LUND UNIVERSITY

Cystamine/cysteamine rescues the dopaminergic system and shows neurorestorative properties in an animal model of Parkinson's disease.

Cisbani, G; Drouin-Ouellet, Janelle; Gibrat, C; Saint-Pierre, M; Lagacé, M; Badrinarayanan, S; Lavallée-Bourget, M H; Charest, J; Chabrat, A; Boivin, L; Lebel, M; Bousquet, M; Lévesque, M; Cicchetti, F

Link to publication

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Accepted Manuscript

Cystamine/cysteamine rescue the dopaminergic system and show neurorestorative properties in an animal model of Parkinson's disease

G. Cisbani, J. Drouin-Ouellet, C. Gibrat, M. Saint-Pierre, M. Lagacé, S. Badrinarayanan, M.H. Lavallée-Bourget, J. Charest, A. Chabrat, L. Boivin, M. Lebel, M. Bousquet, M. Lévesque, F. Cicchetti

Please cite this article as: Cisbani, G., Drouin-Ouellet, J., Gibrat, C., Saint-Pierre, M., Lagacé, M., Badrinarayanan, S., Lavallée-Bourget, M.H., Charest, J., Chabrat, A., Boivin, L., Lebel, M., Bousquet, M., Lévesque, M., Cicchetti, F., Cystamine/cysteamine rescue the dopaminergic system and show neurorestorative properties in an animal model of Parkinson's disease, *Neurobiology of Disease* (2015), doi: 10.1016/j.nbd.2015.07.012
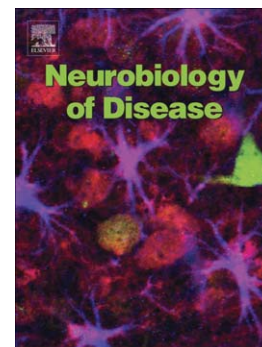
Cystamine/cysteamine rescue the dopaminergic system and show neurorestorative properties in an animal model of Parkinson's disease

[*1]Cisbani G, [*2]Drouin-Ouellet J, [1]Gibrat C, [1]Saint-Pierre M, [1]Lagacé M,
[1]Badrinarayanan S, [1]Lavallée-Bourget MH, [3]Charest J, [3]Chabrat A, [1]Boivin L,
[1]Lebel M, [1]Bousquet M, [3,4]Lévesque M, [*1,4]Cicchetti F

Authors have contributed equally to this work

[1]Centre de Recherche du CHU de Québec (CHUQ), Axe Neurosciences, 2705 Boulevard Laurier,
Québec, QC, Canada, G1V 4G2; [2]Department of Clinical Neurosciences, John van Geest Centre
for Brain Repair, University of Cambridge, Cambridge, United Kingdom, CB2 0PY; [3]Centre de
recherche de l'Institut Universitaire en Santé Mentale de Québec, 2601, Chemin de la Canardière,
Québec, QC, Canada G1J 2G3; [4]Département de Psychiatrie & Neurosciences, Université Laval,
Québec, QC, Canada, G1K 0A6

Running title: Neurorestorative effects of cystamine and cysteamine

Correspondence:
Francesca Cicchetti, Ph.D.
Centre de Recherche du CHU de Québec (CHUQ)
Axe Neuroscience T2-50
2705, Boulevard Laurier
Québec, QC, G1V 4G2, Canada
Tel #: (418) 656-4141 ext. 48853
Fax #: (418) 654-2753
E-mail: Francesca.Cicchetti@crchul.ulaval.ca

ABSTRACT

The neuroprotective properties of cystamine identified in pre-clinical studies have fast-tracked this compound to clinical trials in Huntington's disease, showing tolerability and benefits on motor symptoms. We tested whether cystamine could have such properties in a Parkinson's disease murine model and now provide evidence that it can not only prevent the neurodegenerative process but can even reverse motor impairments created by a 6-hydroxydopamine lesion 3 weeks post-surgery. Importantly, we report that cystamine has neurorestorative properties 5 weeks post-lesion as seen on the number of nigral dopaminergic neurons which is comparable with treatments of cysteamine, the reduced form of cystamine used in the clinic, as well as rasagiline, increasingly prescribed in early parkinsonism. All three compounds induced neurite arborization of the remaining dopaminergic cells which was further confirmed in *ex vivo* dopaminergic explants derived from *Pitx3*-GFP mice. The disease-modifying effects displayed by cystamine/cysteamine would encourage clinical testing.

Keywords: 6-OHDA, neuroprotection, neurorescue, disease-modifying

INTRODUCTION

Parkinson's disease is a neurodegenerative disorder for which existing therapies are restricted to symptom management. The development of novel treatments to prevent or halt dopaminergic neuronal degeneration, which largely underlies this pathology, is still awaited, as most candidate compounds exhibiting neuroprotective properties in pre-clinical studies have failed to demonstrate disease-modifying effects when tested in the clinical setting (la Fuente-Fernandez et al., 2010; Parkinson Study Group QE3 Investigators et al., 2014; Poewe et al., 2012; Postuma et al., 2012). The multitude of cellular and molecular mechanisms involved in Parkinson's disease pathogenesis and the significant proportion of dopaminergic neurons already lost at the time of disease diagnosis (Schapira, 1999) are elements that significantly hinder our efforts to identify a single compound capable of acting on a number of pathways simultaneously.

Amongst the neuroprotective agents emerging from experimental work, we and others have identified that cystamine, a small disulfide-containing chemical, holds such properties. The neuroprotective potential of cystamine was first demonstrated in animal models of Huntington's disease, including the R6/2 (Dedeoglu et al., 2002; Fox et al., 2004; Karpuj et al., 2002; X. Wang et al., 2005) and the YAC128 mice (Van Raamsdonk et al., 2005). In these models, cystamine was shown to prevent brain atrophy, neuronal loss and subside neuropathological features. Prolonged life span and improvements in motor behaviours were further reported in R6/2 mice (Dedeoglu et al., 2002). More recently, cysteine (a molecule of the same family of cystamine) treatment was shown to reverse disease-related features in cultured Huntington's disease tissue as well as in mouse models of the disease (Paul et al., 2014).

3

Given the significant benefits observed in Huntington's disease models, the reduced form of cystamine, cysteamine, quickly moved to clinical trials (Dubinsky and Gray, 2006). The results in a small number of Huntington's disease patients confirmed drug tolerability (Dubinsky and Gray, 2006) and ongoing phase II/III trials show a positive trend towards slower progression of the Total Motor Score in patients treated with a delayed-release cysteamine vs. patients on placebo (Raptor Pharmaceuticals-website). Recent studies conducted in animal models of parkinsonism, such as those induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Gibrat et al., 2010) and 6-hydroxydopamine (6-OHDA) (Stack et al., 2008) have provided evidence that cystamine could also be neuroprotective in Parkinson's disease.

Cystamine has pleiotropic effects on biological processes, but was first proposed as a potential therapeutic strategy for neurodegenerative disorders because of its inhibitory effects on transglutaminase (Junn et al., 2003; Karpuj et al., 2002; Lorand and Conrad, 1984), an enzyme contributing to protein aggregate formation (X. Wang et al., 2005). What further renders cystamine appealing is its antioxidant activity, which improves mitochondrial function (Fox et al., 2004; Stack et al., 2008), its modulation of brain derived neurotrophic factor (BDNF) (Borrell-Pagès et al., 2006; Gibrat et al., 2010) and its capacity to inhibit apoptosis through its action on active caspase-3 (Jókay et al., 1998; Lesort et al., 2003). While the pathophysiology leading to nigral dopamine neuronal loss in Parkinson's disease remains to be fully elucidated, it is believed to result from oxidative stress, mitochondrial dysfunction, diminished trophic factor support, inflammation, apoptosis, as well as impairments in the ubiquitin-proteasome and autophagic systems (for review, see (Hirsch et al., 2012).

Because a number of mechanisms of action of cystamine thus far identified match those driving the pathophysiology of dopaminergic neuronal loss, we assessed whether cystamine, as well as its reduced form cysteamine, could prevent and even reverse ongoing dopaminergic degeneration in a toxin-induced animal model of Parkinson's disease.

MATERIALS AND METHODS

In vivo experiments

Animals

Adult male C57BL/6 mice (9-week old, 25 g) were purchased from Charles River Laboratories. The animals were housed in a temperature and light-controlled room (22°C, a 12 h cycle) and were fed and allowed to drink water *ad libitum*. The health status of all mice was monitored throughout the experimental protocol primarily via weight loss and close observations for signs of any health-related issues. All experiments were performed in accordance with the Canadian Guide for the Care and Use of Laboratory animals and all procedures were approved by the Institutional Policy of the Centre de recherche du CHU de Québec (CHUQ).

Unilateral striatal 6-OHDA lesion

The neurorescue and neurorestorative properties of cystamine were assessed in a mouse model of parkinsonism generated by a 6-OHDA lesion 3 weeks (when dopaminergic degeneration was ongoing) and 5 weeks following the lesion (when the dopaminergic degeneration was stable and had plateaued). Importantly at 5 weeks post-lesion, the effects of cystamine were compared to those of cysteamine, the form used in the clinic (Dubinsky and Gray, 2006), as well as rasagiline, a drug increasingly given to early and *de novo* Parkinson's disease patients (Blandini, 2005; Korchounov et al., 2012).

5

Animals were anaesthetized using isoflurane (Sigma-Aldrich) and placed onto a mouse stereotactic frame (Kopf Instruments). 6-OHDA (Sigma-Aldrich) was dissolved at a concentration of 2 µg/µl in 0.9% saline and 0.02% ascorbic acid and a volume of 2 µl was injected unilaterally into the right striatum at a rate of 0.5 µl/min. The injection was performed using a Hamilton syringe at the following co-ordinates: AP: +0.04 cm, ML: -0.18 cm, DV: -0.31 cm (corresponding to the Allen Brain Atlas (Allen, 2008)). The needle was left in place for 3 min after the injection before complete retraction. Sham mice were subjected to the same surgical procedures but were injected with 2 µl of a vehicle solution (0.9% saline and 0.02% ascorbic acid).

Cystamine, cysteamine and rasagiline treatments

Based on previous publications, cystamine and cysteamine were administered i.p. (10 mg/kg and 20 mg/kg, respectively) (Bousquet et al., 2010) whereas rasagiline was administered s.c. (2.5 mg/kg) (Blandini et al., 2004) commencing 3 or 5 weeks post-lesion and continuing daily for 6 weeks (**Figs. 1A** and **2A**). A total of 100 mice were used in this study (n = 10 per group).

Behavioural measures

Two distinct cohorts of mice were used to assess the effects of cystamine 3 weeks post-6-OHDA lesion. One was assigned to the cylinder and stepping tests, while the other was assigned to the apomorphine-induced rotational test. The cylinder test was performed first. The stepping test was conducted the following day and all tests were performed in the morning. All mice were tested at 3, 6 and 9 weeks post-lesion. Note that only the apomorphine-induced rotational test was conducted when cystamine was investigated as a neurorestorative agent. The test was performed at 5, 8 and 11 weeks following the lesion.

6

Cylinder test. To examine side bias in spontaneous forelimb use during explorative activity, mice were placed individually in a glass cylinder (10 cm diameter, 14 cm height) facing two vertical mirrors which allowed movement evaluation from all angles. Mice were concomitantly video-recorded during the 3 min testing phase. Video-recordings were subsequently examined to count the number of wall contacts made with the forepaws. A measure of forelimb use asymmetry was obtained by expressing the touches performed by the paw contralateral to the lesion (left paw) as a percentage of the total number of touches recorded for each session.

Stepping test. Mice were held by the base of the tail with their hindlimbs suspended above the table and moved backwards at a steady rate so that they traversed 1 meter over 5 sec (Blume et al., 2009). All mice were tested three times and video-recorded. Videos were subsequently analysed to count the number of touches performed by the paw contralateral to the lesion (left paw) so as to obtain a measure of forelimb use asymmetry.

Apomorphine-induced rotation. The apomorphine test was performed in all 6-OHDA lesioned mice, that is in those in which the cystamine treatment begun 3 or 5 weeks post-lesion. Mice were challenged with apomorphine (0.5 mg/kg, Sigma-Aldrich) and rotation assessed for 45 min using an automated rotometer system (San Diego Instruments). Results were averaged and expressed as net rotation (number of contralateral rotations – number of ipsilateral rotations) (Metz and Whishaw, 2002; Moore et al., 2001). Animals were allowed to habituate to their environment for 5 min after the injection before the rotations were recorded. Mice were tested before treatment begun (3 or 5 weeks post-surgery), 3 weeks following the initiation of treatment (6 or 8 weeks post-surgery) and at the end of the study just before sacrifice (9 or 11 weeks post-surgery) (**Figs. 1A** and **2A**).

7

Post-mortem analyses

Perfusion and tissue processing

6-OHDA lesioned animals. All mice were subjected to intracardiac PBS perfusion in RNAse free condition and under deep anaesthesia. Brains were collected and the most posterior area comprising the entire midbrain (AP: -1.155 mm to -7.755 mm) was post-fixed in 4% paraformaldehyde (PFA) for additional immunohistochemistry and *in situ* hybridization experiments while the anterior brain (AP: + 3.245 mm to -1.055 mm), comprising the right and left striata, was snap-frozen in 2-methyl-butane and stored at -80°C. Coronal brain sections of 25-μm thickness were obtained using a freezing microtome (Leica Microsystems), serially collected in anti-freeze solution (monophosphate sodium monobasic 0.2 M, monophosphate sodium dibasic 0.2 M, ethylene glycol 30%, glycerol 20%) and kept at -20°C until use. The frozen samples were prepared from cryostat dissection for HPLC and western blot analyses.

Catecholamine quantification by HPLC

Striatal dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxytryptamine (5HT) concentration were measured by HPLC coupled with electrochemical detection (Bousquet et al., 2011; 2008). Each striatal sample comprised ten 20-μm thick cryostat sections ranging between AP: +0.945 mm and +0.020 mm (Allen, 2008) and was processed and analysed as previously described (Bousquet et al., 2011; 2012; Drouin-Ouellet et al., 2011b).

TH and NeuN immunohistochemistry

To assess neuronal loss, and more specifically loss of the dopaminergic cells, immunohistochemistry against neuronal nuclei (NeuN) and tyrosine hydroxylase (TH) were

performed. For details on the immunohistochemistry protocol, please refer to previously published articles (Bousquet et al., 2011; 2008; Gibrat et al., 2009).

In situ hybridization for Nurr1, DAT and BDNF

Specific [^{35}S]UTP-labeled complementary RNA (cRNA) probes were used to assess tissue mRNA levels for nuclear receptor related 1 protein *(Nurr1)*, dopamine transporter (*DAT)* and *BDNF*. All details relating to these probes are described in (Bousquet et al., 2012, Gibrat et al., 2010). Sense probes were also generated and yielded no specific signal (data not shown). Brain sections were hybridized following the procedures previously published (Bousquet et al., 2009; Gibrat et al., 2010). All *in situ* protocols underwent the same preparation and were conducted in RNAse free conditions. After hybridization, tissue sections were placed against BiomaxMR (Kodak) radioactive sensitive films. Autoradiograms were developed following a 72 h exposure for *Nurr1*, 5 h exposure for *DAT* and a 9-day exposure for *BDNF*. Silver emulsion was further performed for *DAT* to allow for stereological counts of *DAT* positive cells in the substantia nigra pars compacta (SNpc), as previously described (Bousquet et al., 2012).

Densitometric measurements of Nurr1 and BDNF mRNA levels

Levels of autoradiographic labeling were quantified by computerized densitometry. Digitized brain images and their analyses were performed using a video camera (XC-77; Sony) and a constant-illumination light table using computerized densitometry (NIH image v.1.63). Optical density of the autoradiograms was translated into μCi/g of tissue using ^{14}C radioactivity standards (ARC 146-^{14}C standards, American Radiolabeled Chemicals Inc.). *Nurr1* and BDNF mRNA levels were measured in the SNpc using similar antero-posterior levels for all sections. The average labeling for each SNpc level was calculated from 3 adjacent brain sections of the same

mouse. Background intensities taken from white areas of the SN pars reticulata devoid of *Nurr1* or *BDNF* mRNA levels were subtracted from every measurement.

Immunofluorescent staining

For LIM homeobox transcription factor 1-beta (Lmx1b)/TH/Ki67 triple immunofluorescent staining, free-floating tissue sections were incubated in a pre-heated solution of 10 mM sodium citrate (Sigma-Aldrich), pH 6.0 and 0.05% Tween 20 (Thermo Fisher) at 95°C for 15 min to retrieve antigens and allowed to cool down at room temperature (RT). For all stainings, sections were blocked with 0.5% bovine serum albumin (Bioshop), 5% normal goat serum (Wisent), and 0.4% Triton X-100 (Sigma-Aldrich) in KPBS for 30 min. This was followed by an overnight incubation at 4°C in blocking solution with the following primary antibodies: guinea pig anti-Lmx1b (received from Dr. Birchmeier (Germany); 1:200) (Yan et al., 2011) in combination with a mouse anti-TH (Millipore; 1:1000) and a rabbit anti-Ki67 (Abcam; 1:250). For nigral microglial and astroglial cell densities, immunofluorescence was performed using antibodies against Iba1 (ionized calcium binding adaptor molecule 1; Waco Pure Chemicals Industries, 1:1,000) and GFAP (glial fibrillary acidic protein; Sigma-Aldrich, 1:1,000). Following a 2.5 h incubation at RT with appropriate secondary antibodies the nuclei were counterstained with 0.022% 4',6-diamidino-2-phenylindole (DAPI) for 7 min at RT (Molecular Probe). Finally, the sections were mounted on microscope slides, coverslipped with fluoromount-G and sealed with nail polish.

Western blot analyses

Samples were homogenized and processed following identical procedures published in (Bousquet et al., 2012; Drouin-Ouellet et al., 2011a). Membranes generated were immunoblotted with the

following primary antibodies: rabbit anti-TH (Pel-Freez, 1:1,000), rabbit anti-BDNF (Santa Cruz, 1:500), rabbit anti-nerve growth factor (NGF) (Millipore, 1:500), rabbit anti-Neurotrophin 3 (NT3) (Abcam, 1:500), mouse anti-NeuN (Millipore, 1:1,000), mouse anti-βactin (ABM Inc, 1:10,000), and with the secondary antibody goat anti-rabbit or anti-mouse (Jackson Immunoresearch, 1:60,000 or 1:100,000) followed by the addition of chemiluminescence reagents (Luminata, Millipore). Band intensities were quantified using a myECL imager (Thermo Scientific).

Stereological quantifications

The number of nigral TH, NeuN and *DAT* mRNA expressing cells was determined by stereological counts under bright-field illumination, while GFAP, IbaI and TH+/Lmx1b+ were quantified using epifluorescence light. Every fifth section (for TH, NeuN, GFAP and IbaI) and every tenth section (for DAT and TH/Lmx1b) throughout the SNpc (AP: levels of −2.75 mm to −3.58 mm (Allen, 2008)) were analysed using the Stereo investigator software (MicroBrightfield) integrated to a E800 Nikon microscope (Nikon Canada Inc.) (Drouin-Ouellet et al., 2011b; Gibrat et al., 2009).

Dopaminergic neurite analyses on mouse tissue

Arborisation of TH+ neurons in the SNpc of 6-OHDA lesioned mice, visible under brightfield microscopy, was drawn using Neuroexplorer and analysed using Neurolucida. This was performed on the lesioned side using 5 animals per group. Analyses included: the total length of neurites (computed by adding the lengths of each segment, referred to as B1- branch 1, B2- branch 2 and B3- branch 3) (**Fig. 4A**), the % of neurite presenting 0, 1 or 2 branches and the total neurite surface. The volume and the number of intersecting neurites were analysed using the

11

Sholl method (Neurolucida) which consists in placing concentric circles over the cell body (**Fig. 4B**).

BDNF immunoassay

The level of BDNF protein from tissue extracts was determined with the BDNF Emax® ImmunoAssay System (Promega) as per manufacturer's instruction.

Ex vivo **experiments**

Dopaminergic neurite analysis ex vivo

The ventral mesencephalon of E14.5 *Pitx3*-GFP heterozygous embryos were isolated in ice-cold L-15 media (Invitrogen) supplemented with 5% FBS and dissected into small explants with a diameter of approximately 450 μm. The explants were grown for 48 h in collagen gel matrix (Matrigel) in growth medium (86,8% NDS, 5% P/S, 2% B27, 0,2%, 1% NaPyruvate, 5% FBS) at 37°C, 5% CO_2. After 3 days *in vitro*, the medium was replaced by the same complemented medium without FBS. Explants were then challenged for 24 h with 6-OHDA, followed by a treatment of 200 μM cysteamine or of 10 μM rasagiline dissolved in the media without serum for 48 h. Explants were then fixed (in PBS, PFA 3.5%, and sucrose 4%) for 30 min at 4°C (Schmidt et al., 2012).

Immunofluorescent staining of dopaminergic explants

Explants were blocked in 1% normal donkey serum and 0.1% triton in PBS pH 7.4 for 30 min and incubated in a mouse primary anti-tubulin antibody (Sigma, 1: 3 000) (Schmidt et al., 2012) overnight at 4°C. The sections were washed in PBS and stained with the secondary antibody (donkey anti-mouse, Alexa-Fluor-488, 1:200; Jackson immune) for 2 h at RT. Explants were

12

subsequently stained for actin for 40 min at RT with a buffer solution containing 1% rhodamine-phalloidin (Life Technologies) and 1% BSA.

Neurite and growth cone measurements

Neurites and growth cones were quantified in 2 explants per condition. Each explant was subdivided into 4 quadrants and each quadrant into 7 boxes of equal dimension, which were placed at pre-set distances from the explant core. Three boxes were drawn near, and four boxes further from the explant core. This allowed the coverage of the entire explant (see **Suppl. Fig. 2**). Areas more proximal to the core of the explant were not considered because of the high density of neurites which affected the accuracy of the quantification. Distance from the core (proximal = 150 μm and distal = 450 μm) and the size of the boxes (150 μm x 150 μm) were consistent across images, which were acquired using a Zeiss LSM Pascal confocal microscope and assembled using Adobe Photoshop CS5. Quantifications of neurites and growth cones were performed using ImageJ (version 1.45s, NIH).

In vitro **experiments**

Cell lines and culture conditions

In vitro experiments included the use of BV2 (murine microglia) and N2a (murine neuroblastoma) cell lines, as well as primary culture of mouse astrocytes. The N2a and BV2 cells were cultured in DMEM (Sigma-Aldrich) supplemented with 10% heat inactivated FBS (Sigma-Aldrich), 2 mM L-glutamine (Invitrogen) and 1X Penicillin/Streptomycin solution (Sigma-Aldrich). N2a cells were differentiated into a neuronal like phenotype in media containing 0.5% FBS supplemented with 0.5 mM *dibutyryl* cyclic adenosine monophosphate (dbcAMP) (Sigma-Aldrich) for 3 days (Tremblay et al., 2010). Cell viability was assessed by Trypan blue (Sigma-

Aldrich) exclusion using an hemocytometer. Detailed protocols for isolation of murine astrocytes and primary culture are described in (Michaud et al., 2012).

All cell types were plated at concentrations varying from 10^3 to 5×10^5 for 24 h before adding toxins or treatments. After determining the optimal concentrations, cells were treated with 250 µM cystamine and/or 100 µM 6-OHDA (Sigma-Aldrich) for 24 h. To evaluate the effects of cystamine on microglia, BV2 cells were treated for 24 h with either 250 µM cystamine and/or lipopolysaccharide (LPS) 1 µg/ml (Sigma-Aldrich) and further compared to the anti-inflammatory drug dexamethasone (1 µM; Sigma-Aldrich). Control conditions were not treated. Cystamine was added 6 h following (referred to as Post-treatment), 6-OHDA or LPS challenge. The cell culture supernatant was collected to analyse NO and BDNF release, while cells were collected for protein extraction.

Western blot analyses

Proteins were loaded in 4-15% agarose precast gels (Biorad) and electroblotted onto 0.45 µm Immobilon PVDF membranes. Membranes were immunoblotted with rabbit anti-inducible nitric oxide synthase (iNOS; Sigma-Aldrich, 1:1,000), anti-β-actin (ABM Inc, 1:10,000) and GADPH (ABM Inc, 1:1000) primary antibodies as described in the *post-mortem western blot analyses* section. GADPH protein levels were measured as a loading control in the LPS challenge experiments instead of β-actin, since levels of this cytoskeleton protein can be altered by the toxin.

Promega Griess assay

Production of NO was determined by measuring the accumulated level of nitrite (an indicator of

NO) in the cell culture supernatant using the Griess assay (Promega) according to manufacturer's instructions. Total nitrites were measured at 520 nm with a microplate reader. Results were obtained from 6 separate measurements for each experimental condition.

BDNF immunoassay

The BDNF ELISA assay was performed on the extracted proteins derived from the cells as well as on cell media using the BDNF Emax® ImmunoAssay System (Promega) as described in previous sections (see *post-mortem BDNF immunoassay*). Secreted BDNF was concentrated using Amicon Ultra 0.5 ml centrifugal filters for protein purification and concentration (Millipore) prior to performing the assay.

Protrusion length analysis

BV2 cells were grown on a coverslip and fixed for 10 min with 4% PFA pH 7.4. Following 3 additional washes in PBS 1X, the cells were permeabilized with 0.1% Triton X-100 for 30 min at RT and actin filaments were stained with 10 µg/ml Phalloidin-Alexa546 (Sigma) for 1 h at RT. The total protrusion length of 100 BV2 cells per group was measured using ImageJ (version 1.45s, NIH).

Protein extraction and quantification

Each well of a 6-well plate was washed twice with cold PBS 1X and detached in the same solution using a cell scraper. After centrifugation for 5 min at 13,000 *g*, the cells were resuspended in RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, and 0.5% sodium deoxycholate, 1% (v/v) Triton X-100) supplemented with proteases (Roche) and phosphatase inhibitors (Sigma-Aldrich). The cell suspension was then spun for 10 sec, agitated for 10 min at

4°C and spun again for 30 sec. Finally, the samples were centrifuged at 4°C for 20 min at 13,000 g. The supernatant was collected and quantified using a BCA protein assay kit.

Image preparation and statistical analyses

Photomicrographs were taken by Picture Frame software (Microbrightfield) linked to an E800 Nikon microscope (Nikon Instruments). Pictures of immunofluorescent stainings were taken with Simple PCI version 6.0 (Hamamatsu) software linked to a Nikon eclipse 90i microscope (Nikon Instruments). Images were finalized for illustration using Adobe Photoshop and Illustrator CS5. Two-way ANOVAs, crossed or nested, were used to compare 6-OHDA to the saline groups and the different treatments. For behavioural analyses, a repeated measures ANOVA was used to compare toxin, treatment and time effects. For some outcomes, a different variance was estimated for each treatment to correct for the heterogeneity of the variances. Step-down Bonferonni correction was used to ensure that the overall significance level of the multiple comparisons tests was 0.05. Statistical analyses were performed by the *Service de Consultation Statistique* of Université Laval using the MIXED procedure of SAS (version 9.2, SAS).

RESULTS

Cystamine halts toxin-induced nigral dopaminergic neuronal loss and rescues behavioural impairments

We first assessed if cystamine could preserve the dopaminergic system and promote behavioural recovery once the degeneration had been initiated using the unilateral striatal 6-OHDA lesioned mouse model of Parkinson's disease, which generates quantifiable motor impairments (Alvarez-Fischer et al., 2008). Cystamine was thus administered daily 3 weeks following the 6-OHDA lesion for a period of 6 weeks (**Fig. 1A**). 6-OHDA-lesioned mice treated with cystamine

exhibited significant motor improvements when tested on apomorphine-induced rotations 6 and 9 weeks post-lesion, as compared to baseline measures (prior to the initiation of the cystamine treatment) and sham-operated animals at these time points ($p < 0.05$; **Fig. 1B**). Cystamine also induced functional benefits in the stepping test, which measures forelimb akinesia. The percentage of adjusting steps − performed by the forepaw contralateral to the lesion − was re-established to control levels 6 and 9 weeks post-lesion in the cystamine group when compared to baseline ($p < 0.05$), which was similar to sham-operated animals (**Fig. 1C**). At 6 weeks post-lesion, a significant difference ($p < 0.05$) was also observed between the cystamine and saline treated 6-OHDA lesioned groups, suggesting a full recovery in the 6-OHDA lesioned group treated with cystamine at this time point. The assessment of limb-use asymmetry, as measured by the cylinder test, further revealed a positive effect of cystamine on this motor component 9 weeks post-lesion, as opposed to the saline-injected 6-OHDA lesioned mice which demonstrated sustained impairments over time ($p < 0.05$; **Fig. 1D**).

In accordance with the behavioural recovery, the 6-week cystamine treatment in 6-OHDA lesioned animals yielded similar striatal dopamine levels to those found in animals sacrificed at 3 weeks post-lesion (corresponding to the beginning of the cystamine treatment), as measured by HPLC ($p < 0.05$; **Fig. 1E; Suppl. Fig. 1A**). However, striatal TH expression, as quantified by western blots, was similar to levels obtained in the saline-injected group (**Fig. 1F**). While the number of nigral NeuN+ neurons in the 6-OHDA lesioned mice treated with cystamine was similar to sham-operated controls ($p < 0.05$; **Fig. 1G**), the loss of dopaminergic neurons was partially prevented by cystamine, as revealed both by TH+ cell counts and nuclear receptor related 1 protein (*Nurr1*) mRNA expression ($p < 0.01$, $p < 0.05$; **Figs. 1H, I**). A trend towards a partial protection could also be observed for the number of nigral dopamine transporter (*DAT*)+

cells ($p = 0.1031$; **Fig. 1J**).

Cystamine/cysteamine depict neurorestorative properties on nigral dopaminergic neurones

Having established that cystamine exhibits neurorescuing properties, we then tackled the neurorestorative properties of the compound. The drug treatment commenced 5 weeks following the lesion when dopaminergic neuronal degeneration was complete (**Fig. 2A**). We further compared the effects of cystamine to cysteamine, the active form of cystamine in the brain (Bousquet et al., 2010) and which is used in the clinical setting (Dubinsky and Gray, 2006), as well as rasagiline, a drug increasingly prescribed to early (Blandini, 2005; Gaines and Hinson, 2012) and *de novo* (Korchounov et al., 2012) Parkinson's disease patients. Despite the lack of a significant amelioration of the apomorphine-induced rotational motor behaviour following a 6-week treatment of either cystamine, cysteamine and rasagiline (**Fig. 2B**) or of a number of striatal catecholaminergic components (DA, HVA, DOPAC, 5-HT as measured by HPLC – **Fig. 2C**, data not shown) and neurotrophic factor levels (BDNF, NT3 and NGF; as measured by western blots, **Suppl. Table 1**), the loss of nigral TH+ neurons was partially prevented by all three compounds ($p < 0.05$; **Fig. 2F**). Indeed, the total number of nigral TH+ cells was significantly elevated 11 weeks following the 6-OHDA lesion in animals treated with cystamine, cysteamine or rasagiline as compared to the 6-OHDA + saline (i.p.) group (cystamine, $p = 0.0443$; cysteamine, $p = 0.0108$; rasagiline, $p = 0.0115$) (**Fig. 2F**). Despite a trend for an increase in *Nurr1* mRNA levels ($p = 0.091$; **Fig. 2G**) and the number of *DAT* mRNA expressing cells, these measures did not reach significance for any of the treatment conditions (**Fig. 2H**). Importantly, the total number of nigral NeuN+ cells was not significantly different between sham and 6-OHDA lesioned animals indicating that cell loss was restricted to TH+ cells (**Fig. 2E**).

In light of these findings, we evaluated the possibility that new neurons committed to the TH phenotype could be present in the SNpc and triggered by the drug treatment. We thus assessed the expression of the transcription factor Lmx1b, which is essential in guiding cells to the dopaminergic fate (Smidt et al., 2000). However, the number of labelled Lmx1b+ nigral cells that were negative for TH was similar for all treatments (**Fig. 3A, B**) and we did not observe the presence of proliferating cells in the SNpc (**Fig. 3A**) − labelled with Ki67 − although positive staining was observed in the dentate gyrus (**Fig. 3C**), a region known to contain proliferating cells and to continuously generate new neurons even during adulthood (van Praag et al., 1999).

Cystamine/cysteamine and rasagiline impact dopaminergic neurite arborization

Taken together, these results suggest that none of the drug treatments (cystamine, cysteamine nor rasagiline) stimulated the genesis of new nigral neurons. The beneficial effects seen on some behavioural aspects as well as on the nigral dopaminergic system had to be due to other factors. We thus focused on the effects of these three compounds on the remaining nigral TH+ cells, examining the number, length and branching of TH+ neurites (**Fig. 4A, B**). Neurites evaluated in the cystamine group covered longer distances, occupied larger surfaces and volumes when compared to the control, cysteamine and rasagiline groups ($p < 0.05$; **Fig. 4C-E**). Interestingly, the TH+ neurites analysed in the cystamine and rasagiline groups showed a higher degree of arborization/branching and complexity compared to the saline group. Indeed, a higher percentage of neurites depicted one or more branches, while fewer projections were characterized by the absence of branches ($p < 0.05$; **Fig. 4F**). Additionally, when neurites were analysed using the Sholl analysis method (**Fig. 4B**), the neuronal projections of mice treated with cystamine intersected the concentric circles more frequently than in the cysteamine group ($p < 0.05$; **Fig. 4G**), again supporting the higher complexity of the neurites exposed to this compound.

To better understand the effects of cysteamine and rasagiline on the projections of dopaminergic neurons, we turned to an *ex vivo* system of dopaminergic explants which we subjected to these drugs 24 h following a 6-OHDA treatment (**Fig. 5A**). As expected, the explants exposed solely to 6-OHDA presented a drastic loss of neurites when compared to the explants treated with saline (**Fig. 5B, C**). However, both cysteamine and rasagiline significantly increased the numbers of neurites as well as the number of growth cones in regions more proximal ($p < 0.05$; **Fig. 5D-I**) in comparison to more distal regions of the core of the explant (**Fig. 5J, K**).

Trophic and anti-inflammatory/anti-oxidant effects of cystamine following exposure to 6-OHDA

Several mechanisms of action by which cystamine could protect neurons in Parkinson's disease models have been suggested (Gibrat and Cicchetti, 2011), including the increase in BDNF expression (Gibrat et al., 2010). We thus undertook a series of *in vitro* studies to identify factors that could take part in the beneficial effects of cystamine observed post-injury. While cystamine increased BDNF levels in N2a cells (differentiated into neuronal-like cells (Tremblay et al., 2010)) challenged 24 h prior with 6-OHDA ($p < 0.05$; **Fig. 6A**), the compound did not rescue the toxin-induced decrease in BDNF expression in BV2 microglia nor in primary cultures of astrocytes (**Fig. 6B**). In accordance with these *in vitro* data, an increase in BDNF could also be observed in the SN of cystamine treated 6-OHDA lesioned mice of the neurorescue paradigm ($p < 0.05$; **Fig. 6C**), suggesting that the beneficial effects of cystamine observed while the degenerative process is ongoing could be explained by a similar impact on growth factor expression specifically in surviving neurons.

Cystamine has also been reported to reduce iNOS levels in an animal model of systemic lupus erythematosus (H.-P. Wang et al., 2009) and could thus reduce glial cell activation. Given that a significant increase in microglial and astrocytic cell density was quantified in the SN of 6-OHDA lesioned mice as late as 9 weeks post-lesion ($p < 0.05$; **Fig. 7C, D**), we investigated whether cystamine could decrease the production of iNOS dimers and nitrite oxide (NO) in glial cells *in vitro*. This is particularly important given that iNOS and NO are produced by glial cells when in a pro-inflammatory state (Galea et al., 1992; Murphy, 2000) and are often associated with tissue damage (Koprowski et al., 1993; Murphy, 2000). Both the 6-OHDA-induced production of iNOS dimers and NO were partially dampened by cystamine administered 24 h following the 6-OHDA challenge in BV2 microglial cells ($p < 0.05$; **Fig. 7A**), but not in astrocytes.

The inhibition of iNOS and NO production in microglia could thus underlie the positive effect of cystamine on the dopaminergic system and on behavioural impairments associated to the 6-OHDA mouse model when treatment is administered after the degeneration has been initiated. As such, we further investigated the effects of cystamine on the microglial response using LPS and compared its effect with the potent anti-inflammatory drug dexamethasone. Cystamine treatment partially reduced the increase in LPS-induced protrusion length of microglial cells ($p < 0.001$; **Fig. 7B**). The morphological changes induced by LPS were also accompanied by an increase in β-actin protein levels ($p < 0.01$), which was prevented by cystamine when given 24 h following LPS ($p < 0.01$; **Fig. 7B**). In contrast, dexamethasone failed to generate such effects. Both cystamine and dexamethasone reduced iNOS dimer levels ($p < 0.05$ and $p < 0.01$, respectively; **Fig. 7B**), which matched control values and significantly reduced nitrite levels ($p < 0.001$). These results suggest a potent anti-inflammatory effect of cystamine even when administered 24 h following the toxin challenge. Although the 6-week cystamine treatment failed to decrease the

number of nigral microglia (**Fig. 7C**), a very strong trend towards a reduction in the number of nigral astrocytes could be observed in the 6-OHDA lesioned group treated with cystamine when compared to the untreated lesioned group (p = 0.0577; **Fig. 7D**).

DISCUSSION

This study was designed to determine whether cystamine/cysteamine were effective in counteracting behavioural and pathological changes observed in a neurotoxic mouse model of Parkinson's disease when the treatment is administered during an ongoing dopaminergic neuronal degeneration as well as after the degeneration has taken place.

Here we show that a continuous 6-week administration of cystamine following a 6-OHDA lesion (initiated 3 weeks post-surgery) can rescue the remaining neurons within the SNpc as well as part of the striatal DA content. These observations are accompanied with a complete recovery of behavioural impairments characteristic of the 6-OHDA lesioned model. The mechanisms for cystamine-induced behavioural improvements would likely include the re-establishment of the nigro-striatal dopamine levels, although we cannot exclude a symptomatic effect of cystamine on its own.

The second part of this study focused explicitly on the potential disease-modifying properties of cystamine/cysteamine using a time point when dopaminergic degeneration is stable, that is 5 weeks following a 6-OHDA lesion. *Post-mortem* analyses provided evidence that these compounds were able to reverse one of the main pathological hallmarks of Parkinson's disease, that is nigral TH cell degeneration. Importantly, our *in vivo* and *ex vivo* analyses combined clearly demonstrate that cystamine and cysteamine exert a striking effect on neurite sprouting;

modulating the number of neurites and their length as well as the number of growth cones. The more complex and richer arborization observed denotes a healthier cell status and the increased number of growth cones – which are motile structures, sensing the surrounding environment, directing neurites to proper targets to establish functional synaptic contacts (Mortimer et al., 2008) – point to an increased malleability and plasticity of dopaminergic cells following the exposure to cystamine/cysteamine. These observations, combined to the absence of any signs of proliferating cells in the SN following cystamine/cysteamine treatments, suggest that these compounds are likely to exert their beneficial effects, in part, by directly acting on the remaining nigral dopaminergic neurons. In other words, cystamine/cysteamine may be able to boost the capacity of the remaining dopaminergic cells, which may in turn translate into disease-modifying effects.

Another important observation is that the effects of cysteamine on neurite sprouting were similar to those generated by rasagiline. Dopaminergic cells exposed to either cysteamine or rasagiline were characterized by more complex neurite arborisation. Previous reports have suggested that rasagiline prevents damage of neurites and collapse of growth cones in PC12 cells (Abu-Raya et al., 1999) but growth *per se* had not been assessed. Our observations provide the first demonstration that rasagiline, one of the very few drugs that has been claimed to have neuroprotection properties in the clinic, impacts neurite sprouting, a phenomenon which is likely to be responsible for some of the beneficial effects generated by this drug. Prior to our observations, the only mechanism of action identified pointed to the increased expression of the glial cell line-derived growth factor (Maruyama et al., 2004) and anti-apoptotic properties *in vitro* when administered prior to 6-OHDA in the human neuroblastoma SH-SY5Y cells (Maruyama et al., 2001).

Increases in BDNF expression have been observed both *in vitro* and *in vivo* in the brain and plasma of Huntington's disease animal models following cystamine treatment (Borrell-Pagès et al., 2006). We also previously reported an increase in nigral *BDNF* mRNA and protein expression *in vivo* when cystamine was given concomitantly with MPTP (Gibrat et al., 2010). Our *in vitro* data revealed that cystamine rescues the decrease in BDNF levels in neuronal cells 24 h following the toxin challenge, which is likely to represent one factor involved in the pro-survival action that cystamine exerts on the dopaminergic neurons of the SNpc. Furthermore, BDNF enhances plasticity and can promote nigral dopaminergic axonal growth (García Navia et al., 2008; Ostergaard et al., 1996), which could underlie the cystamine-induced neuronal sprouting observed in our study.

To date, only one study reported the capacity of cystamine to reduce the activation of iNOS and neuronal NOS in the brain of animal models of systemic lupus erythematosus (H.-P. Wang et al., 2009). We found that cystamine reduced iNOS levels in microglia challenged with 6-OHDA, which could, to some extent, relate to the anti-oxidative properties of cystamine (Fox et al., 2004), considering that 6-OHDA promotes oxidative stress (Hanrott et al., 2006). To confirm the ability of cystamine to reduce the glial response, we then evaluated the effects of cystamine on microglia primed by LPS. Cystamine modulated cell morphology and reduced microglial activation by regulating iNOS and nitrite release, and was more effective than dexamethasone to lower β-actin protein levels. The ability of cystamine to modulate glial activation, as observed *in vitro,* might constitute one of the mechanisms by which the dopaminergic system is protected *in vivo* (L'Episcopo et al., 2010; Ousman and Kubes, 2012; Saavedra et al., 2006; Swanson et al., 2004). Indeed, the propensity of cystamine to reduce the number of astrocytes in the SNpc

suggests that cystamine could play a protective role on this cell type by reducing the release of detrimental factors onto neurons, an hypothesis which requires further evaluation.

Among the various drugs that have shown a neuroprotective potential in the pre-clinical setting (Poewe et al., 2012), very few have led to measurable benefits once tested in the clinic (e.g. Co-enzyme Q10 (Parkinson Study Group QE3 Investigators et al., 2014), Creatine (parkinson.org website), Caffeine (Postuma et al., 2012)). Rasagiline constitutes, however, a compound which showed promise in *in vitro* and *in vivo* models and which further translated into tangible benefits in parkinsonian patients. Rasagiline is a selective and irreversible inhibitor of type B monoamine oxidase (Gaines and Hinson, 2012) and blocks the oxidative metabolism of dopamine, preventing oxidative stress. Pre-clinical studies described the neuroprotective properties of rasagiline in both MPTP (Kupsch et al., 2001) and 6-OHDA paradigms (Blandini et al., 2004), as we did here with cystamine/cysteamine in the 6-OHDA model. For example, rasagiline treatment prior to MPTP administration in primates attenuated TH cell loss and further prevented motor dysfunction (Kupsch et al., 2001). Similarly, chronic administration of rasagiline in 6-OHDA lesioned mice conferred some neuroprotection of the SN when administered 1 h following the lesion, while more subtle protection of dopaminergic terminals was reported in the striatum (Blandini et al., 2004).

The striatal 6-OHDA lesion produces degeneration of dopaminergic fibers followed by the cell bodies of the SNpc. At 5 weeks post-lesion, neurite sprouting appears to be restricted to the SN given that we did not observe any difference in the level of striatal TH proteins nor dopamine at this time point. In order to replenish the striatum in dopamine content, the new fibers would have to grow several mm. Such process has been shown to take months in mice transplanted with

dopaminergic neurons (Grealish et al., 2014) and up to years in men as exemplified by the neurturin gene therapy trial that did not reveal any beneficial effects at the primary end-point of 12 months, but did show improvements at 15-18 months (Björklund and Kordower, 2013). The fact that some patients in receipt of fetal ventral mesencephalic grafts are still improving more than 15 years post grafting (Politis et al., 2010) provides further evidence that measurable effects may take a considerable amount of time. It is thus likely that the 6-week cystamine/cysteamine/rasagiline treatments used in our study were not sufficient to provide measureable functional benefits. It would therefore be conceivable that the restorative effects of cystamine/cysteamine in patients would be seen months following the beginning of treatment.

The majority of clinical trials performed thus far have reported sustained amelioration of motor functions in early Parkinson's disease patients (Hauser et al., 2009; Mínguez-Mínguez et al., 2013; Olanow et al., 2003). Importantly, some have claimed that rasagiline can slow down the progression of the disease (Blandini, 2005; Gaines and Hinson, 2012). Accordingly, rasagiline is currently administered either in combination with L-DOPA to late-stage patients or as monotherapy in early and *de novo* patients (Korchounov et al., 2012; Olanow et al., 2009; Parkinson Study Group, 2002; Rascol et al., 2011). Taken together, our results suggest that cystamine/cysteamine may be important disease-modifying candidates, which deserve further testing in clinical trials.

Conclusions

We have demonstrated that cystamine can rescue dying dopaminergic neurons and restore motor functions in an animal model of Parkinson's disease. The beneficial effects of cystamine in the context of an ongoing degeneration is most probably multifactorial and could act, at least in part,

through the modulation of BDNF and glial-derived toxic factors. Most importantly, we have unravelled a critical effect of cystamine, cysteamine and rasagiline on dopaminergic neurite sprouting. This is likely to be a key element underlying the benefits generated by these drugs. Taken together, these results reveal a beneficial effect of cystamine/cysteamine on pathological features and deficits created in toxin-induced mouse model of Parkinson's disease. Our results suggest that the effects of cystamine/cysteamine go beyond neuroprotection, putting forward the idea that cystamine/cysteamine could be good candidates for clinical trials in newly diagnosed Parkinson's disease patients and be used, similarly to rasagiline, early in the course of disease evolution.

ACKNOWLEGMENTS

Conflicts of interest

This research was supported in part by Raptor Pharmaceuticals Corp.

FIGURE LEGENDS

Figure 1. Effects of cystamine treatment on the nigrostriatal dopaminergic system and associated behaviours 3 weeks following a 6-OHDA lesion. (**A**) Time line of experimentation. (**B-D**) Throughout the experiment, mice were evaluated using 3 different behavioural tests: apomorphine-induced rotations (**B**), adjusting steps (**C**) and limb-use asymmetry (**D**) at 3 distinct time points: prior to the commencement of the cystamine treatment (3 weeks post-surgery), 6 weeks and 9 weeks post-lesion. Cystamine was shown to reverse behavioural nigro-striatal-related impairments induced by a unilateral injection of 6-OHDA (apomorphine-induced rotations, stepping test and cylinder test). (**B**) $*$ = $p < 0.05$ significant difference compared to shams, $\#$ = $p < 0.05$, significant difference compared to the 6-OHDA + cystamine group; (**C**) $*$ = $p < 0.05$, significant difference compared to the sham group, $\#$ = $p < 0.05$, significant difference compared to 6-OHDA + cystamine group at 3 and 6 weeks post-lesion, $\&$ = $p < 0.05$, significant difference compared to 6-OHDA + cystamine group at baseline; $+$ = $p < 0.05$ significant difference compared to 6-OHDA + cystamine group. (**E** and **F**) HPLC quantification of the striatal dopamine content (**E**) and western blot analysis of striatal TH level (**F**) revealed that cystamine partially rescues the striatal DA levels at 9 weeks following the 6-OHDA lesion. (**G-J**) Post-treatment effects of cystamine on the number of nigral NeuN+ cells (**G**), TH+ neurons (**H**), the densitometric levels of *Nurr1* (**I**) and the number of cells expressing *DAT* mRNA (**J**) in 6-OHDA-lesioned mice. Values are expressed as means ± S.E.M. Statistical analyses were performed using Step-down Bonferroni correction method. $*$ = $p < 0.05$ significant difference compared to sham, $\#$ = $p < 0.05$, significant difference compared to 6-OHDA + cystamine group. Western blot bands seen in this figure were derived from the same experiment and cropped from the same membrane.

29

Figure 2. Comparative effects of cystamine, cysteamine and rasagiline treatments on the nigrostriatal dopaminergic system and associated behaviours 5 weeks following a 6-OHDA lesion. (**A**) Time line of experimentation. (**B**) Apomorphine-induced rotations at baseline and at 8 weeks and 11 weeks following the 6-OHDA lesion. (**C**) HPLC quantification of striatal dopamine level and (**D**) western blot analysis of striatal TH levels. (**E-H**) Cystamine, cysteamine and rasagiline treatment effects on the total number of NeuN+ neurons, TH+ neurons, densitometric levels of *Nurr1* mRNA and the total number of cells expressing *DAT* mRNA in the SNpc of 6-OHDA-lesioned mice and their controls. Values are expressed as means ± S.E.M. Statistical analyses were performed using two-way ANOVA followed by a Bonferroni's multiple comparisons test. * = $p < 0.05$, significant difference compared to Sham-Rasagiline group; & = $p < 0.05$, significant difference compared to 6-OHDA-saline (i.p.) group. Abbreviations. Cys: cystamine; Ce: cysteamine; Ra: rasagiline; S (ip): saline intraperitoneal; S (sc): saline subcutaneous. Western blot bands seen in this figure were derived from the same experiment and cropped from the same membrane.

Figure 3. Comparative effects of cystamine, cysteamine and rasagiline treatments on dopaminergic precursors and nigral proliferating cells 5 weeks following a 6-OHDA lesion. (**A**) Representative images of double immunofluorescent staining for TH (red) and Lmx1b (green) in cysteamine and saline animals in the SNpc of the lesioned and unlesioned side. Insets show TH and Lmx1b staining separately as well as the absence Ki67 immunofluorescent staining (proliferating cells) in the SNpc of the same animals. (**B**) Percent of nigral TH-/Lmx1b+ cells as counted on the lesioned side. (**C**) Immunofluorescent staining for Ki67 in hippocampal slices of the same animals served as positive controls. Values are expressed as means ± S.E.M. Statistical analyses were performed using a one-way ANOVA followed by a Tukey post-hoc test.

Abbreviations. 6-OHDA: 6-hydroxydopamine; Cys: Cystamine; Ce: Cysteamine; ip: intraperitoneal; Ra: Rasagiline. Scale bars: 50 µm.

Figure 4. Comparative effects of cystamine, cysteamine and rasagiline treatments on nigral TH+ neurites 5 weeks following a 6-OHDA lesion. (**A-B**) Schematic of the various analyses of TH+ neurites *in vivo*. (**C**) Total neurite length, (**D**) total neurite surface (μm^2), (**E**) total neurite volume (μm^3), (**F**) % of neurites characterized by 0, 1 or 2 branches and (**G**) total number of intersecting neurites. Values are expressed as means ± S.E.M. Statistical analyses were performed using two-way ANOVA followed by a Bonferroni's multiple comparisons test. * = $p <$ 0.05, ** = $p < 0.01$ significant difference compared to 6-OHDA-cystamine group; # = $p < 0.05$, significant difference compared to 6-OHDA- saline (i.p.) 0 branch; & = $p < 0.05$, significantly different compared to rasagiline ≥2 branches. Abbreviations. 6-OHDA: 6-hydroxydopamine; B1: branch 1; B2: branch 2; B3: branch; Cys: cystamine; Ce: cysteamine; Ra: rasagiline; S (ip): saline intraperitoneal; S (sc): saline subcutaneous.

Figure 5. Comparative effects of cysteamine and rasagiline treatments on neurite growth *ex vivo*. (**A**) Time line of experimentation. (**B-G**) Double staining for two cytoskeleton proteins, actin (rhodamine-phalloidin, red) and tubulin (green) was used to quantify neuritic outgrowth in explants derived from the ventral mesencephalon of *Pitx3*-GFP mice. Scale bars B: 200 µm, B': 50 µm and B": 25 µm. (**H-K**) Total number of neurites and growth cones proximal and distal to the explant core. Values are expressed as means ± S.E.M. Statistical analyses were performed using two-way ANOVA. * = $p < 0.05$, ** = $p < 0.01$, significantly difference compared to the saline group.

31

Figure 6. Cystamine modulates BDNF in a 6-OHDA challenge. (**A**, **B**) ELISA quantification of BDNF in N2a, BV2 and mouse astrocyte cell extracts after a 6-OHDA and/or cystamine treatment. (**C**) Densitometric levels of *BDNF* mRNA in the SNpc of 6-OHDA lesioned mice. Values are expressed as means ± S.E.M. Statistical analyses were performed using Step-down Bonferroni correction method. (**A**) * = $p < 0.05$, significant difference compared to control group; # = $p < 0.05$, significant difference compared to 6-OHDA group; (**B**) ## = $p < 0.01$, significant difference compared to 6-OHDA group; (**C**) * = $p < 0.05$, significant difference compared to the sham + saline, sham + cystamine and 6-OHDA + cystamine groups Abbreviation: Cys = cystamine.

Figure 7. Cystamine modulates the glial response to 6-OHDA and LPS challenges

(**A**) Effects of cystamine on iNOS and nitric levels in BV2 cells and primary culture of murine astrocytes. (**B**) Photomicrographs of BV2 cells stained with Phalloidin (red) and depicting morphological changes following LPS (1 µg/ml) challenge and/or cystamine (250 µM) treatment. Quantification of BV2 cell protrusions, actin and iNOS levels as well as nitrite release following LPS (1 µg/ml) challenge and/or cystamine (250 µM) or dexamethasone (1µM) treatment. Scale bar: 50 µm. (**C, D**) Stereological counts of microglia (Iba1+ cells) (**C**) and astrocytes (GFAP+ cells) (**D**) in the SNpc of 6-OHDA lesioned animals. Values are expressed as means ± S.E.M. Statistical analyses were performed using Step-down Bonferroni correction method. (**A**) ** = $p < 0.01$, *** = $p < 0.001$, significant difference compared to control condition; ## = $p < 0.01$, ### = $p < 0.001$, significant difference compared to cystamine condition; && = $p < 0.01$, significant difference compared to cystamine 6-OHDA condition; (**B**) * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, significant difference compared to control condition; # = $p < 0.05$, ## = $p < 0.01$, ### = $p < 0.001$, significant difference compared to 6-OHDA condition; & = $p < 0.05$, &&& = $p < 0.001$,

significant difference compared to cystamine condition; \$ = $p < 0.05$, significant difference compared to dexamethasone condition; (**C, D**) * = $p < 0.05$, significant difference compared to the sham + saline group. Abbreviation: Cys = cystamine. Abbreviations: Cys = Cystamine; DEX = dexamethasone; LPS = lipopolysaccharide. Western blot bands seen in this figure were derived from the same experiment and cropped from the same membrane.

Supplementary material

Supplementary Table 1. Summary of western blot quantifications for striatal BDNF (precursor and mature forms), NGF, NT3 protein levels in 6-OHDA-lesioned mice and their controls. Values are expressed as means ± S.E.M. Statistical analyses were performed using two-way ANOVA followed by a Bonferroni's multiple comparisons test. * = $p < 0.05$ vs rasagiline.

Supplementary Figure 1. Degeneration of the nigrostriatal dopaminergic system 3 weeks following a 6-OHDA lesion. (**A**) HPLC quantification of striatal dopamine content, (**B**) western blot analysis of striatal TH levels and (**C**) stereological count of nigral TH+ neurons in 6-OHDA-lesioned mice 3 weeks following the lesion. Values are expressed as means ± S.E.M. Statistical analyses were performed using Student's t-test. ** = $p < 0.005$; *** = $p < 0.001$ significant difference compared to sham-operated animals.

Supplementary Figure 2. Analyses of neurites in dopaminergic explants. Double immunofluorescent staining for the cytoskeleton proteins actin (rhodamine-phalloidin, red) and tubulin (green) identifying neuritic outgrowth in dopaminergic explants. Squares labelled 1, 2, 3 were used for the quantification of growth cones and neurites near the explant core (distance of 150 μm) whereas squares labeled 4, 5, 6 and 7 were used to quantify the number of growth cones and neurites distant from the explant core (distance of 450 μm). Scale bar: 200 μm.

References

Abu-Raya, S., Blaugrund, E., Trembovler, V., Shilderman-Bloch, E., Shohami, E., Lazarovici, P., 1999. Rasagiline, a monoamine oxidase-B inhibitor, protects NGF-differentiated PC12 cells against oxygen-glucose deprivation. J. Neurosci. Res. 58, 456–463.

Allen, 2008. Allen Brain Atlas. Seattle (WA): Allen Institute for Brain Science Available from: http://www.brain-map.org.

Alvarez-Fischer, D., Henze, C., Strenzke, C., Westrich, J., Ferger, B., Hoglinger, G.U., Oertel, W.H., Hartmann, A., 2008. Characterization of the striatal 6-OHDA model of Parkinson's disease in wild type and alpha-synuclein-deleted mice. Exp. Neurol. 210, 182–193.

Björklund, A., Kordower, J.H., 2013. Cell therapy for Parkinson's disease: what next? Mov. Disord. 28, 110–115.

Blandini, F., 2005. Neuroprotection by rasagiline: a new therapeutic approach to Parkinson's disease? CNS Drug Rev 11, 183–194.

Blandini, F., Armentero, M.T., Fancellu, R., Blaugrund, E., Nappi, G., 2004. Neuroprotective effect of rasagiline in a rodent model of Parkinson's disease. Exp. Neurol. 187, 455–459.

Blume, S.R., Cass, D.K., Tseng, K.Y., 2009. Stepping test in mice: a reliable approach in determining forelimb akinesia in MPTP-induced Parkinsonism. Exp. Neurol. 219, 208–211.

Borrell-Pagès, M., Canals, J.M., Cordelières, F.P., Parker, J.A., Pineda, J.R., Grange, G., Bryson, E.A., Guillermier, M., Hirsch, E., Hantraye, P., Cheetham, M.E., Néri, C., Alberch, J., Brouillet, E., Saudou, F., Humbert, S., 2006. Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via HSJ1b and transglutaminase. J. Clin. Invest. 116, 1410–1424.

Bousquet, M., Gibrat, C., Ouellet, M., Rouillard, C., Calon, F., Cicchetti, F., 2010. Cystamine metabolism and brain transport properties: clinical implications for neurodegenerative diseases. J. Neurochem. 114, 1651–1658.

Bousquet, M., Gibrat, C., Saint-Pierre, M., Julien, C., Calon, F., Cicchetti, F., 2009. Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. Progress in neuro-psychopharmacology & biological psychiatry 33, 1401–1408.

Bousquet, M., Gue, K., Emond, V., Julien, P., Kang, J.X., Cicchetti, F., Calon, F., 2011. Transgenic conversion of omega-6 into omega-3 fatty acids in a mouse model of Parkinson's disease. J. Lipid Res. 52, 263–271.

Bousquet, M., Saint-Pierre, M., Julien, C., Salem, N., Cicchetti, F., Calon, F., 2008. Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's disease. FASEB J. 22, 1213–1225.

Bousquet, M., St-Amour, I., Vandal, M., Julien, P., Cicchetti, F., Calon, F., 2012. High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. Neurobiol. Dis. 45, 529–538.

Dedeoglu, A., Kubilus, J.K., Jeitner, T.M., Matson, S.A., Bogdanov, M., Kowall, N.W., Matson, W.R., Cooper, A.J., Ratan, R.R., Beal, M.F., Hersch, S.M., Ferrante, R.J., 2002. Therapeutic effects of cystamine in a murine model of Huntington's disease. J. Neurosci. 22, 8942–8950.

Drouin-Ouellet, J., Brownell, A.-L., Saint-Pierre, M., Fasano, C., Emond, V., Trudeau, L.-E., Lévesque, D., Cicchetti, F., 2011a. Neuroinflammation is associated with changes in glial mGluR5 expression and the development of neonatal excitotoxic lesions. Glia 59, 188–199.

Drouin-Ouellet, J., Gibrat, C., Bousquet, M., Calon, F., Kriz, J., Cicchetti, F., 2011b. The role of the MYD88-dependent pathway in MPTP-induced brain dopaminergic degeneration. J Neuroinflammation 8, 137.

Dubinsky, R., Gray, C., 2006. CYTE-I-HD: phase I dose finding and tolerability study of cysteamine (Cystagon) in Huntington's disease. Mov. Disord. 21, 530–533.

Fox, J.H., Barber, D.S., Singh, B., Zucker, B., Swindell, M.K., Norflus, F., Buzescu, R., Chopra, R., Ferrante, R.J., Kazantsev, A., Hersch, S.M., 2004. Cystamine increases L-cysteine levels in Huntington's disease transgenic mouse brain and in a PC12 model of polyglutamine aggregation. J. Neurochem. 91, 413–422.

Gaines, K.D., Hinson, V.K., 2012. Adjunctive therapy in Parkinson's disease: the role of rasagiline. Neuropsychiatr Dis Treat 8, 285–294.

Galea, E., Feinstein, D.L., Reis, D.J., 1992. Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. Proc. Natl. Acad. Sci. U.S.A. 89, 10945–10949.

García Navia, J.T., Burguillos, M.A., Ramón-Cueto, A., Machado, A., Cano, J., Venero, J.L., 2008. Regional-specific regulation of BDNF and trkB correlates with nigral dopaminergic cell sprouting following unilateral nigrostriatal axotomy. J. Neurosci. Res. 86, 2016–2027.

Gibrat, C., Bousquet, M., Saint-Pierre, M., Levesque, D., Calon, F., Rouillard, C., Cicchetti, F., 2010. Cystamine prevents MPTP-induced toxicity in young adult mice via the up-regulation of the brain-derived neurotrophic factor. Progress in neuro-psychopharmacology &

biological psychiatry 34, 193–203.

Gibrat, C., Cicchetti, F., 2011. Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. Progress in neuro-psychopharmacology & biological psychiatry 35, 380–389.

Gibrat, C., Saint-Pierre, M., Bousquet, M., Levesque, D., Rouillard, C., Cicchetti, F., 2009. Differences between subacute and chronic MPTP mice models: investigation of dopaminergic neuronal degeneration and alpha-synuclein inclusions. J. Neurochem. 109, 1469–1482.

Grealish, S., Diguet, E., Kirkeby, A., Mattsson, B., Heuer, A., Bramoulle, Y., Van Camp, N., Perrier, A.L., Hantraye, P., Björklund, A., Parmar, M., 2014. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell 15, 653–665.

Hanrott, K., Gudmunsen, L., O'Neill, M.J., Wonnacott, S., 2006. 6-hydroxydopamine-induced apoptosis is mediated via extracellular auto-oxidation and caspase 3-dependent activation of protein kinase Cdelta. J. Biol. Chem. 281, 5373–5382.

Hauser, R.A., Lew, M.F., Hurtig, H.I., Ondo, W.G., Wojcieszek, J., Fitzer-Attas, C.J., TEMPO Open-label Study Group, 2009. Long-term outcome of early versus delayed rasagiline treatment in early Parkinson's disease. Mov. Disord. 24, 564–573.

Hirsch, E.C., Vyas, S., Hunot, S., 2012. Neuroinflammation in Parkinson's disease. Parkinsonism & related disorders 18 Suppl 1, S210–2.

Jókay, I., Kelemenics, K., Gyuris, A., Minárovits, J., 1998. S-methylthio-cysteine and cystamine are potent stimulators of thiol production and glutathione synthesis. Life sciences 62, PL27–33.

Junn, E., Ronchetti, R.D., Quezado, M.M., Kim, S.-Y., Mouradian, M.M., 2003. Tissue transglutaminase-induced aggregation of alpha-synuclein: Implications for Lewy body formation in Parkinson's disease and dementia with Lewy bodies. Proc. Natl. Acad. Sci. U.S.A. 100, 2047–2052.

Karpuj, M.V., Becher, M.W., Steinman, L., 2002. Evidence for a role for transglutaminase in Huntington's disease and the potential therapeutic implications. Neurochemistry international 40, 31–36.

Koprowski, H., Zheng, Y.M., Heber-Katz, E., Fraser, N., Rorke, L., Fu, Z.F., Hanlon, C., Dietzschold, B., 1993. In vivo expression of inducible nitric oxide synthase in experimentally

induced neurologic diseases. Proc. Natl. Acad. Sci. U.S.A. 90, 3024–3027.

Korchounov, A., Winter, Y., Rössy, W., 2012. Combined beneficial effect of rasagiline on motor function and depression in de novo PD. Clin Neuropharmacol 35, 121–124.

Kupsch, A., Sautter, J., Götz, M.E., Breithaupt, W., Schwarz, J., Youdim, M.B., Riederer, P., Gerlach, M., Oertel, W.H., 2001. Monoamine oxidase-inhibition and MPTP-induced neurotoxicity in the non-human primate: comparison of rasagiline (TVP 1012) with selegiline. J Neural Transm 108, 985–1009.

L'Episcopo, F., Tirolo, C., Caniglia, S., Testa, N., Serra, P.A., Impagnatiello, F., Morale, M.C., Marchetti, B., 2010. Combining nitric oxide release with anti-inflammatory activity preserves nigrostriatal dopaminergic innervation and prevents motor impairment in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. J Neuroinflammation 7, 83.

la Fuente-Fernandez, de, R., Schulzer, M., Mak, E., Sossi, V., 2010. Trials of neuroprotective therapies for Parkinson's disease: problems and limitations. Parkinsonism & related disorders 16, 365–369.

Lesort, M., Lee, M., Tucholski, J., Johnson, G.V., 2003. Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. J. Biol. Chem. 278, 3825–3830.

Lorand, L., Conrad, S.M., 1984. Transglutaminases. Mol. Cell. Biochem. 58, 9–35.

Maruyama, W., Nitta, A., Shamoto-Nagai, M., Hirata, Y., Akao, Y., Yodim, M., Furukawa, S., Nabeshima, T., Naoi, M., 2004. N-Propargyl-1 (R)-aminoindan, rasagiline, increases glial cell line-derived neurotrophic factor (GDNF) in neuroblastoma SH-SY5Y cells through activation of NF-kappaB transcription factor. Neurochemistry international 44, 393–400.

Maruyama, W., Youdim, M.B., Naoi, M., 2001. Antiapoptotic properties of rasagiline, N-propargylamine-1(R)-aminoindan, and its optical (S)-isomer, TV1022. Ann. N. Y. Acad. Sci. 939, 320–329.

Metz, G.A., Whishaw, I.Q., 2002. Drug-induced rotation intensity in unilateral dopamine-depleted rats is not correlated with end point or qualitative measures of forelimb or hindlimb motor performance. Neuroscience 111, 325–336.

Michaud, J.-P., Richard, K.L., Rivest, S., 2012. Hematopoietic MyD88-adaptor Protein Acts as a Natural Defense Mechanism for Cognitive Deficits in Alzheimer's Disease. Stem Cell Rev.

Mínguez-Mínguez, S., Solís-García Del Pozo, J., Jordán, J., 2013. Rasagiline in Parkinson's disease: a review based on meta-analysis of clinical data. Pharmacological research : the official journal of the Italian Pharmacological Society 74, 78–86.

Moore, A.E., Cicchetti, F., Hennen, J., Isacson, O., 2001. Parkinsonian motor deficits are reflected by proportional A9/A10 dopamine neuron degeneration in the rat. Exp. Neurol. 172, 363–376.

Mortimer, D., Fothergill, T., Pujic, Z., Richards, L.J., Goodhill, G.J., 2008. Growth cone chemotaxis. Trends Neurosci. 31, 90–98.

Murphy, S., 2000. Production of nitric oxide by glial cells: regulation and potential roles in the CNS. Glia 29, 1–13.

Olanow, C.W., Goetz, C.G., Kordower, J.H., Stoessl, A.J., Sossi, V., Brin, M.F., Shannon, K.M., Nauert, G.M., Perl, D.P., Godbold, J., Freeman, T.B., 2003. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann. Neurol. 54, 403–414.

Olanow, C.W., Rascol, O., Hauser, R., Feigin, P.D., Jankovic, J., Lang, A., Langston, W., Melamed, E., Poewe, W., Stocchi, F., Tolosa, E., ADAGIO Study Investigators, 2009. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N. Engl. J. Med. 361, 1268–1278.

Ostergaard, K., Jones, S.A., Hyman, C., Zimmer, J., 1996. Effects of donor age and brain-derived neurotrophic factor on the survival of dopaminergic neurons and axonal growth in postnatal rat nigrostriatal cocultures. Exp. Neurol. 142, 340–350.

Ousman, S.S., Kubes, P., 2012. Immune surveillance in the central nervous system. Nat. Neurosci. 15, 1096–1101.

Parkinson Study Group, 2002. A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. Arch. Neurol. 59, 1937–1943.

Parkinson Study Group QE3 Investigators, Beal, M.F., Oakes, D., Shoulson, I., Henchcliffe, C., Galpern, W.R., Haas, R., Juncos, J.L., Nutt, J.G., Voss, T.S., Ravina, B., Shults, C.M., Helles, K., Snively, V., Lew, M.F., Griebner, B., Watts, A., Gao, S., Pourcher, E., Bond, L., Kompoliti, K., Agarwal, P., Sia, C., Jog, M., Cole, L., Sultana, M., Kurlan, R., Richard, I., Deeley, C., Waters, C.H., Figueroa, A., Arkun, A., Brodsky, M., Ondo, W.G., Hunter, C.B., Jimenez-Shahed, J., Palao, A., Miyasaki, J.M., So, J., Tetrud, J., Reys, L., Smith, K., Singer, C., Blenke, A., Russell, D.S., Cotto, C., Friedman, J.H., Lannon, M., Zhang, L., Drasby, E., Kumar, R., Subramanian, T., Ford, D.S., Grimes, D.A., Cote, D., Conway, J., Siderowf, A.D., Evatt, M.L., Sommerfeld, B., Lieberman, A.N., Okun, M.S., Rodriguez, R.L., Merritt, S., Swartz, C.L., Martin, W.R.W., King, P., Stover, N., Guthrie, S., Watts, R.L., Ahmed, A., Fernandez, H.H., Winters, A., Mari, Z., Dawson, T.M., Dunlop, B., Feigin, A.S., Shannon,

B., Nirenberg, M.J., Ogg, M., Ellias, S.A., Thomas, C.-A., Frei, K., Bodis-Wollner, I., Glazman, S., Mayer, T., Hauser, R.A., Pahwa, R., Langhammer, A., Ranawaya, R., Derwent, L., Sethi, K.D., Farrow, B., Prakash, R., Litvan, I., Robinson, A., Sahay, A., Gartner, M., Hinson, V.K., Markind, S., Pelikan, M., Perlmutter, J.S., Hartlein, J., Molho, E., Evans, S., Adler, C.H., Duffy, A., Lind, M., Elmer, L., Davis, K., Spears, J., Wilson, S., Leehey, M.A., Hermanowicz, N., Niswonger, S., Shill, H.A., Obradov, S., Rajput, A., Cowper, M., Lessig, S., Song, D., Fontaine, D., Zadikoff, C., Williams, K., Blindauer, K.A., Bergholte, J., Propsom, C.S., Stacy, M.A., Field, J., Mihaila, D., Chilton, M., Uc, E.Y., Sieren, J., Simon, D.K., Kraics, L., Silver, A., Boyd, J.T., Hamill, R.W., Ingvoldstad, C., Young, J., Thomas, K., Kostyk, S.K., Wojcieszek, J., Pfeiffer, R.F., Panisset, M., Beland, M., Reich, S.G., Cines, M., Zappala, N., Rivest, J., Zweig, R., Lumina, L.P., Hilliard, C.L., Grill, S., Kellermann, M., Tuite, P., Rolandelli, S., Kang, U.J., Young, J., Rao, J., Cook, M.M., Severt, L., Boyar, K., 2014. A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. JAMA Neurol 71, 543–552.

Paul, B.D., Sbodio, J.I., Xu, R., Vandiver, M.S., Cha, J.Y., Snowman, A.M., Snyder, S.H., 2014. Cystathionine γ-lyase deficiency mediates neurodegeneration in Huntington's disease. Nature 509, 96–100.

Poewe, W., Mahlknecht, P., Jankovic, J., 2012. Emerging therapies for Parkinson's disease. Current opinion in neurology 25, 448–459.

Politis, M., Wu, K., Loane, C., Quinn, N.P., Brooks, D.J., Rehncrona, S., Björklund, A., Lindvall, O., Piccini, P., 2010. Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. Sci Transl Med 2, 38ra46.

Postuma, R.B., Lang, A.E., Munhoz, R.P., Charland, K., Pelletier, A., Moscovich, M., Filla, L., Zanatta, D., Rios Romenets, S., Altman, R., Chuang, R., Shah, B., 2012. Caffeine for treatment of Parkinson disease: a randomized controlled trial. Neurology 79, 651–658.

Rascol, O., Fitzer-Attas, C.J., Hauser, R., Jankovic, J., Lang, A., Langston, J.W., Melamed, E., Poewe, W., Stocchi, F., Tolosa, E., Eyal, E., Weiss, Y.M., Olanow, C.W., 2011. A double-blind, delayed-start trial of rasagiline in Parkinson's disease (the ADAGIO study): prespecified and post-hoc analyses of the need for additional therapies, changes in UPDRS scores, and non-motor outcomes. Lancet Neurol 10, 415–423.

Saavedra, A., Baltazar, G., Santos, P., Carvalho, C.M., Duarte, E.P., 2006. Selective injury to dopaminergic neurons up-regulates GDNF in substantia nigra postnatal cell cultures: role of

neuron-glia crosstalk. Neurobiol. Dis. 23, 533–542.

Schapira, A.H., 1999. Science, medicine, and the future: Parkinson's disease. BMJ 318, 311–314.

Schmidt, E.R.E., Morello, F., Pasterkamp, R.J., 2012. Dissection and culture of mouse dopaminergic and striatal explants in three-dimensional collagen matrix assays. J Vis Exp.

Smidt, M.P., Asbreuk, C.H., Cox, J.J., Chen, H., Johnson, R.L., Burbach, J.P., 2000. A second independent pathway for development of mesencephalic dopaminergic neurons requires Lmx1b. Nat. Neurosci. 3, 337–341.

Stack, E.C., Ferro, J.L., Kim, J., Del Signore, S.J., Goodrich, S., Matson, S., Hunt, B.B., Cormier, K., Smith, K., Matson, W.R., Ryu, H., Ferrante, R.J., 2008. Therapeutic attenuation of mitochondrial dysfunction and oxidative stress in neurotoxin models of Parkinson's disease. Biochim. Biophys. Acta 1782, 151–162.

Swanson, R.A., Ying, W., Kauppinen, T.M., 2004. Astrocyte influences on ischemic neuronal death. Current molecular medicine 4, 193–205.

Tremblay, R.G., Sikorska, M., Sandhu, J.K., Lanthier, P., Ribecco-Lutkiewicz, M., Bani-Yaghoub, M., 2010. Differentiation of mouse Neuro 2A cells into dopamine neurons. Journal of neuroscience methods 186, 60–67.

van Praag, H., Kempermann, G., Gage, F.H., 1999. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat. Neurosci. 2, 266–270.

Van Raamsdonk, J.M., Murphy, Z., Slow, E.J., Leavitt, B.R., Hayden, M.R., 2005. Selective degeneration and nuclear localization of mutant huntingtin in the YAC128 mouse model of Huntington disease. Hum. Mol. Genet. 14, 3823–3835.

Wang, H.-P., Hsu, T.-C., Hsu, G.-J., Li, S.-L., Tzang, B.-S., 2009. Cystamine attenuates the expressions of NOS- and TLR-associated molecules in the brain of NZB/W F1 mice. Eur. J. Pharmacol. 607, 102–106.

Wang, X., Sarkar, A., Cicchetti, F., Yu, M., Zhu, A., Jokivarsi, K., Saint-Pierre, M., Brownell, A.L., 2005. Cerebral PET imaging and histological evidence of transglutaminase inhibitor cystamine induced neuroprotection in transgenic R6/2 mouse model of Huntington's disease. J. Neurol. Sci. 231, 57–66.

Yan, C.H., Levesque, M., Claxton, S., Johnson, R.L., Ang, S.-L., 2011. Lmx1a and lmx1b function cooperatively to regulate proliferation, specification, and differentiation of midbrain dopaminergic progenitors. J. Neurosci. 31, 12413–12425.

RP103 for Huntington's disease [Internet]. [cited 2015 Jan 30] Available from:

http://www.raptorpharma.com/pipeline/rp103-dr-cysteamine-for-huntingtons-disease

NPF statement about halting of creatine study [Internet]. Parkinson.org; 2013. [cited 2015 Jan 30] Available from: http://www.parkinson.org/About-Us/Press-Room/Press-Releases/2013/September/NPF-Statement-About-Halting-of-Creatine-Study
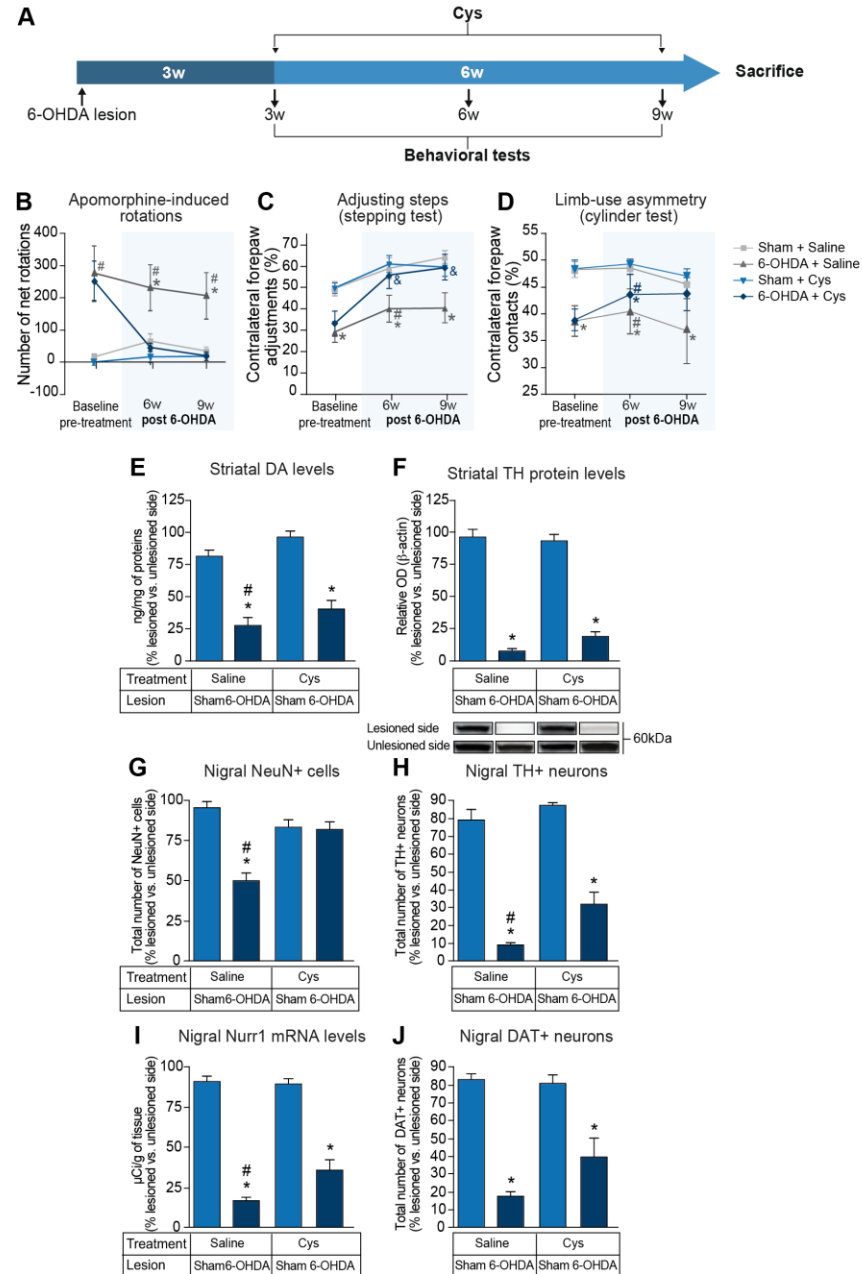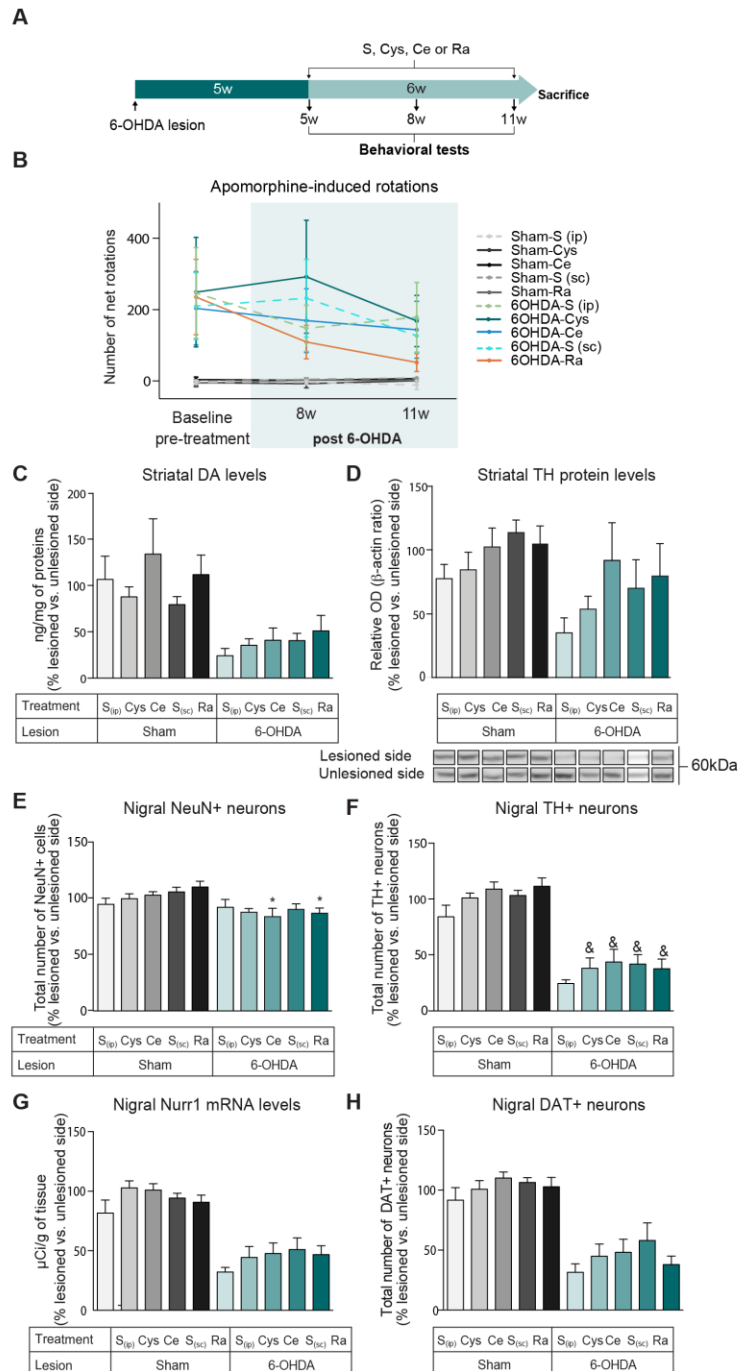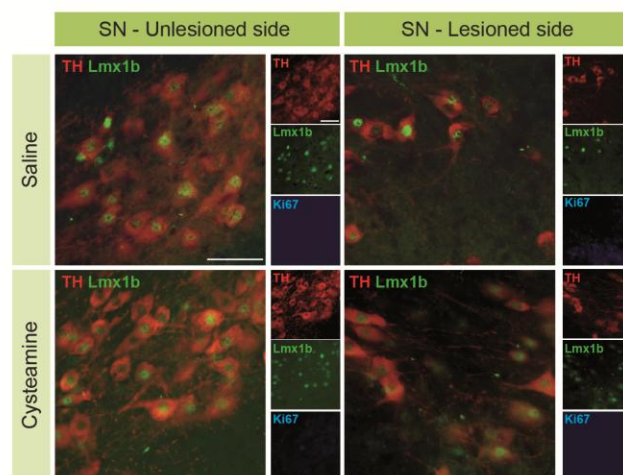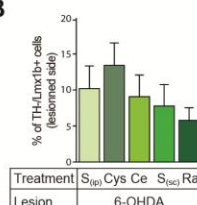
Figure 1.

Figure 2.

Figure 3.

A

B

C

Control - dentate gyrus

Figure 4.

A Neurite analysis

B Sholl analysis

Analyses of TH+ neurite sprouting

Figure 5.

Figure 6.

A Neuronal BDNF levels
B Glial BDNF levels
C Nigral BDNF mRNA expression
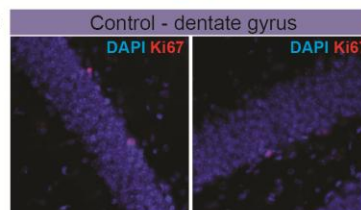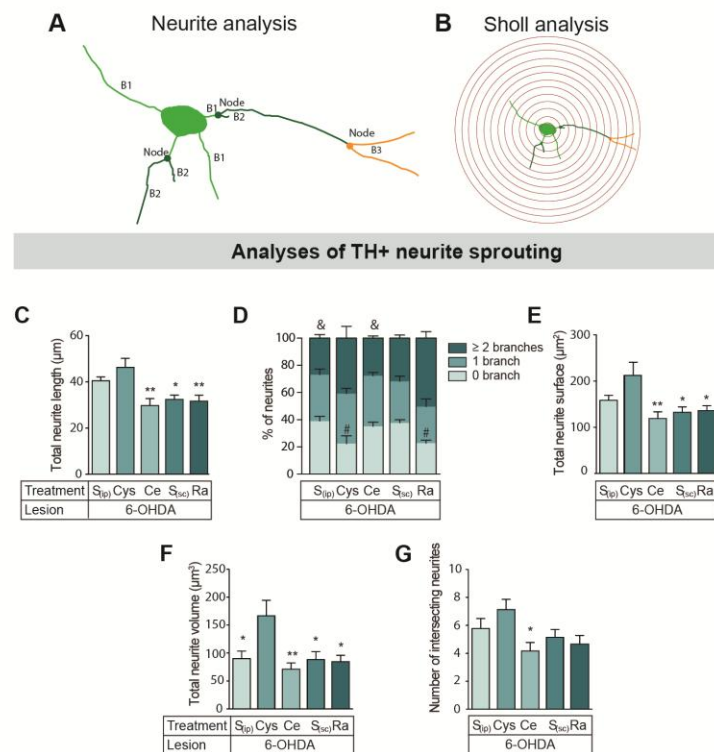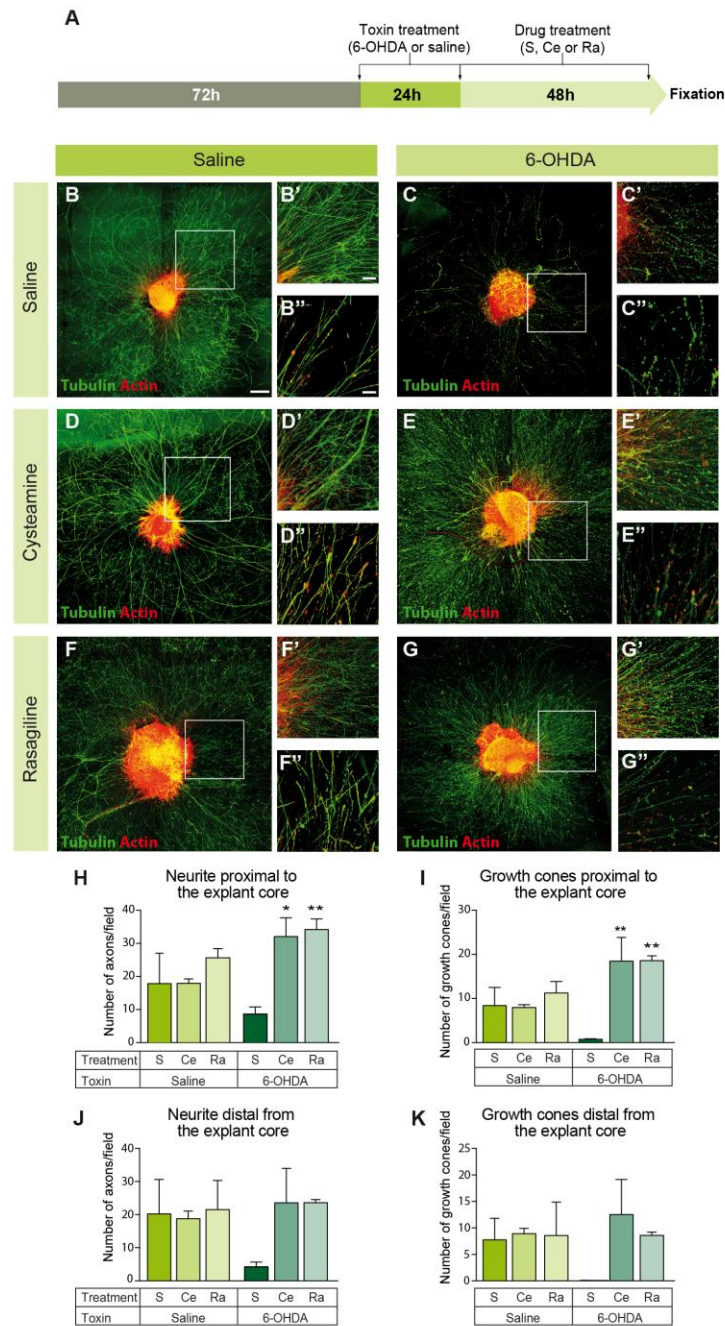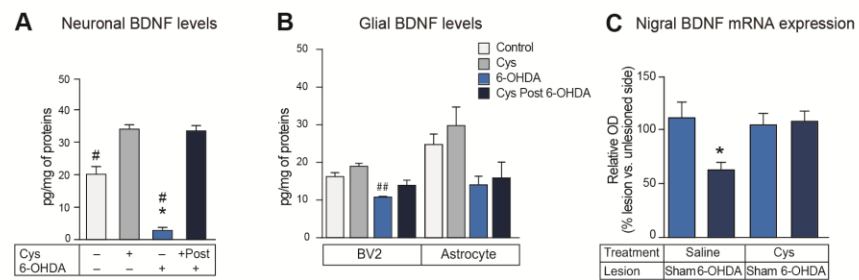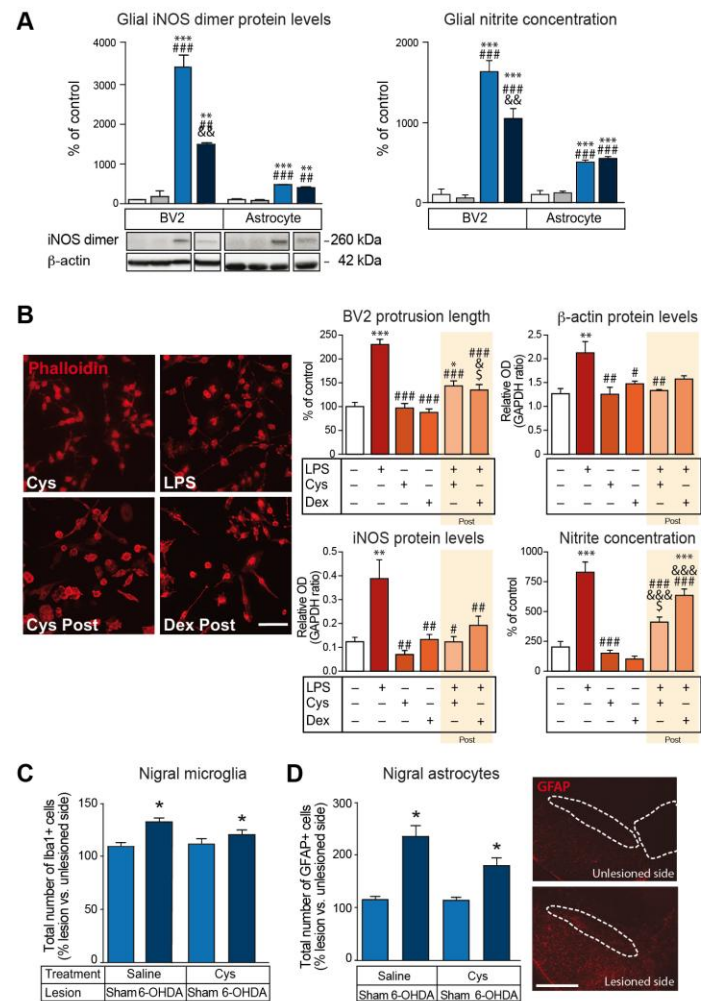
Figure 7.

Highlights

- Cystamine rescues dopamine cells and reverses motor deficits following 6-OHDA.

- Cystamine/cysteamine further show neurorestorative properties.

- Cystamine/cysteamine increase neurite arborization of surviving dopaminergic neurons.

- Cystamine/cysteamine exhibit disease-modifying effects warranting clinical trials.