

Promoting Innate Phagocytosis: Novel Treatment Strategies for Alzheimer’s Disease

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Introduction

In the CNS, innate phagocytosis mediated by microglia is responsible for the recognition and phagocytosis of cellular debris, protein aggregates, and invading bacteria. Unlike the adaptive immune response, innate phagocytosis is not dependent on antibodies or the complement system but is suppressed by as little as 1% of serum. Accordingly, innate phagocytic activity is expected to be maximally active within the CNS environment, where cerebrospinal fluid (CSF) is largely devoid of plasma proteins. Furthermore, since innate phagocytosis decreases with age, this decline may contribute to impaired clearance capacity in age-related neurodegenerative diseases, including AD and age-related macular degeneration (AMD). Therefore, restoration of innate phagocytic competence to remove accumulated Aβ and maintain neuronal homeostasis represents a potential therapeutic strategy for age-related neurodegeneration.

Copaxone® (CPX; also known as glatiramer acetate), a heterogeneous synthetic polypeptide mixture composed of glutamic acid, lysine, alanine and tyrosine, is widely used in the treatment of relapsing forms of multiple sclerosis. Several groups have reported the beneficial effects of CPX in different mouse models of AD, where CPX was used as an immunostimulant and administered peripherally. We have previously shown that innate phagocytosis of peripheral monocytes is defective in AD and age-related macular degeneration (AMD), and that CPX restores phagocytosis *in vitro*. We hypothesised that direct delivery of CPX to the CNS microenvironment may modulate microglial function more effectively than peripheral administration.

In the current study, we first investigated whether CPX enhances Aβ uptake by monocytes/microglia and antagonizes Aβ cytotoxicity. We then evaluated the therapeutic effect of CPX in a mouse model of AD by intracerebroventricular (ICV) delivery of CPX via mini-osmotic pump as this route provided maximum efficacy. To further assess translational relevance, we examined biosafety and pharmacokinetics in a sheep model, evaluated CSF and plasma biomarkers and PET imaging in a non-human primate model of AD.

Methods & Results

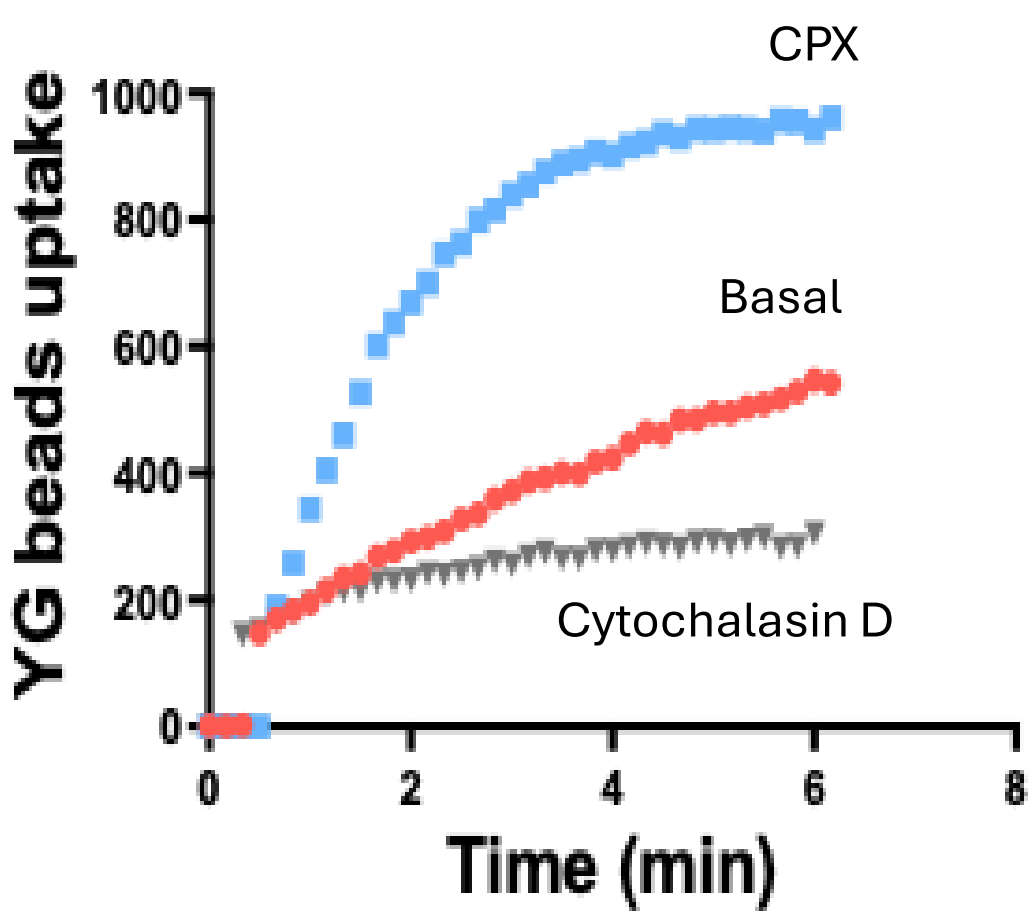


Fig.1: CPX enhances phagocytosis of YG beads by human monocytes *in vitro*. Fresh human CD14⁺ monocytes were pretreated with CPX (100 µg/mL, 10 min, 37°C), followed by addition of YG beads. Uptake over 6 min was quantified by real-time flow cytometry. Cytochalasin D was used to inhibit actin-dependent phagocytosis. Data are representative of independent experiments. A and B show representative traces from independent experiments, consistent with previously reported findings (Gu 2016).

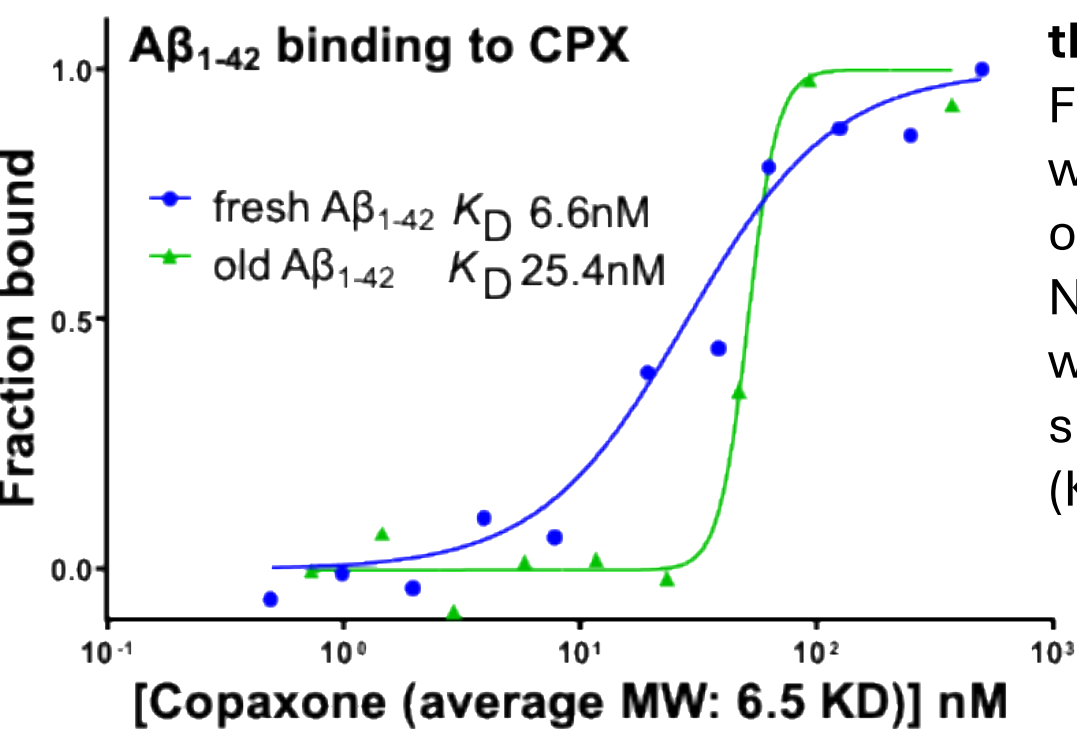


Fig. 2: Binding of CPX to Aβ₁₋₄₂ measured by microscale thermophoresis (MST). Hilyte Fluor488-labelled Aβ₁₋₄₂ (80 nM, PBS) was mixed 1:1 (v/v) with serial dilutions of CPX and analysed using a Monolith NT.115 system. CPX bound fresh Aβ₁₋₄₂ with high affinity (K_D = 6.6 nM) and showed reduced affinity for aged Aβ₁₋₄₂ (K_D = 25.4 nM).

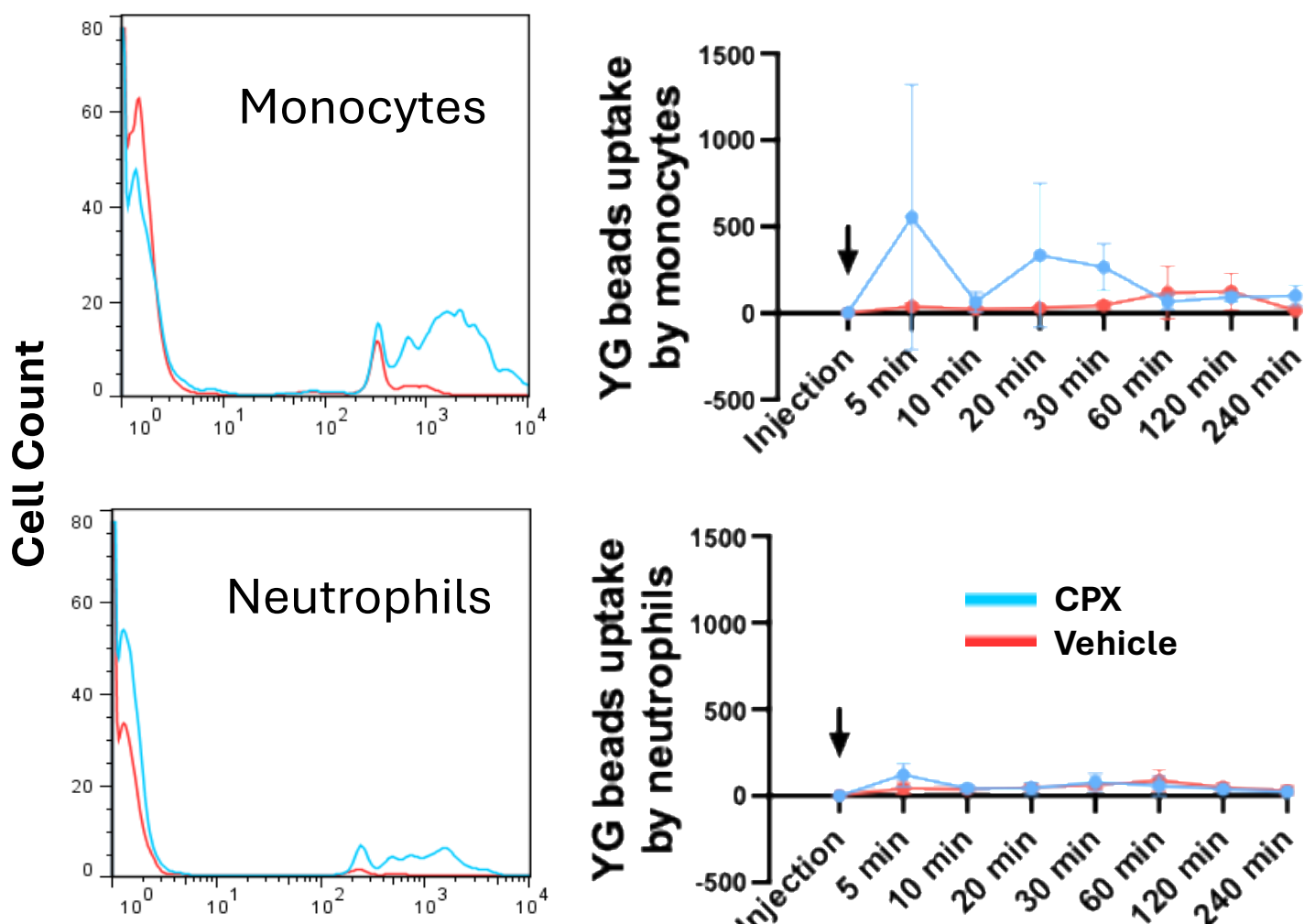


Fig. 3: CPX enhances phagocytosis of YG beads by circulating monocytes *in vivo*. Rabbits received intravenous YG beads followed by CPX or vehicle (4% mannitol). Blood was collected at indicated time points and monocyte-associated YG fluorescence was analysed by flow cytometry. **Left:** Representative histograms at 30 min. **Right:** Time course of YG bead uptake in monocytes (n = 7).

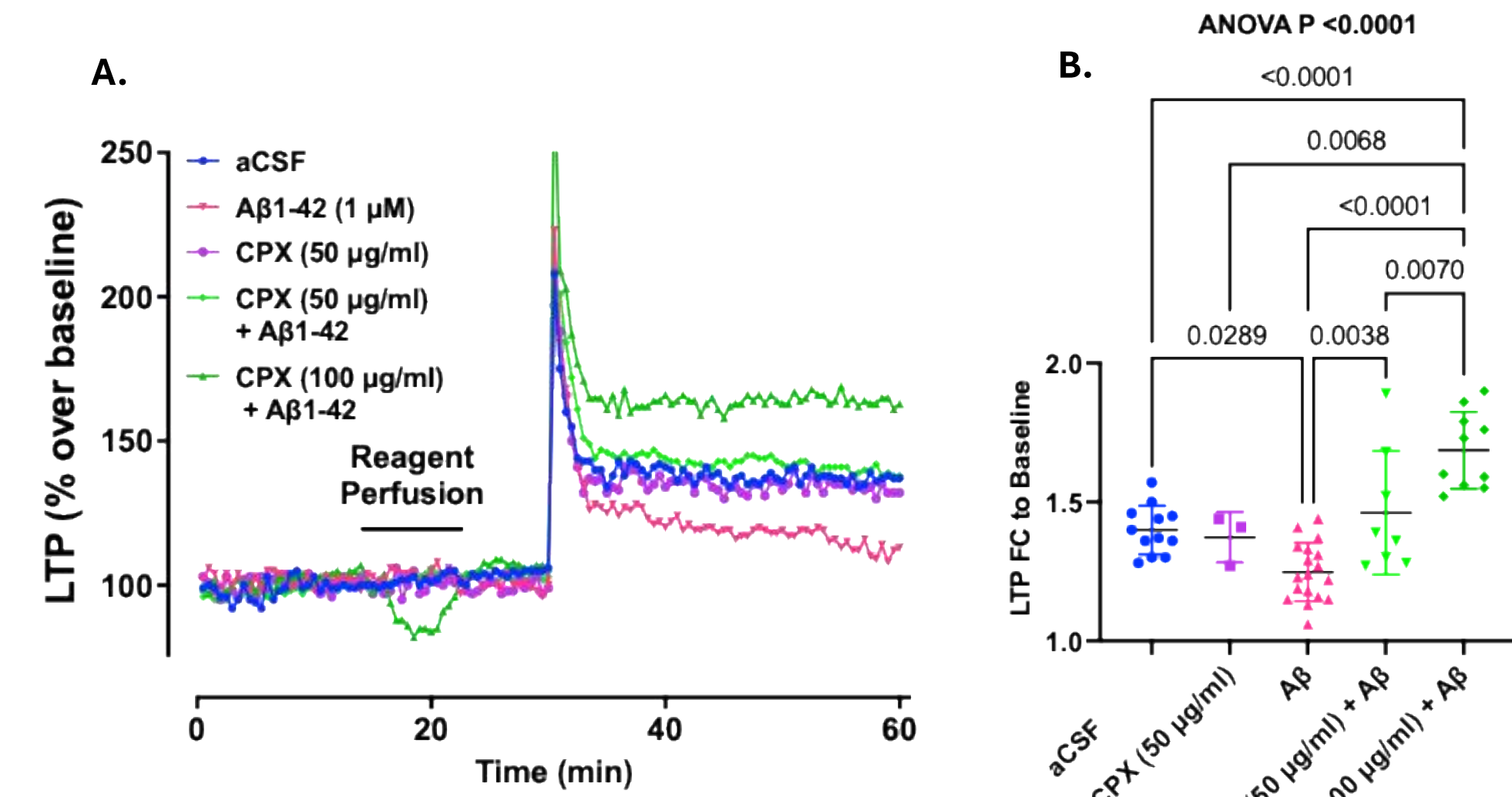


Fig. 4. CPX restores Aβ-induced suppression of hippocampal long-term potentiation (LTP). **A.** Aβ₁₋₄₂ (1 µM) reduced LTP compared with aCSF control. CPX alone (50 µg/mL) had no effect. Co-treatment with CPX attenuated Aβ-induced impairment and restored LTP. Traces are shown as time course (% over baseline; mean responses). **B.** Quantification of LTP at 50 min (30 min post-reagent perfusion) from traces shown in (B). Aβ significantly reduced LTP compared with aCSF, while CPX restored LTP in a dose-dependent manner (one-way ANOVA, P < 0.0001). Data are shown as individual data points with mean ± SD. Sample sizes: aCSF (n = 4 mice / 5 slices / 12 electrodes), CPX 0.05 mg/mL (n = 1 / 1 / 3), Aβ₁₋₄₂ (n = 7 / 8 / 18), CPX 50 µg/mL + Aβ₁₋₄₂ (n = 1 / 1 / 8), and CPX 100 µg/mL + Aβ₁₋₄₂ (n = 3 / 3 / 10).

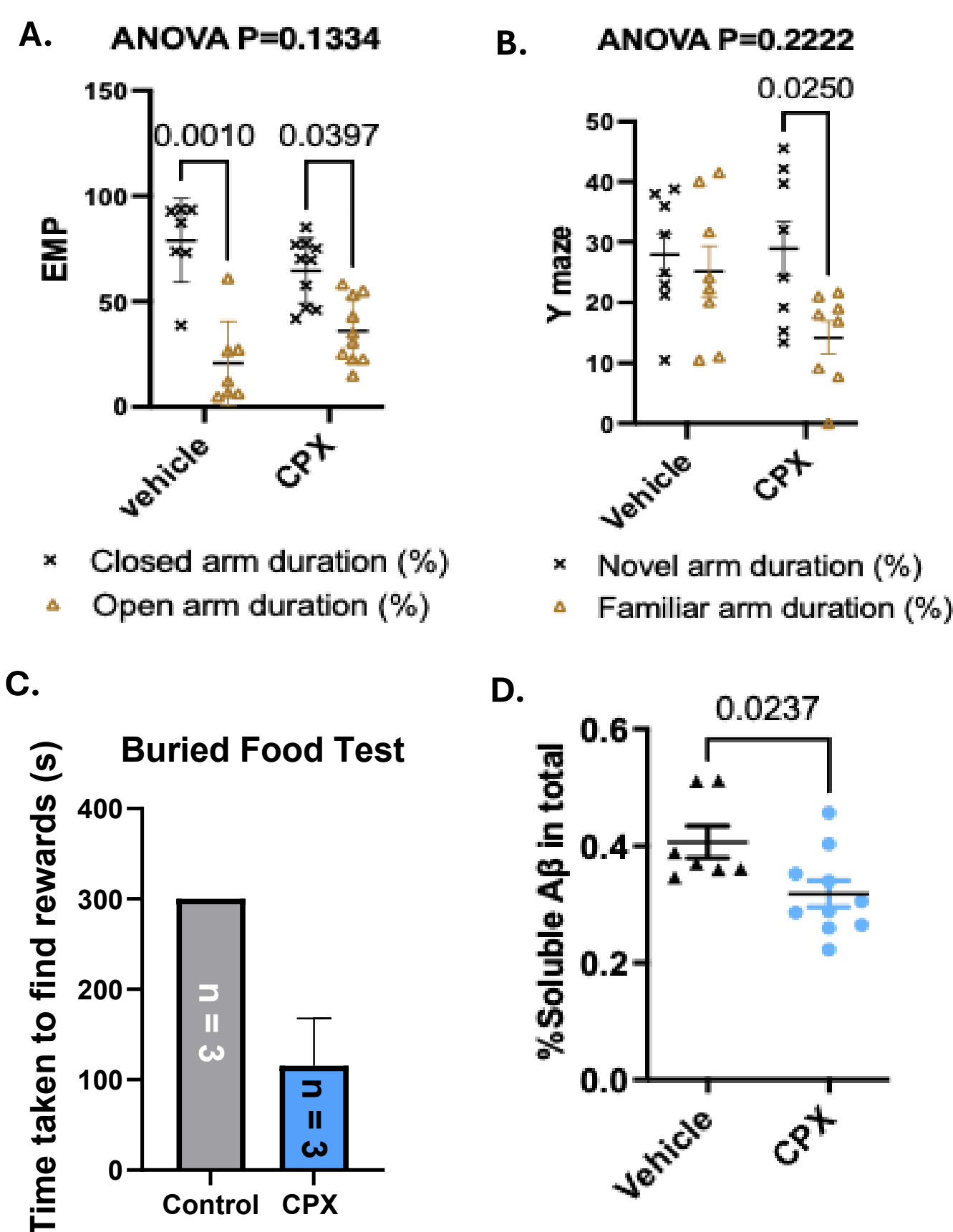


Fig. 5: CPX ameliorates behavior, LTP and brain Aβ clearance of aged APP/PS1 mice. **A.** Elevated plus maze (EPM). Percentage of time spent in open and closed arms. Both groups showed the expected preference for closed arms. **B.** Y-maze. Percentage of time spent in the novel and familiar arms. **C.** Buried food test. **D.** Biochemical quantification of brain Aβ by ELISA. **E.** Etching ring shape Aβ plaques in CPX-treated mouse hippocampus and cortex. **a.-d.** are Aβ IHC using anti-Aβ antibody (clone 1E8). CPX-treated microglia showed tighter association with Aβ plaques compared to control microglia. Microglia-like cells (with small dark-blue nuclei) can be seen in the center of Aβ plaques in CPX-treated mice (white arrows). Some CPX mouse Aβ plaques showed a clear center of circle (black arrows). In contrast, control mouse Aβ plaques had lower level of microglia infiltration into Aβ-plaques (white arrows) and less profound in center-etching phenomenon (black arrow). **e.-f.** Microglial IHC using anti-IBA-1 antibody. Hippocampal microglial cells can be seen under microscope. Some microglial cells joined into a circle (asterisks) in both CPX-treated and control mice. More microglia appeared to be ramified in CPX-treated mice compared to control mice. And microglia in control mice appeared to be rod-shaped. Magnification X40.

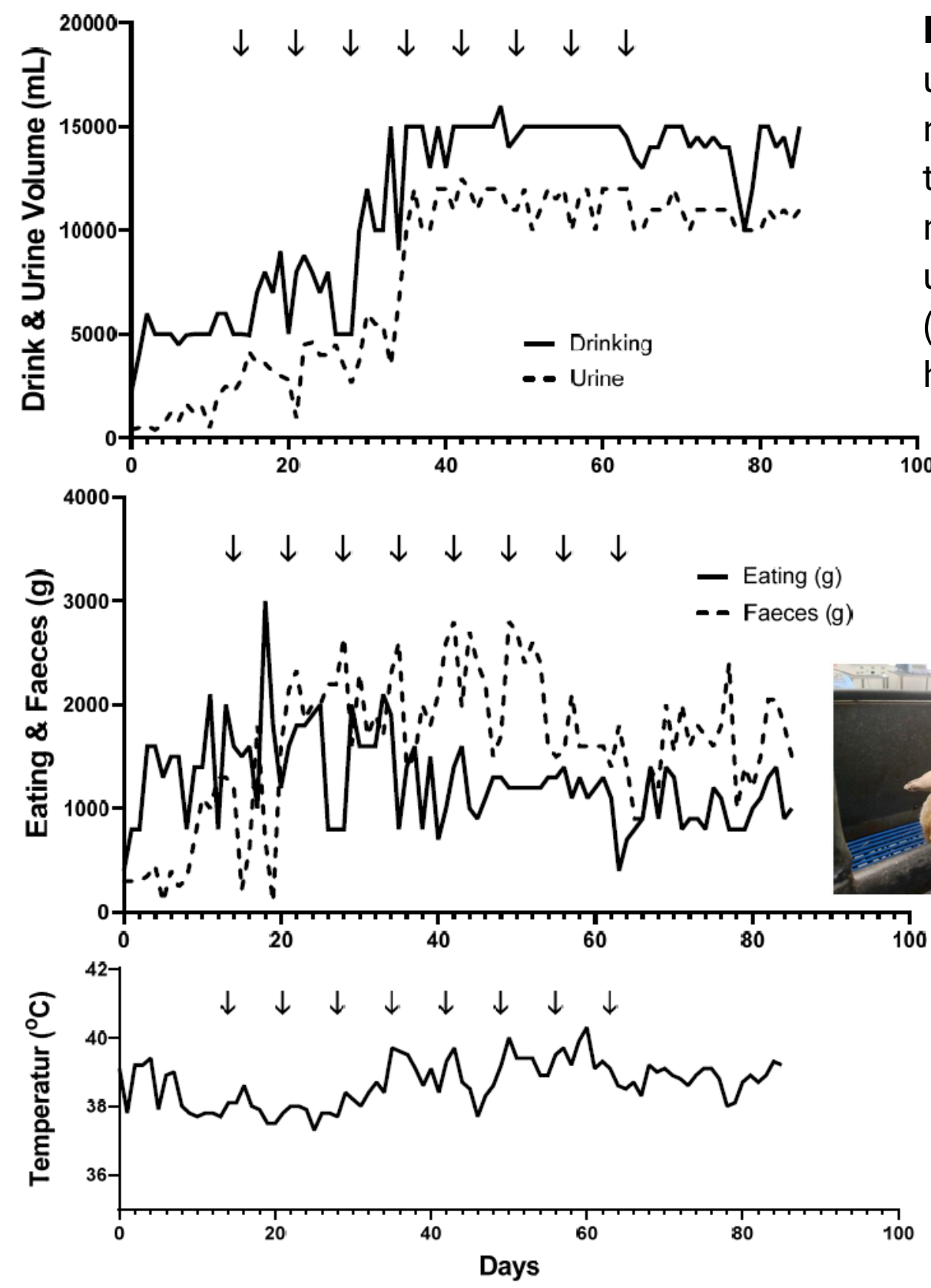


Fig. 6: A typical sheep experiment record. The sheep underwent ventricular cannulation on the first day and received 8 glatiramer acetate (CPX) direct administration via the ventricle (indicated by the arrow) in the following 3 months, 0.5 mL (10 mg) each time. Daily drinking and urinating (top), forage consumption and defecation (middle), anal body temperature (bottom), breathing and heartbeat were recorded.

Fig. 7: The changes of brain A and Tau following CPX treatment in non-human primate model of AD. Two AD monkeys (AD-6 and ADos-1) were administered with CPX. The data from two untreated AD monkeys (AD-2 and AD-7) were provided as control. Fluorescent IHC results of Thioflavin S in hippocampal and cortical tissue. White arrows indicate ThioS*NeuN⁺ signals, and yellow arrows indicate ThioS*NeuN⁺ signals. **A.** AD-2; **B.** AD-7; **C.** AD-6; **D.** ADos-1. **(Right)** Typical Aβ-PET images in CPX treated AD monkeys (AD-6 and ADos-1) before and 8-week and 16-week after CPX administration.

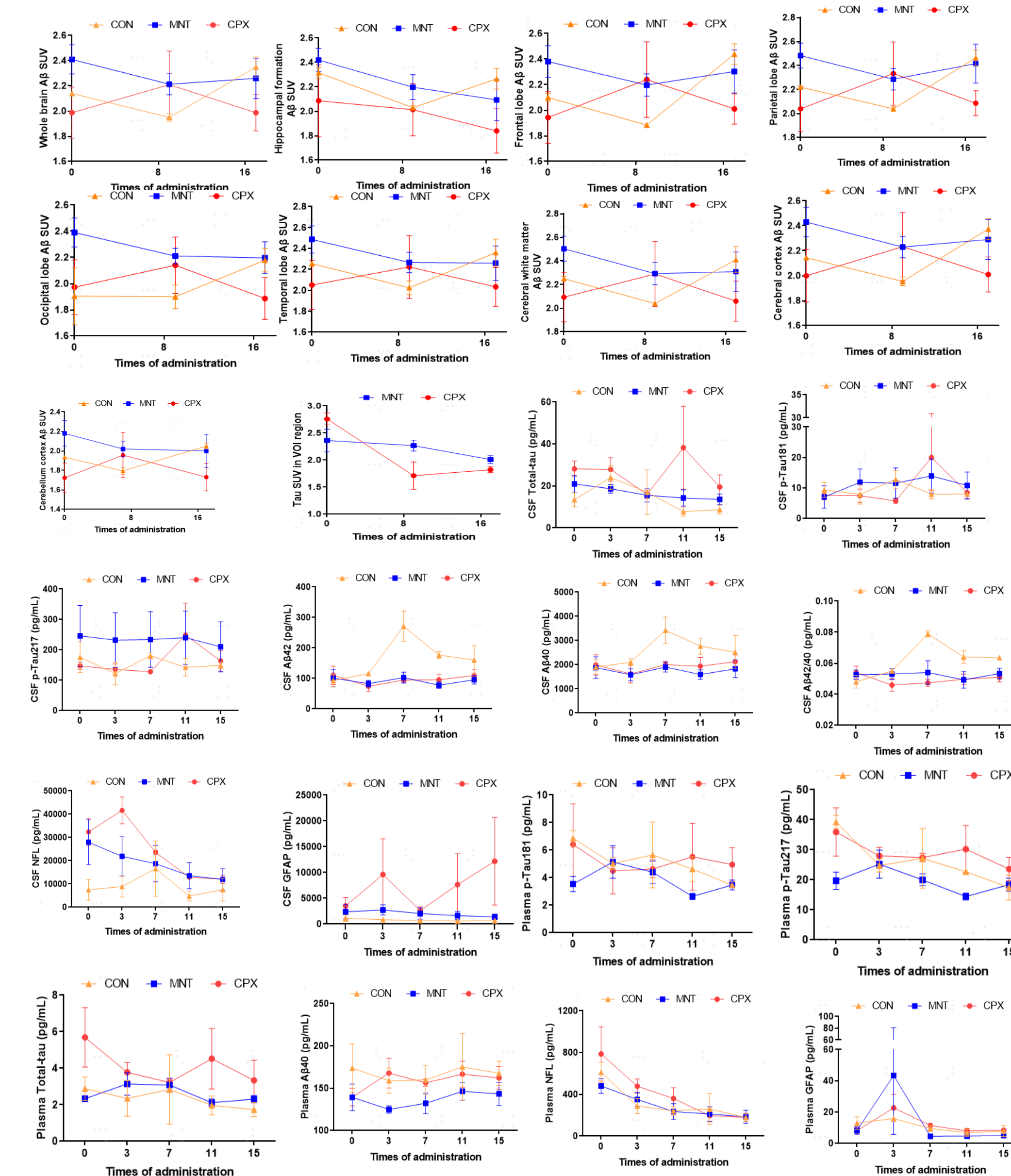
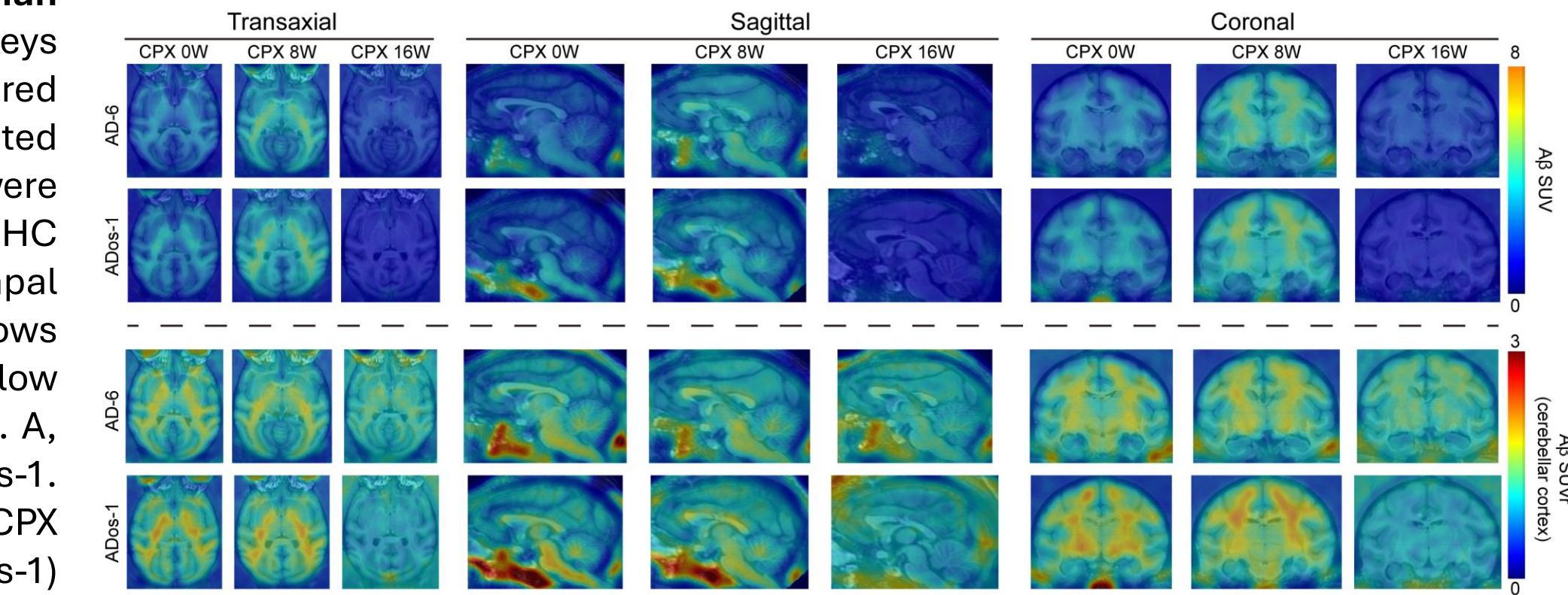
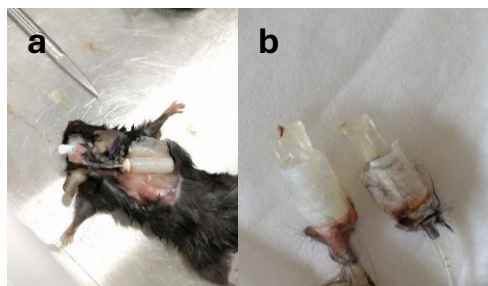


Figure 8: The longitudinal changes of brain imaging, CSF and blood biomarkers following CPX treatment in non-human primate model of AD. Cerebrospinal fluid (CSF) levels of total tau, phosphorylated tau at threonine 217 (pTau217), and phosphorylated tau at threonine 181 (pTau181) were measured longitudinally across repeated dosing time points. Animals were grouped as normal controls (CON), model controls (MNT), and CPX-treated (CPX).

Conclusion: Our proof-of-concept study supports that promoting innate phagocytic function modulates amyloid-driven neuroimmune dysfunction across species. Targeting both innate phagocytosis and Aβ represents a complementary therapeutic strategy to enhance Aβ clearance in addition of current antibody therapy.