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Original Article

Cystic Fibrosis-related neurodegenerative disease associated with tauopathy and cognitive decline in aged CF mice

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ABSTRACT

Background: Highly effective modulator therapies (HEMT) are increasing the lifespan for many people with cystic fibrosis (pwCF), making it necessary to identify and understand CF specific age-related consequences. In this study, we examine the impact of aging on cognitive function and age-related brain pathology in a CF mouse model focusing on phospho-Tau (pTau) pathology.

Methods: Cognitive function was measured by novel object recognition and spontaneous alternation behavior tests. Hippocampal neuronal function was assessed by measuring long-term potentiation (LTP) electrophysiology, the synaptic correlate of learning and memory. Tau pathology was assessed by immunohistochemical analyses and western blot assessment of pTau levels in CF mouse brain, as well as human nasal epithelial cells isolated from pwCF.

Results: Cognitive function declined progressively with age in Cftr (G542X/G542X) (G542X) mice, a model of CF, compared to wild-type (WT) littermate controls. LTP was also deficient in older G542X mice. Increased pTau was observed by staining and western blot analysis in the hippocampus of aged CF mice. Secondary impacts of tauopathy, including increased microglial uptake of cholesterol and reduced neuronal density were also observed. Lastly, human nasal epithelial cells from pwCF were found to display elevated pTau levels compared to non-CF controls.

Conclusions: Aging CF mice develop tauopathy, cognitive decline, LTP impairment, microglial activation, and neurodegeneration that is not experienced by age-matched WT littermates, a condition herein termed cystic fibrosis-related neurodegeneration (CFND). These findings suggest that pwCF may be at risk for tauopathy-related neurodegeneration and cognitive impairment with aging.

1. Introduction

Cystic fibrosis (CF) is a multiorgan disorder caused by mutations in the CF transmembrane conductance regulator gene (*cftr*), with lung disease related to impaired mucus production and clearance being the leading cause of mortality for people with CF (pwCF) (1). Fortunately, the recent advent of highly effective modulator therapy (HEMT) has greatly improved health outcomes of pwCF (2). As these treatments are anticipated to also increase life expectancy, age-related CF health issues need to be considered including neurological health. While CFTR expression is found in many regions of the brain, including the hypothalamus, cortex, and hippocampus (3), little is understood of its role in neurological function.

There is evidence that cognitive decline may begin early in the context of CF. For example, neuroimaging and cognitive assessments have demonstrated mild cognitive impairment in pwCF as early as 30

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years of age (4). When comparing T1 and T2 weighted images from pwCF to healthy age matched controls (average 29.7 years), grey matter tissue damage in the hippocampus and prefrontal cortex and altered T2 relaxation in many brain regions associated with cognition have been reported in pwCF. Additionally, the Beck Depression Inventory (BDI) was found to be significantly higher in pwCF, while the Montreal Cognitive Assessment was significantly lower, mostly on the visuospatial subscale (4). Cognitive changes have also been reported in CF-related diabetes (CFRD) patients, who display cognitive disfunction including problems with verbal and spatial memory, processing speed, and cognitive flexibility (5).

Animal studies also highlight neurological issues associated with CF. In mouse models exploring the cognitive effects of myocardial infarction (MI), Vanherele et al. report that treating wild-type (WT) mice with CFTR modulators (lumacaftor/ivacaftor) increased hippocampal neuron CFTR expression, mitigated MI-induced deficits in hippocampal dendritic arborization and spine density, and improved cognitive function on the novel object recognition (NOR) test (6). This study suggests a specific link between CFTR function and maintenance of normal learning and memory. Our previous work characterized both disrupted circadian rhythms and increased depression and anxiety in the F508del CF mouse model (7–9). These findings are significant in that these phenotypes are often precursors to advancing cognitive decline in Alzheimer's and other neurological diseases (10–12). As these brain disruptions occurred in the absence of obvious systemic inflammation or extensive lung disease, it is reasonable to hypothesize a direct neurological function of CFTR. Given the presence of neurological phenotypes in CF mouse models that are precursors to cognitive decline, we hypothesized that aging CF mice would exhibit reduced performance on learning and memory behavioral assays.

2. Methods

2.1. Mice

Cftr(G542X/G542X) and age-matched WT littermate controls on a C57Bl/6 J background were obtained from the Cystic Fibrosis Mouse Model Core at CWRU. Mice were allowed access to sterile water with osmotic laxative, PEG-3350 with electrolytes (Kremers Urban Pharmaceuticals). Mice were group housed inside a temperature controlled dormitory (22 °C) in a 12:12 light:dark cycle. Mice were provided chow and water ad libitum for the duration of the study. The institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University approved all animal protocols. All methods were conducted according to necessary guidelines and established regulations.

2.2. Novel object recognition test (NOR)

On day 1 mice were allowed to habituate to the open field for 10 min. On day 2 mice were placed into an open field with 2 identical objects (A-A) for 10 min of exploration. After 24 h, mice were returned to the arena with one familiar object and one novel object for 5 min of video-recorded exploration to assess object recognition memory. The videos were analyzed at a later time and the mouse interaction with the objects (nose oriented towards object, within ~2 cm of the object while not climbing on the object were counted as interaction time) was scored using event tracking software (OD Log, Macropod software). The results display the time spend interacting (seconds) with the familiar and novel object. The discrimination index is the (time spent interacting with the novel object) – (time spent interacting with familiar object) / (total object interaction time).

2.3. Spontaneous alternation test (SA)

Mice were placed in the home chamber (10 cm x10cm x 20 cm) at the far end of the alley way for 1 min, then released to explore the rest of the maze for 7 min. A sliding door separated the home chamber from the rest of the alley way (40 cm) that leads to the choice point, to enter either the left or right arm of the maze (30 cm x 10 cm x 20 cm). A divider panel (20 cm) was positioned at the intersection of the T and extended (10 cm) into the alley way. An alternation attempt was scored when all four feet of the mouse entered one of the lateral arms, reentered the alley way (past the central divider) and then entered the lateral arm opposite the one previously chosen. Reentry into the same arm is a non-alternation. Performance was quantified by calculating the percentage of alternations within the trial (Number of alternations/ the number of alternation attempts x 100). The T-maze was cleaned with 30 % ethanol between animal trails.

2.4. Histology

Mice were euthanized with CO_2 and perfused transcardially with 30 mL of 0.01 M phosphate-buffered saline (PBS; pH ~7.4) followed by 30 mL of 4 % paraformaldehyde in PBS (PFA; pH~7.4). Brains were removed and postfixed overnight in 4 % PFA then cryoprotected in 20 % sucrose in PBS overnight. The tissue was sectioned (30μ m) into a 1:8 series using a cryostat into PBS and stored at 4 °C until immunohistochemical processing.

2.5. Phosphorylated Tau (AT8) staining

Hippocampal sections were washed in PBS and placed in 0.3 % H_2O_2 in PBS for 30 min. Following rinses tissue was blocked in 10 % normal donkey serum (NDS; Jackson Immunoresearch, 017–000–121). Tissue was then incubated in the appropriate primary antibodies overnight (mouse anti-phospho-Tau 1:250, Thermofisher, MN1020). Tissue was then rinsed in PBSx and incubated in secondary Ab for 2 hrs.

2.6. Western blots

Standard procedures were used. Specific protocols are provided in supplemental material.

2.7. In vitro electrophysiology recording

Electrophysiology recording in mice hippocampal slices was described previously by Woo, J.A. (13). Further details are in Supplemental material.

2.8. Sex as a biological variable

No sex differences were found so data are cumulatively reported.

2.9. Statistical analysis

Statistical analyses were performed with Deltagraph Prism software. For behavior studies, Western blot analysis, and regional pTau (AT8) staining intensity analyses, Student's *t*-test was used to compare directly between WT and CF (G542X or F508del) groups. For LTP studies, significance was determined by two-way ANOVA. The evoked fEPSP measured by the initial slope after the TBS was normalized to the averaged baseline slope of each hippocampal slice. For the paired pulse facilitation (PPF), the second fEPSP sloe was normalized to the first fEPSP slope. For the input-output experiment, the amplitude of fiber volley (FV) and the fEPSP slope were measured. A stimulus that evoked the half maximal fEPSP response would define a FV, with the amplitude of this fiber volley was normalized to all the FV value. The maximal fEPSP slopes will be used to normalize the fEPSP slope. A sigmoidal function (FV vs fEPSP) for each slice and a predicted value from this curve was shown. Significance for microglial lipid uptake, neuronal density, and human nasal epithelial pTau levels were also determined by *t*-test, directly comparing WT and CF groups for each assay. Data are

shown as the mean ± SEM for all studies.

3. Results

3.1. Learning and memory impairment in aged G542X CF mice

To assess cognitive function in CF mice, both short-term and long-term learning and memory were examined in the CF model G542X CF mice at three ages, 3, 7, and 15 months. Spontaneous Alternation (SA) was used to assess short-term working memory by evaluating mouse exploratory choices. Mice will typically equally explore two novel environments unless their short-term memory is impaired. In that case, the impaired mouse will repeatedly explore one environmental choice. The NOR assay was used to measure long-term object recognition memory. As described in the methods, this assay takes place across three days. Mice are habituated to an open field on day 1, on day 2, mice are placed in the same open field with two identical novel objects (A,A) and allowed to explore the objects. On day 3, mice are placed in the open field with one object they saw previously (object A) and a new object (B). Mice show an innate preference for novelty and should explore the novel object more, if they have a memory of the previously viewed object (A). At each age WT and G542X mice explored the maze equally, with no differences in overall arm entries between genotypes, nor differences in the distance travelled during NOR between genotypes (Fig. S1). Overall arm entries and distance travelled were measured to ensure that novel object exploration impairment was not due to differences in motivation to explore.

At 3 months of age no difference between G542X and WT mice was observed on either the SA or NOR (Fig 1A-B). At 7 months, G542X mice showed significant deficits in short-term memory on the SA test compared to WT controls t(8)=3.056, $p = 0.0078$ (Fig 1C), but performed comparably to WT mice in the NOR test suggesting that object recognition memory remained intact (Fig 1D). At 15 months, G542X mice displayed further cognitive decline with significant deficits on both the SA and NOR tests compared to WT (Fig 1E-F). SA was impaired in G542X mice compared to WT controls t(33)=2.817, $p = 0.0035$. On the

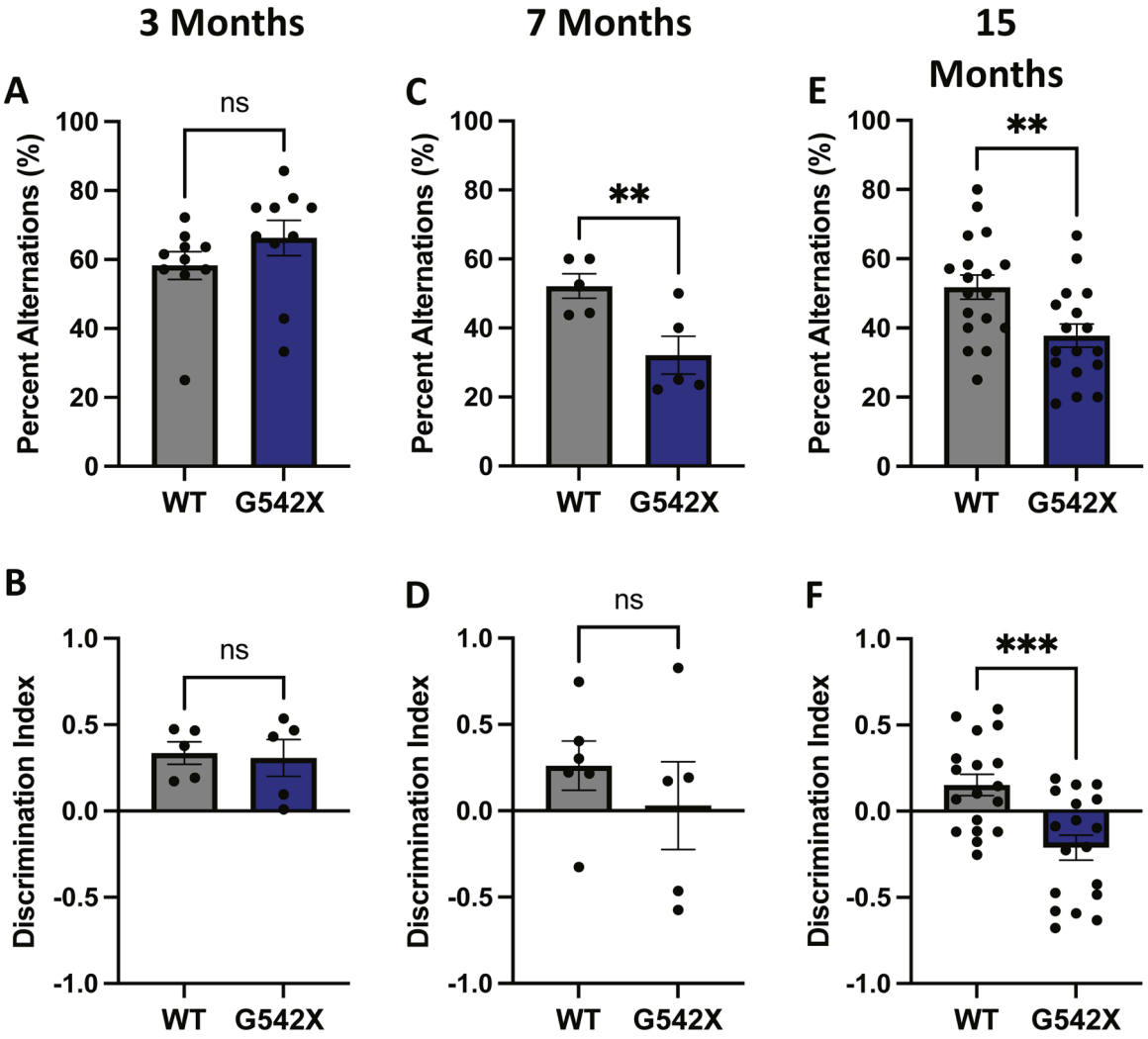

Fig. 1. Spontaneous Alternation and Novel Object Recognition in CGX vs WT mice at 3, 7 and 15 months of age. Different cohorts of mice were run at 3 ($n = 10$ per genotype), 7 ($n = 5$ per genotype) and 15 ($n = 18$ per genotype). SA: mice of both genotypes explored the maze equally at all ages. No difference in total arm entries was observed across genotypes (Supplemental data) (Suppl Fig 1. A-B) At 3 months no difference on SA was observed between genotypes t(18)=1.229, $p = 0.1174$, 95 % (−5.684–21.72). Similarly no difference on NOR was observed between genotypes t(8)=0.23, $p = 0.4119$, 95 % (−0.3165 to 0.2591). (Fig 1. C-D) At 7 months, G542X mice were significantly impaired on SA with significantly fewer alternating arm entries observed in the G542X mice t(8)=3.056, $p = 0.0078$, 95 % (−35.13 to −4.912). However performance on NOR was preserved with no differences between genotypes t(8)=0.8286, $p = 0.2144$, 95 % (−0.8596 to 0.3987). (Fig 1. E-F) At 15 months, G542X were significantly impaired on SA t(33)=2.871, $p = 0.0071$, 95 % (−23.90 to −4.06) and NOR t(33)=3.813, $p = 0.0006$, 95 % (−0.5575 to −0.1698) compared to age matched WT controls. G542X engaged in significantly fewer alternating arm entries and exhibited a significantly reduced discrimination index compared to WT controls. Significance determined by student *t*-test: $p > 0.05$ NS, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$.

NOR test, the amount of time spent with the novel object during the testing phase was significantly lower in G542X mice, resulting in a significantly lower discrimination index t(34)=3.813, $p = 0.0006$. These data demonstrate a progressive loss of cognitive function with age in G542X mice, with short-term working memory showing earlier deficits than long-term recognition memory. More rapid loss of short-term memory could indicate pathological involvement of the prefrontal cortex in addition to other regions of the brain. Long-term memory deficits are indicative primarily of hippocampus pathology, although the storage of memory information is complex. Together these data indicate a pathological process in the CF mouse brain that likely encompasses multiple brain regions.

A second CF mouse model carrying the F508del mutation was also tested in the SA test at 1-year of age to determine if the findings were consistent across mouse models. One-year old F508del mice also exhibit cognitive impairment in the SA test (Fig. S2) showing these effects are not specific to one model. These mice are being aged for further study.

3.2. Loss of CFTR function is associated with increased total and pTau in the hippocampus

Based on the observed cognitive impairments in CF mice, we hypothesized that these declines would be associated with CNS pathology compatible with neurodegeneration. Tau is a microtubule binding protein that stabilizes microtubules. In pathological conditions where Tau is hyperphosphorylated and dissociated from microtubules, it can from insoluble aggregates that can contribute to neuronal toxicity and cognitive decline (13). Since we have previously published microtubule

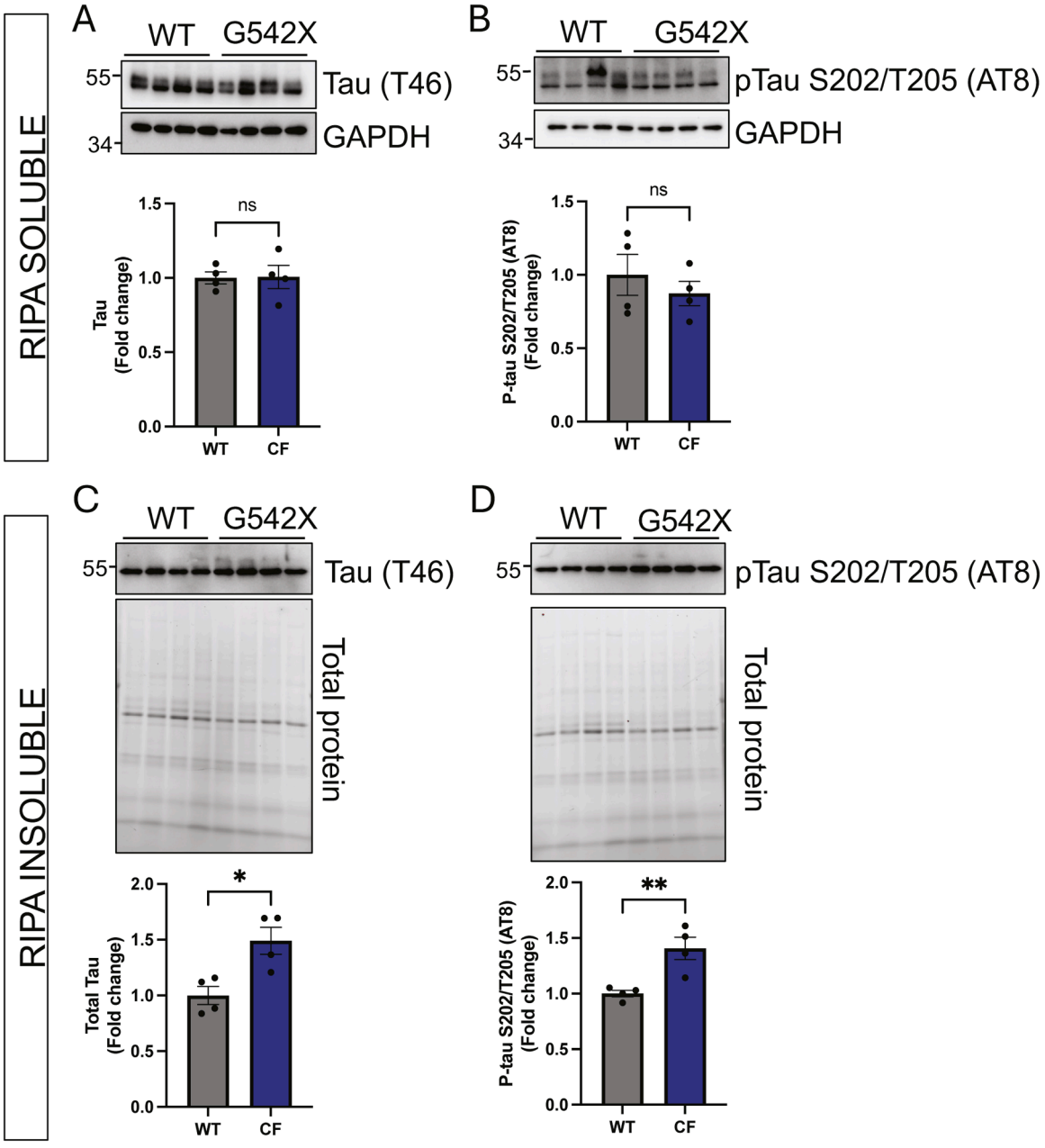

Fig. 2. Total and phosphorylated tau (AT8) in RIPA soluble and insoluble hippocampal fractions. (A) No significant changes were found in the RIPA soluble fraction for total Tau (A) t(6)=0.07294, $p = 0.9442$, 95 %(−0.2080 to 0.2208) or P-tau (B) t(6)=0.7845, $p = 0.4626$, 95 %(−0.5228 to 0.2689). In the RIPA insoluble fraction, total (C) t(6)=3.349, $p = 0.0154$, 95 % (0.1320 t0 0.8480) and p- tau (D) t(6)=3.851, $p + 0.0085$, 95 % (0.1483 to 0.6653) were significantly higher in G542X compared to WT. Significance determined by paired students *t*-test $^*p < 0.05$, $^{**}p < 0.01$.

alterations in CF cells consistent with instability (14), we hypothesized that Tau could be released from microtubules and then hyper-phosphorylated coincident with the observed cognitive decline. First, total and phosphorylated (S202/T205, AT8) tau were quantified in RIPA soluble and insoluble fractions derived from hippocampal lysates of 15-month-old G542X and WT mice. In the RIPA soluble fraction, there were no differences in total Tau or pTau between the G542X and WT mice littermates (Fig 2A,B). In the insoluble fraction, both total Tau and pTau were significantly elevated in the G542X mice $p < 0.05$ and 0.01 respectfully, suggesting that tau is accumulating intracellularly in G542X mice (Fig 2C,D).

Immunohistochemistry was used to spatially visualize pTau accumulation within the hippocampus. Mean fluorescence intensity (MFI) was measured using FIJI in anterior and posterior sections of the hippocampus corresponding to figures 70 and 80 from the Allen Brain reference atlas. At 9 months of age pTau was found to be significantly elevated in the anterior aspect of the dentate gyrus $p < 0.05$ (Fig 3A). No significant differences were observed in the posterior section of the dentate gyrus at 9 months of age, At 15 months of age AT8 staining within the anterior dentate gyrus was 3.5 times more intense in G542X mice than in WT littermates $p < 0.01$ (Fig 3C,F). It was also significantly elevated in G542X mice in posterior dentate gyrus $p < 0.01$(Fig 4D). The three fields of the cornu ammonis (CA1–3) were also quantified, and in every region except the posterior CA1 subfield AT8 was significantly elevated in G542X mice compared to WT littermates (Fig S3). These data also indicated an age-related progressive development of tauopathy.

3.3. Loss of CFTR function is associated with decreased hippocampal long-term potentiation (LTP)

LTP underlies learning and memory and given the clear deficit in cognitive function observed in G542X mice we decided to examine LTP in the oldest cohort of mice. To assess whether loss of CFTR function is associated with changes in short term or long-term synaptic plasticity, electrophysiological recordings from CA1 to CA3 in *ex vivo* hippocampal slices were assessed in 20-month-old WT and G542X littermate mice (Fig 4A). There were no differences observed in the input/output (I/0) curves of the field excitatory postsynaptic potential (fEPSP) between WT and G542X littermates suggesting normal baseline synaptic function (Fig 4B). In paired-pulse facilitation, which assesses short-term plasticity, there was no significant difference between WT and G542X mice (Fig 4C). We then applied theta burst stimulation to induce LTP and observed that G542X mice showed significantly attenuated LTP compared to WT littermates over the 1hr recording period (Fig 4D). The clear deficits in

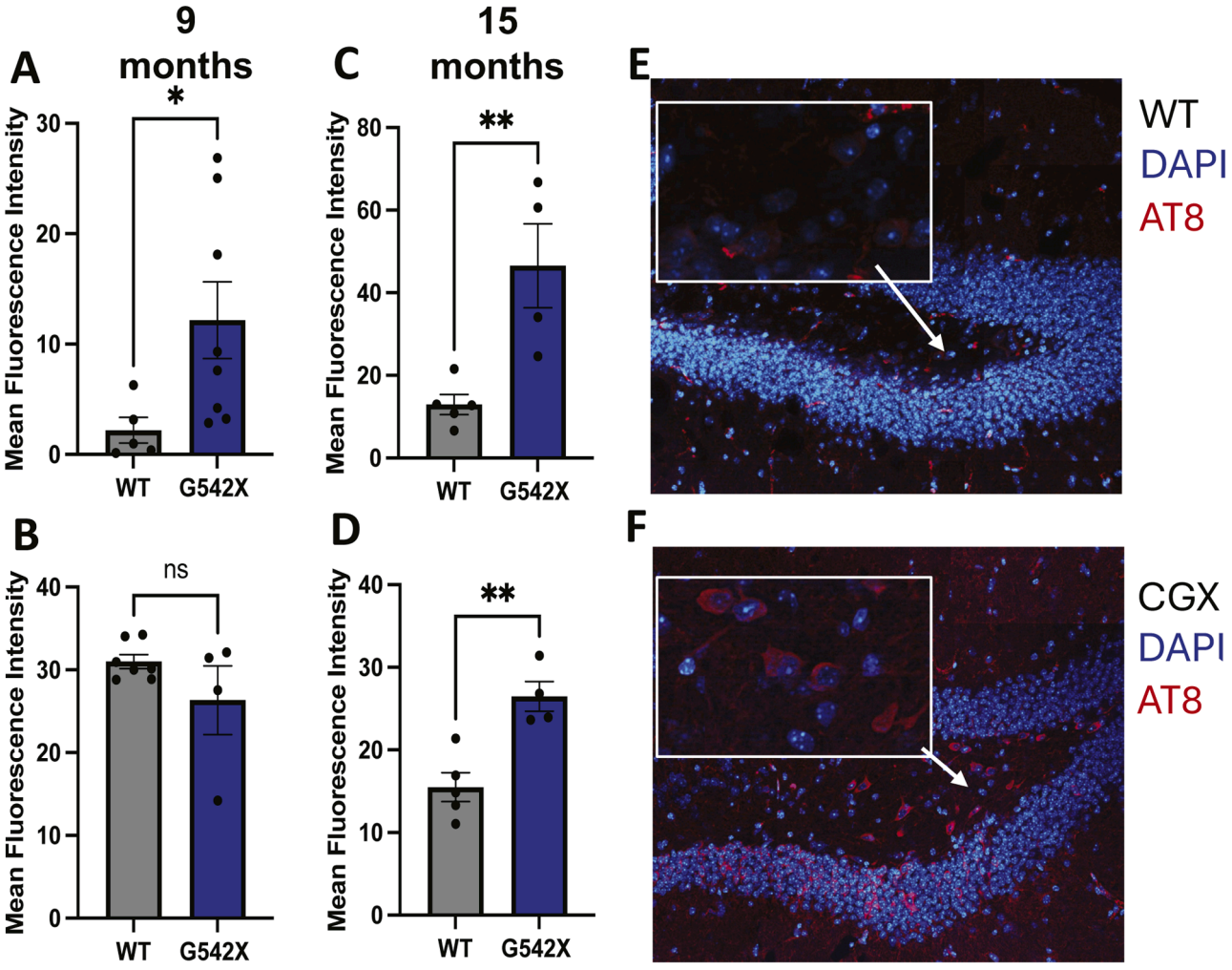

Fig. 3. Phosphorylated tau (AT8) in the dentate gyrus of the hippocampus in WT and G542X mice at 9 and 15 months. Mean fluorescence intensity of phosphorylated tau (AT8) in the anterior (A,C) and posterior (B,D) dentate gyrus of the hippocampus. AT8 intensity was significantly elevated in the anterior DG at 9 months t(11)= 2.187, $p = 0.0256$, 95 %(−0.0655 to 19.99). At 15 months of age there is significantly more phosphorylated tau within the dentate gyrus in the G542X mice in both the anterior (C) t(7)=3.590, $p = 0.0089$, 95 % (11.46 to 55.71) portions of the hippocampus (D) t(7)=4.312, $p = 0.0035$, 95 % (4.955 to 16.99). Representative micrographs at 40X of the anterior dentate gyrus showing DAPI stained cell bodies and phosphorylated tau (AT8) in red anterior section from a WT (E) and G542X mouse (F).

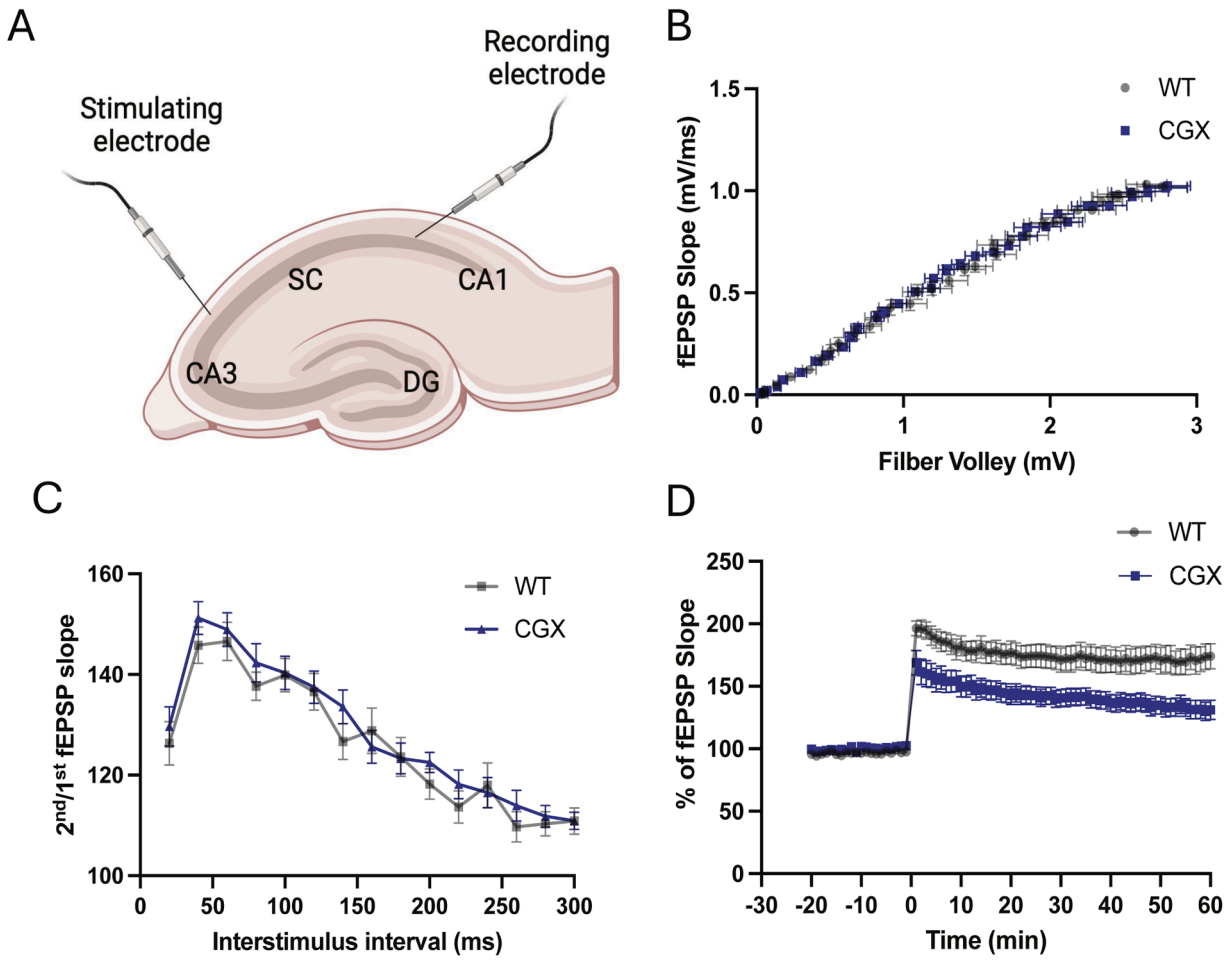

Fig. 4. Long-term potentiation in WT and CF (CGX) mouse brain. (A) Diagram of electrophysiological recordings from the schaffer collateral in *ex vivo* hippocampal sections (https://biorender.com) (B) Quantification of In-Out curves from 20-month-old female mice (two-way ANOVA, not significant, $n = 28$ slices from 5 WT, $n = 32$ slices from 5 CGX). (C) Quantification of Paired Pulse Facilitation from 20-month-old female mice. (two-way ANOVA, not significant, $n = 28$ slices from 5 WT, $n = 32$ slices from 5 CGX). (D) Quantification of Long-Term Potentiation after theta-burst stimulation from 20-month-old mice. (two-way ANOVA, F(79,4640)=2.515, $P < 0.0001$; posthoc Tukey main genotype effect: $P < 0.0001$, 95 % (21.84 to 26.36); posthoc Tukey main genotype effect: ****$p < 0.0001$; $n = 28$ slices from 5 WT, $n = 32$ slices from 5 CGX).

LTP in G542X mice are consistent CF-specific neuropathology underlying the cognitive impairment in these mice.

3.4. Hippocampal neutral lipid in aged G542X mice

Given our previous work showing altered lipid processing and increased inflammatory signaling in CF epithelial cells and the established link between altered lipid processing in neurodegenerative disease we hypothesized that similar changes in lipid processing and inflammation may occur in CF neuronal cells (15–19). In order to determine if CF neuronal tissues display altered lipid processing and microglia uptake, borondiprromethene (BOPIDY 493/503) co-staining with the microglia marker ionized calcium binding adaptor molecule (Iba1) was performed on hippocampal sections of 15 month-old WT and G542X mice (Fig 5A,B). BOPIDY 493/503 stains neutral lipids, including cholesterol, and provides direct information on lipid processing in CF neuronal tissues. This staining revealed significantly increased lipid uptake by microglia in the hippocampus of G542X mice compared to WT littermates (Fig 5A-C). These data demonstrate lipid-related pathology in microglia of CF mice, consistent with previously observation in CF epithelial cells and in other tauopathies and suggest a neuronal inflammatory response in CF (17–21).

3.5. Loss of CFTR function is associated with reduced neuron density

Based on the increased intracellular pTau observed in G542X mice and the lipid accumulation in microglia within the hippocampus, we hypothesized that these mice would also show hippocampal atrophy. The neuron specific cell marker NeuN was used to assess cell density in CA1–3 and the dentate gyrus in anterior and posterior sections of the hippocampus of 15-month CGX and WT mice (Fig 5D-G). This showed a significant reduction in all subfields CA1–3 in the anterior section in G542X mice, but not in the dentate gyrus. (Fig S4) In the posterior sections no significant differences were noted. These findings indicate that tauopathy in G542X mice is associated with neurodegeneration and serves as a likely mechanism of LTP impairment and cognitive decline.

3.6. Increased pTau levels in primary CF human nasal epithelial cells

It has been shown that levels of pTau in oral epithelium are increased in people with Alzheimer's disease as well as in nasal smears (22,23). To assess whether increased pTau levels were also found in pwCF, as we observed in CF mice, primary HNE cells isolated from three non-CF control subjects and five pwCF (4 F508del homozygous and 1 G542X homozygous samples) were examined for pTau. CF HNE cells showed a significant increase in pTau (AT8) levels ($p = 0.03$) compared to non-CF controls (Fig 5H-K), but primarily in soluble fractions. Imaging revealed

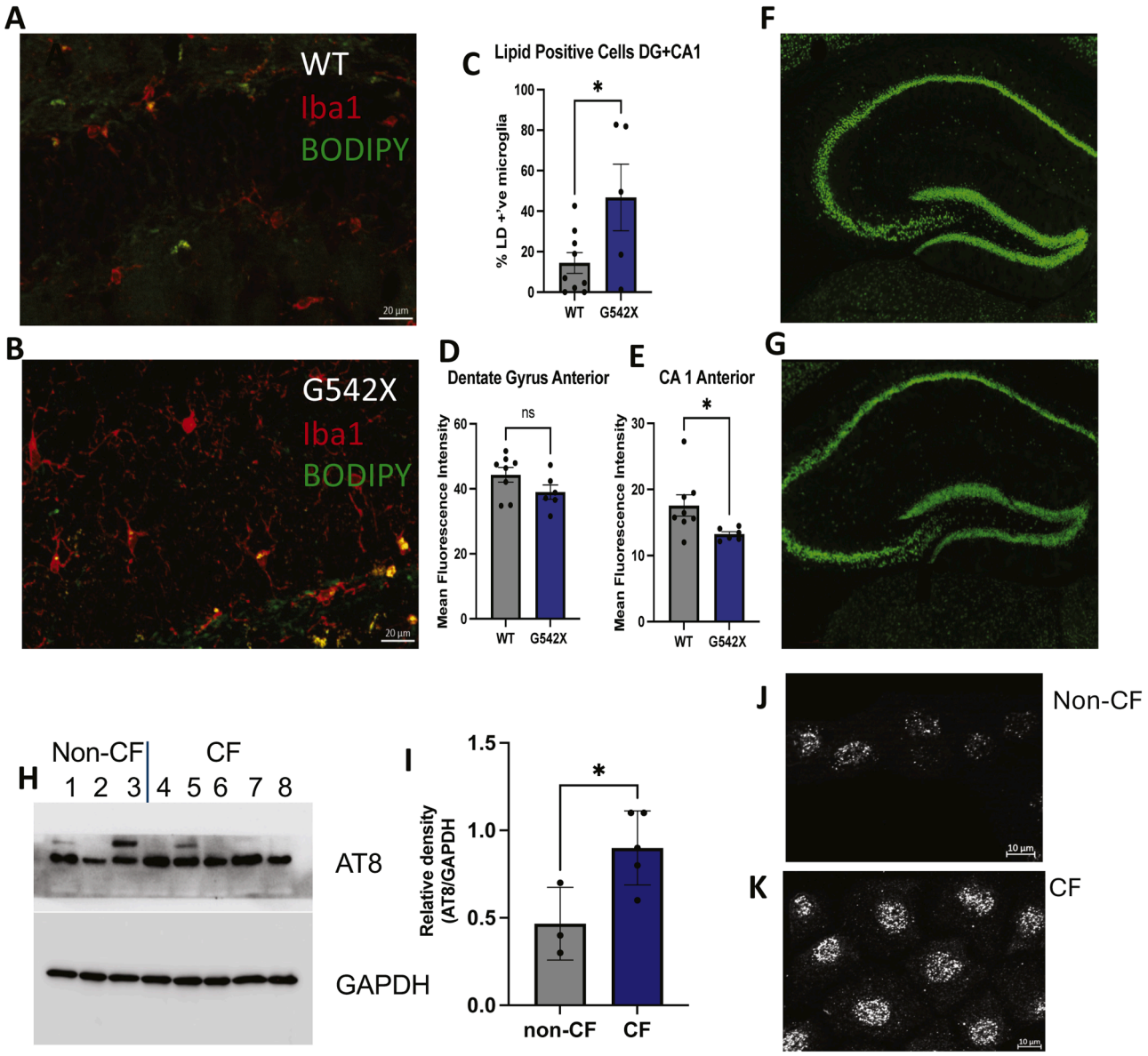

Fig. 5. Cellular changes in the brain of G542X mice. Co-staining of the hippocampus for ionized calcium binding adaptor molecule (Iba1) and borondipyrromethene (BODIPY) suggests that macrophages are taking up more cholesterol in aged (15 month) G542X mice compared to controls t(12)=2.354, $p = 0.0365$, 95 % (2.405 to 62.28) (A-C) To assess if the observed changes in hyperphosphorylated tau and cholesterol accumulation and uptake by microglia in the hippocampus was associated with neuronal cell loss, NeuN staining was quantified in the hippocampus, were slight decreases in NeuN signal were observed in G542X mice in the dentate gyrus(D) t(12)=1.645, $p = 0.0626$, 95 % (−12.40 to 1.730), and CA1 (E) t(12)=2.283, $p = 0.0207$, 95 % (−8.487 to −0.197). Representative photomicrographs of NeuN staining in the anterior hippocampus from a WT (E) and G542X (F) mouse at 20X. Significance determined by *t*-test, $^*P < 0.05$. (H) Western blot of pTau (AT8) in soluble fraction of HNE cells; lanes 1–3 non-CF subjects, lanes 4–7 F508del homozygous CF subjects, lane 8 G542X homozygous CF subject. (I) Densitometry analysis of AT8 and GAPDH. Significance determined by *t*-test; t(6)=2.815, $p = 0.0306$, 95 %(0.05661 to 0.8101). (J-K)Representative images of immunostain of pTau (AT8) in non-CF and F508del (CF) HNE cells showing nuclear accumulation.

that most pTau is localized to the nucleus, consistent with pathogenic pTau localization in neurons (24). These data suggest that aberrant tau regulation is inherent to CF cells and indicate that the CF mouse findings are relevant to pwCF.

4. Discussion

New HEMT therapies for treating CF are significantly increasing lifespan for pwCF, rendering it imperative to identify and understand CF-specific complications of aging (25). Neurological complications of aging are particularly important as it is not clear whether modulators cross the blood-brain barrier to correct neuronal CFTR function. In this study, progressive cognitive decline, brain pathology, and reduced measures of hippocampal function are shown. Taken together these findings suggest that declining cognitive function related to pTau pathology is a significant consequence of aging in the absence of CFTR function.

A key feature of age-related neurodegeneration is the progressive loss of cognitive function. Here, we investigated the functioning of the hippocampus and associated pathology. Two well-characterized assays of cognition, the SA and NOR assays, were performed at 3, 7 and 15 months of age in WT and CF, with CF mice displaying a progressive decline in cognition. The SA assay measures novelty driven exploratory behavior dependent on memory of a previous decision (26). Within the NOR assay, we assessed object recognition memory by evaluating the discrimination index between a known and novel object across a

training and testing phase separated by 24 hrs, and multiple lines of evidence (i.e. electrophysiology, lesions, and imaging) suggest roles for the hippocampus and perirhinal cortex (PRC) in this assay (27). Changes in pTau and neuronal loss in the hippocampus were examined here, and future studies should also assess whether these forms of pathology also occur in the PRC of CF mice.

We hypothesize two potential mechanisms that could link CFTR function to progressive cognitive decline. The first potential mechanism is a direct effect of CFTR on neuronal function. In this study, the electrophysiological properties of neurons in the hippocampus were assessed. While we observed clearly reduced LTP in CF mice, it is not known whether this is a direct or indirect result of CFTR dysfunction. CFTR is a cAMP-activated ion channel that directly regulates chloride and bicarbonate transport and indirectly influences intracellular Ca^{2+} homeostasis (28). The absence of CFTR could potentially alter the resting membrane potential and subsequent depolarization of neurons. Wu et al. have also demonstrated that CFTR directly impacts hypothalamic neuron excitability and hormonal cycles in females (29). Patch clamp experiments of cultured hypothalamic cells from F508del mice showed significantly reduced frequency and amplitude of electrical spikes. Similar influences on hippocampal neuronal function would be consistent with behavior changes observed in this study. However, it is not clear if these direct neuronal functions would lead to the progressive loss of cognitive function observed in this study as opposed to a more inherent loss of cognitive function with the loss of CFTR activity.

The second potential mechanism linking loss of CFTR function to the progressive neurological disease presented here is an indirect effect of CFTR function on microtubule stability leading to Tau pathology. Woo et al. have shown identical changes in LTP and pTau pathology in a mouse model of microtubule disruption that we observe here in CF mice (13). Phosphorylation of tau at S202/T205 detects pretangles and neurofibrillary tangles in tauopathies and is thought to represent early stages of tau pathology (30,31). This finding is consistent with our previous work showing impaired intracellular transport in CF epithelial cells due to altered microtubule regulation (14). It is hypothesized that the instability of microtubules in CF cells that we have shown would lead the release of Tau and its subsequent phosphorylation, thereby promoting progressive Tau pathology. However, further study is required to determine if these same microtubule changes occur in neurons lacking CFTR activity. Continued study on the role of CFTR in regulating Tau pathology in neurons and non-neuronal brain cells is needed to elucidate specific mechanisms and to highlight future therapeutic options. Finally, the presence of elevated pTau in primary HNE cells from pwCF suggests that the pathological process we identified in mice is relevant to human patients. Further analysis of human samples and imaging is needed to further characterize this phenomenon.

The hypothesis of CF-related microtubule instability leading to Tau pathology and cognitive decline suggests that cognitive decline is directly related to the pTau accumulation in CF mouse brain presented in this study. This study does not directly show that Tau pathology is the driving force of the progressive cognitive decline seen in CF mice, only that Tau pathology is coincident with the behavior findings. Though we propose that Tau pathology is the likely mechanism of cognitive decline, chronic neuronal inflammation as indicated by microglial cholesterol uptake could be another mechanism leading to learning and memory changes.

Therapeutically, the impact of HEMT on the development of these cognitive issues needs to be determined. It is unclear if modulator compounds cross the blood-brain barrier (BBB) and are even available for CFTR correction in the brain. A study by Li et al. in the CF rat model shows clear transport of all components of elezacaftor/tezacaftor/ivacaftor (ETI) across the fetal BBB, but this is more restricted with adult rats (32). It also must be noted that adverse events associated with HEMT include anxiety, depression, and cognitive issues (33). Lastly, it must be considered that modulator metabolites or even components of ETI could be exacerbating symptoms associated with areas of the brain that have pre-existing, early-stage pathology.

In conclusion, this study establishes the presence of the previously unknown condition of CFND, which consists of tauopathy, microglial pathology, neurodegeneration, and progressive cognitive impairment. While neurological phenotypes associated with human CF disease are faithfully reproduced in these CF mice including anxiety and depression-like behaviors as well as altered circadian timing regulation, it remains to be determined to what extent pwCF are at increased risk to develop CFND as they age.

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Author contributions

DP, AP, and TK contributed to the concept development of the project. DP, XW, SB, ER, DC, NA, and WW contributed experimental data for the manuscript. RD, DP, AP, DEK, and TK analyzed and interpreted data. DP, AP, and TK contributing to writing the manuscript. All authors reviewed the manuscript.

Conflict of interest statement

The authors have declared that no conflict of interest exists.

Declaration of competing interest

No authors have any conflicting interests with this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2025.04.003.

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