or prepared anaerobically. To determine the optimal dose of each treatment on motor function post-MPTP insult, distance covered (**C**) and mean speed (**D**) within the actimetry arena was assessed after i.p. L-dopa (25-100 mg/kg/day) or i.c.v. dopamine prepared anaerobically (A-dopamine)(40-120 µg/day) or aerobically (O-dopamine) (60-120 µg/day). Neuropathological outcomes were also assessed within the same parameters by measuring ipsilateral and contralateral TH-ir + optical density in dorsal striatum (**E**) and TH-ir + neuronal counts in SNpc (**F**). Data are expressed in percentage from saline mice, means ± SEM ($n = 6$/group for **A&B**, 12/group for **C&D**, 8/group for **E&F**). Significant differences * *vs.* saline mice, # *vs.* untreated MPTP mice, $p < 0.05$ (one-way ANOVA and LSD Fisher post-hoc tests or Mann-Whitney comparison test).

(Fig. 2A). Very low intensity in brown discoloration of the brain's ventricular walls was observed in some mice (~30%).

3.3. Functional recovery induced by A-dopamine

The safety profile of A-dopamine was further studied in MPTP-treated mice using a broad dose range (40–120 µg/day) and compared to O-dopamine (60–120 µg/day) and i.p. L-dopa (twice daily, 25–100 mg/day) (Fig. 2 C—F). After 7 days, A-dopamine functionally restored mean speed and distance covered by the mouse and had a broader therapeutic index than peripheral L-dopa treatment (*i.e.* only 50 mg/kg/day L-dopa was beneficial)(Fig. 2C–D). Dose-effect on SNpc and striatal TH-ir + neuron survival by A-dopamine was u-shaped, without hemispheric preference in the SNpc (Fig. 2 E–F). The increase in TH-ir + neurons induced by A-dopamine at 60 and 80 µg/day was determined to be optimal whereas 120 µg/day also reversed TH-ir

+ terminal loss in striatum. Conversely, neither O-dopamine nor L-dopa prevented reduced TH-ir counts after MPTP in the SNpc and striatum.

3.4. Diffusion and metabolism of A-dopamine within the brain

Lateral ventricular administration of 80 and 120 μg/day A-dopamine demonstrated good diffusion into the ipsilateral striatum post MPTP intoxication. Similar results were observed upon peripheral L-dopa administered at 50 and 100 mg/day, with 120 μg/day A-dopamine and 100 mg/day L-dopa having comparable maximal diffusion (Fig. 3A).

The reduction in DOPAC and HVA metabolites caused by MPTP in the striatum (75% loss) was significantly restored by 60 to 120 μg/day of A-dopamine and in a dose-dependent manner with L-dopa (Fig. 3B–C). Of note, A-dopamine preferentially increased HVA whereas L-dopa favored a DOPAC increase, potentially revealing alternate metabolism pathways.

As bilateral distribution from the lateral ventricle through the third ventricle to both striatum is not evident in mice, A-dopamine did not alter the contralateral striatal level of dopamine or metabolites (except for the highest dose which significantly increased HVA).

3.5. Changes to A-dopamine oxidative metabolism

The oxidative metabolism of dopamine was determined by 5-cysteinyl dopamine; a glutathione-dependent covalent modification of dopamine quinones derived from dopamine oxidization (Chen et al., 2008). Levels were not modified with doses of A-dopamine below 120 μg/day (*i.e.* ≤ 0.3 nmol/mg) or with the peripheral L-dopa treatment (undetectable) (Suppl. Table 1). Glutathione was strongly oxidized by MPTP intoxication (as indicated by low GSH/GSSG ratio) but the GSH/GSSG ratio was significantly increased by both A-dopamine and L-dopa treatments. This suggested that both were equally protective in regard to the redox state of dopaminergic neurons.

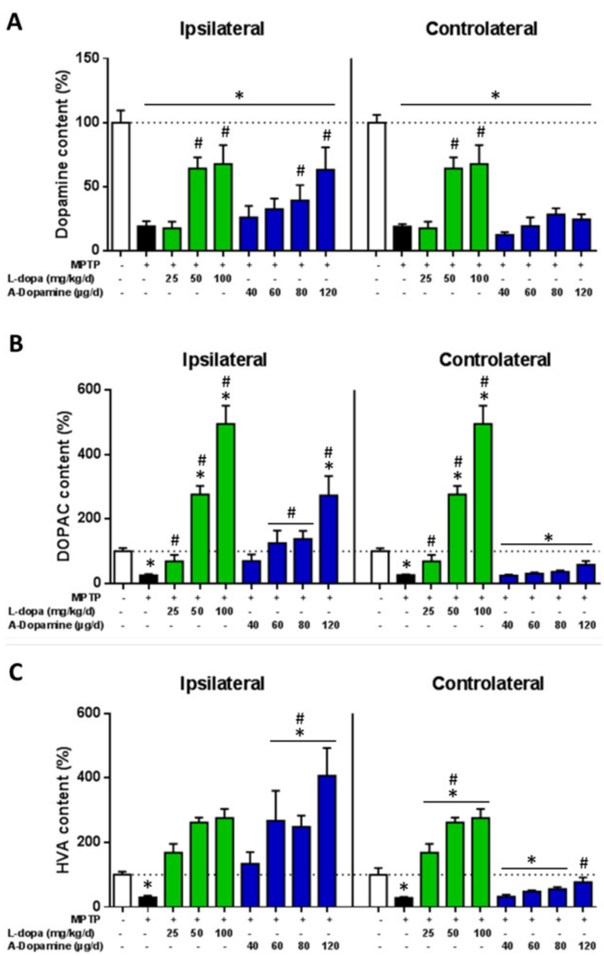

Fig. 3. Diffusion and metabolization of striatal A-dopamine. Within the striatum, dopamine (A) 3,4 dihydroxyphenylacetic acid (DOPAC) (B) and homovanillic acid (HVA) (C) were measured in each hemisphere after 7 days of L-dopa or anaerobic dopamine (A-dopamine). Ipsilateral and contralateral represents the side in which i.c.v. dopamine was infused. Data are expressed in percentage from saline mice, means ± SEM ($n = 6$ animal per group). * *vs.* saline mice, # *vs.* untreated MPTP mice, $p < 0.05$ (one-way ANOVA and LSD Fisher post-hoc tests).

3.6. Lack of tachyphylaxia following chronic A-dopamine stimulation in 6-OHDA rats

In 6-OHDA treated rats, A-dopamine was chronically administered by i.c.v. for 30 days (16 h/day) at 3 doses (1, 2 and 3 mg/day). This regime was compared behaviorally to peripherally administered L-dopa (6 mg/kg twice daily) over the same time period. For A-dopamine, 'stepping' and the 'cylinder' tests were consistent with actimetry analysis. At both 15 and 30 days post-neurotoxin injection, a dose response effect was observed for A-dopamine, whereby 1 mg/day was considered ineffective, 2 mg/day restored the mean locomotive speed and distance of the rat and 3 mg/day induced over-activity (Fig. 4A–D). Observationally, it was noted that differences between 2 and 3 mg/day at 15 days were reduced after 30 days of treatment. As previously reported 6-OHDA rats responded well to L-dopa treatment in all three motor tests (Laloux et al., 2012) despite an over compensation after 30 days of treatment of the contralateral paw in the cylinder and stepping test (Fig. 4C & D).

3.7. Lack of dyskinesia and dopaminergic sensitization following A-dopamine stimulation

Chronic peripheral treatment with L-dopa (6 mg/kg twice daily for 30 days) induced a consistent and high dyskinesia score compared to sham or 6-OHDA-treated rats (Table 1). However throughout the treatment period, all doses of A-dopamine (1–3 mg/day) produced minimal abnormal locomotive movements and dyskinesias was undetectable (Video 1). Moreover, as previously reported (Bordet et al., 2000), by the end of the 30 day treatment period behavioral dopaminergic sensitization associated with dyskinesia was evident in L-dopa treatment (Fig. 4E). Conversely, a reduced number of apomorphine-induced rotations were observed with A-dopamine administration.

3.8. Good safety profile of A-dopamine

Neither L-dopa or A-dopamine had supplementary deleterious effects on the remaining 10% TH-ir + neurons after ipsilateral 6-OHDA injection (Fig. 4G & H). Highest doses of A-dopamine may even have had a positive impact (Fig. 4G). A significant body weight gain was observed in higher doses of A-dopamine and may be attributed to dopamine effect on motivation to eat (*via* its action on mesolimbic system) or an orexigen effect (*via* the hypothalamic system). In L-dopa treated rats this gain was reduced after 30 days compared to shams (Fig. 4F) and could be associated with higher daily energy expenditure as a result from the observed dyskinesias. No overall harmful effects could be attributed to body weight changes and no anatomopathological alteration of heart, liver, pancreas, spleen, kidney, spinal cord, eye, and brain were observed after 30 days of either treatment.

4. Discussion

Results obtained in these three different models of PD demonstrate a promising therapeutic regime for A-dopamine treatment. *In vitro*, a positive effect of dopamine was observed on LHUMES cell survival. *In vivo*, A-dopamine restored motor function and induced a dose dependent increase of nigro-striatal tyrosine hydroxylase positive neuron survival in mice after 7 days of MPTP insult, suggesting a strong safety profile that

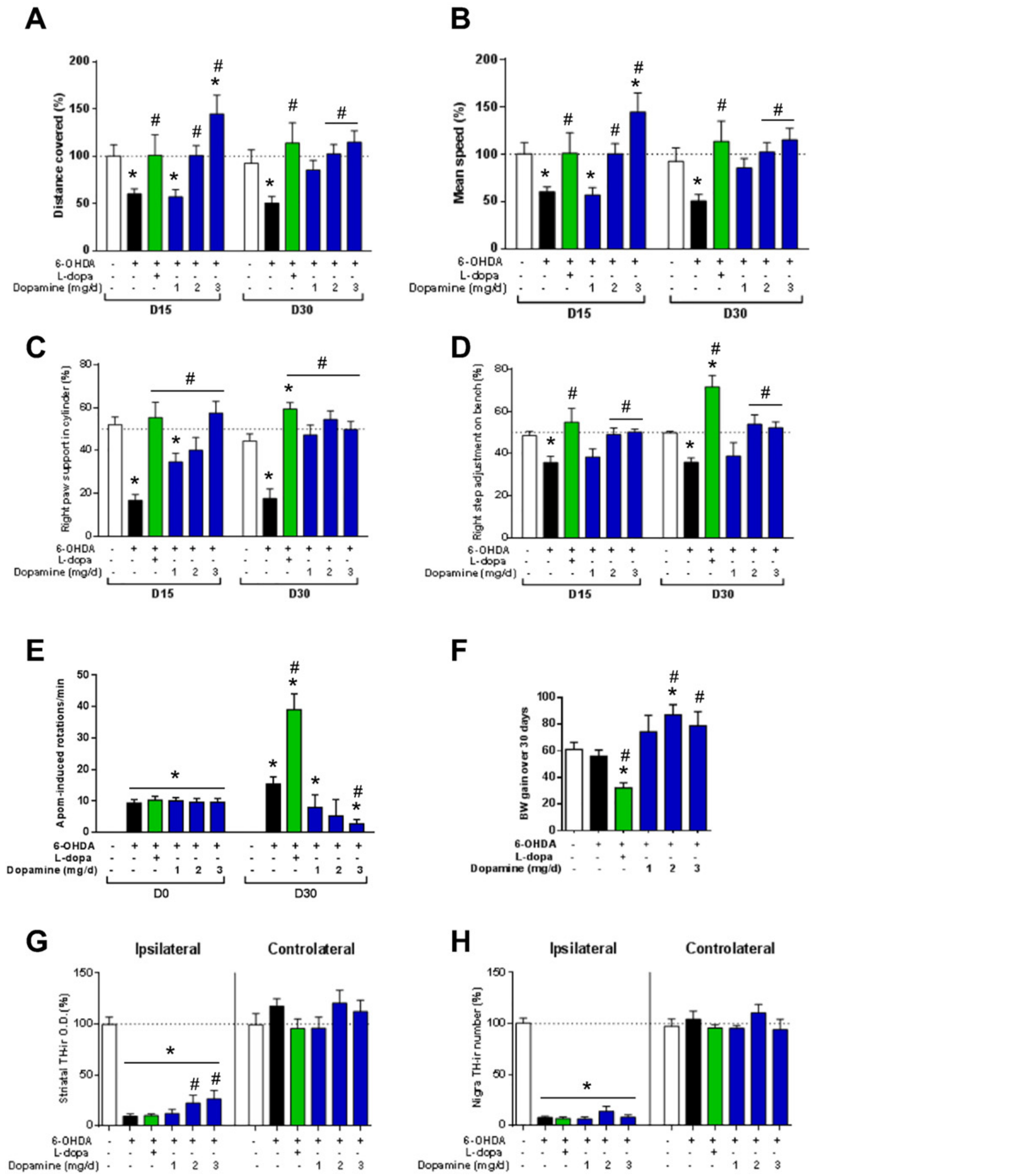

Fig. 4. Functional recovery without tachyphylaxia or dopaminergic sensitization following chronic circadian A-dopamine stimulation in 6-OHDA rats. 6-OHDA rats were administered i.p. L-dopa (12 mg/kg/day) or i.c.v. anaerobically prepared dopamine (A-dopamine; 1–3 mg/day) and motor skills assessed after 15 or 30 days. Distance covered (**A**) and mean speed (**B**) in the actimetry arena as well as right paw support in the cylinder test (**C**) and right step adjustment in the stepping test (**D**) were evaluated. All data was compared to vehicle control set at 100% within the actimetry arena (**A&B**) or 50% representing equal forelimb preference (**C&D**). For apomorphine induced rotation, measurements were taken immediately after 6-OHDA insult but before treatment (D0) as well as 30 days after treatment (D30) in the same treatment parameters (**E**). Rat body weight gain during the 30 days of treatment (**F**) as well as TH-ir + optical density in dorsal striatum (ipsilateral and contralateral) (**G**) and TH-ir + neuronal count in SNpc (**H**) were also evaluated. All data are expressed in percentage from Vehicle rats, means ± SEM ($n = 10$/group). Significant differences * *vs.* vehicle rats, # *vs.* untreated 6-OHDA rats, $p < 0.05$ (one-way ANOVA and LSD Fisher post-hoc tests or Mann-Whitney comparison test).

was not evident with either O-dopamine or L-dopa. In a chronic rat model using 6-OHDA-lesioning, continuous circadian i.c.v. injection of A-dopamine over 30 days also improved motor activity without occurrence of tachyphylaxia. Significantly, A-dopamine did not induced dyskinesia or behavioral sensitization as observed with peripheral L-dopa treatment.

Similar to L-dopa treatment in previous clinical and preclinical studies, inconsistencies are reported in the use of dopamine, whereby high doses are described as neurotoxic (Hastings et al., 1996) but sub-toxic concentrations have neuroprotective and neurotrophic effects (Jia et al., 2008). We confirm that the positive impact of dopamine on TH-ir + neurons of the nigro-striatal pathways was predominantly dependent on dose, but the oxidation state of dopamine is also paramount. As well as illustrating that O-dopamine was detrimental to neurons, higher A-dopamine concentrations in the presence of MPTP also induced significant neurotoxic 5-cysteinyl-DA production. However a low A-dopamine dose promoted TH-ir + neuronal counts with no evidence of oxidized dopamine.

To our knowledge, this is the first to describe such a SNpc and striatal TH-ir + neuronal plasticity with dopamine administration. We suggest

Table 1
Lack of dyskinesia during cerebral infusion of A-dopamine in 6-OHDA rats.
The table differentiates dyskinesia scores (Limb, axial and orolingual) and abnormal involuntary movements (AIM) in locomotion each 20 min over 2 h. At 7, 15 and 30 days of treatment, measurement began 30 min after i.p. L-dopa or during A-dopamine i.c.v. Data are expressed in means ± SEM ($n = 10$ animal per group). Significant differences * *vs.* untreated 6-OHDA rats, # *vs.*L-dopa treated 6-OHDA rats, $p < 0.05$ (non-parametric Kruskal-Wallis variance analysis and Mann-Whitney comparison).

Exp time	6-OHDA rat groups	Dyskinesia types			Dyskinesias score	Locomotive AIM
		Limb	Axial	Orolingual		
D7	Vehicle	0	0	0	0	0
	L-dopa	11.1 ± 1.99 *	8.4 ± 1.67 *	9.9 ± 1.66 *	29.40 ± 5.25 *	4.8 ± 1.39 *
	A-DA 1 mg/d	0	0	0	0	1 ± 1 #
	A-DA 2 mg/d	0	0	0	0	2.14 ± 0.91
	A-DA 3 mg/d	0	0	0	0	5.4 ± 1.12 *
D15	Vehicle	0	0	0	0	0
	L-dopa	12.2 ± 1.67 *	7.9 ± 1.38 *	10.9 ± 1.75 *	31.0 ± 4.63 *	6.1 ± 1.0 *
	A-DA 1 mg/d	0	0	0	0	0.7 ± 0.7 #
	A-DA 2 mg/d	0	0	0	0	1.56 ± 0.67 #
	A-DA 3 mg/d	0	0	0	0	2.71 ± 1.02 #
D30	Vehicle	0	0	0	0	0
	L-dopa	13.6 ± 2.03 *	8.6 ± 1.85 *	13.5 ± 2.15 *	35.7 ± 5.89 *	7.2 ± 1.33 *
	A-DA 1 mg/d	0	0	0	0	1 ± 1 #
	A-DA 2 mg/d	0	0	0	0	2.25 ± 1.31 #
	A-DA 3 mg/d	0	0	0	0	0.6 ± 0.4 #

that this could be explained by a culmination of events. Low A-dopamine dose prevented TH stained neuronal death caused by synaptic inactivity (Jeon et al., 1995), restored dopaminergic cell markers required for maintained dopamine production (Datla et al., 2001), scavenged rather than promoted ROS formation (Agil et al., 2006) and may have enabled a switch in striatal neurons from a serotonergic to dopaminergic phenotype (Carta et al., 2007).

A viable optimal therapeutic regime would be to continuously compensate the deficit in dopamine that mediates neuronal communication within the SNpc. A continuous non-pulsatile i.c.v. administration better mimics the physiological released of dopamine caused by the tonic background activity of SNpc neurons (Olanow et al., 2006). In animal models of PD, several studies have proven the efficacy of continuous dopamine infusion. Hargraves and Freed (1987) showed that striatal injection of 12 or 120 μg/day dopamine with SMBS (during 13 days) reduced locomotive abnormalities, whereas others studies confirmed this result with alternative administration parameters (*e.g.* i.c.v. injection, dose at 240 μg/day or time over 7 days) (De Yebenes et al., 1987; Kroin et al., 1991). The first human case report (Venna et al., 1984) described a good tolerance to dopamine infusion over 1 year. A subsequent human case report (Horne et al., 1989) supports our current data by illustrating that long-term i.c.v. dopamine provides a smooth control of motor symptoms. However until now, concerns regarding dopamine oxidation and patient tachyphylaxia have restricted further development. Of note, PD patients from previous studies received O-dopamine and at the same dose throughout a 24 h cycle. Prior experience obtained from the use of an apomorphine pump and duodopa® has identified the need to differentiate between diurnal and nocturnal minimum efficient dose in order to avoid worsening motor fluctuations (Devos and French DUODOPA study group, 2009; Drapier et al., 2016).

In summary, far from being deleterious, we observe that when dopamine levels and oxidative state are harnessed it can be beneficial to neuron survival. Minimizing dopamine administrative dose, focusing delivery location and restricting the oxidative states all had a positive impact on the nigro-striatal pathways. Long-term i.c.v. of A-dopamine using a circadian cycle also dramatically reduced the detrimental side effects of the current therapeutic regime of L-dopa (*i.e.*L-dopa related complications with dyskinesia).

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Authors's roles

Ch.L., JC.D., D.D. conceived, managed the project and designed experiments; Ch.L., F.G., Cé.L., K.T. performed most experiments and analysed data; B.DV, A.J., M.P., G.G., N.R. helped for the experiments. Ch.L., JC.D., D.D., J.A.D. wrote the manuscript text and prepared figures; C.M., R.B. review and critique of the paper.

Full financial disclosures of all authors for the past year

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