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INDIVIDUAL AND FAMILIAL SUSCEPTIBILITY TO MPTP IN A COMMON MARMOSET MODEL FOR PARKINSON'S DISEASE

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ABSTRACT

Insight into susceptibility mechanisms underlying Parkinson's disease (PD) would aid the understanding of disease etiology, enable target finding and benefit the development of more refined disease modifying strategies.

We used intermittent low-dose MPTP (0.5 mg/kg/week) injections in marmosets and measured multiple behavioural and neurochemical parameters. Genetically diverse monkeys from different breeding families were selected to investigate inter- and intra-family differences in susceptibility to MPTP treatment.

We show that such differences exist in clinical signs, in particular non-motor PD related behaviours, and that they are accompanied by differences in neurotransmitter levels. In line with the contribution of a genetic component, different susceptibility phenotypes could be traced back through genealogy to individuals of the different families.

Our findings show that low-dose MPTP treatment in marmosets represents a clinically relevant PD model, with a window of opportunity to examine the onset of the disease, allowing detection of individual variability in disease susceptibility, which may be of relevance for the diagnosis and treatment PD in humans.

INTRODUCTION

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder of the central nervous system [33]. PD is initially characterized as a movement disorder due to the progressive loss of dopaminergic neurons in the substantia nigra, involving potentially different but most likely overlapping pathogenic mechanisms. Although the actual underlying mechanisms are still unclear, disease onset is probably driven by insufficient cellular repair mechanisms and the incapacity to maintain homeostasis as a result of mitochondrial dysfunction, protein misfolding, protein aggregation, oxidative stress, proteasome dysfunction or inflammatory processes [33, 40, 123]. The fact that pathological events may act separately or synergistically may make certain individuals more susceptible for developing PD than others. Although much research has been done trying to identify the causes of PD, there is no answer to the question why some individuals are more susceptible to the disease than others. A small subgroup of patients is genetically predisposed and is diagnosed with a heritable familial form of PD [40], but the majority of cases are categorized as sporadic/ idiopathic. The most important risk factor involved in PD is age [144], however, external environmental factors, such as pesticide exposure or a medical history of constipation and depression, are also associated with PD [122]. Providing further insight into the mechanisms that are relevant for PD susceptibility would aid the understanding of the etiology of this disease and enable finding targets for developing disease modifying strategies.

Animal models offer a useful experimental platform for this, and models to study PD often involve neurotoxins such as MPTP that ablate dopamine production [31]. Consequently, MPTP causes behavioural, neurochemical as well as pathological features related to PD [31, 47, 81, 96, 97]. Whereas many researchers aim for a phenotypically homogeneous group of animals in their experiments, and thus prefer to work with inbred mice or rats, outbred animals are known to respond dissimilar to an equal dose of MPTP [53, 137, 183, 184] and thus offer the advantage to study individual differences in MPTP susceptibility. Here we investigated how individual and/or familial differences determine susceptibility to Parkinson-related disease manifestation after MPTP administration in marmoset monkeys. We used animals of the Biomedical Primate Research Centre (BPRC) breeding facility, which offers the unique opportunity to select monkeys from different families and to investigate response differences within and between families. Low doses of MPTP (0.5 mg/kg once weekly) are used to slowly induce Parkinson-related symptoms. This slow induction of disease symptoms mimics the slow progression of idiopathic PD as opposed to the more acutely induced cell death using high doses of MPTP [47]. Here we report the behavioural and neurochemical differences that were observed in these animals. Our experimental setup uniquely models the genetic diversity as observed in humans, and further enables future studies directed to revealing the mechanisms that contribute to PD pathology.

METHODS

Animals

Twenty common marmoset monkeys (*Callithrix jacchus*), obtained from five different breeding families (A-E), between 3 and 4 years of age (10M/10F; from each family two males and two females) were selected. One female died prior to the start of the study during anesthesia, and her brain was used in the control group. For post-mortem analysis one extra group of 7 healthy control monkeys was included (3M/4F). Animals were purchased from the purpose-bred colony of the Biomedical Primate Research Centre (BPRC) in the Netherlands, and were pair-housed, preferably together with their twin-siblings, in spacious marmoset cages (76 x 71 x 190 cm) enriched with branches, ropes, and toys with padded shelter provided on the floor, under constant humidity (60%), temperature (23-25°C) and lighting (12h light/dark cycles; lights on at 7:00 am) conditions. The daily diet consisted of commercial food pellets for New World monkeys (Special Diet Services, Witham, Essex, UK), supplemented with raisins, peanuts, biscuits, fresh fruit, Arabic gum and mealworms. Drinking water was provided *ad libitum*. All study protocols and experimental procedures were reviewed and approved by the BPRC ethics committee before the start of experiments, according to Dutch law.

Study design

Marmosets were repeatedly injected with a low dose of MPTP (0.5 mg/kg) on every Friday for a period of 5 weeks (total MPTP dose: 2.5 mg/kg). Behavioural abnormalities were examined prior to and during MPTP treatment. Behavioural tests were performed weekly on Monday to Thursday, thus excluding acute effects of MPTP due to the injections on Fridays. All tests were executed in the morning and early afternoon between 9:00h and 14:00h. Each animal was scheduled for a fixed time slot, whereby each task was planned within one day for all animals, i.e. hourglass test on Monday and Bungalow on Tuesday. Every task was conducted once a week, with the exception clinical scores and the periodic 24h measurement of home-cage activity. All animals were habituated in the different test setups prior to the start of the experiment. Baseline values were obtained over multiple weeks before disease induction. Previous studies showed no effect of vehicle treatment [90, 126, 181], and all current animals showed stable baseline performance. Therefore, each monkey served as its own control.

Drug treatment

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride, purchased from Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% saline to a concentration of 1 mg/ml (as a free base). Injection volume was 0.5 ml/kg, and administered via subcutaneous injections in the abdominal area.

Observational clinical signs

Marmosets were scored 2-3 times during the week for the presence and severity of parkinsonian symptoms. We recorded clinical scores (CS) using the validated observational scoring scale described elsewhere [177, 182], scoring apathy, tremors, immobility, inadequate

grooming, mask forming, smack and muscle rigidity. Clinical signs were scored on a scale form zero (normal/healthy) to four (severely affected). The sum of separate observations formed a clinical score outcome. Bodyweight was measured weekly as an independent clinical parameter.

Human threat test (HTT)

To test the emotional mood of the monkeys, the HTT was applied. The HTT is a documented test of fear and anxiety in non-human primates [177]. In brief, the HTT scores arousal and fear of an animal in the home-cage in response to the presence of a human observer in front of the cage. The observer assessing the behaviour stood approximately 30–50 cm from the cage front and made eye contact with the marmoset during a 2-min test period. Throughout this period, movement, behaviour and position of the marmoset in the cage were scored as fear postures (rearing, twisting, split staring, jumps in the backside of the cage) and arousal postures (scent marking, pilo-erection, presenting genitals, forward jumps). This task was executed in duplo, by two different individuals and scores were subsequently averaged.

Home-cage activity

Home-cage activity for measuring effects on the circadian rhythm was measured using telemetry allowing continuous remote and non-invasive monitoring of spontaneous activity in the monkey's home cage over the total period of the experiment. An external actimetry device (Remo 200series, Remo Technologies, Wiltshire, UK) was attached to the collar of each animal. An antenna mounted on the ceiling received signals from each device. Data were stored and real-time displayed using eDacq software (EMMS, Bordon, UK). Results are expressed as an averaged arbitrary unit. In addition, periods of resting were derived from these activity patterns. Periods during awake-time lasting for a minimum of 5 min with an activity count below 10 were regarded as resting periods. Thus four parameters were measured in this test, i.e. nighttime and daytime activity and number and duration of resting periods.

Hand-Eye Coordination (HEC)

Reward-related hand-eye coordination (HEC) performance for measuring the fine motor skills was tested with an automated robot-guided test setup [132, 177, 182]. Animals were placed in a test cage in the experimental setup in front of a small panel with a sliding door (5 x 8 cm). A robot arm presented a small marshmallow behind the door, which opened at the beginning of each trial. Subsequently, the monkey had to grab the reward. A balanced design was offered to the monkeys with different speeds of the robot arm: non-moving (0 cm/sec), slow moving (2 cm/sec) and fast moving (4 cm/sec). The test consisted of 24-30 trials depending on the individual performance of the animal. Monkeys were trained to perform > 80% correct trials at 0 cm/sec before the start of the experiment. In addition to accuracy, reaction times needed to grab the marshmallow were recorded.

Hourglass

The Hourglass was used to test the marmoset's natural righting reflex as a measure of rigidity, analogous to axial motor behaviour in humans. The currently used method was described previously [182]. In short, animals were placed in a Plexiglas cylinder (11 x 27 cm) in front of a camera. One trial consisted of a manual 180° turn of the cylinder. Time needed for the marmoset to turn back in the upright position was measured using non-automated video analysis. When an animal did not return to the upright position, the cylinder was turned back after 30 seconds allowing the animal to sit in their natural postural position. Each session consisted of 10 consecutive trials and sessions were carried out once a week.

Tower

The Tower was used to test natural jumping behaviour of the animals as described previously [182]. Minor adjustments were made for this study. In short, a trespa tower (35 x 35 x 250 cm) with a Plexiglas front contained 5 different horizontal levels. The levels varied in distance from each other, from 10 cm at the lowest levels to 70 cm at the highest levels. Animals entered the tower at the bottom and could freely move for 10 minutes throughout the tower. A camera was placed in front of the tower allowing the scoring of position of the animal using manual video-analysis. Both the location (level) of the animals and the time spent at each location were recorded.

Bungalow

Spontaneous locomotor activity was tested using the bungalow. This experimental setup has been well-validated and described [132, 182, 198]. The apparatus consisted of four horizontally placed non-transparent boxes (23 x 23 x 23 cm), all interconnected by six PVC tubes (inner diameter 9.5 cm). Each animal was placed in the same compartment at the start of each session and allowed to move freely throughout all compartments in the bungalow for a period of 10 minutes. Manual video-analysis was used to score the location of the animal and time spent in each of the compartments.

Tissue preparation

The euthasate (sodium pentobarbital, intracardiac) was administered after deep sedation with Alfaxan (0.6 ml, intramuscular.), brain tissue of all animals was collected and stored at -80°C until sample preparation. The left hemisphere was cut into sections of 7 μm for immunohistochemistry. Individual brain areas were isolated from the right hemisphere for immunoblotting and biochemistry. Tissue of substantia nigra (SN), caudate nucleus (CN) and putamen was homogenized in a sucrose buffer (0.32 M sucrose, 5 mM HEPES and 1 tablet of EDTA-free protein inhibitor).

Immunohistochemistry

The substantia nigra was analysed for the presence of tyrosine hydroxylase (TH)- neuronal profiles as previously described [134]. In short, serial sections of 7 μm were stained for TH (Sigma Alderich, 1:100). Every 30st section was taken for analysis. Within seven corresponding sections, the total number of TH positive neurons was manually counted and expressed as a

percentage of the number of cells found in healthy untreated control marmoset brains. Subsequently, sections were analysed using the software package Stream Essentials (OLYMPUS Corporation, Tokio, Japan) with a morphometric method to estimate TH-positive neuronal density. Images at 200x magnification were taken (2560 x 1920 pixels) and the covered area of TH-positive structures were measured using the count and measure module of the program. Cell body size was measured by the size in pixels of TH-positive staining with a minimum area of 400 pixels (software parameters: hue 20-53, saturation 125-256 and intensity value 125-256). The area of neurites was measured as TH-positive staining of a minimum of 10 pixels minus area of cell bodies (software parameters: hue 20-53, saturation 25-256 and intensity value 50-256).

Cortisol measurements

Hair from the posterior vertex region of the neck was shaved, collected into aluminum foil and stored at -20 °C until use. The cortisol measurements of marmoset hair were adapted from procedures previously described by Davenport et al. [32]. Hair samples were washed twice with isopropanol and dried for 5 days in clean protected area. Then, the hair was grounded to powder using a Bead neater MBB-24 (Lab Services, Breda, The Netherlands). 1 ml of methanol was added to 50 mg of powdered hair and incubated for 24 hours with slow rotation. Next, the samples were spun and 0.6 ml liquid was taken and dried in a heating block for approximately 5-6 hours (38-45 °C). Subsequently, the dried extracts were reconstituted with 0.4 ml of phosphate buffer provided in the assay kit (Salimetrics, Suffolk, United Kingdom). The hair cortisol levels were analysed using an EIA kit designed for cortisol measurements in saliva as described by manufacturer (Salimetrics).

Immunoblotting

Protein concentrations were determined using a Bradford assay. Immunoblotting was performed following a standard protocol [178]. In short, samples were heated for 5 min at 95 °C in Laemmli sample buffer and 7 µg protein was loaded on a TGX stain-free gradient gel (Biorad, CA, USA). After running of the gel, proteins were blotted onto a PVDF membrane for 2h (90V). Subsequently, blots were washed and blocked with 5% non-fat dry milk dissolved in TBS-T for 1h. Primary antibody (rabbit anti-tyrosine hydroxylase, Abcam, Cambridge, UK, 1:10000) was incubated overnight at 4°C and secondary anti-rabbit antibody was incubated for 1h at room temperature. Visualization of the bands was performed using FEMTO and a LICOR-scanning device. Intensity of the bands was analysed using Image Studio 2.0.

Monoamine measurement

The monoamines noradrenalin (NA), dopamine (DA) and serotonin (5-HT), and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxy-phenylacetic acid (HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA) were determined by high-pressure liquid chromatography (HPLC). Tissue of CN was measured using HPLC-ECD (Column: Phenomenex C18-XB150x4.6 mm, ECD potential: 460 mV) as described elsewhere [177]. Peaks were identified to calculate monoamine concentrations using a standard solution of 5,06 ng/ml NA (A7257, Sigma Aldrich), 5,1 ng/ml DA (H8502, Sigma Aldrich), 4,79 ng/ml

5-HIAA (H8876, Sigma Aldrich), 9,76 ng/ml 5-HT (H7752, Sigma Aldrich), 4,04 ng/ml HVA (H1252, Sigma Aldrich). 3 µl of standard solution for Metanephrines (Clinrep, Recipe, Munich, Germany) was used as internal standard and added to each sample of 33 µl protein mixture (1,5 mg/ml protein concentration). Subsequently, this internal standard was used for normalization.

Statistics

Statistical analyses of behavioural data and post-mortem analyses were performed using R (version 3.0.2). Hierarchical cluster analysis was done using Euclidian distance. Data in all graphs are expressed as averaged values with a standard error of the mean (SEM). Data were first tested for normal distribution using a Shapiro-Wilk test. All test showed non-parametric distribution of one ore more test parameters, excluding repeated measures ANOVA for data analysis [52]. In order to examine both time effects and differences between treatment groups we choose to us linear mixed modeling to reduce behavioural data [165, 194]. The R-package 'nlme' (Linear and non linear mixed effect models, version 3.1-118) was used to acquire slopes and intercepts for each parameter over the two different phases (baseline and MPTP exposure) [95, 107, 138]. Subsequently, intercepts and slopes were tested for significant differences between stages. Slope and intercept data were first tested for normality (Shapiro-Wilk). Parametric data was then analysed using ANOVA and Tukey as post-hoc test. Non-parametric data were tested using Friedman and Mann-Whitney tests. Immunohistochemical data were tested using ANOVA, with Tukey as posthoc analysis. Data on cortisol were tested using a Students t-test. Results were regarded significant when $p < 0.05$.

RESULTS

Clinical scores

All monkeys developed a mild form of parkinsonism. Although all monkeys received the same dose of MPTP, some showed higher parkinsonian scores than others. First, hierarchical cluster analysis was performed with clinical scores and individual animals as factors, on the basis of which animals were divided into three groups (Figure 1A). The six highest responding animals (hereafter named high responders; HR) and the six lowest responding animals (hereafter named low responders; LR) were selected for this study. The remaining animals (the intermediate responders) were used in another study and are described elsewhere [58]. Differences in clinical scores between the high and low responders were significant (slope analysis, df=10, p<0.001), and were present already in the second week after the first MPTP injection (Figure 1B). Monkeys from the same family had a strong tendency to cluster (Figure 1C), suggesting that susceptibility to MPTP is in part determined by family relationships and may have an underlying genetic component.
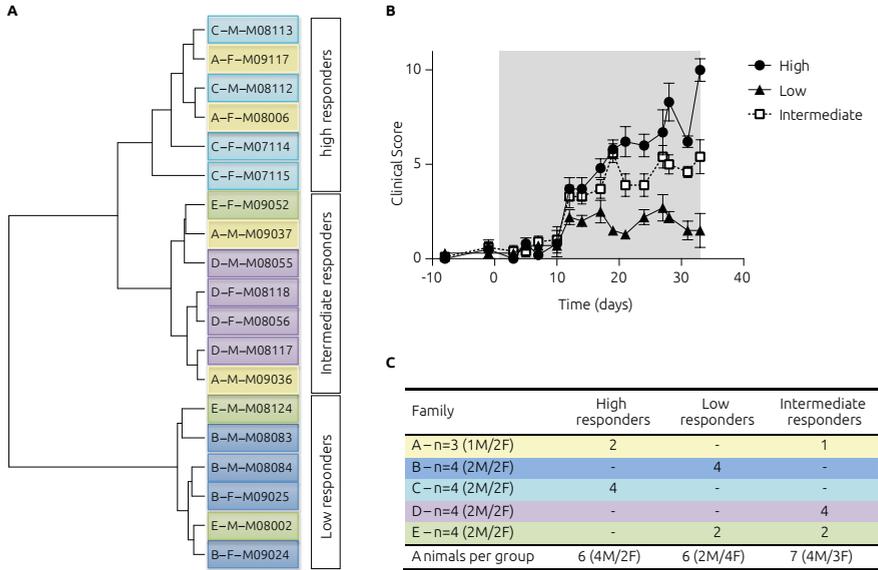
Figure 1: Parkinsonian symptoms. (A) Clustering of clinical symptoms. Different colors reflect different breeding families. Labels are organized as "Family (A-E)"- "Gender (M/F)"- "Individual name monkey". (B) Shaded area indicates MPTP exposure. Round closed circles reflect high responders, closed triangles reflect low responders and intermediate responders are depicted as open squares. Data are expressed as average ± SEM, with significant differences between intermediate animals and high responders, intermediate animals and low responders, and low and high responders. (C) Synopsis of genealogy among the groups.

## Factors potentially confounding the genetic component

We tested several factors that potentially could have confounded the underlying genetic component. All groups had an equal age distribution (slope analysis, df=10, p=0.327). Blood analysis was performed at the start of the experiment; clinical chemistry as well as hematology did not show any differences between the groups after correction for multiple testing and values did not deviate from standard values (Table 1, next page). Also bodyweights were not different between groups at the start of the study (average weight HR: 0.37±0.01 kg, LR: 0.38±0.01 kg). Cortisol levels were measured in hair samples and did not differ between high and low responders (Students t-test, df= 10, p=0.671, HR: 2751±829 pg/mg, LR: 2587±401 pg/mg). From this we conclude that differences between LR and HR are not due to these potentially confounding factors that were present before disease induction.

Table 1: Hematology and clinical chemistry in blood at the start of the experiment

Parameters		Low responders	High responders	P-Value
WBC	White blood cells	4.10 ± 0.37	5.05 ± 0.80	0.913
RBC	Red blood cells	7.33 ± 0.24	5.96 ± 1.13	0.913
HGB	Hemoglobin	10.08 ± 0.13	9.57 ± 0.41	0.989
HCT	Hematocrit	0.50 ± 0.01	0.42 ± 0.08	0.989
MCV	Mean volume of erythrocytes	68.37 ± 2.33	72.12 ± 2.55	0.913
MCH	Mean content of hemoglobin per erythrocyte	1382.00 ± 41.83	1410.20 ± 27.53	0.972
MCHC	Mean concentration of hemoglobin per erythrocyte	20.20 ± 0.14	57.63 ± 37.51	0.994
PLT	Platelets	516.00 ± 32.12	416.83 ± 89.41	0.972
RDW-SD	Relative distribution width of erythrocytes	34.98 ± 0.68	37.52 ± 0.61	0.430
RDW-CV	Relative distribution width of erythrocytes per volume of erythrocytes	16.63 ± 0.96	16.62 ± 0.65	1.000
PDW	Relative distribution width of platelets	10.70 ± 0.53	10.62 ± 0.90	1.000
MPV	Mean platelet volume	9.52 ± 0.29	9.80 ± 0.21	0.913
P-LCR	Platelet larger cell ratio	21.87 ± 2.33	24.45 ± 2.12	0.790
PCT	% of blood that is occupied by platelets	0.49 ± 0.02	0.41 ± 0.09	0.989
NEUT	Neutrophils	1.20 ± 0.19	1.23 ± 0.24	1.000
LYMPH	Lymphocytes	2.60 ± 0.29	3.49 ± 0.62	0.913
MONO	Monocytes	0.27 ± 0.03	0.19 ± 0.04	0.790
EO	Eosinophils	0.00 ± 0.00	0.01 ± 0.01	0.913
BASO	Basophils	0.02 ± 0.00	0.12 ± 0.07	0.559
NEUT%	% Neutrophils	28.96 ± 3.08	24.88 ± 3.68	0.913
LYMPH%	% Lymphocytes	64.04 ± 3.67	68.45 ± 3.96	0.913
MONO%	% Monocytes	6.60 ± 0.57	4.03 ± 0.67	0.430
EO%	% Eosinophils	0.00 ± 0.00	0.13 ± 0.13	0.913
BASO%	% Basophils	0.42 ± 0.05	2.50 ± 1.16	0.913
ALB2	Albumin	43.03 ± 1.49	47.06 ± 0.57	0.559
ALP2S	Alkaline phosphatase	75.92 ± 4.81	103.13 ± 14.04	0.790
ALTPL	Alanine aminotransferase	15.70 ± 3.81	22.15 ± 5.95	0.913
ASTPL	Aspartate aminotransferase	158.90 ± 14.44	173.20 ± 38.44	0.989
BILTS	Bilirubin	1.42 ± 0.09	1.42 ± 0.13	1.000

MPTP-induced alterations in non-motor behaviours

Homecage activity was measured with a telemetry device and showed significant differences between the high and low responders on all four measured parameters, i.e. nighttime and daytime activity and number and duration of resting periods, after MPTP exposure but not in baseline activity (Figure 2). Daytime activity decreased in both high and low responders after MPTP exposure (slope analysis; baseline HR: 147±49, MPTP exposure HR: 77±23, df=10, p=0.001, baseline LR: 170±17, MPTP exposure LR: 137±6, p=0.010; Figure
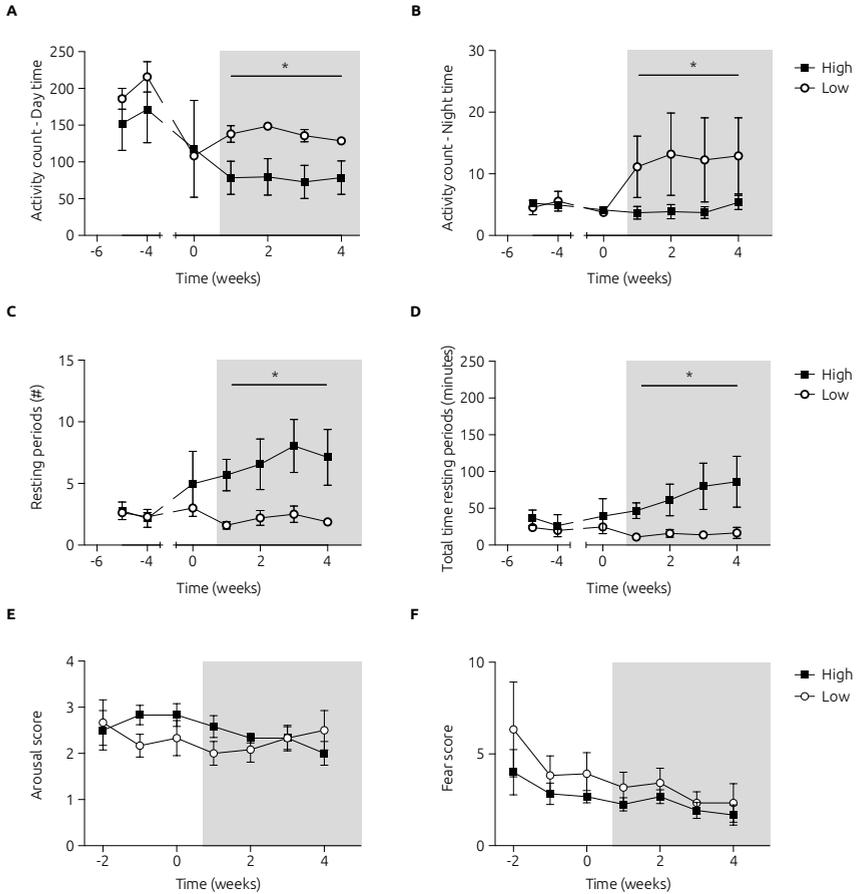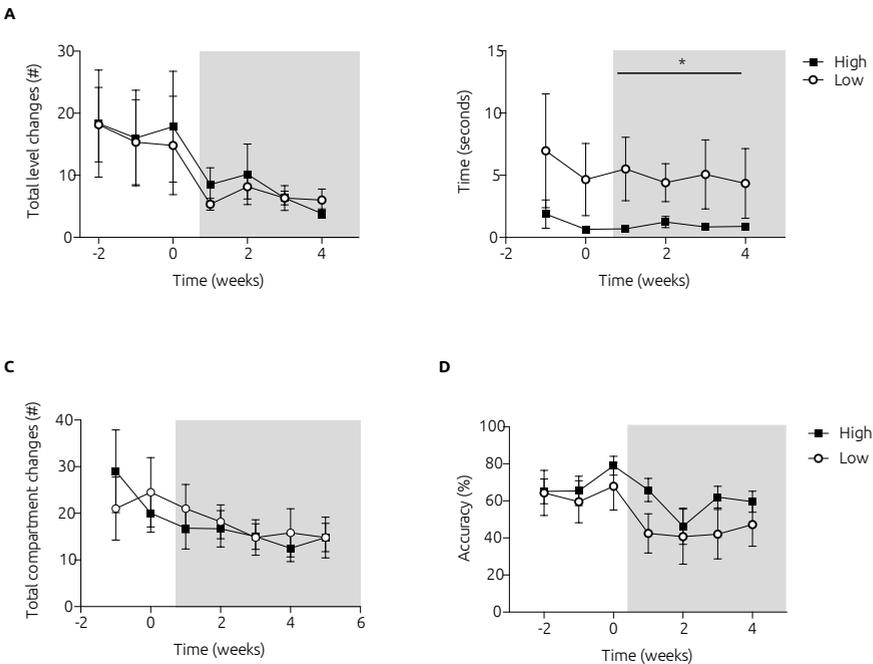
Figure 2: Non-motor related parkinsonian symptoms. Shaded area indicates MPTP exposure. Round closed squares and closed bars reflect high responders and open circles and bars reflect low responders. Data are expressed as average ± SEM, * represents significant difference between the slope of low and high responders. (A-D) Homecage activity measured using telemetry. (E-F) Arousal and fear related postures in the HTT.

2A), but significantly less activity was observed in the HR compared with the LR during treatment (slope analysis, df=10, p=0.036). Nighttime activity was also lower in HR than in LR during MPTP exposure (slope analysis, df=10, p=0.035), but not before exposure (slope analysis; baseline HR: 5±0, MPTP exposure HR: 5±1, df=10, p=0.296; baseline LR: 5±1, MPTP exposure LR: 12±6, df=10, p=0.357; Figure 2B). Both the number (Figure 2C) and total duration (Figure 2D) of resting periods were higher in HR compared with LR after MPTP exposure (slope analysis, number: baseline HR: 2±1, LR: 3±0, MPTP exposure HR: 6±2, LR: 2±0, df=10, p=0.036; duration: baseline HR: 34±16min, LR: 23±1min, MPTP exposure HR:

69±25min, LR: 14±5min, df=10, p=0.036). Arousal (Figure 2E) and fear-related behaviour (Figure 2F) were not affected after MPTP exposure (slope analysis; arousal score: baseline HR: 3.2±0.7, MPTP exposure HR: 2.1±0.4, df=10, p=0.424; baseline LR: 4.7±1.6, MPTP exposure LR: 2.8±0.8, df=10, p=0.123; fear score: baseline HR: 2.7±0.3, MPTP exposure HR: 2.3±0.2, df=10, p=0.424; baseline LR: 2.3±0.4, MPTP exposure LR: 2.2±0.3, df=10, p=0.067) and did not differ between groups (slope analysis; arousal score: df=10, p=0.857, fear score: df=10, p=0.370).

MPTP-induced alterations in motor behaviours

Jumping behaviour in the tower setup was not different between high and low responders (slope analysis, df=10, p=0.283) (Figure 3A), and both groups showed a similar decrease in the total number of level changes upon MPTP exposure (slope analysis; baseline HR: 13±8, MPTP exposure HR: 7±3, df=10, p=0.310; baseline LR: 16±7, MPTP exposure LR: 6±1, df=10, p=0.469). In the hourglass test (Figure 3B), LR needed more time to turn back in an upward position than HR, but this difference was already apparent during the baseline period (slope analysis; baseline HR: 1.3±0.7s, LR: 5.8±3.8s, df=10, p=0.012, MPTP exposure HR: 0.9±0.3s,


Figure 3: Motor related parkinsonian symptoms. Shaded area indicates MPTP exposure. Closed squares and bars reflect high responders and open circles and bars reflect low responders. Data are expressed as average ± SEM, * represents significant difference between the slope of low and high responders. (A) Total level changes in the tower-setup. (B) Time needed to turn into an upward position in the hourglass. (C) Total number of compartment changes in the bungalow. (D) Accuracy in the hand eye coordination- task.

LR: 4.8±2.4s, df=10, p=0.012). Spontaneous locomotor activity in the bungalow test was decreased after MPTP exposure (slope analysis; baseline HR: 24±6, MPTP exposure HR: 15±3, df=10, p<0.001; baseline LR: 23±7, MPTP exposure LR: 17±4, df=10, p=0.001), but not differently between groups (slope analysis, df=10, p=0.605) (Figure 3C). Hand-eye coordination was not affected after MPTP exposure (slope analysis; baseline HR: 70±7%, MPTP exposure HR: 58±7%, df=10, p=0.958; baseline LR: 64±12%, MPTP exposure LR: 43±13%, df=10, p=0.132) and there was also no difference in accuracy between the groups (slope analysis, df=10, p=0.749) (Figure 3D).

MPTP-induced anatomical and neurochemical alterations in the nigrostriatal system

No difference was observed between high and low responders compared with untreated controls in the number of TH-positive cells in the substantia nigra (ANOVA; HR: 104±10%, df=16, p= 0.5662; LR: 108±9%, df=16, p= 0.8382), the total cell body area (ANOVA; HR: 115±23%, p= 0.3681; LR: 126±20%, df=16, p= 0.6310) or the total area of neurites (ANOVA; HR: 99±18%, df=16, p= 0.7575; LR: 108±18%, df=16, p= 0.9680) (Figure 4A). Immunoblotting for TH also did not reveal a difference in TH levels between treatment groups and control, neither in the substantia nigra (ANOVA; HR: 102±14%, df=16, p=0.9549; LR: 86±15%, df=16,
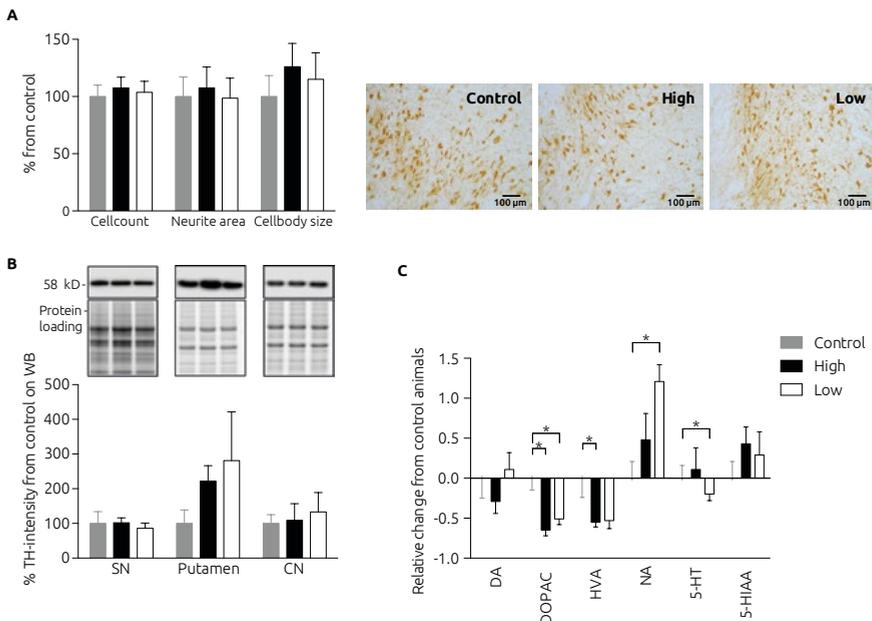
Figure 4: Histological analysis. Grey bars represent control animals, solid black bars represent high responders and open bars low responders. Data are represented as mean ± SEM. (A) Immunohistochemistry with tyrosine hydroxylase staining in substantia nigra (magnification: 200x). (B) Immunoblotting of TH in substantia nigra, putamen and caudate nucleus. (C) Neurotransmitters of the caudate nucleus measured with HPLC.

p=0.6975), nor in the putamen (ANOVA; HR: 222±44%, df=16, p= 0.0727; LR: 281±140%, df=16, p= 0.2861), nor caudate nucleus (HR: 109±48%, df=16, p= 0.8794; LR: 133±56%, df=16, p= 0.6285) (Figure 4B).

We also measured the levels of several neurotransmitters and their metabolites in the caudate nucleus. No significant differences in monoamines were detected between low and high responders, although trends were observed for lower levels of NA and higher levels of 5-HT in high responders compared with low responders (Figure 4C). Both groups differed significantly from control animals for some of the neurotransmitters and their metabolites. In particular, DOPAC and HVA were decreased in both low and high responders compared with control animals (ANOVA; DOPAC: HR: -0.65±0.07 fold change, df=16, p=0.002, LR: -0.51±0.07 fold change, LR, df=16, p=0.008; HVA: HR:-0.55±0.06 fold change, df=16, p=0.039, LR: -0.53±0.10 fold change, df=16, p=0.06). NA and 5-HT were only significantly affected for the LR compared with the control animals; NA levels were increased (ANOVA; NA: HR: 0.48±0.33 fold change, df=16, p= 0.128, LR: 1.21±0.21 fold change, df=16, p=0.026), whereas 5-HT levels were decreased (ANOVA; 5-HT: HR: 0.11±0.27, df=16, p= 0.288, LR: -0.20±0.08 fold change, df=16, p=0.005). Levels of DA and 5HIAA were not different from control animals (ANOVA; DA: HR: -0.29±0.15 fold change, df=16, p=0.331, LR: 0.11±0.21 fold change, df=16, p=0.743; 5HIAA: HR: 0.43±0.21 fold change, df=16, p=0.733; LR: 0.29±0.29 fold change, df=16, p=0.245).

DISCUSSION

This study relates behavioural and neurochemical parameters to individual differences in susceptibility to the neurotoxin MPTP in the common marmoset. We demonstrate that parkinsonian symptoms differ between individuals, and that low or high response of individual marmosets to MPTP depends on family history.

In non-human primates it is not uncommon to have relatively high variation in the response to the same dose of MPTP [53, 183, 184]. The unique response of each individual animal might hold a key to solving the issue of susceptibility to MPTP, which in turn might lead to novel insights in the pathogenesis of PD and explain why some individuals are more prone to develop the disease than others.

We found that animals with similar disease scores also shared family history, suggesting that a familial component, i.e. shared genetic background or nurturing, is responsible for the observed individual differences in susceptibility to MPTP. Importantly, several potentially confounding factors, such as age, cortisol levels, body weight, hematology and blood biochemistry did not play a role in susceptibility differences accordingly to current results. Moreover, baseline activity of all behavioural parameters was similar for all animals across the different behavioural tests, except for the hourglass test.

Post-mortem analyses showed no DA cell loss for both groups, however DA-turnover in our model is apparently affected in the absence of neuronal damage, most likely due to mitochondrial stress. Differences between high and low responders could alternatively be explained by differences in synaptic efficacy. Synaptic alpha-synuclein aggregates are

associated with degeneration of dendritic spines in humans, which in turn leads to disturbed vesicle turnover and reduced neurotransmitter release [155, 156]. In addition, significant increases of NA-levels measured in caudate nucleus of low responders possibly explain the decreased MPTP susceptibility and might be neuroprotective [147]. Others showed NA to be of crucial influence to MPTP susceptibility in mice [87, 147]. Trends observed in levels of DA, NA and 5-HT between high and low responders might be related to behavioural differences between groups, although diurnal effects could obscure the effects on DA-release of the high responders [50]. Individual differences in monoamine levels within species were previously reported [85, 204]. Furthermore, it is known that concentrations of monoamine metabolites in the cerebrospinal fluid are highly heritable [113]. Higher levels of DA, NA and D2 receptor binding, and lower levels of 5HT, are all associated with dominance, aggressiveness and higher social status [91, 98, 113, 161, 202, 204]. Non-human primate studies show that animals from different levels in hierarchy display differences in behaviour, neurochemical composition and physical characteristics [91, 98, 168, 204]. In humans, levels of specific neurotransmitters are associated with personality traits [102], and certain personality traits in patients are indeed linked to the severity of PD symptoms, such as increased neuroticism and decreased extroversion [193]. Strikingly, the monkeys from the low responders originated from an assertive and aggressive family, which is in line with the findings in literature about the personality and monoamine levels and might indicate different catecholamine levels in these animals.

In conclusion, in this study we show differences in susceptibility to MPTP in the common marmoset in clinical signs, in particular in non-motor PD related behaviours, and accompanied by differences in neurotransmitter levels. Different behavioural phenotypes can be traced back through genealogy into the different families, suggesting a genetic component to susceptibility. From a diagnostic and treatment perspective, future research might also include testing of L-Dopa efficacy in low and high responders to identify possible differences in dosing requirements and in L-Dopa-induced dyskinesia.

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