Comparison of the behavioural and histological characteristics of the 6-OHDA and α-synuclein rat models of Parkinson's disease

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A R T I C L E   I N F O

Article history:
Received 7 January 2012
Revised 12 February 2012
Accepted 20 February 2012
Available online xxxx

Keywords:
Behavioural test
Neurodegeneration
Axonopathy
AAV
Animal models

A B S T R A C T

Development of relevant models of Parkinson’s disease (PD) is essential for a better understanding of the pathological processes underlying the human disease and for the evaluation of promising targets for therapeutic intervention. To date, most pre-clinical studies have been performed in the well-established rodent and non-human primate models using injection of 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Overexpression of the disease-causing protein α-synuclein (α-syn), using adeno-associated viral (AAV) vectors, has provided a novel model that recapitulates many features of the human disease. In the present study we compared the AAV-α-syn rat model with models where the nigro-striatal pathway is lesioned by injection of 6-OHDA in the striatum (partial lesion) or the medial forebrain bundle (full lesion). Examination of the behavioural changes over time revealed a different progression and magnitude of the motor impairment. Interestingly, dopamine (DA) neuron loss is prominent in both the toxin and the AAV-α-syn models. However, α-syn overexpressing animals were seen to exhibit less cell and terminal loss for an equivalent level of motor abnormalities. Prominent and persistent axonal pathology is only observed in the α-syn rat model. We suggest that, while neuronal and terminal loss mainly accounts for the behavioural impairment in the toxin-based model, similar motor deficits result from the combination of cell death and dysfunction of the remaining nigro-striatal neurons in the AAV-α-syn model. While the two models have been developed to mimic DA neuron deficiency, they differ in their temporal and neuropathological characteristics, and replicate different aspects of the pathophysiology of the human disease. This study suggests that the AAV-α-syn model replicates the human pathology more closely than either of the other two 6-OHDA lesion models.

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Introduction

Generation of predictable and relevant models for the human neurodegenerative disorders is crucial for a better understanding of the pathophysiology of the disease and the development of efficient therapies. The progressive loss of nigral dopamine (DA) neurons and the presence of α-synuclein (α-syn)-positive inclusions are characteristic features that define Parkinson’s disease (PD) (Spillantini et al., 1997; Braak and Braak, 2000). Modeling PD for pre-clinical studies remains to date a critical challenge. While the canonical degeneration of the nigro-striatal pathway can be achieved in various ways, inducing it in a progressive and age-dependent manner in order to mimic the early pre-symptomatic and advanced symptomatic stages of the disease is more problematic. In addition, the formation of α-syn-containing aggregates in surviving nigral neurons, which is characteristic of human PD, is only observed in a limited number of models.

Among the animal models of PD, the toxin-based models, i.e. the 6-hydroxydopamine (6-OHDA) rat model and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse and primate models, have been the most extensively studied and have provided considerable insights into the physiology and function of midbrain DA neurons and the pathological process leading to their loss. Applying 6-OHDA in striatum or the medial forebrain bundle (MFB) results in different extent and development of the lesion: the striatal lesion being moderate and protracted over several weeks while the MFB lesion is severe and develops rapidly over 1–2 weeks (for review, see Cannon and Greenamyre, 2010). This degenerative process, mediated through the generation of reactive oxygen species and mitochondrial damage, however, does not replicate any of the α-syn-linked pathology seen in human PD.

More recently, the identification of several mutations causing familial forms of PD has led to the generation of novel models based on overexpression of the disease-causing protein α-syn. However, none of the transgenic mice characterised to date represent a true model of PD mainly because of the absence of profound DA neuron loss and phenotype (for review, see Magen and Chesselet, 2010). Viral vector-mediated overexpression of wild type (wt) or...
mutated human α-syn has allowed the specific targeting of the nigro-striatal system, and induces a substantial loss of DA neurons and the presence of α-syn aggregates recapitulating cardinal features of the human disease (Kirik et al., 2002; Klein et al., 2002; Lo Bianco et al., 2002; Yamada et al., 2004; Ulusoy et al., 2010; Decressac et al., 2011). We recently reported that overexpression of wt human α-syn using adeno-associated viral (AAV) vectors, where the expression of the transgene is driven by a synapsin-1 promoter and amplified by a WPRE sequence, is an efficient method to induce progressive development of spontaneous and drug-induced motor deficits associated with a pronounced DA neuron loss (Decressac et al., 2012).

In the present study, we compared the lesion of the nigro-striatal system induced by AAV-mediated overexpression of α-syn with the well-established toxin rat models induced by injection of 6-OHDA in either MFB (complete lesion) or striatum (partial lesion). Using different behavioural assessments we compared the development of motor deficits in these three models and matched the magnitude of impairment against the severity of neuronal and terminal loss assessed by histological analysis. We found that these models differ from each other and present characteristic features in terms of development of the pathology and extent of the final damage. While the motor deficit correlates well with the extent of DA neuron loss, and loss of striatal innervation in the 6-OHDA lesion models, the deficit seen in the AAV-α-syn model reflects a combination of DA neuron loss and α-syn-induced axonal pathology in the surviving neurons.

Material and methods

Production of recombinant AAV viral vector

The AAV6-α-syn vector is similar to the one we previously described (Decressac et al., 2011). The expression of the transgene is driven by the synapsin promoter and enhanced using a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). Vector production was performed as described previously (Decressac et al., 2012). Briefly, transfer plasmids carrying AAV2 ITRs coding for a human wt α-syn, downstream to a synapsin-1 promoter, were generated. The transfection into HEK 293 cells was carried out using the calcium-phosphate method, and included the packaging plasmids pDP6 encoding AAV6 capsid proteins (Zolotukhin et al., 1999; Grimm, 2002). The cells were treated with a lysis buffer (50 mM Tris, 150 mM NaCl, pH 8.4) and by performing freeze-thaw cycles in dry ice/ethanol bath. The crude lysates were purified first by ultracentrifugation (1.5 h at 35000 × g at 18 °C) in a discontinuous iodixanol gradient and the virus containing fractions were purified with ion-exchange chromatography using FPLC. Genome copy titre, as determined by real time quantitative PCR, was 3.7 × 10^{12} genome copies/ml, and 3.1 × 10^{8} gc/3 μl were injected.

Animals

Adult female Sprague Dawley rats, 225–250 g at the time of surgery, were housed two to three per cage with ad libitum access to food and water during a 12 h light/dark cycle. All procedures were approved and conducted in accordance with guidelines set by the Ethical Committee for the use of laboratory animals in the Lund–Malmö region and the European Ethical Committee (86/609 EEC).

Surgical procedure

All surgical procedures were performed under general anaesthesia using a 20:1 mixture of fentanyl citrate (Fentanyl) medetomidin hydrochloride (Dormitor) (Apoteksbolaget, Sweden) injected i.p. Rats were placed in a stereotaxic frame (Stoelting) and vector solution or 6-OHDA were injected using a 10 μl Hamilton syringe fitted with a glass capillary (outer diameter of 250 μm). Each of the three groups comprised 12 rats. Three additional rats received injections of the AAV-α-syn vector and were sacrificed at 3 weeks for assessment of α-syn expression by immunostaining.

In the AAV-α-syn group, 3 μl of the vector solution was infused at a rate of 0.2 μl/min and the needle was left in place for an additional 3 min period before it was slowly retracted. Injection was carried out unilaterally on the right side, above the substantia nigra (SN), at the following coordinates (flat skull position, as recently described by Torres et al., 2011): antero-posterior: −5.3 mm, medio-lateral: −1.7 mm, dorso-ventral: −7.2 mm below dural surface, calculated relative to bregma according to the stereotaxic atlas of Paxinos and Watson (1986).

Severe lesion of the nigro-striatal pathway was obtained by injection of 3 μl of 6-OHDA (3.5 μg/μl free base dissolved in a solution of 0.2 mg/ml L-ascorbic acid in 0.9% w/v NaCl) (Sigma) (0.2 μl/min) in the medial forebrain bundle (MFB) at the following coordinates (flat skull, as above): antero-posterior: −4.4 mm, medio-lateral: −1.1 mm, dorso-ventral: −7.8 mm.

Partial lesion of the nigro-striatal pathway was obtained by injection of 2 × 7 μl of 6-OHDA in the striatum at the following coordinates (Kirik et al., 1998): antero-posterior: +1.2 mm, medio-lateral: −2.5 mm, dorso-ventral: −5.0 mm and antero-posterior: +0.2 mm, medio-lateral: −3.8 mm, dorso-ventral: −5.0 mm (7 μg/3 μl per site; 0.2 μl/min).

Behavioural testing

Assessment of behavioural function was performed 1 week before, and 3 and 8 weeks after injection of AAV-α-syn vector or 6-OHDA using four different tests. Tests were performed in the following order:

(1) Corridor test: lateralised sensorimotor integration was measured using a task that was first established in rats by Dowd et al. (2005). The equipment and procedure were similar as described previously (Torres et al., 2008). Rats were food-restricted and maintained at 85% free-feeding bodyweight throughout habituation and testing. At the first time point, rats were habituated to the corridor by scattering sugar pellets (40 mg; TestDiet) along the floor and allowing them to freely explore for 10 min on two consecutive days prior to testing. When testing began, hungry rats were first placed in an identical, but empty, corridor and allowing them to freely explore for 5 min, before being transferred to one end of the testing corridor. The number of ipsilateral and contralateral retrievals made by each rat was counted and trials were terminated when the rat made a total of 20 retrievals or a maximum time of 5 min had elapsed. A ‘retrieval’ is defined as the animal poking its nose a pot, whether or not a pellet is eaten, and a new retrieval can only be made by investigating a new pot. Data are expressed as percentage contralateral retrievals, calculated as the number of contralateral retrievals expressed as a percentage of the total retrievals made from both sides of its body axis.

(2) Cylinder test: the rats were tested for forelimb use asymmetry by the cylinder test and for amphetamine-induced rotational behaviour, as previously described (Winkler et al., 2000). For cylinder test, rats were put in a glass cylinder, a total of 20 forepaw touches were counted and the percentage of left paw touches was determined.

(3) Stepping test: the animals were tested for forelimb akinesia using the stepping test as described earlier (Kirik et al., 2001). On the 2 days preceding the test, the animals were handled by the experimenter to familiarise them with the test procedure. The test was performed on three consecutive
days by an experimenter unaware of the group identity of the tested rats. Briefly, the rat was held by the experimenter fixing both hind limbs with one hand and the forelimb not to be monitored with the other, while the unrestrained forepaw was touching the table. The number of adjusting steps was counted while the rat was moved sideways along the table surface (90 cm in 5 s), in the forehand direction. This procedure was repeated twice for each forelimb. The mean of data obtained on three testing days constituted the final dependent variable.

(4) Rotational behaviour: Analysis of rotations was performed in automated rotational bowls (AccuScan Instruments) coupled to the Rotameter software as described previously (Ungerstedt and Arbuthnott, 1970). Rats received an i.p. injection of α-aminomethanesulphate (2.5 mg/kg) (Apoteksbolaget, Sweden). Right and left full body turns were recorded over a period of 90 min. Data are expressed as net full turns per minute, with turns ipsilateral to the injection side being assigned a positive value.

Tissue processing and immunohistochemistry

All animals were sacrificed 30 min after the last rotation test, i.e. 2 h after amphetamine injections, to measure c-Fos activation in the striatum. The rats were deeply anaesthetised with 1.2 ml sodium pentobarbital i.p. (Apoteksbolaget, Sweden) and then perfused through the ascending aorta with 50 ml saline (0.9% w/v) at room temperature, followed by 250 ml ice-cold paraformaldehyde (4% w/v in 0.1 M phosphate buffered saline). The brains were removed, post-fixed for 2 h in 4% paraformaldehyde and cryoprotected overnight in sucrose (25% w/v in 0.1 M phosphate buffered saline) before being sectioned on a freezing microtome (Leica). Coronal sections were collected in 6 series at a thickness of 35 μm.

Immunohistochemical stainings were performed on free-floating sections using antibodies raised against tyrosine hydroxylase (TH) (rabbit, 1:1500; Chemicon), vesicular monoamine transporter-2 (VMAT) (rabbit, 1:5000; Abcam), dopamine transporter (DAT) (rat, 1:1000, Chemicon), c-Fos (rabbit, 1:1000, Calbiochem), synaptophysin (mouse, 1:5000, Chemicon) and human alpha-synuclein (mouse, 1:5000; courtesy of Dr. Virginia M. Lee, University of Pennsylvania). Sections were rinsed three times in potassium-phosphate buffer (KPBS) between each incubation period. All incubation solutions contained 0.25% Triton X-100 in KPBS. The sections were quenched for 10 min in 3% H2O2/10% methanol. One hour of pre-incubation with 5% normal goat serum, normal horse serum or normal rabbit serum was followed by incubation overnight with the primary antibody in 2% serum at room temperature and incubation with 1:200 dilution of biotinylated goat anti-rabbit antibody, rabbit anti-rat or horse anti-mouse (Vector Laboratories, Burlingame, CA), followed with avidin–biotin–peroxidase complex (ABC Elite; Vector Laboratories, Burlingame, CA), and visualised using 3,3-diaminobenzidine (DAB) as a chromogen, mounted and coverslipped using the DPX mounting medium.

For the c-Fos immunostaining, no quenching step was performed, the procedure was done in Tris buffer (TBS 0.1 M), and a solution of DAB-Ni (0.1%) was used for the revelation.

Cell counting and optical densitometry analysis

Assessment of the total number of TH+ and VMAT-2+ neurons in the SN was made according to the optical fractionator principle, using the Olympus Denmark A/S (Alberstlund, Denmark) CAST-Grid system, as described (Decressac et al., 2011). Every 6th section covering the entire extent of the SN was included in the counting procedure. A coefficient of error of <0.10 was accepted.

Striatal TH+ and DAT+ fibre density was measured by densitometry at four coronal levels (+1.2, 0.8, 0.00 and −0.4 mm relative to bregma) using the ImageJ software (Version 1.32j, National Institutes of Health, USA). The measured values were corrected for non-specific background staining by subtracting values obtained from the cortex (Decressac et al., 2011). The data are expressed as a percentage of the corresponding area from the intact side.

Quantification of c-Fos immunoreactivity

High-resolution images were captured from two sections, at +0.20 and −0.30 mm from bregma, using a ScanScope GL system with ImageScope v8.2 software. A 0.8 × 0.8 mm2 area from the medial, lateral and central part of the striatum was selected for analysis using the ImageJ software. The intense staining of c-Fos+ nuclei and low background allowed for software-automated calculation of the total number of c-Fos+ cells based on optical density upon defining the threshold for specific signal (Grealish et al., 2010).

Statistical analysis

All statistics were conducted using the GraphPad Prism software (version 5.0). Interaction time × treatment was determined by a 2-way ANOVA analysis. One-way ANOVA analysis followed by Bonferroni post hoc test was used to determine inter-group and intra-group differences. All values are presented as mean ± standard error of mean. Difference in the number of c-Fos+ cells between sides was determined by a Student t-test. Correlation analysis was performed using non-linear (one-phase decay) regressions. Statistical significance was set at P<0.05.

Results

Transgene expression following AAV-vector injection

We first examined the expression of α-syn in the nigro-striatal system at 3 weeks after AAV vector injection. Histological analysis showed that overexpression of wt human α-syn resulted in strong immunoreactivity on the injected side of the midbrain (SN pars compacta and reticulata, and VTA) (Fig. 1A), and no staining was observed on the contralateral side. High levels of expression were also seen in axons along the nigro-striatal pathway, and in the terminals in the ipsilateral striatum, indicating that the proteins produced in the nigral DA neurons were actively transported anterogradely towards the terminals (Fig. 1A). Double immunofluorescent staining specific for human α-syn confirmed that the vast majority of the TH+ or VMAT-2+ DA neurons in the SN (>90%) were transduced (Figs. 1B–D).

Behavioural changes following 6-OHDA injection or α-syn overexpression

Since behavioural deficits are known to develop differently between the models we used here, we assessed motor function 1 week before, and 3 and 8 weeks after surgery. Four different tests we performed to evaluate various aspects of behavioural impairment. Akinesia and forelimb use were examined using the cylinder and stepping tests. Drug-induced rotational behaviour was assessed after amphetamine injection, and sensorimotor integration was studied using the corrotor test.

Two-way ANOVA analysis revealed that all three lesions had an effect on behavioural performance over time [F4,66 =10.12 for corrotor test, F4,66 =11.3 for cylinder, F4,66 =15.94 for stepping test, and F4,66 =20.85 for rotations test; time × treatment interaction] (Figs. 2A–D). We found that MFB injection of 6-OHDA leads to a rapid and profound impairment in all tests as soon as 3 weeks...
(16.1±3.0% in corridor, 16.9±2.7% in cylinder, 2.0±0.6 steps in the stepping test and 7.9±0.6 turns/min in the rotation test) compared to the pre-test (51.5±3.4% in corridor, 48.3±3.6% in cylinder, 10.4±0.4 steps in the stepping test, and 0.9±0.9 turns/min in the rotation test; all P<0.001). This pronounced deficit was maintained at 8 weeks (11.0±4.4% in corridor, 8.3±1.7% in cylinder, 1.1±0.5 steps in the stepping test, and 9.8±0.6 turns/min in the rotation test; all P<0.001) ([black circles in Figs. 2A–D]).

Partial lesion of the nigro-striatal system induced by intra-striatal injection of 6-OHDA (2×7 μg) led at 3 weeks to a significant deficit only in the stepping test (6.7±0.9 vs 9.3±0.8 steps in the pre-test; P<0.05), while no impairment was observed in the other tests at this early time-point (39.2±3.8% in corridor, 39.0±3.5 in cylinder, and 2.9±1.0 turns/min in the rotation test; vs. 48.7±3.6% in corridor, 47.0±5.2% in cylinder and 0.6±1.1 turns/min in the pre-test; all P>0.05). At 8 weeks, significant reduction in motor performance was seen in the corridor test (31.2±4.2%; P<0.05) and stepping test (6.1±0.7 steps; P<0.05), while the score recorded in the cylinder test (33.9±6.0%) and the rotation test (3.7±1.4 turns/min) were not statistically different from the pre-test (all P>0.05) ([grey circles in Figs. 2A–D]).

Overexpression of α-syn in nigral DA neurons resulted in decreased performance in the stepping test at 3 weeks (5.8±0.7 vs. 9.2±0.7 steps in the pre-test; P<0.05), while no impairment was seen in the other tests at this time-point (44.1±3.0% in corridor, 45.0±2.3 in cylinder, and 1.6±0.7 turns/min in rotation test; all P>0.05) ([pre-test: 47.2±4.2% in corridor, 51.2±2.6% in cylinder and 0.2±0.6 turns/min in rotation test]). Eight weeks after vector
injection, significant motor deficits had developed in 3 of the 4 tests (29.3 ± 4.2% in cylinder, P < 0.05; 2.9 ± 0.6 steps in the stepping test, P < 0.001; and 6.1 ± 0.9 turns/min in the rotation test; P < 0.01 compared to pre-test). Difference in the corridor test remained non-significant (36.6 ± 3.7%; P > 0.05 compared to pre-test) (open circles in Figs. 2A–D).

When comparing the groups at the 2 time-points post surgery, no statistical difference was found between the AAV-α-syn and striatal 6-OHDA groups in all tests (all P > 0.05) except in the stepping test performed at 8 weeks (P < 0.05). Conversely, behavioural scores from the MFB 6-OHDA group was significantly different in all tests (all P < 0.05) compared to the α-syn overexpressing and striatal 6-OHDA group, except in the stepping test at 8 weeks when compared to the AAV-α-syn group (P > 0.05) (Figs. 2A–D).

Cell loss and striatal fibre degeneration in the three models

The animals were sacrificed at 8 weeks after AAV-α-syn or 6-OHDA injection, midbrain sections were stained for TH or VMAT-2 and forebrain sections for TH or DAT. Histological analysis revealed a marked loss of nigral DA neurons and their terminals on the side of injection compared to intact side, but to a different extent depending on the lesion type (Figs. 3A and B). Using a stereological approach, we estimated the number of TH+ and VMAT-2+ nigral neurons and compared it to that recorded from the contralateral intact side. Quantifications revealed that all lesion methods induced a pronounced loss of TH+ and VMAT-2+ neurons in the SN [F$_{2,33}$ = 15.33 for TH+ cells, F$_{2,33}$ = 15.87 for VMAT-2+ cells, P < 0.001]. Post hoc analysis indicated a severe loss of nigral neurons 8 weeks after injection of 6-OHDA in the MFB (6.4 ± 1.2% for TH+ cells and 6.5 ± 1.1% for VMAT-2+ cells; P < 0.001 compared to intact side) (Figs. 3C and D). The lesion was less severe when 6-OHDA was delivered in the striatum (30.8 ± 5.0% for TH+ cells and 32.0 ± 5.6% for VMAT-2+ cells; P < 0.001 compared to intact side) (P < 0.001 for 6-OHDA MFB compared to 6-OHDA Str: both markers). Overexpression of wt human α-syn induced a loss of nigral neurons comparable to that obtained with 6-OHDA in the striatum (32.6 ± 3.8% for TH+ cells and 33.5 ± 3.1% for VMAT-2+ cells; P < 0.001) (P < 0.0001 for AAV-α-syn compared to 6-OHDA MFB for both markers; for AAV-α-syn compared to 6-OHDA Str: P = 0.78 for TH+ cells; P = 0.82 VMAT-2+ cells) (Fig. 3C).

Degeneration of striatal DA fibres was assessed at 8 weeks by optical densitometry of TH and DAT immunostained forebrain sections (Figs. 3A and B). Similar to the stereological cell counts, ANOVA analysis revealed a significant effect of lesion type on the density of surviving DA terminals [F$_{2,33}$ = 15.26 for TH+ fibres, F$_{2,33}$ = 13.42 for DAT+ fibres, P < 0.001] (Figs. 3C and D). Post hoc analysis revealed a severe loss of terminals when 6-OHDA was injected in the MFB (6.5 ± 0.9% for TH+ fibres; 6.4 ± 1.3% for DAT+ fibres; P < 0.001 compared to intact side), while striatal 6-OHDA injection and α-syn overexpression induced a more moderate loss of striatal fibres (striatal 6-OHDA: 30.4 ± 5.1% for TH+ fibres; 29.1 ± 4.9% for DAT+ fibres; P < 0.001 compared to intact side; and AAV-α-syn: 32.3 ± 3.7% for TH+ fibres; 33.4 ± 4.5% for DAT+ fibres; P < 0.001 compared to intact side). The lesion induced by injection of 6-OHDA in the MFB was significantly more damaging than the two

Fig. 3. Stereological counting of dopamine neurons in the SN. A and B: Immunolabelling of striatal TH+ fibres (A) and midbrain TH+ neurons (B) 8 weeks after 6-OHDA in MFB, 6-OHDA in striatum or AAV-α-syn injection in SN. A marked reduction in striatal fibre density and nigral DA neurons is observed on the side of injection (right) in all groups. Scale bar: 2 mm (A and B). C: Quantification of the number of TH+ and VMAT-2+ neurons in the SN by stereology. D: Quantification of striatal TH+ and DAT+ fibre immunoreactivity 8 weeks after 6-OHDA or AAV-α-syn injection. Data are presented as percentage of neurons (C) and percentage of striatal fibre immunoreactivity (D) compared to intact side, and expressed as mean ± SEM. *P < 0.05 compared to intact side; **P < 0.01 compared to intact side; ***P < 0.001 compared to intact side; ^P < 0.0001 compared to intact side; #P < 0.05 compared to 6-OHDA MFB group (1-way ANOVA followed by Bonferroni post hoc test).
other methods (P<0.001), while no difference was observed between 6-OHDA injection in the striatum and AAV-α-syn delivery in the SN (P=0.76 for TH+ fibres and P=0.53 for DAT+ fibres) (Fig. 3C).

Correlations and impairment thresholds

First we analysed the correlations between the behavioural performances and the loss of DA neurons (Figs. 4A-D) or striatal terminals (Figs. 4E-H) using non-linear regression analysis since these parameters are known to correlate only when a certain threshold of damage is achieved. We found that the extent of the cell and terminal loss correlated well with motor function in the two neurotoxin models (ranging from 0.83<r<0.91; partial and full lesion taken as a single group for 6-OHDA-induced lesion) (open squares and circles). A lower but still significant correlation was also found between these parameters in the AAV-α-syn model (ranging from 0.71<r<0.86) (black triangles).

Secondly, we defined a threshold score of significant motor impairment for each of the four tests: ≤35% in corridor, ≤35% in cylinder, ≤4 steps in the stepping test, and ≥6 turns/min in the rotation test. Having defined these cut-offs, we determined at which percentage of TH+ cell and fibre loss these performance thresholds were reached in the 6-OHDA model (partial and full lesion taken as a single group) and the AAV-α-syn model. We found that the minimum damage necessary to obtain motor impairment is markedly different between the 6-OHDA and α-syn overexpression models. In the 6-OHDA lesioned animals, projections revealed that a behavioural deficit is observed in the four tests when about 20% of TH+ neurons and fibres remained (red dashed lines in Fig. 4).

In the AAV-α-syn model, similar deficits were obtained when 30–35% of the TH+ neurons and 35–40% of the TH+ fibres survived (blue dashed lines in Fig. 4).

Thirdly, we compared the behavioural scores obtained in the animals representing the median value of TH+ cell and fibre loss in the AAV-α-syn treated animals and in the intra-striatal 6-OHDA lesion group (the MFB 6-OHDA lesioned animals were excluded from this analysis since all animals were impaired in all tests). The median values of cell and fibre loss were similar, close to 70% (i.e. 30% remaining) in both groups. In all four tests, the animals displaying this level of cell and fibre loss (denoted by the encircled yellow areas in Fig. 4) exhibited a more pronounced motor deficit in the AAV-α-syn group compared to the animals in the partial 6-OHDA lesion group. Thus, the animals with a 70% TH+ cell loss in the AAV-α-syn group scored 6–7 turns/min in the amphetamine rotation test and 3 steps in the stepping test, compared to 3 turns/min and 6–7 steps in the 6-OHDA group (Figs. 4C and D). These observations suggest that, in addition to cell death and axonal degeneration, another factor contributes to the functional impairment of the nigro-striatal pathway in the model of α-syn overexpression.

Characterisation of cell and axonal pathology

The presence of α-syn-containing inclusions in the cell bodies and axonal terminals of surviving nigral DA neurons, i.e. Lewy bodies and neurites, is a major histological hallmark of human PD (Spillantini et al., 1997; Braak and Braak, 2000). We investigated whether we could detect signs of axonopathy in these animal models. Previous studies have revealed a pronounced α-synucleinopathy in the AAV-α-syn model (Kirik et al., 2002; Chung et al., 2009; Decressac et al., 2012).

Consistent with these previous findings, we detected TH+ and α-syn+ swellings of different sizes (ranging from 3–70 μm) in the striatal fibres and accumulation of beaded structures in chains in single axonal terminals suggesting a defect in axonal transport. These structures were prominent not only in the severely denervated medial part, but also in the lateral part where α-syn expressing axons and terminals remained in large numbers (see Figs. 3C–F). Some of them are also immunoreactive for VMAT-2 and synaptophysin suggesting an effect on synaptic function. None of these structures were seen in the control striatum (insert in Fig. 5C). Similar signs of pathology were observed in the dendrites in the SN pars reticulata (Figs. 5J–K) as well as in nigral DA neurons in the SN pars compacta (Figs. 5L–N).

In the partial 6-OHDA lesion model, TH immunostaining also revealed signs of cellular stress. Within the denervated, dorsolateral part of the striatum surviving axonal terminals appeared distorted and truncated compared to the intact control striatum, while no aggregated structures were observed (Figs. 5A and B). By contrast, the TH+ innervation seen in the spared parts of the striatum, medially and ventrally as illustrated in Fig. 3A, appeared totally unaffected.

Evaluation of post-synaptic activity in striatal target neurons

Depletion of the nigro-striatal pathway induces the development of compensatory events in the post-synaptic striatal targets, reflecting the development of hyper-sensitisation of DA receptors that translates into increased post-synaptic activity (Cenci et al., 1992). Here, we used immunohistochemistry of the immediate-early gene product c-Fos to monitor the extent of these post-synaptic changes in the three lesion models. At 8 weeks, rats were sacrificed 30 min after completion of the 90 min rotation test, i.e. 2 h after injection of 2.5 mg/kg amphetamine, and striatal c-Fos expression was analysed by immunohistochemistry (Fig. 6A). In this test, the activation of c-Fos in the striatal target neurons is caused by the release of DA from the remaining nigro-striatal terminals, acting on supersensitive post-synaptic receptors.

As shown in Fig. 6, the reduction in amphetamine-induced c-Fos expressing cells was about twice as large in the 6-OHDA MFB lesioned rats (Figs. 6B and D) as in the striatal 6-OHDA lesioned ones (Figs. 6B and E) (90±11 cells for MFB 6-OHDA; 263±28 cells for striatal 6-OHDA), as compared to contralateral side (Figs. 6B and C) (439±15 cells for MFB 6-OHDA; 489±18 cells for striatal 6-OHDA; P<0.001). In the AAV-α-syn model, the reduction in the number of c-Fos+ cells was intermediate between the two 6-OHDA lesion types (Figs. 6B, F) (186±29 cells) (intact side: 486±13 cells; P<0.001). This decrease was significantly different from the 6-OHDA MFB group (P<0.05) but not from the 6-OHDA Str group (P>0.05).

Discussion

In the present study, we report that the 6-OHDA and the AAV-α-syn models of PD have distinct characteristics. As described previously, injection of 6-OHDA into the MFB results in a rapid and profound loss of the nigro-striatal DA neurons accompanied by severe motor deficits and post-synaptic modifications. Delivery of the toxin into the striatum leads to more moderate changes in motor behaviour despite a significant damage to the dopaminergic pathway.

Fig. 4. Correlations between dopaminergic parameters and behavioural scores. A–D: Scatter-plots showing the correlation between the percentage of TH+ nigral neurons (A–D) or percentage of striatal TH+ fibres (E–H) and the scores in corridor test (A and E), cylinder test (B and F), stepping test (C and G), and the number of amphetamine-induced rotations (D and H). Continuous grey line in corridor, cylinder and stepping tests illustrate normal performance. Hatched lines mark the selected threshold for behavioural impairment (≤35% in corridor test, ≥35% in cylinder test, ≤4 steps in the stepping test and ≥6 turns/min in the amphetamine-induced rotations test), Red (6-OHDA-induced lesion) and blue (α-syn-induced lesion) vertical hatched lines indicate the threshold of surviving DA neurons and fibres under which behavioural impairment was observed. Symbols encircled by the yellow area represent the median values of TH+ cell and fibre loss for the striatal 6-OHDA and the AAV-α-syn models.

Please cite this article as: Decressac, M., et al., Comparison of the behavioural and histological characteristics of the 6-OHDA and α-synuclein rat models of Parkinson’s disease, Exp. Neurol. (2012), doi:10.1016/j.expneurol.2012.02.012
Overexpression of human wt α-syn induces a DA neuron cell loss that resembles that observed in the intrastriatal 6-OHDA model. The overall behavioural impairment, however, is more profound and closer to that obtained in the MFB 6-OHDA full lesion model. In addition, the AAV-α-syn model showed a more progressive development of the behavioural deficits that occurred over several weeks, being non-significant to moderate after 3 weeks and well established after 8 weeks for 3 of the 4 tests performed.

This difference in disease progression reflects the nature of the toxic insult. It is well known that 6-OHDA mediates its toxic effect mainly through the perturbation of mitochondrial function, leading to high oxidative stress levels in the DA neurons (for review, see Cannon and Greenamyre, 2010). In contrast, α-syn toxicity is known to result from a progressive build-up of toxic oligomers and a combination of several secondary phenomena, including inflammatory reaction (Chung et al., 2009), impairment of proteasome and lysosomal systems (Ebrahimi-Fakhari et al., 2011), ER-Golgi traffic (Cooper et al., 2006) and synaptic function (for review, see Venda et al., 2010). In the AAV-α-syn model, neuronal degeneration is preceded by a pre-symptomatic phase where DA neurons survive but present severe signs of axonal damage and synaptic dysfunction, such as impairment in DA release and re-uptake (Lundblad et al., 2012;...
Decressac et al., in press). At early stages (3 weeks), therefore, the behavioural impairment seen in the AAV-α-syn model is primarily caused by dysfunction of still surviving neurons, and at later stages (8 weeks), when neurodegeneration is complete, the magnitude of impairment reflects a combination of DA neuron cell loss and dysfunction in surviving neurons affected by α-syn induced axonopathy (Decressac et al., in press).

The loss of DA neurons and striatal TH+ innervation induced by the intra-striatal 6-OHDA lesion used here (2 × 7 μg) were, on average, similar in magnitude to that seen in the AAV-α-syn treated animals. Analysis of the scatter plots in Fig. 4 revealed an interesting difference between the 6-OHDA and the AAV-α-syn models: in all four behavioural tests, the threshold of significant impairment is reached with a greater number (about 15–20%) of surviving DA neurons and terminals in the AAV-α-syn model compared to the toxin model. Likewise, for a similar magnitude of cell loss, α-syn overexpressing animals displayed more severe motor alterations. These observations highlight the difference between these two models that results from the nature of the damage: in the 6-OHDA model the loss of dopaminergic innervation, and in the AAV-α-syn model a combination of cell loss and prominent axonopathy. As illustrated schematically in Fig. 7, this will result in a shift of the cell-loss-vs.-impairment curve, such that the motor dysfunction seen in the AAV-α-syn model will start to appear at a lower level of DA neuron cell loss. The difference, estimated at about 15–20% in the present experiment, is likely to be due to α-syn induced axonal and/or synaptic dysfunction in the surviving neurons. In a previous study (Lundblad et al., in press) we have shown that storage, release and re-uptake of DA in the striatum are markedly impaired in α-syn overexpressing DA neurons, suggesting that α-syn induced axonal damage leads to impaired synaptic DA function. This is further supported by the observation that the postsynaptic activation of c-Fos induced by injection of amphetamine, was as impaired in the AAV-α-syn treated animals as in the animals that received 6-OHDA in the MFB, despite a significant difference in spared striatal innervation.

The combination of cell loss and axonopathy seen in the AAV-α-syn model is reminiscent of the changes seen in human PD. PD is classically described as a neurodegenerative disease clinically diagnosed by the cardinal motor symptoms when about 30–50% of the nigral DA neurons and 60–70% of the striatal terminals are lost, as assessed by post-mortem analyses and imaging techniques (Cheng et al., 2010). Following this definition, our study suggests that the AAV-α-syn model replicates the human pathology more closely than either of the two 6-OHDA lesion models. The α-synucleinopathy seen in the AAV-α-syn model at early and late stages after vector injection is similar to that seen in PD patients, i.e. Lewy bodies and Lewy neurites, suggesting that the surviving neurons remain in a dysfunctional state. This is an interesting feature of the AAV-α-syn model which makes it particularly useful for studies of pathological mechanisms that are difficult to investigate in any of the available neurotoxin models.

The use of viral vectors to overexpress the disease causing-protein is advantageous in that it can be applied in a wide range of species,
including both rodents and non-human primates, and when administered unilaterally, it produces a progressive unilateral degeneration of the nigro-striatal system allowing for internal comparison with the contralateral intact side (Kirik et al., 2002; Klein et al., 2002; Lo Bianco et al., 2002; Yamada et al., 2004; Decressac et al., 2012).

Another advantage of the AAV-α-syn model for preclinical testing is the progressive nature of the model that allows for therapeutic interventions at different stages of the degenerative process. Some specific features, such as axonopathy and synaptic dysfunction, are likely to be important elements of the human disease that can be explored individually in the AAV-α-syn model.

The AAV-α-syn model offers an interesting alternative and complement to the common and widely used toxin-based models. While 6-OHDA and MPTP model aspects of free radical damage and mitochondrial function, the damage induced by α-syn overexpression involves also other pathogenic mechanisms, such as production of toxic oligomers, impaired protein degradation and ER-Golgi trafficking (Venda et al., 2010). For this reason it is possible that agents that have proved efficacious in toxin models may fail to provide benefit in α-syn overexpression models, due to, for example, impaired axonal transport or down-regulation of synaptic transmission or signalling pathways in the affected DA neurons. This may be a particular concern for studies of neuroprotective and restorative approaches that have been explored almost exclusively in toxin-based models of PD. In future studies it seems advisable to test candidate therapeutic interventions in several models that complement each other with respect to individual and interacting mechanisms underlying the pathogenesis of the human disease (Meissner et al., 2011).

Acknowledgments

The study was supported by grants from the Swedish Research Council [grant no. 604-3874 and the BAGADILICO program], the Swedish Foundation for Strategic Research, the EU 7th Framework project REPLACES, and Parkinsonfonden. The authors thank Björn Anzelius, Ulla Jarl and Michael Sparrenius for excellent technical assistance.

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