Early and progressive microstructural brain changes in mice overexpressing human α-Synuclein detected by diffusion kurtosis imaging

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Abstract

Diffusion kurtosis imaging (DKI) is sensitive in detecting α-Synuclein (α-Syn) accumulation-associated microstructural changes at late stages of the pathology in α-Syn overexpressing TNWT-61 mice. The aim of this study was to perform DKI in young TNWT-61 mice when α-Syn starts to accumulate and to compare the imaging results with an analysis of motor and memory impairment and α-Syn levels. Three-month-old (3mo) and six-month-old (6mo) mice underwent DKI scanning using the Bruker Avance 9.4 Tesla magnetic resonance imaging system. Region of interest (ROI) analyses were performed in the gray matter; tract-based spatial statistics (TBSS) analyses were performed in the white matter. In the same mice, α-Syn expression was evaluated using quantitative immunofluorescence.

Mean kurtosis (MK) was the best differentiator between TNWT-61 mice and wildtype (WT) mice. We found increases in MK in 3mo TNWT-61 mice in the striatum and thalamus but not in the substantia nigra (SN), hippocampus, or sensorimotor cortex, even though the immunoreactivity of human α-Syn was similar or even higher in the latter regions. Increases in MK in the SN were detected in 6mo mice. These findings indicate that α-Syn accumulation-associated changes may start in areas with a high density of dopaminergic nerve terminals. We also found TBSS changes in white matter only at 6mo, suggesting α-Syn accumulation-associated changes start in the gray matter and later progress to the white matter.

MK is able to detect microstructural changes induced by α-Syn overexpression in TNWT-61 mice and could be a useful clinical tool for detecting early-stage Parkinson's disease in human patients.

1.0 Introduction

For the past decade, diffusion tensor imaging (DTI), which is a non-invasive magnetic resonance imaging (MRI) technique, has played an important role in detecting and quantifying neurodegeneration in Parkinson's disease (PD). It has been reported that by measuring both directionality of diffusion (fractional anisotropy, FA) and overall movement of water molecules (mean diffusion, MD), DTI might be able to differentiate PD patients from healthy controls. Several studies of DTI in early-stage PD patients have observed decreased FA and increased MD in the SN. Vaillancourt et al. reported remarkable diagnostic accuracy with DTI if the SN is accurately delineated; they also found a decrease in FA in the SN of early-stage, unmedicated PD patients (Vaillancourt *et al.* 2009). Another recent DTI imaging study found a decrease in FA in the anterior olfactory system in early-stage PD (Rolheiser *et al.* 2011). It has been reported that DTI has potential for differentiating atypical PD (i.e. multiple system atrophy and progressive supranuclear palsy) from PD and healthy controls with regional FA and diffusivity changes (Prodoehl *et al.* 2013; Schocke *et al.* 2002). However, recent clinical and pre-clinical studies with DTI have shown some ambiguities with nigral FA changes, as some reports show an increase in FA (Van Camp *et al.* 2009; Wang *et al.* 2011; Lenfeldt *et al.* 2015) and others show a decrease in FA (Vaillancourt *et al.* 2009; Boska *et al.* 2007; Soria *et al.* 2011; Cochrane and Ebmeier 2013; Zhang *et al.* 2015; Langley *et al.* 2016; Loane *et al.* 2016); some studies did not find any significant changes (Schwarz *et al.* 2013; Hikishima *et al.* 2015; Schuff *et al.* 2015). A recent study by Schuff et al. involving a large population of patients (around 220 subjects) failed to validate the sensitivity of nigral FA changes in PD despite using the same ROI approach reported by Vaillancourt (Schuff *et al.* 2015; Vaillancourt *et al.* 2009).

DTI has intrinsic drawbacks. This method presumes that water diffusion in the brain is not restricted and that it follows Gaussian distribution (Basser 1995; Pierpaoli and Basser 1996); however, the brain is a complex structure and water diffusion is restricted due to cell membranes and organelles as well as liquid compartments and it follows a non-Gaussian distribution. To overcome this limitation, diffusion kurtosis imaging (DKI) was developed. DKI is an extension of DTI for quantifying non-Gaussian diffusion (Jensen *et al.* 2005; Hui *et al.* 2008; Jensen and Helpern 2010). With DKI data, both diffusion parameters (FA, MD, axial and radial diffusion) and extra kurtosis parameters, including mean kurtosis (MK), axial kurtosis (AK), and radial kurtosis (RK), can be obtained. The dimensionless kurtosis parameters provide the microstructural information that results from restrictions to the free diffusion of water occurring due to cellular and subcellular barriers present in tissue and

calculates the degree of diffusion restriction. Higher kurtosis values suggest increased tissue complexity or greater hindrance to the diffusion of water molecules. Unlike DTI, DKI sensitivity and specificity are not limited to the anisotropic environment; hence, DKI is able to quantify the microstructural integrity of crossing fibers in white matter and isotropic gray matter regions (Jensen *et al.* 2005; Coutu *et al.* 2014; Umesh Rudrapatna *et al.* 2015; Zhu *et al.* 2015; Guglielmetti *et al.* 2016). Both clinical and pre-clinical DKI studies in PD patients and animal models have reported changes in MK in the SN, basal ganglia, and cingulate fibers (Wang *et al.* 2011; Kamagata *et al.* 2013; Khairnar *et al.* 2015a; Khairnar *et al.* 2015b; Kamagata *et al.* 2014). Therefore, MK in the SN seems to be a better diagnostic performer than the diffusion tensor parameter FA. Zhang et al. recently reported similar results and although they found FA and MK specificity in the SN to be similar, the sensitivity of MK (94.4%) was higher than the DTI-derived FA parameter (86.1%) (Zhang *et al.* 2015).

Even though clinical studies with DTI and DKI in PD have found significant alterations in diffusivity and kurtosis parameters, no study has yet explained the mechanism behind these changes. Pre-clinical studies employing DTI in neurotoxin-based animal models of PD, known to induce neuronal loss in the SN, have thus far found only inconclusive alterations of FA (Van Camp *et al.* 2009; Boska *et al.* 2007; Soria *et al.* 2011). It is well established that other factors, such as α-Syn accumulation and glial cell activation, contribute to human PD pathology. None of the DTI or DKI studies performed to date have considered these factors.

Evidence can be provided by transgenic animal models showing α-Syn overexpression. The aim of the present study was to evaluate whether DKI is able to detect very early changes induced by α-Syn accumulation in 3mo and 6mo transgenic mice (TNWT-61) and to compare these data with the α-Syn loads evaluated by immunohistochemistry. A comprehensive behavioral profile was assessed in order to evaluate the potential age-dependent developments of the PD-like phenotype in motor performance and memory.

We used TNWT-61 mice because it is a well-established and extensively studied genetic model of PD (Masliah *et al.* 2000; Fleming *et al.* 2004; Fleming *et al.* 2008; Watson *et al.* 2012; Magen *et al.* 2012; Chesselet *et al.* 2012; Delenclos *et al.* 2014). Our group recently reported that MK is a significant marker for detecting late stage α-Syn accumulation-induced microstructural changes in this model (Khairnar *et al.* 2015a; Khairnar *et al.* 2015b). No animal model mimics all the pathological hallmarks of human PD. The TNWT-61 model shows a progressive decrease in striatal dopamine release, α-Syn accumulation, and early motor and non-motor deficits prior to typical manifestations of PD. The limitation of this model is the lack of neurodegeneration of dopaminergic neurons in the SN in young animals;

this pathology is present only in the striatum at 14mo. Another limitation is the lack of Lewy body formation and the lack of brain atrophy. Taken together, the characteristics of the TNWT-61 model make it particularly well suited for studying the early prodromal stages of PD, before major dopaminergic neuron loss occurs, and at the same time well suited for developing an innovative imaging biomarker.

2.0 Methods

2.1 Animals

Transgenic mice overexpressing human wildtype α-Synuclein under the murine Thy-1 promoter (TNWT-61) were provided by QPS Austria, where the model is bred under license. The line was developed at the University of California at San Diego (Rockenstein et al., 2002), crossed into a mixed C57BL/6-DBA/2 background, and maintained by breeding mutant females with wildtype (WT) males. Only male mice were included in the study: n=15 TNWT-61 mice and n=15 WT littermates at 3mo and the same group sizes at 6mo, i.e. 60 mice altogether. The mice were housed in sections of 1 to 3 mice at the Central Animal Facility of Masaryk University, Brno, Czech Republic, and maintained on a normal 12/12-hour light/dark cycle (lights on at 6 a.m.) with a constant relative humidity of 50-60% and temperature of 22±1ºC. Water and food were available *ad libitum*. Later they were transported to the animal house of the Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno, Czech Republic and maintained under the same conditions as in the previous location. All procedures were performed in accordance with EU Directive no. 2010/63/EU and approved by the Animal Care Committee of the Faculty of Medicine, Masaryk University, Czech Republic and the Czech Governmental Animal Care Committee, in compliance with the Czech Animal Protection Act No. 246/1992.

2.2 Behavioral assessments

The behavioral studies were not always performed in all mice in order to limit the physical strain on the transgenic mice weakened by their condition. The number of mice differs in some tests; this is indicated separately for every behavioral assessment in the Results section. All behavioral apparatuses and arenas were wiped with 1% acetic acid between the individual runs to avoid olfactory cues.

2.2.1 Locomotor activity test

Open-field test: In a brightly lit room, mice were individually tested for locomotor activity using the Actitrack system (Panlab, Spain). Each Plexiglas arena (45×45×30 cm) was surrounded by two frames equipped with photocells located one above another at 2 and 7 cm over the cage floor. The mice were placed in the center of arena and the spontaneous behavior was tracked for 30 min. In the test, horizontal locomotor activity (the trajectory calculated by the system as beam interruptions that occurred in the horizontal sensors) and vertical activity

(number of rearing episodes breaking the photocell beams of the upper frame) were recorded. At the end of the session, the mice were returned to their home cages.

2.2.2 Motor performance tests

Challenging Beam Traversal Test: Motor performance was measured with a challenging beam traversal test (Fleming *et al.* 2004; Fleming *et al.* 2006; Schintu *et al.* 2009). Briefly, the mice were trained to traverse a 1 m non-reflective gray hardened polyvinyl chloride (PVC) beam consisting of four sections (25 cm each) with different widths (3.5 cm to 0.5 cm by 1 cm decrements) leading to the mice's home cage. After a day of training, a mesh grid (1 cm squares) of corresponding width was placed over the beam surface, leaving approximately a 1 cm space between the grid and the beam surface. The mice were then videotaped while traversing the grid-surfaced beam for a total of three trials. Videotapes were manually scored in slow motion for the number of slips and the time to traverse across three trials by an experienced investigator blind to the mouse genotype. Scores were calculated across all three trials and averaged for each mouse.

Square and Round Beam Walk Tests: The beam walk tests were set up as described in a previous study (Suidan *et al.* 2013). Briefly, the mice transverse two 1 m beams raised approximately 50 cm above the surface: the first beam is 10 mm wide and square, the second beam is 16 mm wide and round. To motivate the mice to cross, the home cage was placed at the end of the beam. Each mouse was placed on the far end of the beam and allowed to cross to the home cage once.

Grid test: The inverted grid test was used to assess neuromuscular abnormalities. Mice were placed in the center of a horizontal square (12x12 cm) grid consisting of wire mesh (mesh loop of 0.5 cm^2) surrounded by non-reflective gray hardened PVC walls. The grid was placed 20 cm above a table-top and was rotated upside down, allowing the mouse to move freely. The test is performed by inverting the grid. The latency to fall off the grid is recorded with maximum cut-off duration of 60 s (Fleming *et al.* 2004; Sgado *et al.* 2011; Tillerson and Miller, 2003).

2.2.3 Memory tests

Test of spontaneous alternations in Y maze: The mice were placed in the center of a Y maze (three identical arms 5 cm wide, 25 cm long, with 120° angles between them) and their behavior was videotaped for 10 min. Later the exploratory activity was scored and the order of the arms visited by the subject was registered in triplets. The proportion of spontaneous alternations (arm A, B, and C in any order) was calculated as a percent of the total number of the triplets (Yadav *et al.* 2013; Belforte *et al.* 2010).

Novel object recognition test (NORT): The NORT was performed analogously as already described (Bevins and Besheer 2006; Leger *et al.* 2013). In brief, the mice were placed in the center of a Plexiglas arena (45×45×30 cm) with two identical objects and allowed to explore the environment for 5 min. Then they were returned to their home cage for 30 min. The arena was cleaned and one of the objects was replaced with a new one. The mouse was then allowed to explore the objects again for 5 min and videotaped. All objects were made of colored wooden bricks toys. The videos were scored by a skilled observer and the total time spent exploring each object was measured. The discrimination index was calculated as the time exploring the known object / time exploring the new object.

Barnes maze: We used an adapted Barnes maze protocol (Barnes 1979) to examine spatial learning and memory (Hall *et al.* 2015; Magen *et al.* 2012). Mice received 5 consecutive days of testing consisting of 3 trials per day, with an inter-trial interval of approximately 15 min. Prior to the first testing trial, the mice received 3 training sessions, during which they were placed in the center of the maze in a start tube and released after 10 s. After two hole-explorations, mice were gently guided to the escape hole and left in the shelter chamber for 90 s. During each test trial, the mice were placed in the center of the maze and left to spontaneously explore until they found the escape hole, with a cut-off time of 3 min, and then left in the chamber for 1 min. Over the course of the trials, the mice could use presented environmental cues to navigate to the shelter. The number of errors (the number of visits to any of the 19 non-escape holes) and the latency (time taken to locate the single target escape hole) was measured for each trial. Following the test, the mice received a single probe trial 24 h after the last test trial to assess memory retention of the shelter. During the probe trial, mice received one 90 s trial that was identical to the training trials except that the chamber was removed, thus appearing identical to the other 19 holes of the maze. The total time visiting the previous shelter location and the number of errors were recorded. All of the sessions were videotaped and later scored by an experienced researcher.

2.3 Diffusion-weighted MR data acquisition

DKI data were obtained with a Bruker Avance 9.4T MRI system equipped with a gradient system delivering up to 660 mT/m. All experiments were performed using a quadrature volume coil (inner diameter 86 mm) for transmission and a four-channel surface phased-array head coil as a receiver. Mice were anesthetized using isoflurane inhalation (1.5–2%) and monitored to maintain constant physiological parameters. Fast low angle shot (FLASH) scout images were used to localize the mouse brain. Reference T2-weighted brain scans were

acquired using the 2D RARE (rapid acquisition with relaxation enhancement) sequence with the following acquisition parameters: 24×24 mm field of view (FOV), 256×256 acquisition matrix size, and fifteen adjacent slices of 0.5 mm slice thickness. The echo-train length for each of the echoes was set to eight, and the repetition time (TR) was 2500 ms with four averages for a total acquisition time of ~6 min. For the DKI acquisition, diffusion-weighted images were acquired with two-shot spin-echo echo-planar imaging (SE-EPI). Respiratory gating was used to prevent motion artifacts. The generalized auto-calibrating partially parallel acquisitions (GRAPPA) with an acceleration factor of 2 was used to improve image quality and diminish susceptibility-caused artifacts. The DKI protocol included the acquisition of six b-values (b=0, 500, 1000, 1500, 2000, and 2500 s/mm^2) along with 30 non-collinear directions, δ=4 ms, Δ=11 ms, with seven averages used for b=0 acquisition and four averages for each other b-value. The SE-EPI sequence was recorded using the following parameters: FOV=24×24 mm, acquisition matrix = 98×128, echo time TE=25 ms using 300 kHz bandwidth and TR ~5 s depending on respiratory rate, and fifteen adjacent slices of 0.5 mm slice thickness, for a total acquisition time of approximately 100 min.

Data analysis

MRI data were converted to NIFTI from the Bruker format with a Matlab script programmed locally. Diffusion data were corrected for eddy currents and movement artifacts to the first non-diffusion-weighted image (Jenkinson and Smith 2001). The following parametric maps were calculated in ExploreDTI v4.8.4. Software (Leemans A *et al.* 2009): MK, AK, RK, MD, AD, RD, and FA using the robust estimation of tensors by outlier rejection (RESTORE) fitting method. For further data analysis, two different approaches were applied:

2.3.1 Region of interest (ROI) analysis

Averaged diffusion, FA, and kurtosis parameters were obtained from multiple regions: the SN (one slice), striatum (average of three slices), sensorimotor cortex (average of five slices), hippocampus (average of three slices), and thalamus (average of two slices). We chose these specific ROIs based on published histology results showing a substantial accumulation of α-Syn in these brain areas (Chesselet *et al.* 2012) and on our previous studies with 9mo and 14mo TNWT-61 mice (Khairnar *et al.* 2015a; Khairnar *et al.* 2015b). The ROI selection on b=0 images was drawn manually according to the mouse brain atlas (Paxinos *et al*. 2001) with the help of FA maps using ImageJ® software for various brain regions.

2.3.2 Tract-based spatial statistics (TBSS)

For white matter (WM) analysis, TBSS from FSL Software was used. Brain extraction was carried out with BET (Smith 2002), then all diffusivity maps were checked visually for voxel-

wise statistical analysis. TBSS (Smith *et al.* 2006) was implemented and modified according to the protocol for rodent brains (Sierra *et al.* 2011). All animal data were affine-registered into a common 3D space to make the comparison of affected WM tracts easier. The following steps were taken: (1) all of the individual FA maps were co-registered and the best registration target was chosen using the free-search method to find the most representative brain – this step minimized the image warping; (2) the calculated best target was used as a template for the final transformations; (3) the mean FA map was calculated with all of the maps registered to the template, and the mean FA skeleton was created at the threshold of 0.2 to represent the core of all tracts; (4) each mouse's FA data was projected onto the skeleton; (5) these steps were performed for all of the diffusivity maps using the co-registration warp-fields and tract projection information resulting from FA map processing; and (6) permutation-based non-parametric testing with 10,000 permutations was used to compare groups with multiple comparison correction, and $p<0.05$ was deemed significant. The results of the TBSS analysis were identified using the mouse brain atlas (Paxinos and Franklin 2001).

2.4 Immunohistochemistry of α-Syn and glial fibrillary acidic protein (GFAP)

Mice were anesthetized after MRI acquisition intraperitoneally with ketamine hydrochloride (100 mg/kg, Narketan®) and xylazine (10 mg/kg, Rometar®) and transcardially perfused with paraformaldehyde (4% in 0.1 M phosphate buffer, pH 7.4) for immunohistochemical studies.

2.4.1 Preparation of samples

The right-brain hemispheres of 24 mice were cryo-sectioned at 10 μm thickness (Leica CM 1950). The sectioning levels were chosen according to the mouse brain atlas (Paxinos and Franklin 2001). The collection of sagittal sections of the cerebral cortex, the striatum, and the thalamus started at a level 0.2 mm lateral from the midline and extended laterally through the hemisphere in order to ensure sampling through all targeted brain structures. The SN was sectioned frontally. Sections were stored at -20°C until used in IHC.

2.4.2 Histological labeling

The histological labeling experiments were executed on a uniform systematic random set of five sections per mouse. Antibodies against GFAP (Dako, # Z0334, dilution 1:500) and human α-Syn (Enzo Life Sciences, # ALX-804-258, dilution 1:10) were used according to the manufacturer's instructions. The binding of primary antibodies was visualized using highly cross-absorbed secondary antibodies that were fluorescently labeled with Alexa fluorophores. Sections of TNWT-61 mice incubated without primary antibodies served as a negative control.

2.4.3 Imaging

Mosaic images of the stained sections were recorded on a Zeiss AxioImager Z1 microscope, equipped with a Zeiss AxioCam MRm camera and Zeiss AxioVision 4.8 software.

2.4.4 Evaluations

Immunoreactive structures were identified by threshold-based detection and subsequent morphological filters and were quantified using semi-automated rater-independent macros running in ImagePro Plus 6.2.

The target area was identified by drawing an area of interest (AOI) on the images. A second AOI excluded artifacts, such as wrinkles, air bubbles, or any other inconsistency interfering with the measurement, and defined the area for quantitative image analysis. We used background correction (subtraction of low pass-filtered image) and detected immunoreactive objects (for example: somata, neurites) by thresholding and morphological filtering (size, shape). Readouts were the size and intensity of objects, the number of objects per mm^2 (numerical object density), and the percentage of the AOI area covered by immunopositive objects. Once the parameters of the targeted objects had been defined in a test run, the quantitative image analysis ran automatically by a macro so that the results would be rater-independent and fully reproducible. All measurements were done using ImageProPlus (v6.2) software. Raw data were organized and sorted in Excel, and then transferred to GraphPad Prism for statistical analysis and preparation of graphs.

2.5 Statistical analysis

Both behavioral and MRI data were averaged using arithmetic mean and are expressed as mean ± SEM in the figures. The time points were evaluated separately, i.e. two sample tests were employed as appropriate because the study was cross-sectional by design and different mice were used in individual time points (3mo and 6mo). Therefore, the genotype effect (TNWT-61 mice versus WT littermates) was assessed for all behavioral tests and DKI/DTI parameters using an independent two-tailed Student's t-test or a Mann-Whitney U (MWU) test if the dataset showed significant results in the Kolmogorov-Smirnov normality test. Barnes maze data were analyzed by repeated measures ANOVA (factor: group; repeated variable: day) followed by a Bonferroni post-test for group-day interaction. The Pearson's correlation between immunohistochemical and DKI variables was assessed in the TNWT-61 group in the ROIs. The level of statistical significance was set at $p<0.05$. A receiver operating characteristic (ROC) curve analysis was performed to calculate the specificity and sensitivity

of predefined DKI parameters in 3mo mice. Statistics were calculated using the software packages Statistica 12 and SPSS20.

3.0 Results

3.1 Behavioral assessments

Figure 1 summarizes all of the behavioral measures in both groups of transgenic mice (3mo and 6mo) and their WT littermates. The TNWT-61 mice show highly significantly increased locomotion in distances traveled (MWU test, 3mo: $p=0.0003$, n=10 per group, 6mo: $p=0.004$; n=13 WT and n=10 TNWT-61) at both time points. The number of rearing episodes differed at 3mo only (t-test, $p=0.0135$); this effect was no longer significant at 6mo (t-test, n.s., $p=0.4626$; n=13 WT and n=10 TNWT-61). Therefore, hyperactivity is not an age-dependent characteristics of the transgenic model.

The results of the motor performance tests showed a severe motor impairment detected in all tests at both time points (3mo and 6mo). The performance did not develop over time, i.e. the 3mo TNWT-61 mice exhibited a similar extent of motor disability as the 6mo group. At 3mo, the *challenging beam walk test* detected significant increases in both time to cross and number of slips in the TNWT-61 mice (t-test, $p=0.0105$ and $p<0.001$ respectively; n=12 WT, n=13 TNWT-61 mice). At 6mo, the time to cross was not different between the groups but the number of slips remained highly increased (t-test, $p<0.001$; n=15 WT, n=10 TNWT-61 mice). The *square beam walk test* detected significant increases in both time to cross and number of slips in the TNWT-61 mice at both time points: at 3mo, MWU test, in both measures $p<0.001$ (n=16 WT, n=15 TNWT-61); at 6mo, the t-test for time to cross $p=0.0005$, MWU test for number of slips $p<0.001$ (n=15 WT, n=9 TNWT-61). Analogously, the *round beam walk test* detected significant motor impairment in the TNWT-61 mice. At 3mo, the t-test detected significant increases in time to cross ($p=0.002$; n=16 WT, n=12 TNWT-61) and MWU test showed significant increases in number of slips ($p<0.001$). At 6mo, the t-test revealed increased time to cross ($p<0.001$; n=15 WT, n=9 TNWT-61) and MWU test showed significantly increased number of slips ($p<0.001$). *Grid test* performance was worsened in the TNWT-61 at both 3mo (MWU test, $p=0.0017$; n=12 WT, n=10 TNWT-61) and 6mo (t-test, $p<0.001$; n=18 WT, n=13 TNWT-61).

The memory tests showed impaired performance in older mice only. The Y maze score in 3mo mice was not significant (MWU test; n=16 WT, n=21 TNWT-61); at 6mo, the detrimental effect of the genotype in the TNWT-61 was apparent (t-test, $p=0.015$; n=16 WT, n=13 TNWT-61). Similarly, the discrimination index in the novel object recognition test was not significant at 3mo; it reached a high significance at 6mo (t-test: n.s.; n=10 in both groups and $p<0.001$; n=14 WT, n=10 TNWT-61, respectively). The Barnes maze test assessed retention memory by the probe trial. The data from the early time point revealed no

differences between TNWT-61 and WT mice. However, the test detected impaired memory retention in the TNWT-61 mice at 6mo in terms of decreased time exploring the shelter location (t-test, p=0.0312) and increased number of errors (t-test, p=0.0608).

3.2 DKI MRI results

3.2.1 ROI analysis

Moderate changes were found in the analysis of ROIs at 3mo. Data are shown in **Figure 2**. The t-test identified significant increases of MK and RK in the striatum (p=0.0173 and p=0.0497 respectively) and of MK in the thalamus (p=0.0307). Mean diffusivity in the striatum was decreased (p=0.0494), which corresponds to the increased kurtosis values. ROC curve analysis for MK in the thalamus and striatum was performed to calculate the sensitivity and specificity as the earliest marker of pathology in this model (Suppl. Figure 1). For the striatum, the area under the curve (AUC) was 0.852 (sensitivity – 66.67%, specificity – 77.78%); for the thalamus, AUC was 0.778 (sensitivity – 77.78%, specificity – 55.56%) with a 95% confidence interval.

In the later time point (**Figure 3**), the changes were apparent in more regions and DKI variables. The striatum and thalamus were again the most affected; the t-test detected significantly increased MK and RK in both the striatum (p=0.0022 and p=0.0127 respectively) and thalamus (p=0.00132 and p=0.0021) respectively. These findings were confirmed by a significant decrease of radial diffusivity in both regions: striatum (p=0.0495) and thalamus (p=0.0057). MK was also elevated in the SN (p=0.0129), as was FA (p=0.0315). A highly significant elevation of FA was also found in the hippocampus (p=0.0041).

3.2.2 TBSS analysis

TBSS detected no WM alterations in 3mo transgenic mice. At 6mo, increased MK (p=0.004) was found in the anterior and posterior part of the anterior commissure, in the internal capsule, left habenular commissure, medial lemniscus, mammillothalamic tract, external capsule, and reticular formation. Furthermore, increased AK (p=0.039) was detected in the right-sided external capsule and decreased AD (p=0.025) was found in the ventral hippocampal commissure, trajection from/to septofimbrial nucleus, corpus callosum, external capsule, and in the fornix. Significant data from 6mo mice are shown together with a graphic presentation of the changes in **Figure 4**.

3.3 Immunohistochemistry

3.3.1 α-synuclein

Fluorescence intensity and immunoreactive area were assessed in all ROIs in both time points. Both transgenic groups exhibited highly significant accumulations of total α-Syn in all ROIs in terms of both measured variables. The specific values and results of the t-test are summarized in **Table 1**. We also performed an age-dependent comparison of the transgenic groups to assess potential development in α-Syn accumulation. As shown in **Figure 5**, the only significant result was an increase of the immunoreactive area in the hippocampus (t-test, p=0.0244).

3.3.2 GFAP

We did not find activation of astroglial cells in TNWT-61 mice compared to WT mice (data not shown).

3.3.3 Correlation between DKI and α-Syn immunohistochemistry

The Pearson's correlation was calculated for all significant DKI measures in the ROIs in TNWT-61 mice at both time points (n=6 in each group). There were no significant correlations between DKI measures and total α-Syn accumulation in any of the ROIs (data not shown).

4.0 Discussion

This study evaluated the significance of diffusivity and kurtosis metrics in detecting α-Syn accumulation-induced microstructural changes in young TNWT-61 mice. Significant changes were detected in MK in the striatum and thalamus in TNWT-61 mice already at 3mo; no alterations were observed with DTI parameters. This is in accordance with clinical findings (Zhang *et al.* 2015; Wang *et al.* 2011) indicating that MK might become an early clinical biomarker for PD diagnoses. The results of this study are in line with our previous reports and the hypothesis proposed by Giannelli et al., 2012, that accumulation of α-Syn or Lewy body like structures might play an important role in MK changes (Giannelli *et al.* 2012). However, unlike in our previous study with 14mo TNWT-61 mice (Khairnar *et al.* 2015a; Khairnar *et al.* 2015b), in this study we did not find a significant correlation between the amount of α-Syn and any diffusion values in our ROIs. This may be caused by the small numbers of mice evaluated post mortem using relevant immunohistochemistry approaches (n=6) or by the fact that α-Syn-induced changes or specific subtypes of α-Syn rather than an amount of total α-Syn per se may cause DKI increases early in the disease progression.

Since we did not observe any changes with DTI parameters at a very early stage, it is only possible to conclude that DKI was more useful than DTI in detecting early pathologies affecting the microstructural changes in gray matter. DTI has a proven value in detecting white matter changes (Steven *et al.* 2014). TBSS analysis in white matter found changes in kurtosis and diffusivity parameters only at 6mo and not at 3mo in TNWT-61 mice, suggesting that α-Syn accumulation-induced changes might first start in gray matter and later in white matter, at least in this animal model. Despite the known lack of motor impairment progression (Chesselet *et al.* 2012; Fleming *et al.* 2006; Fleming *et al.* 2004), the behavioral assessments revealed certain age-dependent effects in the memory performance that were present only at 6mo. This fits well with the increase in FA observed in the hippocampus in only the 6mo and not in the 3mo TNWT-61 mice.

TNWT-61 mice start to show α-Syn accumulation in the brain as early as post-natal day 10 (Chesselet *et al.* 2012). In 3mo TNWT-61 mice, we found a high expression of α-Syn in all evaluated brain regions; this is in line with previous studies (Delenclos *et al.* 2014). In the present study, we found an increase in MK in the thalamus at 3mo and in the SN at 6mo. This may be explained by a difference in regional specificity of proteinase K-resistant α-Syn aggregates. It has been reported that at 1mo, these mice show large aggregates of proteinase K-resistant α-Syn in the thalamus, locus coeruleus, and cerebellum; in the SN, they are detectable at 5mo (Chesselet *et al.* 2012; Delenclos *et al.* 2014). Therefore, it seems that the

presence of proteinase K-resistant α-Syn may have created a hindrance to water diffusion and hence to kurtosis and diffusivity changes in TNWT-61 mice. This finding is very important clinically, since α-synucleopathies proteinase K-resistant α-Syn aggregates correlate with Lewy body pathology, supporting the role of these aggregates in PD (Tanji *et al.* 2010; Neumann *et al.* 2004; McNaught *et al.* 2003). It is important to note that in TNWT-61 mice, the thalamus is the substantially affected region, as we have seen prominent changes in kurtosis within this ROI starting from 3mo TNWT-61 which persisted up to 14mo (Khairnar *et al.* 2015a; Khairnar *et al.* 2015b). Furthermore, although the thalamus is multifunctional, it is engaged in the motor basal ganglia circuitry vital for movement preparation and execution (Surova *et al.* 2016). This could be the reason why TNWT-61 mice start to show motor impairment when they are only 2mo (Fleming *et al.* 2004). In accordance with this study, we also observed motor impairment in TNWT-61 mice at both time points. In line with previous studies, TNWT-61 mice show sustained hyperactivity in the open field, possibly due to increased extracellular striatal dopamine (Lam *et al.* 2011; Chesselet *et al.* 2012). Memory impairment developed with age in this study; this is similar to clinical findings observed in PD patients (Emre *et al.* 2007). In our animal model, memory impairment reflected hippocampal involvement as DKI changes within this ROI were present only at 6mo. Hippocampal involvement (with its volume decreases) was also described in human PD-dementia cases (Rektorova *et al.* 2014; Weintraub *et al.* 2012).

It has been reported that changes in tissue complexity or heterogeneity arising from protein accumulation or glial cell activation can be sensitively detected by MK (Hui *et al.* 2008; Vanhoutte *et al.* 2013; Falangola *et al.* 2013; Umesh Rudrapatna *et al.* 2015; Guglielmetti *et al.* 2016). In our study, α-Syn accumulation might have led to increased structural complexity that may have restricted water diffusion in the striatum and thalamus of young (3mo) TNWT-61 mice, possibly causing an increase in MK. Surprisingly, there was no increase in MK in the SN, hippocampus, or sensorimotor cortex, although we observed that α-Syn accumulation there was similar to or higher than in the striatum and thalamus. From this evidence, we may conclude that the kurtosis changes we observed cannot be attributed only to α-Syn accumulation but rather to α-Syn accumulation-induced changes, and that kurtosis imaging does not detect α-Syn per se but α-Syn accumulation-induced changes. In this study, increased kurtosis was observed first in the striatum in 3mo TNWT-61 mice, and in the SN only in 6mo mice. This suggests that α-Syn-induced changes occur first in nigrostriatal dopaminergic nerve terminals and later in dopaminergic cell bodies in our animal model of PD.

A potential source of kurtosis signal may be associated with astroglial activation. Therefore, we performed an immunohistochemistry for GFAP in TNWT-61 mice. In line with previous studies, no astroglial cell activation was detected in the TNWT-61 mice (Chesselet *et al.* 2012; Watson *et al.* 2012). Furthermore, it was reported by Watson et al., 2012, that TNWT-61 mice exhibit microglial cell activation in the striatum as early as 1mo without activation in the SN (Watson *et al.* 2012). Moreover, TNWT-61 mice showed the greatest microglial cell activation in the SN at 5mo-6mo, with microglial morphology resembling that of PD patients (Watson *et al.* 2012; McGeer *et al.* 1988). Several studies confirmed that microglial cell activation increases the membrane barrier to diffusion of water and thus leads to increases in kurtosis (Falangola *et al.* 2013; Guglielmetti *et al.* 2016). In accordance with this, we observed an increase in MK first in the striatum and later in the SN, suggesting microglial cell activation may have played some role in changes in kurtosis metrics.

Another possible mechanism behind kurtosis changes in the basal ganglia could be iron deposition. Wang et al., 2011, reported that increases in MK in the SN of PD patients can be caused by increased iron content, which may lead to artifactual increases in MK due to a reduced signal-to-noise ratio (Wang *et al.* 2011). It has been reported that TNWT-61 mice show progressive increases in iron deposits in the SN, although their level is not different from the WT controls (Chesselet *et al.* 2012). Therefore, it is improbable that the MK signal originates from the iron content in the tissue.

In the last decade, the importance of DTI in the diagnosis of PD increased when Chan et al., 2007, found an inverse correlation of nigral FA with disease severity, whereas no significant changes of FA were found in other basal ganglia regions (Chan *et al.* 2007). Currently, nigral FA is one of the important parameters in DTI with respect to PD diagnosis. Several clinical studies using DTI have found decreased FA in the SN of PD patients (Cochrane and Ebmeier 2013; Zhang *et al.* 2015; Langley *et al.* 2016; Loane *et al.* 2016); other studies reported opposite results (Wang *et al.* 2011; Lenfeldt *et al.* 2015). In the present study, we did not detect early nigral FA alterations. We found an increase in FA in the SN only in 6mo TNWT-61 mice. This is in line with Wang et al., 2011, who found an increase in MK and FA in the SN of PD patients (Wang *et al.* 2011). However, the exact mechanism behind this change is unknown and hence further research in this direction needs to be conducted. Furthermore, DTI studies in neurotoxin-based animal models have yielded different results related to nigral FA changes: some studies found a decrease in FA (Vaillancourt *et al.* 2009; Boska *et al.* 2007; Soria *et al.* 2011), and others found an increase in FA (Van Camp *et al.* 2009).

TBSS analysis detected no white matter changes at the very early stage of the pathology. We found an increase in MK in the anterior commissure, internal and external capsule, and mammillothalamic tract in 6mo TNWT-61 mice. The α-Syn accumulation-induced changes start in gray matter and occur later in white matter, as α-Syn deposits first in gray matter in TNWT-61 mice (Chesselet *et al.* 2012; Delenclos *et al.* 2014). We also found an increase in axial kurtosis and a decrease in axial diffusivity in the external capsule. As repeatedly confirmed, the changes in kurtosis and diffusivity are always opposite at any age whether it is gray matter or white matter, thus suggesting α-Syn accumulation-induced changes have increased the hindrance to diffusion of water which may lead to an increase in kurtosis and a decrease in diffusivity (Khairnar *et al.* 2015a; Khairnar *et al.* 2015b). We still do not know much about α-Syn-induced changes in white matter in TNWT-61 mice. There is only one study which reported axonal transport deficits in TNWT-61 mice due to axonal α-Syn aggregates (Games *et al.* 2013). Further research in relation to α-Syn accumulation-induced changes in white matter and their impact on kurtosis and diffusivity changes needs to be done. To summarize, MK remains the best differentiator in both gray and white matter between TNWT-61 mice and WT mice. MK was able to significantly capture the α-Syn accumulation-induced changes at a very early stage in the striatum and thalamus of TNWT-61 mice. These kurtosis changes might have occurred due to the presence of proteinase K-resistant α-Syn or activated microglial cells, which are also present in PD patients. Thus, the present study supports the importance of kurtosis imaging as a future early clinical biomarker for differentiating PD patients from controls.

Conflicts of interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1. Behavioral studies. Graphs represent behavioral changes in 3mo and 6mo TNWT-61 mice. TNWT-61 mice were shown to be hyperactive in both time points in the open-field test. Motor impairment induced by the TNWT-61 genotype was tested with challenging beam walk, square beam walk, round beam walk, and grid test. TNWT-61 mice showed significant motor impairment at both time points in all tests. Spatial memory, tested by a Y maze, was found to be impaired in TNWT-61 mice at 6mo; at 3mo there was no between-group difference. Recognition memory, tested by novel object recognition test (NORT), showed analogous findings. There was no difference between TNWT-61 mice and WT controls at 3mo; memory impairment was shown at 6mo. Memory retention, tested with Barnes maze and probe trial, had no difference at 3mo; there was a significant impairment in the TNWT-61 mice at 6mo, showing a shorter time of exploring the correct escape hole. All data are expressed as mean values ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Figure 2. Region of interest based analysis in 3mo TNWT-61 mice. Bar graphs represent diffusion kurtosis and diffusion parameters. Note that to make the error bars visible, the y-axis does not start at 0. The ROIs are as follows: SN, substantia nigra; STR, striatum; HIPP, hippocampus; THAL, thalamus; SmCTX, sensorimotor cortex. Kurtosis and FA are dimensionless units. Diffusivity values are given in mm^2/s. Data are expressed as mean values ± SEM, n = 10 for WT and n = 8 for TNWT-61, *p<0.05.

Figure 3. Region of interest based analysis in 6mo TNWT-61 mice. Bar graphs represent diffusion kurtosis and diffusion parameters. Note that to make the error bars visible, the y-axis does not start from 0. The ROIs are as follows: SN, substantia nigra; STR, striatum; HIPP, hippocampus; THAL, thalamus; SmCTX, sensorimotor cortex. Kurtosis and FA are dimensionless units. Diffusivity values are given in mm^2/s. Data are expressed as mean values ± SEM, n=10 for WT and n=8 for TNWT-61, *p<0.05, **p<0.01.

Figure 4. Immunofluorescence of human α-Syn and GFAP and quantitative data on human α-Syn immunofluorescence. A) Brain cryosections were labeled by double-immunofluorescence for human α-synuclein (hAsyn, red channel) and GFAP (green channel); nuclei were stained with DAPI. The images show examples of immunoreactivity in the hippocampal formation and the SN of transgenic mice. Single channel images were obtained where indicated by the rectangles. Abbreviations: Cornu ammonis regions 1 and 3

(CA1/CA3), dentate gyrus (DG), substantia nigra pars compacta/reticulata (SNc/SNr), **B)** Graphs comparing immunoreactive area and fluorescence intensity of human α-Syn between 3mo and 6mo TNWT-61 mice. There is a general tendency towards higher immunofluorescent signal in 6mo (n=6) TNWT-61 mice compared to 3mo (n=6) TNWT-61; however, the increase is statistically significant in the hippocampus. Data are expressed as mean values ± SEM. *$p<0.05$.

Figure 5. Tract Based Spatial Statistics in 6mo TNWT-61 mice: On the brain slice (z=7.5 mm), the affected voxels are enlarged for easier visualization. Mean kurtosis changes are depicted in red (anterior commissure, internal capsule, left habenular commissure, medial lemniscus, mammillothalamic tract, external capsule, reticular formation), axial kurtosis changes in copper (right-sided external capsule) and axial diffusivity changes in blue (ventral hippocampal commissure, trajection from/to septofimbrial nucleus, corpus callosum, external capsule, fornix) at $p<0.05$. Box plots represent the related parameter's difference between groups. On the box plots, the central mark shows the mean, the 25 and 75 percentiles are represented. Whiskers show the highest and lowest values, which are no greater than 1.5 times the interquartile range. Outliers are depicted with black rings. n=10 for WT and n=8 for TNWT-61.

Table 1. Analysis of human α-Syn immunoreactivity. The table represents the actual values of immunoreactive area and fluorescence intensity and the results of the t-test between WT (n=6) and TNWT-61 (n=6) mice at both time points. TNWT-61 mice showed highly significant increases in both variables. Data are expressed as mean values ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Barnes C. A. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* **93**, 74–104.

Basser P. J. (1995) Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR Biomed.* **8**, 333–344.

Belforte J. E., Zsiros V., Sklar E. R., Jiang Z., Yu G., Li Y., Quinlan E. M., Nakazawa K. (2010) Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* **13**, 76–83.

Bevins R. A., Besheer J. (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study "recognition memory." *Nat. Protoc.* **1**, 1306–1311.

Boska M. D., Hasan K. M., Kibuule D., Banerjee R., McIntyre E., Nelson J. A., Hahn T., Gendelman H. E., Mosley R. L. (2007) Quantitative diffusion tensor imaging detects dopaminergic neuronal degeneration in a murine model of Parkinson's disease. *Neurobiol. Dis.* **26**, 590–596.

Chan L.-L., Rumpel H., Yap K., Lee E., Loo H.-V., Ho G.-L., Fook-Chong S., Yuen Y., Tan E.-K. (2007) Case control study of diffusion tensor imaging in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **78**, 1383–1386.

Chesselet M.-F., Richter F., Zhu C., Magen I., Watson M. B., Subramaniam S. R. (2012) A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurother. J. Am. Soc. Exp. Neurother.* **9**, 297–314.

Cochrane C. J., Ebmeier K. P. (2013) Diffusion tensor imaging in parkinsonian syndromes: a systematic review and meta-analysis. *Neurology* **80**, 857–864.

Coutu J.-P., Chen J. J., Rosas H. D., Salat D. H. (2014) Non-Gaussian water diffusion in aging white matter. *Neurobiol. Aging* **35**, 1412–1421.

Delenclos M., Carrascal L., Jensen K., Romero-Ramos M. (2014) Immunolocalization of human alpha-synuclein in the Thy1-aSyn ("Line 61") transgenic mouse line. *Neuroscience* **277**, 647–664.

Emre M., Aarsland D., Brown R., Burn D. J., Duyckaerts C., Mizuno Y., Broe G. A., et al. (2007) Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* **22**, 1689–1707; quiz 1837.

Falangola M. F., Jensen J. H., Tabesh A., Hu C., Deardorff R. L., Babb J. S., Ferris S., Helpern J. A. (2013) Non-Gaussian diffusion MRI assessment of brain microstructure in mild cognitive impairment and Alzheimer's disease. *Magn. Reson. Imaging* **31**, 840–846.

Fleming S. M., Salcedo J., Fernagut P.-O., Rockenstein E., Masliah E., Levine M. S., Chesselet M.-F. (2004) Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. *J. Neurosci. Off. J. Soc. Neurosci.* **24**, 9434–9440.

Fleming S. M., Salcedo J., Hutson C. B., Rockenstein E., Masliah E., Levine M. S., Chesselet M.-F. (2006) Behavioral effects of dopaminergic agonists in transgenic mice overexpressing human wildtype alpha-synuclein. *Neuroscience* **142**, 1245–1253.

Fleming S. M., Tetreault N. A., Mulligan C. K., Hutson C. B., Masliah E., Chesselet M.-F. (2008) Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. *Eur. J. Neurosci.* **28**, 247–256.

Games D., Seubert P., Rockenstein E., Patrick C., Trejo M., Ubhi K., Ettle B., et al. (2013) Axonopathy in an α-synuclein transgenic model of Lewy body disease is associated with extensive accumulation of C-terminal-truncated α-synuclein. *Am. J. Pathol.* **182**, 940–953.

Giannelli M., Toschi N., Passamonti L., Mascalchi M., Diciotti S., Tessa C. (2012) Diffusion kurtosis and diffusion-tensor MR imaging in Parkinson disease. *Radiology* **265**, 645-646-647.

Guglielmetti C., Veraart J., Roelant E., Mai Z., Daans J., Van Audekerke J., Naeyaert M., et al. (2016) Diffusion kurtosis imaging probes cortical alterations and white matter pathology following cuprizone induced demyelination and spontaneous remyelination. *NeuroImage* **125**, 363–377.

Hall K., Yang S., Sauchanka O., Spillantini M. G., Anichtchik O. (2015) Behavioural deficits in transgenic mice expressing human truncated (1-120 amino acid) alpha-synuclein. *Exp. Neurol.* **264**, 8–13.

Hikishima K., Ando K., Komaki Y., Kawai K., Yano R., Inoue T., Itoh T., et al. (2015) Voxel-based morphometry of the marmoset brain: In vivo detection of volume loss in the substantia nigra of the MPTP-treated Parkinson's disease model. *Neuroscience* **300**, 585–592.

Hui E. S., Cheung M. M., Qi L., Wu E. X. (2008) Advanced MR diffusion characterization of neural tissue using directional diffusion kurtosis analysis. *Conf. Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. IEEE Eng. Med. Biol. Soc. Annu. Conf.* **2008**, 3941–3944.

Jenkinson M., Smith S. (2001) A global optimisation method for robust affine registration of brain images. *Med. Image Anal.* **5**, 143–156.

Jensen J. H., Helpern J. A. (2010) MRI quantification of non-Gaussian water diffusion by kurtosis analysis. *NMR Biomed.* **23**, 698–710.

Jensen J. H., Helpern J. A., Ramani A., Lu H., Kaczynski K. (2005) Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. *Magn. Reson. Med.* **53**, 1432–1440.

Kamagata K., Tomiyama H., Hatano T., Motoi Y., Abe O., Shimoji K., Kamiya K., et al. (2014) A preliminary diffusional kurtosis imaging study of Parkinson disease: comparison with conventional diffusion tensor imaging. *Neuroradiology* **56**, 251–258.

Kamagata K., Tomiyama H., Motoi Y., Kano M., Abe O., Ito K., Shimoji K., et al. (2013) Diffusional kurtosis imaging of cingulate fibers in Parkinson disease: comparison with conventional diffusion tensor imaging. *Magn. Reson. Imaging* **31**, 1501–1506.

Khairnar A., Latta P., Drazanova E., Ruda-Kucerova J., Szabó N., Arab A., Hutter-Paier B., et al. (2015a) Diffusion Kurtosis Imaging Detects Microstructural Alterations in Brain of α-Synuclein Overexpressing Transgenic Mouse Model of Parkinson's Disease: A Pilot Study. *Neurotox. Res.*

Khairnar A., Ruda-Kucerova J., Drazanova E., Szabó N., Latta P., Arab A., Hutter-Paier B., et al. (2015b) Late-stage α-synuclein accumulation in TNWT-61 mouse model of Parkinson's disease detected by diffusion kurtosis imaging. *J. Neurochem.*

Lam H. A., Wu N., Cely I., Kelly R. L., Hean S., Richter F., Magen I., et al. (2011) Elevated tonic extracellular dopamine concentration and altered dopamine modulation of synaptic activity precede dopamine loss in the striatum of mice overexpressing human α-synuclein. *J. Neurosci. Res.* **89**, 1091–1102.

Langley J., Huddleston D. E., Merritt M., Chen X., McMurray R., Silver M., Factor S. A., Hu X. (2016) Diffusion tensor imaging of the substantia nigra in Parkinson's disease revisited. *Hum. Brain Mapp.* **37**, 2547–2556.

Leger M., Quiedeville A., Bouet V., Haelewyn B., Boulouard M., Schumann-Bard P., Freret T. (2013) Object recognition test in mice. *Nat. Protoc.* **8**, 2531–2537.

Lenfeldt N., Larsson A., Nyberg L., Birgander R., Forsgren L. (2015) Fractional anisotropy in the substantia nigra in Parkinson's disease: a complex picture. *Eur. J. Neurol.* **22**, 1408–1414.

Loane C., Politis M., Kefalopoulou Z., Valle-Guzman N., Paul G., Widner H., Foltynie T., Barker R. A., Piccini P. (2016) Aberrant nigral diffusion in Parkinson's disease: A longitudinal diffusion tensor imaging study. *Mov. Disord. Off. J. Mov. Disord. Soc.* **31**, 1020–1026.

Magen I., Fleming S. M., Zhu C., Garcia E. C., Cardiff K. M., Dinh D., De La Rosa K., et al. (2012) Cognitive deficits in a mouse model of pre-manifest Parkinson's disease. *Eur. J. Neurosci.* **35**, 870–882.

Masliah E., Rockenstein E., Veinbergs I., Mallory M., Hashimoto M., Takeda A., Sagara Y., Sisk A., Mucke L. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science* **287**, 1265–1269.

McGeer P. L., Itagaki S., Boyes B. E., McGeer E. G. (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **38**, 1285–1291.

McNaught K. S. P., Belizaire R., Isacson O., Jenner P., Olanow C. W. (2003) Altered proteasomal function in sporadic Parkinson's disease. *Exp. Neurol.* **179**, 38–46.

Neumann M., Müller V., Kretzschmar H. A., Haass C., Kahle P. J. (2004) Regional distribution of proteinase K-resistant alpha-synuclein correlates with Lewy body disease stage. *J. Neuropathol. Exp. Neurol.* **63**, 1225–1235.

Pierpaoli C., Basser P. J. (1996) Toward a quantitative assessment of diffusion anisotropy. *Magn. Reson. Med.* **36**, 893–906.

Rektorova I., Biundo R., Marecek R., Weis L., Aarsland D., Antonini A. (2014) Grey matter changes in cognitively impaired Parkinson's disease patients. *PloS One* **9**, e85595.

Rolheiser T. M., Fulton H. G., Good K. P., Fisk J. D., McKelvey J. R., Scherfler C., Khan N. M., Leslie R. A., Robertson H. A. (2011) Diffusion tensor imaging and olfactory identification testing in early-stage Parkinson's disease. *J. Neurol.* **258**, 1254–1260.

Schintu N., Frau L., Ibba M., Garau A., Carboni E., Carta A. R. (2009) Progressive dopaminergic degeneration in the chronic MPTPp mouse model of Parkinson's disease. *Neurotox. Res.* **16**, 127–139.

Schuff N., Wu I.-W., Buckley S., Foster E. D., Coffey C. S., Gitelman D. R., Mendick S., et al. (2015) Diffusion imaging of nigral alterations in early Parkinson's disease with dopaminergic deficits. *Mov. Disord. Off. J. Mov. Disord. Soc.* **30**, 1885–1892.

Schwarz S. T., Abaei M., Gontu V., Morgan P. S., Bajaj N., Auer D. P. (2013) Diffusion tensor imaging of nigral degeneration in Parkinson's disease: A region-of-interest and voxel-based study at 3 T and systematic review with meta-analysis. *NeuroImage Clin.* **3**, 481–488.

Sierra A., Laitinen T., Lehtimäki K., Rieppo L., Pitkänen A., Gröhn O. (2011) Diffusion tensor MRI with tract-based spatial statistics and histology reveals undiscovered lesioned areas in kainate model of epilepsy in rat. *Brain Struct. Funct.* **216**, 123–135.

Smith S. M. (2002) Fast robust automated brain extraction. *Hum. Brain Mapp.* **17**, 143–155.

Smith S. M., Jenkinson M., Johansen-Berg H., Rueckert D., Nichols T. E., Mackay C. E., Watkins K. E., et al. (2006) Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *NeuroImage* **31**, 1487–1505.

Soria G., Aguilar E., Tudela R., Mullol J., Planas A. M., Marin C. (2011) In vivo magnetic resonance imaging characterization of bilateral structural changes in experimental Parkinson's disease: a T2 relaxometry study combined with longitudinal diffusion tensor imaging and manganese-enhanced magnetic resonance imaging in the 6-hydroxydopamine rat model. *Eur. J. Neurosci.* **33**, 1551–1560.

Steven A. J., Zhuo J., Melhem E. R. (2014) Diffusion kurtosis imaging: an emerging technique for evaluating the microstructural environment of the brain. *AJR Am. J. Roentgenol.* **202**, W26-33.

Suidan G. L., Duerschmied D., Dillon G. M., Vanderhorst V., Hampton T. G., Wong S. L., Voorhees J. R., Wagner D. D. (2013) Lack of tryptophan hydroxylase-1 in mice results in gait abnormalities. *PloS One* **8**, e59032.

Surova Y., Lampinen B., Nilsson M., Lätt J., Hall S., Widner H., Swedish BioFINDER study, Westen D. van, Hansson O. (2016) Alterations of Diffusion Kurtosis and Neurite Density Measures in Deep Grey Matter and White Matter in Parkinson's Disease. *PloS One* **11**, e0157755.

Tanji K., Mori F., Mimura J., Itoh K., Kakita A., Takahashi H., Wakabayashi K. (2010) Proteinase K-resistant alpha-synuclein is deposited in presynapses in human Lewy body disease and A53T alpha-synuclein transgenic mice. *Acta Neuropathol. (Berl.)* **120**, 145–154.

Umesh Rudrapatna S., Hamming A. M., Wermer M. J. H., Toorn A. van der, Dijkhuizen R. M. (2015) Measurement of distinctive features of cortical spreading depolarizations with different MRI contrasts. *NMR Biomed.* **28**, 591–600.

Vaillancourt D. E., Spraker M. B., Prodoehl J., Abraham I., Corcos D. M., Zhou X. J., Comella C. L., Little D. M. (2009) High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease. *Neurology* **72**, 1378–1384.

Van Camp N., Blockx I., Verhoye M., Casteels C., Coun F., Leemans A., Sijbers J., Baekelandt V., Van Laere K., Van der Linden A. (2009) Diffusion tensor imaging in a

rat model of Parkinson's disease after lesioning of the nigrostriatal tract. *NMR Biomed.* **22**, 697–706.

Vanhoutte G., Pereson S., Delgado Y Palacios R., Guns P.-J., Asselbergh B., Veraart J., Sijbers J., Verhoye M., Van Broeckhoven C., Van der Linden A. (2013) Diffusion kurtosis imaging to detect amyloidosis in an APP/PS1 mouse model for Alzheimer's disease. *Magn. Reson. Med.* **69**, 1115–1121.

Wang J.-J., Lin W.-Y., Lu C.-S., Weng Y.-H., Ng S.-H., Wang C.-H., Liu H.-L., Hsieh R.-H., Wan Y.-L., Wai Y.-Y. (2011) Parkinson disease: diagnostic utility of diffusion kurtosis imaging. *Radiology* **261**, 210–217.

Watson M. B., Richter F., Lee S. K., Gabby L., Wu J., Masliah E., Effros R. B., Chesselet M.-F. (2012) Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. *Exp. Neurol.* **237**, 318–334.

Weintraub D., Dietz N., Duda J. E., Wolk D. A., Doshi J., Xie S. X., Davatzikos C., Clark C. M., Siderowf A. (2012) Alzheimer's disease pattern of brain atrophy predicts cognitive decline in Parkinson's disease. *Brain J. Neurol.* **135**, 170–180.

Yadav R., Hillman B. G., Gupta S. C., Suryavanshi P., Bhatt J. M., Pavuluri R., Stairs D. J., Dravid S. M. (2013) Deletion of glutamate delta-1 receptor in mouse leads to enhanced working memory and deficit in fear conditioning. *PloS One* **8**, e60785.

Zhang G., Zhang Y., Zhang C., Wang Y., Ma G., Nie K., Xie H., Liu J., Wang L. (2015) Diffusion Kurtosis Imaging of Substantia Nigra Is a Sensitive Method for Early Diagnosis and Disease Evaluation in Parkinson's Disease. *Park. Dis.* **2015**, 207624.

Zhu J., Zhuo C., Qin W., Wang D., Ma X., Zhou Y., Yu C. (2015) Performances of diffusion kurtosis imaging and diffusion tensor imaging in detecting white matter abnormality in schizophrenia. *NeuroImage Clin.* **7**, 170–176.