IDENTIFICATION OF COGNITIVE IMPAIRMENTS IN MPTP-TREATED MARMOSETS USING THE WISCONSIN GENERAL TESTING APPARATUS

[1]Rose S., [1]Jackson MJ., [1]Fisher R., [1] Lincoln L., and [2] D. Virley.

[1] Neurodegenerative Diseases Research Group, Faculty of Life Sciences and Medicine, Guy's Campus, King's College London, London SE1 1UL, UK.

[2]Transpharmation Ltd, London Bioscience Innovation Centre, 2 ,Royal College Street, London. NW1 0NH, UK.

Introduction

- Most patients with Parkinson's disease (PD) develop dementia, including impairments in attention, executive, and visuospatial functions.
- Motor-asymptomatic MPTP-treated monkeys show cognitive impairment similar to that seen in MPTP intoxicated humans and idiopathic PD (Stern et al 1990; Schneider and Kovelowski, 1990).
- Motor symptoms of PD are well modelled in animals, however, cognitive dysfunction is poorly defined, particularly in motor-impaired animals, and there are limited treatment strategies.
- Therefore, the purpose of this study was to characterise cognitive performance in the MPTP-treated marmoset showing motor deficits as a model of PD-dementia using the Wisconsin General Testing Apparatus (WGTA).

Methods

Animals

Male and female common marmosets were used throughout. Regulated procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986, with approval of the King's College London Ethical Review Panel under Project Licence PPL 70/7146 and were compliant with the minimal standards as defined by the European Communities Council Directive (85/609/EEC).

MPTP Treatment:

- 1 group of marmosets (n=6) were treated with (MPTP: 2.0 mg/kg sc once daily for 5 days,; Sigma, UK), 3 – 5 years prior to the start of the studies according to established protocols (Smith et al., 2003).
- Animals were treated with L-DOPA (8 - 12.5 mg/kg) daily, for up to 30 days, to demonstrate L-DOPA responsiveness and induce the expression of dyskinesia.
- MPTP-treated animals were not test drug naïve.

Assessment of cognitive function

- Naïve (n=6) and MPTP-treated (n=6) common marmosets were tested on a series of different cognitive tasks using the WGTA (Virley et al 1999).
- Animals were habituated to the WGTA and trained to displace 1 of 2 objects covering food wells to obtain a food reward. The position of the rewarded object on each trial was based on pseudorandom schedule (Gellermann, 1933).
- Following successful shaping, marmosets were evaluated on
  - a simple visual discrimination (VD) task (object set B),
  - 2 reversals of this newly learnt VD task
  - a visuospatial conditional discrimination task (VSCD) .
- The mean number of trials and response latencies to criteria (90% correct i.e. 27 correct responses out of 30) were recorded for each marmoset.
- The maximum number of trials per daily session was 30.
- Animals which did achieve criteria in 300 trials were deemed to have failed the task.

Assessment of Motor dysfunction

- MPTP-treated marmosets were placed individually into automated test units for a 60-minute acclimatisation period.
- Animals treated with L-DOPA (8 mg/kg po + benserazide 10 mg/kg po) or vehicle
- Basal and drug-induced locomotor activity, motor disability and dyskinesia was assessed as follows:

Locomotor activity

- The number of interruptions of light beams in activity cages was automatically recorded for 5 hours following drug treatment (Smith et al., 2003).

Motor Disability and Dyskinesia

- Disability and dyskinesia were scored once every 30 minutes before and after drug treatment for 5 hours using an established rating scale (Smith et al., 2003).

Statistical analysis

- Behaviour data was analysed by 2 way ANOVA and post hoc Wilcoxon to compare the effect of L-DOPA and vehicle.
- WGTA data (trials to criteria) and (time to criteria) were analysed by 2 way ANOVA followed by Mann-Whitney to compare MPTP-treated with naïve animals.
- Differences were considered to be significant at $p < 5\%$.

MPTP-treated marmosets were L-DOPA responsive

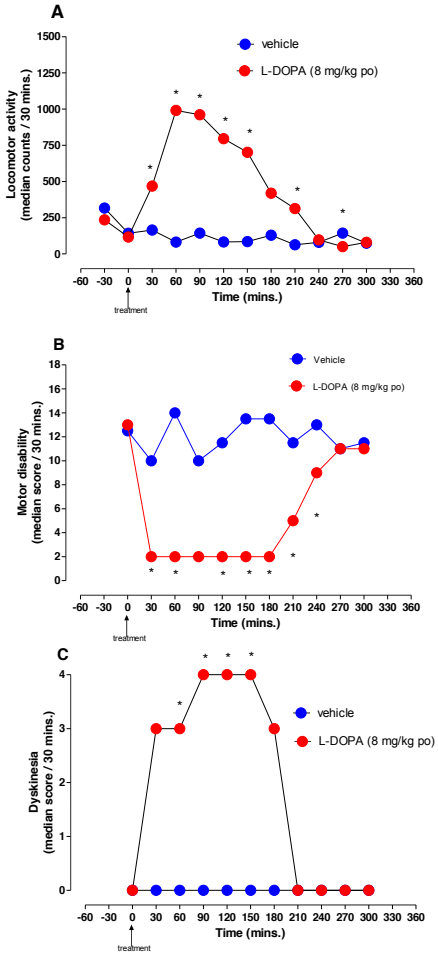

Figure 1. (A) Locomotor activity, (B) motor disability scores and (C) dyskinesia scores in MPTP-treated common marmosets following L-DOPA (8 mg/kg po + benserazide , 10 mg/kg po). Data are median. * P<0.05 compared to vehicle (Wilcoxon)

Impaired cognitive function in MPTP-treated marmosets compared to naïve controls

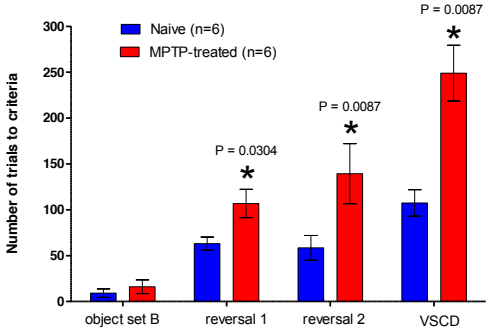

Figure 2. Total number of trials to criteria for naive and MPTP-treated common marmosets following a simple VD task, two reversals and a VS conditional discrimination task (VSCD). Data are mean ± SEM (n=6). * P<0.05 vs naïve (2-way ANOVA and Mann-Whitney).

No difference in time taken per trial in MPTP-treated marmoset compared to naïve control

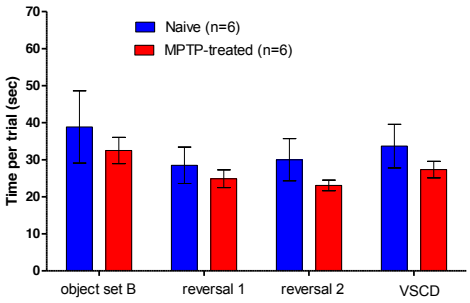

Figure 3. Time per trial for naive and MPTP-treated common marmosets following a simple VD task, two reversals and a VS conditional discrimination task. Data are mean ± SEM (n=6). Data analysed by 2 way ANOVA (P>0.1 NS).

Results 1: Motor disability

- MPTP-treated animals exhibited stable motor deficits including a marked reduction of locomotor activity, poor coordination of movement, abnormal and/or rigid posture, reduced alertness and head-checking movements.
- All MPTP-treated animals were L-DOPA responsive, showing the expected increase in locomotor activity, reversal of motor disability and expression of dyskinesia when challenged with L-DOPA (8 mg/kg po) (Figure 1 A-C)
- Naïve marmosets were mobile, co-ordinated, and alert with repetitive head checking, and sporadic activity in the home cage.

Results 2: Cognitive function

- Both groups of marmosets performed the VD task similarly to criteria (P>0.1, NS).
- When the VD task was reversed, MPTP-treated marmosets were significantly impaired on both the first (P<0.05) and second reversal (P<0.01).
- During the VSCD test, MPTP-treated marmosets demonstrated robust impairments in learning this task compared to naïve animals (P<0.01).
- Response times per trial for each test were not different between the groups (P>0.1, NS), suggesting that MPTP-treated marmosets were motivated and not impeded by their motor deficit to perform the cognitive tasks.

Conclusions

- MPTP-treated marmosets with significant L-DOPA-responsive motor impairment also demonstrated cognitive deficits on tasks sensitive to executive function and visuospatial conditional learning using the WGTA, consistent with PD.
- Importantly, response times per trial for each test were not different between the groups, suggesting that MPTP-treated marmosets were motivated and not impeded by their motor deficit to perform the cognitive tasks using the WGTA
- Reduced dopamine transmission in the prefrontal cortex as a result of MPTP intoxication results in a loss of excitatory synapses (Elsworth et al 2013), which may in part explain the cognitive deficits, although impairments in performance of the VSCD task by marmosets has also been associated with selective bilateral lesions of the CA1 region of the hippocampus (Virley et al., 1999).
- This cognitive battery in the MPTP-treated primate may offer the opportunity of validating novel therapeutic strategies for PD-dementia.

References

1. Stern, Y., et al. (1990). "Cognitive change following MPTP exposure." Neurology **40**(2): 261-264.
2. Schneider, J.S., and Kovelowski, C.J. 2nd (1990). "Chronic exposure to low doses of MPTP. I. Cognitive deficits in motor asymtomatic monkeys". Brain Research 519 (1-2) : 122-128.
3. Virley, D., et al. (1999). "Primary CA1 and conditionally immortal MHP36 cell grafts restore conditional discrimination learning and recall in marmosets after excitotoxic lesions of the hippocampal CA1 field." Brain **122 (Pt 12)**: 2321-2335.
4. Gellermann L.W. (1933). "Chance orders of alternating stimuli in visual discrimination experiments". J. Genet. Psychol 42: 206 – 208.
5. Smith, L. A., et al. (2003). "Effect of pulsatile administration of levodopa on dyskinesia induction in drug-naive MPTP-treated common marmosets: effect of dose, frequency of administration, and brain exposure." Mov Disord **18**(5): 487-495.
6. Elsworth, J. D., et al. (2013). "Loss of asymmetric spine synapses in prefrontal cortex of motor-asymptomatic, dopamine-depleted, cognitively impaired MPTP-treated monkeys." Int J Neuropsychopharmacol **16**(4): 905-912.