

# ApoE isoform-specific differences in behavior and cognition associated with subchronic MPTP exposure

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Parkinson's disease (PD) is characterized clinically by progressive motor dysfunction; overt parkinsonism is often preceded by prodromal symptoms including disturbances in the sleep–wake cycle. Up to 80% of patients with PD also develop dementia. In humans, there are three major apolipoprotein E isoforms: E2, E3, and E4. Increased rate of dementia in PD may be associated with E4 isoform. To better understand prodromal changes associated with E4, we exposed young (3–5 mo) male and female mice expressing E3 or E4 via targeted replacement to a subchronic dosage of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). We hypothesized that E4 mice would be more susceptible to MPTP-related behavioral and cognitive changes. MPTP-treated E4 mice explored novel objects longer than genotype-matched saline-treated mice. In contrast, saline-treated E3 mice preferentially explored the novel object whereas MPTP-treated E3 mice did not and showed impaired object recognition. MPTP treatment altered swim speed of E4, but not E3, mice in the water maze compared to controls. Thus, E4 carriage may influence the preclinical symptoms associated with PD. Increased efforts are warranted to study early time points in this disease model.

[Supplemental material is available for this article.]

Parkinson's disease (PD) is the most prevalent neuromotor disorder. It is characterized by progressive motor dysfunction and dopaminergic cell loss in the substantia nigra (Ehringer and Hornykiewicz 1960; Cheng et al. 2010; Braak and Del Tredici 2017). In addition to the primary motor symptoms, nonmotor symptoms, including sleep, and circadian rhythm dysfunctions and cognitive impairment are of increasing concern and highly problematic to patients' quality of life (Muller et al. 2013). Therapies thus far remain focused on targeting symptoms while researchers continue to study underlying mechanisms for the development of improved therapeutic strategies.

In humans, apolipoprotein E (apoE) exists as 3 distinct isoforms-E2, E3, and E4. E3 is the most common form, while E2 and E4 are found at lower prevalence rates. While less common than E3, E4 has been heavily studied in neurodegeneration due to its association as the strongest genetic risk factor for Alzheimer's disease (Farrer et al. 1997). ApoE transports lipids throughout the body and plays a role in the brain's response to injury; for example, after brain injury an increase in glial fibrillary acidic protein (GFAP) is followed by a progressive increase in apoE mRNA and protein levels and a subsequent increase of low-density lipoprotein receptor binding in neurons (Domenger et al. 2012). Clinical studies suggest that E4 might also be a predictor for increased cognitive decline in PD (Morley et al. 2012; Mata et al. 2014; Paul et al. 2016; Guerreiro et al. 2018), underscoring the clinical relevance of apoE in the context of PD. PD patients often show cognitive changes early in the disease and 40%–80% also develop dementia, termed PD with dementia (PDD) (Hely et al. 2008; Jellinger 2018). There is also a strong genetic association of E4 with PDD (Monsell et al. 2014; Guerreiro et al. 2018; Sun et al. 2019). Recent studies have assessed a potential role of APOE

genotype in regulating disease progression in PD focusing on alpha-synuclein, the primary protein found aggregated in Lewy bodies, the pathological hallmark of PD (Davis et al. 2020; Zhao et al. 2020). Although this contributes to increased understanding of the risk associated with PDD due to APOE genotype, the underlying mechanisms are still unclear.

Since its accidental discovery, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used to induce profound neuronal loss in the substantia nigra and corresponding motor impairment (Langston 2017). MPTP models of PD have contributed much to the understanding of molecular mechanisms involved in PD (Klemann et al. 2016). Compared to humans and nonhuman primates, mice show high resistance to effects of MPTP (Franke et al. 2016). In order to model the progressive loss seen clinically, previous work has applied the strategy of increasing the MPTP dosage to better understand changes resulting in neuronal loss in the substantia nigra (Goldberg et al. 2011; Torres et al. 2018).

Recently, we assessed the role of the metabotropic glutamate receptor 8 (mGlu8) in response to subchronic-MPTP treatment using wild-type (WT) and *mGlu8* knockout mice, revealing genetic ameliorations of MPTP-induced behavioral and cognitive changes (Torres et al. 2018). Here we use a similar experimental design as in the mGlu8 study to determine whether there are apoE isoform-specific differences in response to subchronic-MPTP treatment. We hypothesized that E4 mice would be more susceptible to

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MPTP-induced behavioral and cognitive changes, and related molecular changes in the striatum.

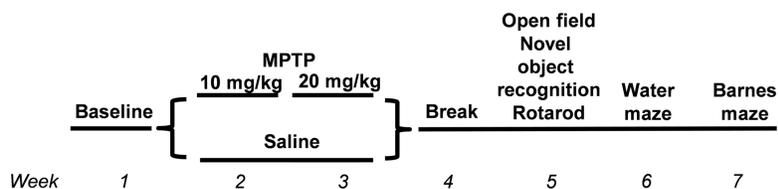
## Results

### General health and home cage activity

Body weights were monitored to assess general health throughout the experiment (see Fig. 1 for experimental design, Table 1 for group sizes). When analyzing body weights at the start of the injections and at the beginning of behavioral testing, male mice weighed more than female mice, as expected for this age range, regardless of genotype (Fig. 2, A. E3:  $F_{(1,33)}=67.312$ ,  $P<0.001$ ; B. E4:  $F_{(1,35)}=53.318$ ,  $P<0.001$ ). Mice treated with MPTP did not show differences in body weight compared to saline treated mice and appeared to tolerate the injections. Within the E3 groups, there was a significant time point  $\times$  sex interaction ( $F_{(1,33)}=9.274$ ,  $P=0.005$ ) suggesting that females increased in body weight over the experiment whereas males slightly decreased. There was also a time point  $\times$  sex  $\times$  treatment interaction ( $F_{(1,33)}=6.629$ ,  $P=0.015$ ) showing both males and females that were given MPTP maintained body weight throughout testing whereas saline-treated females increased while males slightly decreased.

During the baseline period light cycle, no significant differences in activity were found due to sex or the future treatment assignments, that is, whether an animal ended up in the MPTP or control saline group (Supplemental Fig. 1A,B). There was a significant difference due to sex in both the E3 and E4 groups, with females moving significantly more during the dark cycle (Supplemental Fig. 1, C. E3:  $F_{(1,29)}=6.553$ ,  $P=0.016$ ; D. E4:  $P=0.007$ ;  $F_{(1,32)}=8.421$ ,  $P=0.007$ ). Analyses of weekly light cycle averages across all 4 wk of activity monitoring showed no significant effects of MPTP treatment (Supplemental Fig. 1E,F). Mice in both genotype groups showed habituation (Supplemental Fig. 1, E. E3: main effect of week- $F_{(1.685,48.866)}=9.123$ ,  $P<0.001$ ; F. E4: main effect of week- $F_{(1.645,52.627)}=25.546$ ,  $P<0.001$ ). E4 male mice moved less than E4 female mice in the light cycle (Supplemental Fig. 1F.  $F_{(1,32)}=5.414$ ,  $P=0.026$ ). Dark cycle averages showed a similar pattern (Supplemental Fig. 1G,H); E3 and E4 mice showed decreased levels of activity over time in their home cage (Supplemental Fig. 1, G. E3: main effect of week- $F_{(1.266,36.719)}=18.848$ ,  $P<0.001$ ; H. E4: main effect of week- $F_{(1.803,57.692)}=7.001$ ,  $P=0.003$ ). Analysis in the E3 mice also revealed greater changes in activity over time due to MPTP (week  $\times$  treatment effect:  $F_{(1.266,36.719)}=4.945$ ,  $P=0.025$ ). E3 and E4 female mice moved more overall in the dark cycles compared to male mice (Supplemental Fig. 1, G. E3:  $F_{(1,29)}=7.281$ ,  $P=0.011$ , H. E4:  $F_{(1,32)}=8.344$ ,  $P=0.007$ ).

To account for individual differences in general home cage activity, we assessed the average dark cycle activity to light cycle activity ratio during treatment weeks. Female E3 mice displayed higher ratios than E3 males (Fig. 2C.  $F_{(1,29)}=34.421$ ,  $P<0.001$ ). E3 mice also showed a week  $\times$  treatment interaction (Fig. 2C).



**Figure 1.** Experimental design. Mice were habituated to single housing in activity monitoring equipment during the baseline period. MPTP or saline were administered over the course of 2 wk. Behavioral and cognitive testing commenced after a week-long break. During week 5, morning testing consisted of open field for 2 d, followed by object habituation and novel object testing the next 2 d. Rotarod was completed in the afternoons.

**Table 1.** Group sizes for experiment

|        | E3      |       | E4      |       |
|--------|---------|-------|---------|-------|
|        | Females | Males | Females | Males |
| Saline | 8       | 10    | 11      | 7     |
| MPTP   | 9       | 10    | 12      | 10    |

$F_{(1,29)}=6.510$ ,  $P=0.016$ ). E4 mice showed a similar difference between sex (Fig. 2D.  $F_{(1,32)}=8.364$ ,  $P=0.007$ ). Additionally, there was no difference between dark/light ratios of male E4 MPTP-treated mice compared to male E4 saline-treated mice and female E4 MPTP-treated mice compared to their control counterparts (Fig. 2D. sex  $\times$  treatment:  $F_{(1,32)}=3.734$ ,  $P=0.062$ ).

### Locomotor performance and general exploration

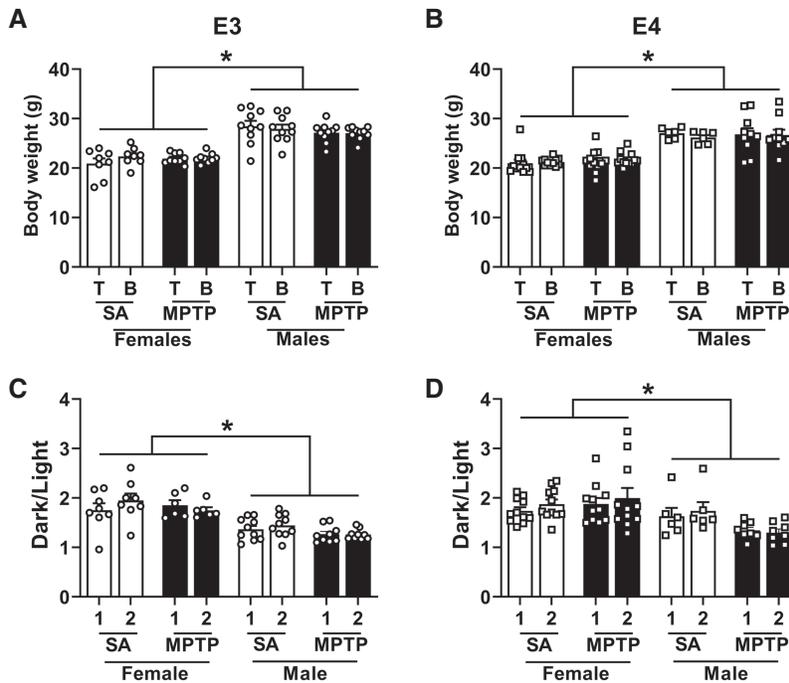
Locomotor performance on the rotarod was not affected by MPTP treatment in either E3 or E4 mice (Supplemental Fig. 2) using this particular subchronic treatment paradigm. Both genotype groups improved over the 3 d of training (Supplemental Fig. 2A, C. E3:  $F_{(2,66)}=74.876$ ,  $P<0.001$ ; B, D. E4:  $F_{(2,70)}=45.849$ ,  $P<0.001$ ) and E3 females performed better than E3 males (Supplemental Fig. 2A, C.  $F_{(1,66)}=22.907$ ,  $P<0.001$ ). We also analyzed the difference in performance of the mice on the first and last day of the rotarod test. There were no differences in the change score, day 3 average—day 1 average (Supplemental Fig. 2E,F).

Behavioral performance in the open field support the lack of MPTP-related motor dysfunction on the rotarod in both genotypes (Supplemental Fig. 3). Both genotype groups showed habituation to the open field arena in the total distance moved over the two trials (Supplemental Fig. 3, A. E3:  $F_{(1,33)}=4.284$ ,  $P=0.046$ ; B. E4:  $F_{(1,33)}=6.985$ ,  $P=0.012$ ). A similar pattern was seen for habituation in center entries (C. E3:  $F_{(1,33)}=25.471$ ,  $P<0.001$ ; D. E4:  $F_{(1,34)}=10.016$ ,  $P=0.003$ ).

### Novel object recognition and spatial learning and memory

There were no significant treatment effects on the total distance moved during the two trials with the objects (Supplemental Fig. 4A,B) nor did animals show a side preference during object habituation (Supplemental Fig. 4C–F). However, while there were no differences between E3 treatment groups (Fig. 3A), E4 MPTP-treated mice explored the objects more during the learning and memory trials (Fig. 3B,  $F_{(1,33)}=5.152$ ,  $P=0.030$ ). On the test day, only E3 saline-treated mice showed a significant preference for the novel object compared to the familiar object (Fig. 3C. paired Student's  $t$ -test,  $P=0.0416$ ). E4 mice did not show a preference for the novel object (Fig. 3D). The discrimination index (Fig. 3E–F.  $DI=[\text{time exploring novel object} - \text{familiar object}]/\text{total time exploring objects}$ ) revealed no differences due to sex or treatment in either genotype.

Working memory was assessed in the water maze using the paradigm illustrated in Figure 4A. There was no effect of MPTP on swim speeds in E3 mice in either the training sessions (Fig. 4B) or the probe trials (Fig. 4D). However, MPTP-treated E4 mice swam significantly faster than saline-treated E4 mice during training (Fig. 4C.  $F_{(1,34)}=19.085$ ,  $P<0.001$ ) and probe trials (Fig. 4E.  $F_{(1,33)}=9.563$ ,  $P=0.004$ ). Overall, E4 mice



**Figure 2.** Effects of MPTP on general health and home cage locomotion throughout the experiment. (A,B) Body weights measured prior to the first saline or MPTP treatment (T) and at the start of behavior (B) showed no differences due to MPTP. E3 and E4 male mice weighed significantly more than females of the same genotype ( $*P < 0.001$ ). (C,D) Average activity during the dark and light cycles of each week are shown as a ratio. (C) Female E3 mice displayed higher ratios than E3 males ( $*P < 0.001$ ). E3 mice also showed a week  $\times$  treatment interaction ( $P = 0.016$ ). (D) E4 females showed dark/light ratios compared to E4 males ( $*P = 0.007$ ).

showed decreasing swim speed across the two probe trials (Fig. 4E.  $F_{(1,33)} = 7.709$ ,  $P = 0.009$ ).

Since sex was not a significant main effect, sex was removed from our model. In E3 mice, there was no effect of MPTP to locate the hidden or visible platform locations as shown by the cumulative distance to the target platform (Fig. 4F). During hidden platform trials, swim speed was a significant covariate in E3 mice (Fig. 4F.  $F_{(1,34)} = 21.527$ ,  $P < 0.001$ ), but not in E4 mice (Fig. 4G). In the first reversal training sessions of E3 and E4 mice, swim speed was not significant and including it did not change the overall findings (Fig. 4F,G). In E3 mice, swim speed was the only significant variable during the second reversal training (Fig. 4F. E3:  $F_{(1,34)} = 6.233$ ,  $P = 0.018$ ). It was also a significant variable during visible training for both E3 mice (Fig. 4F.  $F_{(1,34)} = 11.009$ ,  $P = 0.002$ ) and E4 mice (Fig. 4G.  $F_{(1,35)} = 19.553$ ,  $P < 0.001$ ). In E4 mice, the learning curve during the second reversal training and visible platform training appear visually better in MPTP-than saline-treated mice, but this was not statistically significant.

Pairwise comparisons with Sidak corrections showed that mice performed better throughout the course of hidden platform training (Fig. 4, D. E3: Session 1 vs. 2- $P = 0.010$ , Session 1 vs. 3- $P < 0.001$ ; E. E4: Session 1 vs. 2- $P = 0.019$ , Session 1 vs. 3- $P = 0.005$ ). Mice also improved during the first reversal training (Fig. 4, D. E3: Session 1 vs. 2- $P = 0.027$ ; E. E4: Session 1 vs. 2- $P = 0.004$ ) and second reversal training (Fig. 4, D. E3: Session 1 vs. 2- $P = 0.010$ ; E. E4: Session 1 vs. 2- $P = 0.021$ ). E4 mice also improved during visible training (Fig. 4E. Session 1 vs. 2- $P = 0.011$ ). This was not seen in the E3 mice during visible platform training (Fig. 4D. Session 1 vs. 2- $P = 0.106$ ) likely since the E3 mice performed very well even by the second visible trial in the first session of visible platform training.

Cumulative distance to the target during the probe trials was not significantly affected by sex or MPTP treatment in either genotype (Supplemental Fig. 5A, B). In order to determine whether mice learned the location of the target location, we assessed the percent time in each quadrant mice spent during the probe trials. Results from Probe 1 (Supplemental Fig. 5C,D) show that none of the E3 groups, regardless of sex or genotype showed a preference for the target quadrant. E4 female mice, however, did show preference (Saline: ANOVA,  $F_{(1,70,15,29)} = 11.23$ ,  $P = 0.001$ ; MPTP: ANOVA,  $F_{(1,52,15,19)} = 10.10$ ,  $P = 0.003$ ) while E4 male mice did not. No groups showed preference during the second probe trial (data not shown).

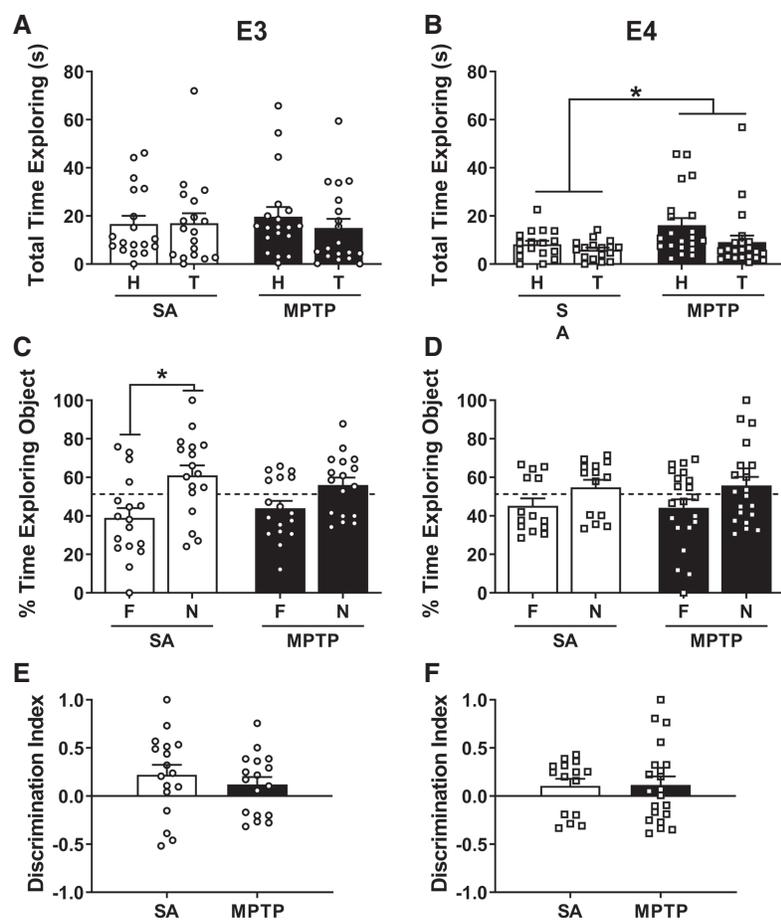
### Tissue measures

Since tyrosine hydroxylase (TH) is enzyme for the rate-limiting step in dopamine synthesis, striatal TH levels were assessed as well (Supplemental Fig. 6A; Haavik and Toska 1998). There were no significant differences due to genotype or MPTP treatment. Furthermore, nicotinamide adenine dinucleotide phosphate (NADPH) (Supplemental Fig. 6B) and microtubule associated protein 2 (MAP-2) (Supplemental Fig. 6C) were assessed as potential markers for molecular mechanisms related to the behavioral

changes. These analyses also did not reveal significant treatment or genotype effects.

### Discussion

Animal models typically reflect aspects of human neurodegeneration but often there is no single animal model that reflects all aspects of the human condition. This is especially pertinent in animal models of PD. Unlike humans that develop PD, animals do not, including aged nonhuman primates. While fine motor skills are affected in aged Rhesus macaques, parkinsonian symptoms are not seen. Consistent with the species differences, aged rodents show little, if any, dopamine cell loss (Meshul, unpublished findings). In the current study, there was no obvious loss of striatal TH protein expression. Based on other animal studies, it has become clear that until there is 80% loss of dopamine terminals, there is continued sprouting of new dopamine terminals (Finkelstein et al. 2000). Therefore, it is conceivable that the lack of loss of TH protein levels might be due to sprouting of existing terminals and/or an up-regulation of TH protein in the existing terminals, as we have previously reported (Churchill et al. 2019). Consistent with this notion, an increase in TH expression in the midbrain was observed following drug treatment in the substantia nigra/midbrain (Churchill et al. 2019). These data suggest that the remaining dopamine cells are increasing their TH levels. Until motor symptoms are seen in PD patients, the brain is constantly adapting to the loss of dopamine. So a major question of translational relevance is as to why only humans develop PD and aged rodents, specifically mice on the C57BL6 background strain (Sedelis et al. 2000; Ciesielska et al. 2007), and nonhuman primates are protected against developing PD. Regardless, clinical treatments have



**Figure 3.** Effects of MPTP on object recognition. (A) There were no effects of MPTP on the time E3 mice spent exploring the objects. (B) E4 MPTP-treated mice explored the objects more during the habituation and test days than E4 saline-treated mice (\*  $P=0.030$ ). (C) On the test day, E3 saline-treated mice showed a significant preference for the novel object compared to the familiar object (Student's  $t$ -test, \*  $P=0.0416$ ); this was not seen in E3 MPTP-treated mice. (D) Saline- and MPTP-treated E4 mice did not spend significantly more time exploring the novel object. There was no effect of MPTP on the discrimination index of E3 mice (E) or E4 mice (F).

been based on findings from these environmental neurotoxin models, supporting the validity of these preclinical PD animal models.

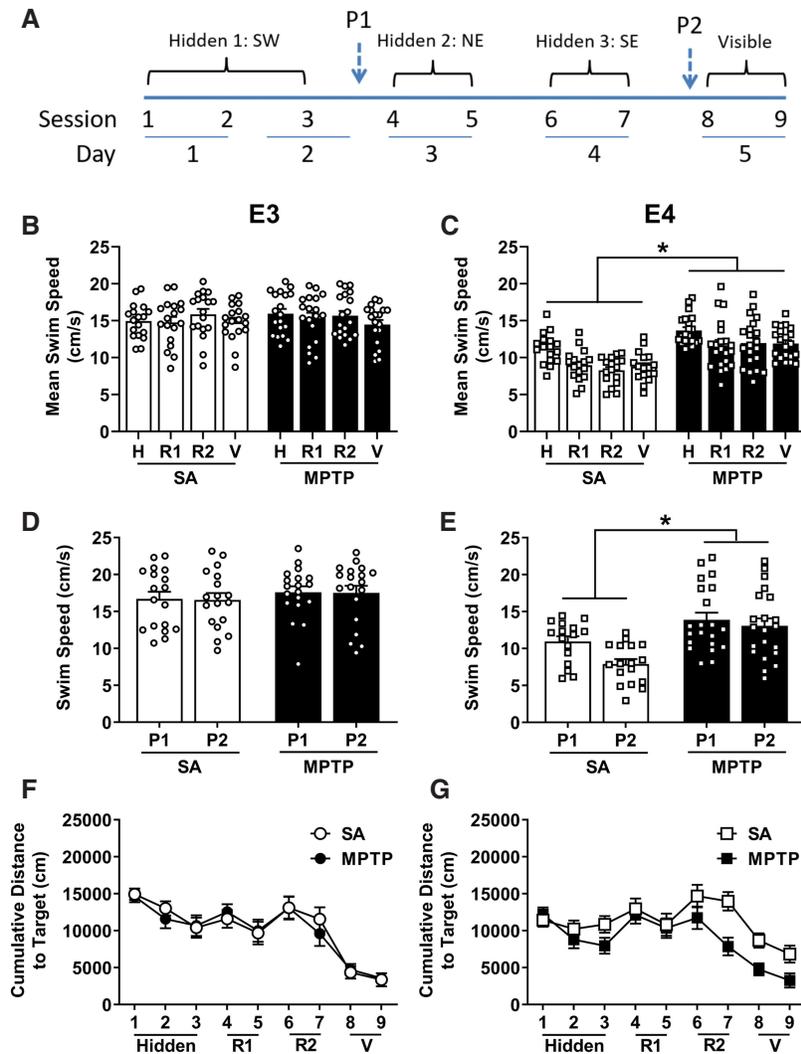
Using this 2-wk MPTP dosage allows us to model PD-related changes before overt motor deficits occur. In our previous research, we lowered the MPTP treatment period from 4 to 2 wk to avoid profound motor deficits that could affect performance on cognitive tests. Here, we again found that this 2-wk regimen did not result in overt health problems. It did, however, result in behavioral changes, most of which were specific to the *APOE* genotype. Object recognition testing revealed MPTP-treated E4 mice explored the objects more than saline-treated mice. This was not dependent on how much time the mice spent in the center or how much they traveled overall and was not seen in E3 mice either. Furthermore, MPTP-treated E4 mice swam faster than saline-treated E4 mice throughout the water maze. These data indicate that E4 mice are more affected by MPTP treatment compared to similarly treated E3 mice. The direction of change seems counterintuitive considering the human condition. The increased swim speeds and increased exploration times might be part of a compensatory response that highlights changes in motor activity and is separate from cognitive performance.

Effects of MPTP on cognitive measures were subtle compared to those on activity measures. In the object recognition test, MPTP treatment resulted in a lack of preference for the novel object in the E3 mice. E4 mice did not show an object preference regardless of MPTP-treatment, although untreated E4 mice at this age typically do show object recognition (Haley et al. 2012). The lack of significant object recognition in the E4 saline control mice in this study might be due to the stress of receiving daily saline injections for a total of 10 d. We recognize that increased activity levels in E4 mice might have contributed to their performance in cognitive tests and as a result complicates the interpretation of the cognitive data in E4 mice.

While we originally intended to analyze the cognitive performance of the mice in the absence and presence of MPTP, not all of our saline-treated mice showed preference for the target quadrant during the probe trial in the water maze. This suggests that, like the Barnes maze, this particular water maze testing paradigm may have been too challenging. Nevertheless, the water maze test provided evidence that motor performance (i.e., swim speed) is altered by MPTP. MPTP-treated E4 mice swam faster than saline-treated genotype-matched controls both in training sessions and the probe trials of the Morris water maze. One study suggests that the increase in swim speed may relate to greater stress exposure (Gehring et al. 2015). While there were no significant differences in TH levels, it is conceivable that in E4 mice increases in glutamate levels contributed to the increases in motor performance like seen for measures like swim speed in the water maze. Previous work using unilateral

injections of 6-hydroxydopamine showed increased striatal glutamate levels 1 mo after injection (Meshul et al. 1999). Acute MPTP treatment also causes similar increases in striatal glutamate levels (Robinson et al. 2003). Differences in swim speed, however, did not necessarily correspond with differences in overall task performance in the water maze. While E4 mice treated with MPTP showed overall enhanced swim speeds during Hidden, Reversal, Visible, and Probe trials, improved performance was only seen during the second reversal and visible platform training. Therefore, enhanced swim speeds are not necessarily sufficient to cause enhanced performance. Haley et al. previously found that E4 mice took longer to show preference for the target locations during probe trials (Haley et al. 2012). This suggests that E4 mice may need more time to learn the water maze and supports the hypothesis that E4 mice may be more susceptible to the effects of stress on cognitive performance.

Compared to the aSyn/apoE model that was recently published, we did not find anxiety-like behavior in MPTP-treated mice (Zhao et al. 2020). This may be due to the influence of using AAV-aSyn to induce parkinsonism or the age at which the animals were tested. Taken with our subchronic dosage of MPTP, our findings likely highlight aspects of early stages of PD before serious motor complications occur.



**Figure 4.** Effects of MPTP on water maze performance. Sexes are shown collapsed within genotypes. (A) Water maze design. P1 = Probe 1; P2 = Probe 2. (B) There were no effects of MPTP on the swim speeds of E3 mice. (C) E4 MPTP-treated mice swam significantly faster than saline-treated E4 mice during all phases of the training ( $* P < 0.001$ ). H = Hidden; R1 = Reversal 1; R2 = Reversal 2; V = Visible. (D) There were no effects of MPTP on the swim speeds of E3 mice during the probe trials. (E) E4 mice treated with MPTP swam significantly faster than saline-treated E4 mice ( $* P = 0.004$ ). E3 (F) and E4 (G) mice showed no difference due to MPTP treatment on the ability to perform the task in any of the individual session types (Hidden, Reversal 1, Reversal 2, Visible), shown by the cumulative distance from the target location.

MPTP effects on behavioral and cognitive performance we previously reported in wild-type mice (Torres et al. 2018) are summarized in Table 2. Notably, despite exposure to the same dosages and similar behavioral and cognitive testing paradigm, we did not find similar MPTP-related changes in the rotarod task, a measure of locomotor ability, or distance traveled in the open field. Moreover, E3 and E4 mice did not present with the striking sex  $\times$  MPTP interactions in the rotarod performance that we saw previously in WT mice (Torres et al. 2018). This does however correspond to previous work using the C57Bl6J strain and MPTP exposure (four injections of 15 mg/kg MPTP spread over 8 h) that also did not find sex differences associated with sensitivity to MPTP (Sedelis et al. 2000). Another study has found conflicting data showing that C57Bl6J females are more susceptible to the neurotoxic effects of MPTP (Ookubo et al. 2009) while yet another provides evidence that

male mice are more susceptible compared to females (Ciesielska et al. 2007) suggesting that more research is needed to understand clearly the influence of sex. Taken with these data, our current findings suggest that human apoE mice, regardless of isoform, may be relatively protected against the effects of MPTP exposure. Compared to our findings in WT mice treated with the same MPTP regimen, E3 mice did not show similar differences to WT mice in behavior due to MPTP exposure.

There are potential reasons why we may not see similar differences in the E3 mice. For example, mouse apoE acts as if it only has one binding domain, which is more similar to E4 than E3, and has the highest binding capacity whereas E3 is the lowest (Nguyen et al. 2014). Yet, mouse apoE does not replicate apoE4 domain interactions and behaves more like E3 and binds preferentially to high density lipoproteins (vs. low density lipoproteins and very low density lipoproteins in E4) (Raffai et al. 2001). Synthesis of apoE drives production of astrocyte lipoproteins, and the type of lipoproteins depends on the isoform of apoE. This lipid: apoE ratio is higher in mouse than human apoE (Fagan et al. 1999), although cholesterol and phospholipids appear to be about the same in ratio (DeMattos et al. 2001) suggesting that specific lipids may be critical to deficits associated with PD. In fact, cholesterol has been shown to contribute to increased dopamine reduction and striatal neuron loss associated with acute MPTP treatment (Paul et al. 2017). Although we hypothesized that the lack of MPTP-related changes in the human apoE mice may be due to NADPH interacting with apoE (Craigie et al. 2015) as well as MAP-2 (Zhou et al. 2018, 2019), we did not find evidence to support this. Moreover, we did not find differences in TH levels suggesting the behavioral changes observed in this study are most likely not related to alterations in

**Table 2.** Summary and comparison of previous findings in WT mice and E3 and E4 mice in current study

| Measure <sup>a</sup>                                       | WT |   | E3 |   | E4 |   |
|--|----|---|----|---|----|---|
|  | ♀  | ♂ | ♀  | ♂ | ♀  | ♂ |
| AM—dark cycle activity average for treatment weeks 1 and 2 | ↓  | ↓ | -  | - | -  | - |
| OF—total distance moved <sup>b</sup>                       | ↑  | ↑ | -  | - | -  | - |
| NO—total distance moved                                    | ↑  | ↑ | -  | - | -  | - |
| NO—total time exploring objects                            | ↑  | ↑ | -  | - | ↑  | ↑ |
| Rotarod improvement score                                  | ↓  | ↑ | -  | - | -  | - |

<sup>a</sup>Directional change is compared to saline controls.

<sup>b</sup>Open field for WTs was three 5-min trials versus the two 10-min trials used here.

TH levels. Since MPTP treatment only lasted 2 wk, there are likely changes in other neurotransmitter/receptor levels that were not measured. It is possible that the behavioral changes may be related more to the motor cortex versus the striatum, which we assessed TH in for this study.

Thus, the behavioral findings in this study highlight the usefulness of MPTP treatment for modeling aspects of preclinical motor symptoms in PD and suggest that E4 may modulate the effects of MPTP. Increased efforts are warranted to elucidate the molecular changes involved in these behavioral differences associated with E4.

## Materials and Methods

### Animals

Procedures for this study followed the ARRIVE guidelines and were approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University. Male and female targeted replacement mice expressing either the human E3 or E4 isoform under the control of the mouse apoE promoter backcrossed on the C57Bl6J strain (the same as the wild-type strain used in our previous study) and bred in our colony were used (Sullivan et al. 1997; Knouff et al. 1999; Torres et al. 2018). Mice were 3–5 mo of age and were group housed in standard vivarium conditions until the start of the study. They were then singly housed for home cage activity monitoring and remained singly housed for the duration of the experiment. The vivarium was maintained at 20°C–21°C and food (PicoLab Rodent Diet 20, no. 5053; PMI Nutrition International) was available *ad libitum*. Lights were kept on a 12 h light: 12 h dark cycle. MPTP or saline treatment and behavioral and cognitive testing (except activity monitoring) were performed during the light cycle, between 1 h after lights on until 1 h before lights off. Activity monitoring was measured noninvasively for the entire day during the weeks of recording.

Table 1 lists the group sizes; Figure 1 depicts the behavioral and cognitive testing schedule the animals underwent. Mice were checked daily and body weight was monitored throughout. Mice were brought into the adjoining testing room, immediately tested, and then returned to the housing room. The behavioral equipment was cleaned with 0.5% acetic between trials except for the water maze testing. The experiment was conducted over three cohorts based on the availability of appropriately aged E3 and E4 mice that were counterbalanced for sex and MPTP treatment. A single experimenter conducted all the behavioral testing for a single cohort, and two experimenters completed the testing for three individual cohorts. Two mice were treated for malocclusions during the testing period (female E3 saline-treated). One mouse was euthanized after open field testing due to severe dermatitis (female E4 MPTP-treated).

### MPTP treatment

Saline or MPTP (Santa Cruz Biotechnology) dissolved in saline was administered via intraperitoneal injections daily for 10 d over the course of 2 wk as described (Torres et al. 2018). The first week consisted of 5 d of MPTP at 10 mg/kg/day or saline, followed by a 2-d break and subsequently 5 d of MPTP at 20 mg/kg/day or saline. Control animals were administered saline at the same time MPTP was delivered. Mice were given a 1-wk break in-between the second week of treatment and the start of behavioral and cognitive testing. The experimenters remained blind to genotype and MPTP treatment group throughout testing and during analyses.

### Home cage activity

Home cage activity was continuously measured using noninvasive home cage monitors (BioBServe) during the baseline period prior to treatment, the treatment weeks, and the break week after the second week of treatment. Data were recorded every second with MLog software (BioBServe) and averaged across 30-min bins for analysis.

Activity monitoring is shown as averages during the light and dark cycles across the days. Due to computer malfunction, only activity monitoring data from days that had at least four mice from each group were included from analysis. As a result, both the baseline and break week were analyzed including only a single light and dark cycle (the end of baseline and the middle of the break week). Light and dark cycles were analyzed separately. To normalize individual differences, a ratio of activity during the dark and light cycles was also analyzed.

### Open field and novel object recognition tests

General locomotor activity was assessed in the open field, which consisted of a plastic enclosure (40.6 cm in length) with transparent walls (300 lux). Mice were allowed to explore in this open field for two 10-min trials separated by 24 h. The subsequent day, two identical orange wooden octagonal prisms were placed equidistant from the walls in the center of the open field. Mice were then allowed to explore these objects for 15 min. The next day, one of the orange blocks was replaced with a green triangular block (novel object) and again mice were allowed to explore this new environment for 15 min. Performance of the mice during these trials was recorded and mouse movement was analyzed using Ethovision XT 7 software (Noldus Information Technologies). Total distance traveled, average velocity, and duration in the center of the open field were analyzed. During novel object trials, the total distance and average velocity were measured. Raters blind to the treatment and genotype scored the videos to determine total amount of time mice explored each object. Mice that explored less than 1 sec were excluded for the analysis of object preference.

### Rotarod

Locomotor ability and balance were assessed using the rotarod test with Rotamex-5 software (Columbus Instruments). Mice were trained to remain on a rotating rod (diameter: 3 cm, elevated: 45 cm) with an increasing speed. The training consisted of three trials per day for three sequential days. Each trial started at a speed of 1 rpm with an interval increase of 1 rpm every 3 sec until the mouse falls off the rod or up to a maximum duration of 300 sec.

### Water maze

Mice were tested for spatial learning and memory using the Morris Water Maze (Morris 1984), similar to as previously described (Weiss et al. 2017). The maze was 140 cm in diameter and was filled with opaque water using nontoxic, white chalk. Large visual cues surrounded the maze to make this a hippocampus-dependent task. An escape platform was submerged 1 cm below the water surface. Training sessions consisted of two trials each, with a 10-min intertrial interval. During each training trial, mice were dropped off in counterbalanced locations and allowed to explore the water maze. The trial ended when the mouse located the escape platform and remained on it for 3 sec. Mice that did not find the platform within the 60-sec trial time, were gently led to the platform by the experimenter. Each day of water maze testing included two sessions, except for the second day that included one session. Animals were first tested in trials in which the platform was “Hidden.” For the first 2 d of testing, the platform location remained the same. The platform location was then changed on day 3 and then again on day 4. After Hidden platform locations 1 and 3, the mice were tested for spatial memory retention during a 1-min “Probe” trial in which the platform was removed. On day 5, ability to learn the task, that is, locating the escape platform, was assessed by placing a conical tube wrapped with colored tape as the visual cue to mark the target platform. See Figure 4A for the water maze paradigm used.

Average swim speed during each session type (Hidden, Reversal 1, Reversal 2, Visible) was included as a covariate. Sex was originally included in the model and then dropped for both genotypes when it was shown to be nonsignificant.

## Barnes maze

Mice were also tested for spatial learning and memory in the dry land Barnes maze, as described (Raber et al. 2004). A circular table (diameter 122 cm) with 40 holes along the circumference. The perimeter was lit by an adverse light (2100 lumens) and an elevated floor fan was used to try motivating the performance of the mice. One hole was designated as the escape with an attached tunnel. Prior to the first trial, mice were gently lowered in the escape tunnel and allowed to habituate to the escape tunnel for 10 sec. During each trial, mice were placed in the center of the maze and allowed to explore freely for 5 min or until they entered the hole. Mice were trained for two sessions each day for a total of 6 d. If mice did not find the escape tunnel, they were gently moved to the escape and encouraged to enter. Movement was recorded using Ethovision XT 7 software. Latency to escape was the primary measure. Perhaps due to motivation-related issues, mice preferred to remain on the edge of the escape hole rather than enter and did not learn this task with the paradigm used. Of the three cohorts of mice tested, the last cohort did not go through the Barnes maze testing as performance of the mice of the first two cohorts was very poor (data not shown).

## Tissue collection and analyses

Mice were euthanized the day after the last day of behavioral testing. Brain tissues were dissected and prepared for analyses. Previously unprocessed striatal tissues from WT mice (Torres et al. 2018) were included to compare WT and human apoE mouse tissues. Briefly, tissues were homogenized with lysis buffer and protease inhibitor (Roche, catalog no: 11836153001). Homogenates were briefly sonicated and then frozen at  $-80^{\circ}\text{C}$ . They were then centrifuged ( $10,000\times 10$  min) and aliquoted for assays.

Due to previous findings showing lower beta-actin levels in striatum of male WT mice following MPTP treatment (Torres et al. 2018), we chose to assess striatal TH levels by ELISA (MyBioSource, catalog no: MBS738113). ELISAs for MAP2 (MBS 725632) and NADPH (MBS2605848) were also performed according to manufacturer instructions (MyBioSource). Target protein levels were normalized to total protein levels, determined by BCA (Thermo Scientific, catalog no: 23235).

## Statistical analyses

Behavioral and cognitive data and biochemical tissue measures are reported as mean  $\pm$  SEM. All data were analyzed using SPSS v.25 software (IBM). Graphs were created using GraphPad software v.8.2.0. We a priori hypothesized that E3 and E4 mice would show different responses to MPTP and thus analyzed these two genotypes separately, including sex and treatment as factors. Analyses consisted of two-way analyses of variance (ANOVAs) with repeated measures noted when appropriate. We set statistical significance to  $P < 0.05$ . Greenhouse–Geisser corrections were used if sphericity was shown to be violated with Mauchly's test. Paired Student *t*-tests were used to assess object preference in the object recognition test. Graphs show the data with sexes collapsed if sex was not a significant factor.

## Competing interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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