Acquisition and Reversal of Visual Discrimination Learning in MPTP-treated C57BL/6J Mice

Taneli Heikkinen[1], Joni Jarvenpää[1,2], Maksym Kopanitsa[1,3], Artem Shatillo[1], Jussi Rytkönen[1], Lauri Remes[1], Jukka Puolivali[1]

[1]Charles River Discovery, Kuopio, Finland; [2]University of Eastern Finland, Kuopio, Finland; [3]UK Dementia Research Institute, Imperial College, London, U.K

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UK Dementia Research Institute

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1 BACKGROUND

Injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice cause a significant dopaminergic neuronal loss, biochemical and cellular changes and behavioural symptoms similar to those observed in individuals with Parkinson's disease.

The c-Abl tyrosine kinase inhibitor nilotinib was previously shown to protect dopaminergic neurons and rescue motor deficits in MPTP-treated mice. Here we explored if:

a) MPTP treatment affected acquisition and reversal of visual discrimination learning in a translational touch screen test and

b) whether administration of nilotinib before and after MPTP injections would prevent putative cognitive deficits in the touch screen test and motor impairment assayed by the tapered beam test.

2 METHODS

In total, 46 8-week-old male C57Bl/6J mice were used for the experiments. The mice were housed at 22 ± 1 °C in a humidity-controlled environment with lights on at 07:00 am and off at 8:00 pm. Mice were kept in groups of 3 to 5 animals per cage. The following experimental groups were used (**Figure 1**):

Group 1 (control): 14 mice were treated once daily with 10 mL/kg of nilotinib vehicle (90% PEG 300/10% NMP) p.o. from day −5 to day 8. Additionally, MPTP vehicle (saline) was administered i.p. on days 0 and 1 in two doses at 3-hour intervals.

Group 2 (MPTP): 16 test mice were treated once daily with 10 mL/kg of nilotinib vehicle (90% PEG 300/10% NMP) p.o. from day −5 to day 8. Additionally, MPTP in saline was administered i.p. on days 0 and 1. MPTP was administered in two doses of 20 mg/kg (total dose 80 mg/kg) at 3-hour intervals.

In group 3 (MPTP+nilotinib), 16 test mice were treated once daily with nilotinib dissolved in 90% PEG 300/10% NMP (10 mL/kg) p.o. from day −5 to day 8. Additionally, MPTP in saline was administered i.p. on days 0 and 1, similarly to group 2.

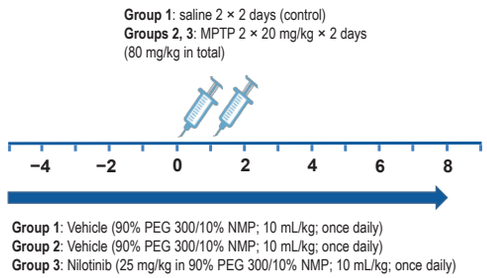

Figure 1. Schematic illustration of the treatment schedule.

Following Day 8, mice were gradually adjusted to a restricted food regimen, at 85–90% of their free-feeding weight, in order to maintain their motivation for performing touch screen tasks, with water ad libitum. Two weeks after last MPTP/saline injection, mice started to receive one operant training session per day, ~between 11 am and 2 pm, 5–7 days per week. Experiments were conducted in 24 Campden Instruments touch screen chambers (Campden Instruments, Loughborough, UK) located in a dedicated room. Before visual discrimination testing, mice were trained on basic touch screen task requirements introduced in several consecutive stages ("Initial touch", "Must touch", "Must Initiate" and "Punish Incorrect" as described by Horner *et al*. in Nat Protoc. 2013 8(10):1961-84. Once the mice completed all pretraining criteria, they were moved on to Visual Discrimination task (Horner *et al*., 2013). During each trial, the mouse was presented with a choice between "Lines Grid-Right" and "Lines Grid-Left" images, one in each response window (**Figure 2**). A touch to one image was rewarded and a touch to the other was not rewarded and triggered a short 5-s flash of the house light. The mice were considered to have acquired discrimination when they reached a performance criterion of at least 80% of trials correct (not including correction trials) in two consecutive daily 30-trial sessions. Mice were moved on to the reversal phase of the task individually, immediately after they attained the acquisition criterion. During the reversal stage, mice had to learn the opposite contingency. All mice acquired visual discrimination and fulfilled reversal learning criteria in <2 months .

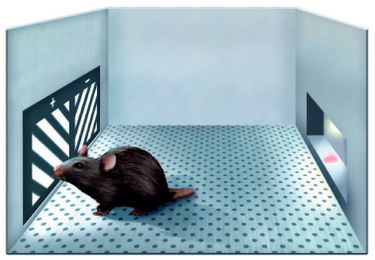

Figure 2. Schematic illustration of the setting for the pairwise Visual Discrimination task in a Bussey-Saksida touch screen operant chamber.

3 RESULTS

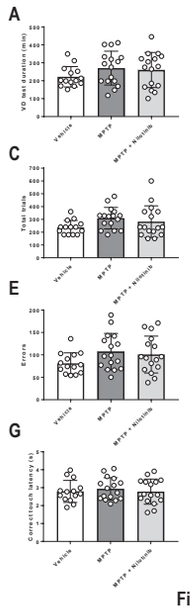

Figure 3

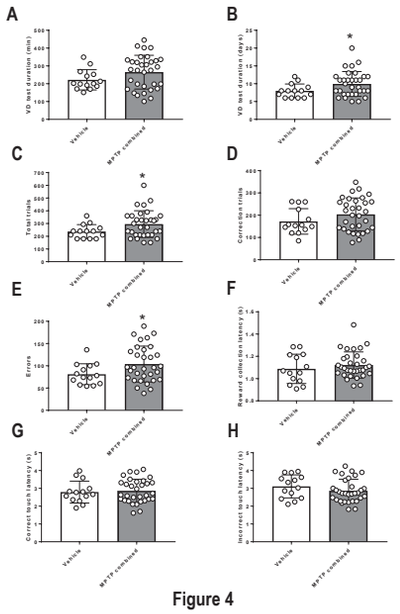

Figure 4

Figures 3 and 4. Time in minutes (A) or days (B) required, trials made (C), correction trials received (D), errors committed (E) before mice achieved the visual discrimination criterion. (F–H) Latencies to collect the reward (F) and to respond to correct (G) and incorrect (H) stimuli in different groups of mice. For comparisons in **Figure 4**, MPTP-treated mice from groups 2 and 3 in **Figure 3** were pooled. Circles indicate summed (B–E) or median (F–H) data for individual mice. Bar charts plot the mean ± standard deviation (*N* = 14–16, **Figure 3**; *N* = 14 and 32, **Figure 4**).

There were no statistically significant differences between saline-injected mice, MPTP-injected mice and MPTP-injected mice that received nilotinib before and after MPTP injections during instrumental pretraining and acquisition of visual discrimination (**Figure 3**). We have noticed, however, that some parameters in MPTP-treated mice in groups 2 and 3 (receiving vehicle$_{nilotinib}$ and nilotinib, respectively, **Figure 1**) were nominally different from those in saline-injected control mice. Therefore, we also compared quantitative parameters of the visual discrimination test in control mice with those in the pooled set of all MPTP-treated animals (**Figure 4**). This comparison demonstrated that MPTP-treated mice overall made more errors and therefore required slightly longer time to achieve visual discrimination criterion (**Figure 4**).

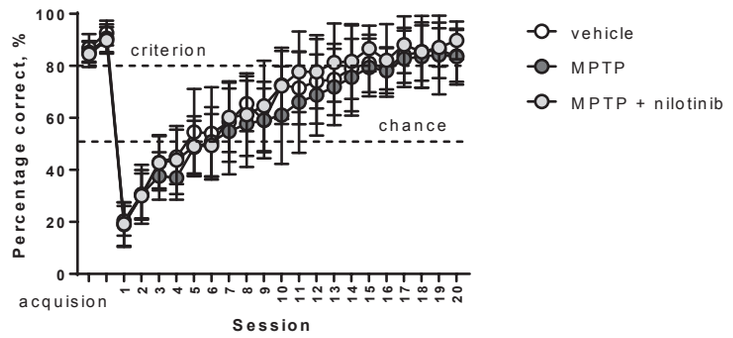

Figure 5. Reversal of visual discrimination learning in mice from groups 1–3 is illustrated as percentage of correct responses across 20 days of reversal learning sessions. The first two symbols indicate performance during the last two visual discrimination acquisition sessions. Criterion (80%) and chance performance (50%) are indicated by dotted and dashed lines, respectively. Data are presented as the mean ± standard deviation.

Once the animal reached the visual discrimination criterion, the contingency reversed so that a touch to a previously rewarded image was not rewarded, whereas a touch to the previously unrewarded image earned a reward. Reversal learning curves were similar in all three groups (**Figure 5**). Furthermore, no statistically significant differences in reversal learning parameters were detected between saline- and MPTP-treated animals, when the latter were pooled from groups 2 and 3 (data not shown).

3 RESULTS CONT'D

After completing touch screen based assessment of cognitive functions, in 2.5 months after MPTP/saline injection, we assessed motor performance of animals in tapered beam test. For this, a wooden 100-cm beam was used, which was tapered from 1.5 cm to 0.5 cm. A 0.5-cm ledge was positioned 2 cm below the top of the beam. At the end of the beam, set at an angle of 17°, a black wooden box was placed as shelter to encourage mice to traverse the full length of the beam. On the training day, the mouse was acclimated to the box for 5 min and then placed on the beam at gradually increasing distances from the box until it fully traversed the length of the beam. On the next (test) day mice received three trials (2-min maximum) separated by a 30 s intertrial interval. The primary performance measures scored from the replay of the videotapes were: 1) latency to turn towards, 2) latency to traverse the beam, and 3) the number of forelimb and hindlimb footslips on the beam.

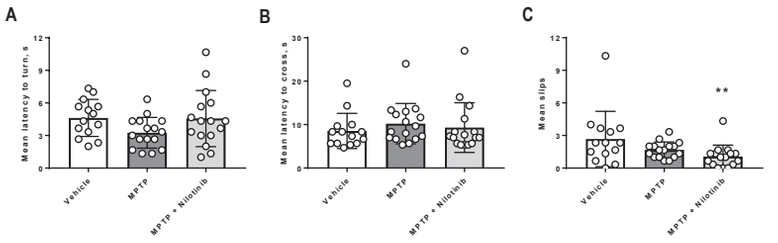

Figure 6. Mean latency to turn towards the box (A), mean latency to traverse the beam (B) and the mean number of forelimb and hindlimb slips (C) in different groups of mice in 2,5 months after injection of saline (vehicle) or MPTP. ***P* < 0.01, Dunn's multiple comparisons test (in relation to "Vehicle").

Surprisingly, we have not observed pronounced impairments in motor coordination in MPTP-treated animals compared to locomotor parameters in control animals that received saline injection. Such deficits have been routinely detected by us and others previously in tapered beam as well as in rotarod tests, however, the gap between exposure to MPTP and motor function assessment was shorter in the majority of such studies (2–4 weeks). Furthermore, at 5 months after saline/MPTP injection, we performed ^{1}H-MRS imaging and have not revealed increased glutamate and decreased lactate levels in the striatum typically seen at ~12–35 days post-treatment (data not shown). HPLC analysis of striatal samples done after ^{1}H-MRS imaging showed expectedly decreased levels of dopamine and its metabolites in MPTP-treated animals, although the changes were smaller compared to historical data obtained at earlier periods after MPTP challenge.

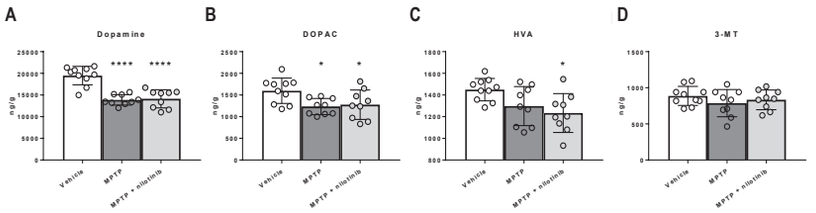

Figure 6. Striatal levels of dopamine (A), 3,4-dihydroxyphenylacetic acid (DOPAC, B), homovanillic acid (HVA, C) and 3-methoxytyramine (3-MT, D) detected at 5.5 months in different groups of mice after injection of saline (vehicle) or MPTP. **P* < 0.05; *****P* < 0.0001, Dunnett's multiple comparisons test (in relation to "Vehicle").

4 CONCLUSIONS

- MPTP treatment may have a very weak negative effect on the accuracy of visual discrimination in the first 3–4 weeks after the injection.
- Reversal learning was unperturbed within 5–8 weeks after the injection of MPTP.
- Motor performance was not impaired in 2.5 months after the injection of MPTP.
- Dopamine and DOPAC levels were lower in MPTP-treated animals at 5.5 months after MPTP injection, but the extent of the decrease was not as large as that observed usually within the first 2-4 weeks.
- We conclude that to detect behavioural disturbances in the MPTP model of PD, the tests have to be relatively short and administered in the first couple of weeks following MPTP treatment. Otherwise, gradual recovery of dopaminergic function may lead to the normalisation of behavioural performance.