

INTRODUCTION

Multi-infarct dementia (MID), the most common form of vascular cognitive impairment and dementia (VCID), is characterized by widespread cerebral microinfarcts (MMIs) that affect both cortical and subcortical regions. Current treatment remains largely supportive, as no disease-modifying therapies are available. Chronic cerebral hypoperfusion and hypoxia trigger neuroinflammatory pathways that contribute to progressive cognitive decline.

Epoxyeicosatrienoic acids (EETs), generated by cytochrome P450 (CYP450) epoxygenases, are potent vasodilatory and anti-inflammatory lipid mediators. We investigated whether astrocyte-specific overexpression of CYP2C11, a key EET-producing enzyme, could enhance cerebral perfusion and cognitive outcomes in a bilateral MMI mouse model.

MATERIALS AND METHODS

Experimental procedures were approved by the Henry Ford Hospital Institutional Animal Care and Use Committee (IACUC protocol 1580) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Twenty middle-aged (12 months old) C57BL/6J male mice were used. To induce the MMI model, mice were anesthetized with isoflurane, and the bilateral common carotid arteries (CCAs) and right external carotid artery (ECA) were isolated via a midline neck incision. After the ECA was ligated distally and tied loosely at its origin, the right CCA and internal carotid artery (ICA) were temporarily occluded. The ECA was then incised, and a modified PE-50 catheter containing 4,000 microspheres (25 μ m diameter; Polysciences) suspended in 100 μ L PBS was advanced into the ICA. Microspheres were slowly infused following temporary occlusion of the left CCA to induce bilateral MMIs.

To verify AAV-mediated transduction, mice (n = 4) received an intravenous injection of AAV-PhP.eB encoding mCherry under the control of the astrocyte-specific gfaABC1D promoter (1×10^{11} vg/mouse in 100 μ L PBS) three days post-MMI. Three weeks later, brains were collected, sectioned, and immunostained with anti-GFAP antibody (1:500; Dako). Sections were imaged via confocal microscopy to evaluate mCherry and GFAP colocalization.

Remaining mice were randomized to receive either AAV-mCherry or AAV-CYP2C11 (n = 8/group). Cognitive function was assessed at 1, 3, and 6 weeks post-MMI using the novel location recognition (NLR) test and Y-maze; Morris water maze (MWM) testing was performed at 5 weeks post-MMI. Regional cerebral blood flow (CBF) was measured longitudinally via arterial spin labeling MRI (ASL-MRI) at baseline, 2 weeks, and 4 weeks post-MMI. At 6 weeks, brain tissues were analyzed for EET levels via liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Michigan State University Mass Spectrometry and Metabolomics Core Facility.

Data are expressed as mean $\pm$ SD; statistical significance was assessed via ANOVA or Student's t-test (p < 0.05).

RESULTS

1. Verification of Astrocyte-Specific Transfection

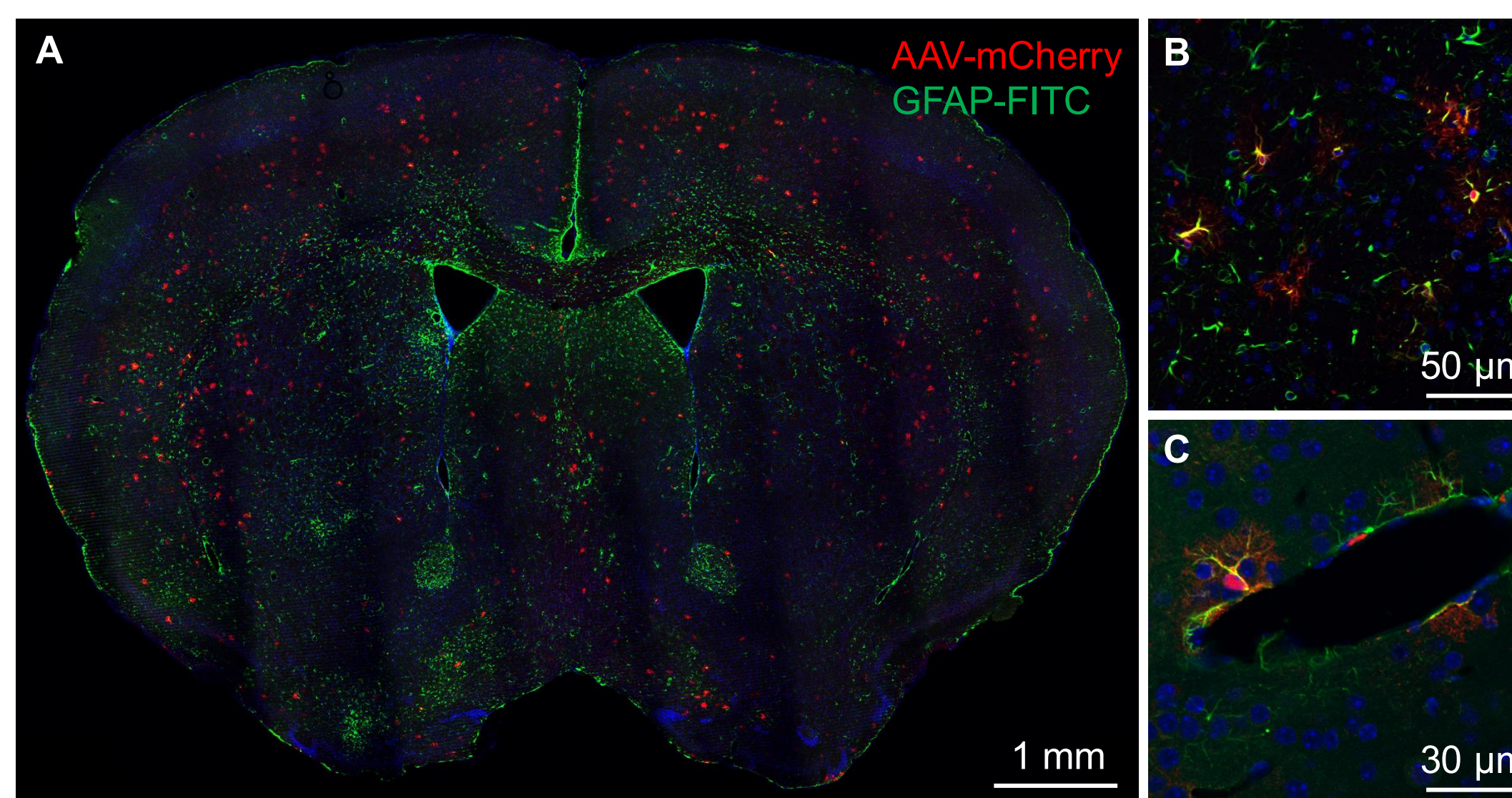


Figure 1. AAV-PhP.eB mediates robust, astrocyte-specific gene expression. (A) Representative confocal tiling image demonstrate widespread cortical and subcortical distribution of AAV-PhP.eB-GFAP-mCherry (red) and microinfarcts identified by GFAP immunostaining (Green) 3 weeks after MMI. High-magnification confocal images show colocalization of mCherry (red) with GFAP-positive astrocytes in the (B) cortex and (C) perivascular regions, confirming targeted astrocytic delivery.

2. CYP2C11 Overexpression Elevates EET Levels

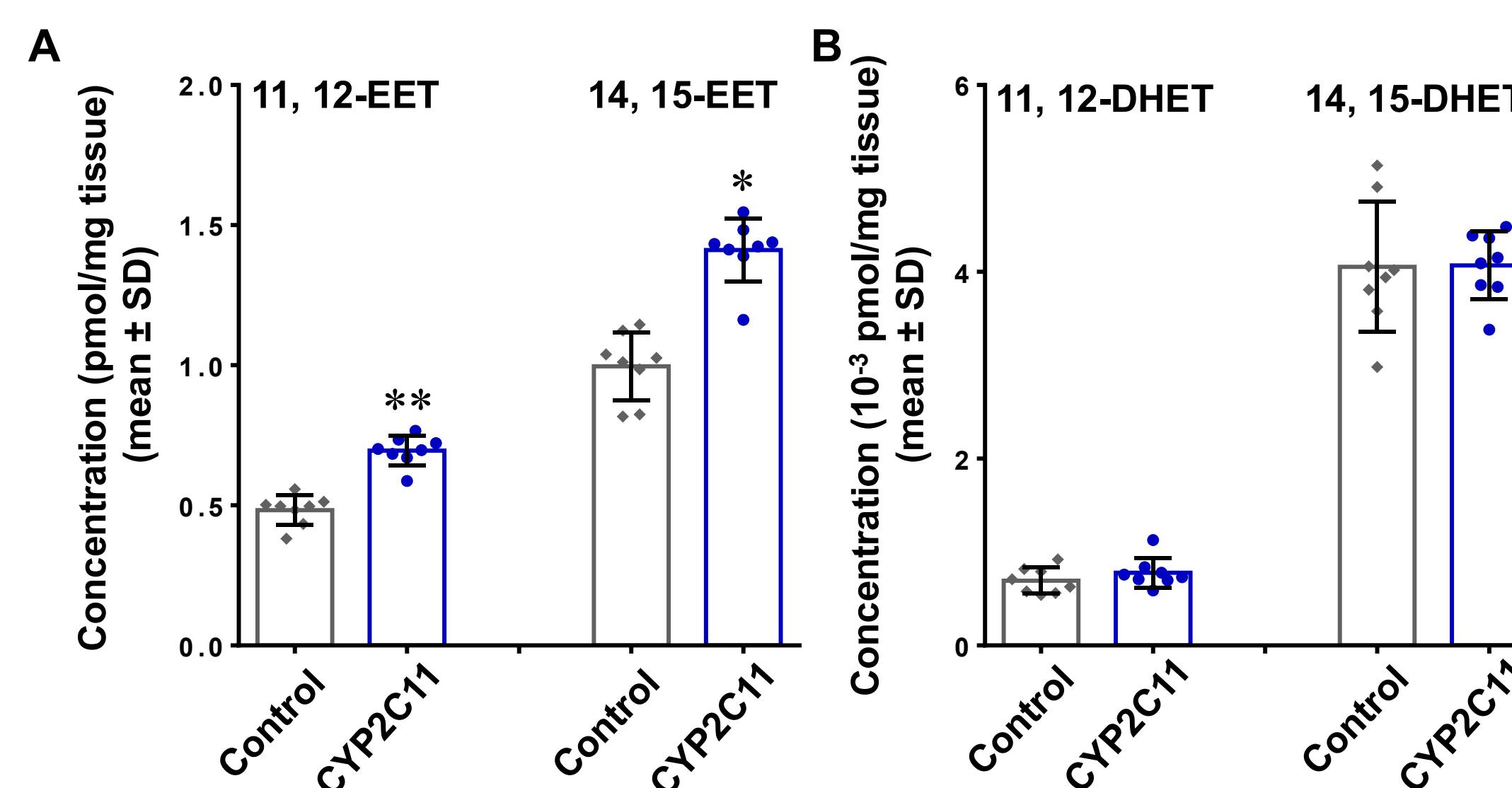


Figure 2. Biochemical validation of CYP2C11 activity using LC-MS/MS analysis of brain tissue homogenates (n = 8/group) demonstrates (A) a significant increase in 11, 12- and 14, 15-EET levels in AAV-CYP2C11 mice compared to AAV-mCherry controls, (B) AAV-CYP2C11 mice exhibit significantly elevated levels of 11, 12-EET and 14, 15-EET compared to AAV-mCherry controls. (B) Levels of 11, 12-DHET and 14, 15-DHET, inactive metabolites generated by soluble epoxide hydrolase (sEH), remain unchanged between groups. *p < 0.01, **p < 0.001 vs control.

3. CYP2C11 Preserves Cerebral Hemodynamics Post-MMI

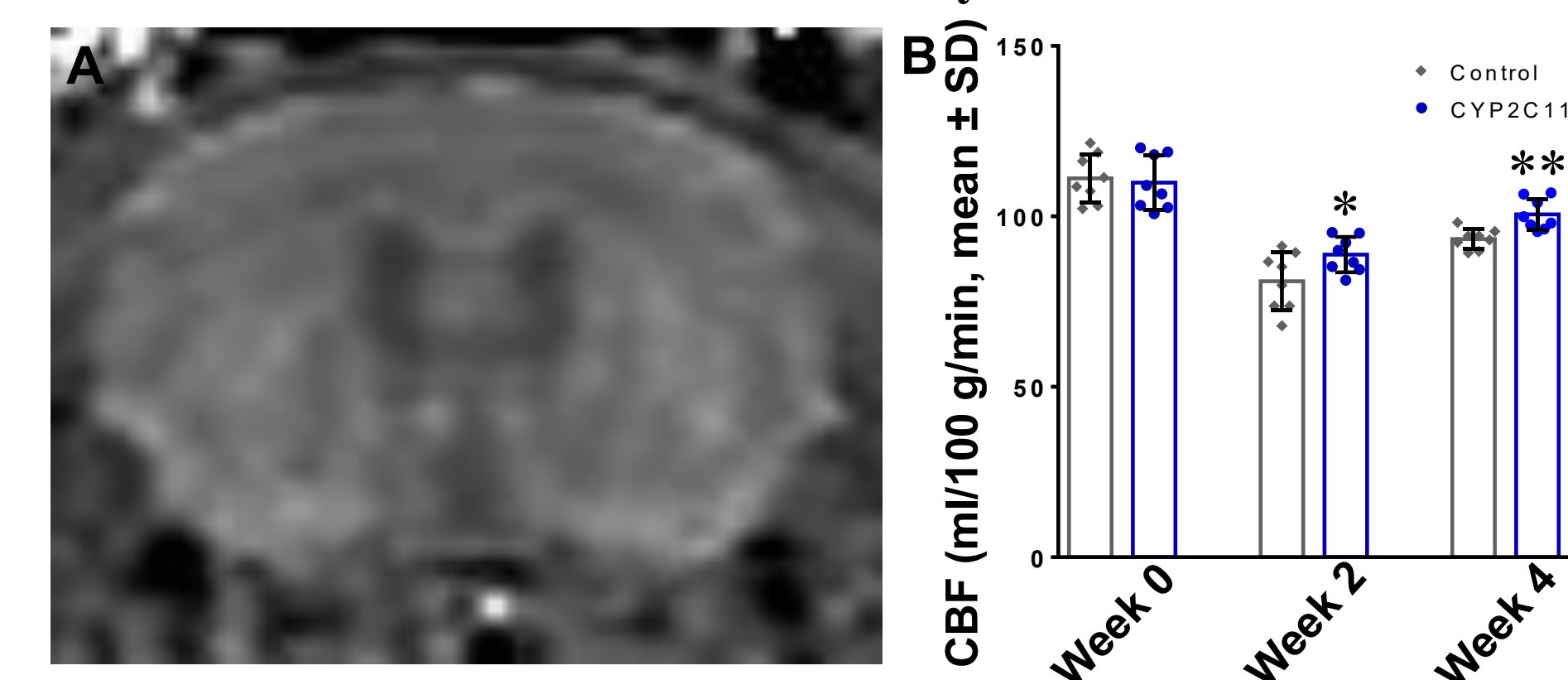


Figure 3. CYP2C11 mitigates MMI-induced hypoperfusion. (A) Representative ASL-MRI perfusion heat maps. (B) Longitudinal quantification of CBF confirms that AAV-CYP2C11 treatment significantly attenuates the reduction in regional blood flow post-MMI compared to controls (n = 8/group). *p < 0.05, **p < 0.01 vs control.

4. Improvement of Cognitive Performance

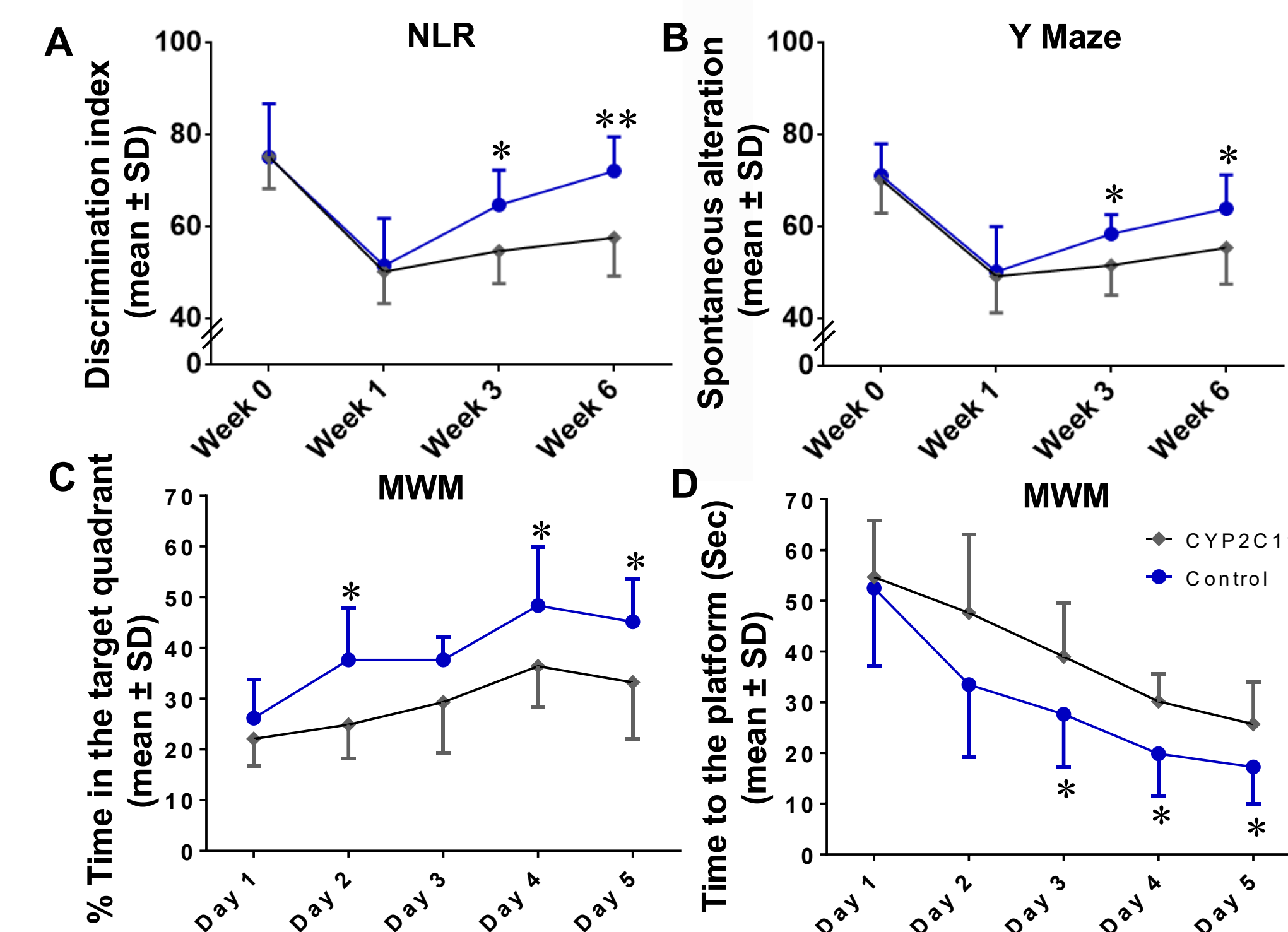


Figure 4. CYP2C11 improves cognitive outcomes. Recovery of cognitive function in AAV-CYP2C11 mice as measured by (A) NLR discrimination index and (B) Y-maze spontaneous alternation at 1, 3, and 6 weeks post-MMI. Morris Water Maze (MWM) performance at 5 weeks post-MMI, shows (C) increased time spent in the target quadrant and (D) reduced escape latency in AAV-CYP2C11 mice (n = 8/group). *p < 0.05, **p < 0.01.

CONCLUSION

Astrocyte-targeted overexpression of CYP2C11 restores cerebral perfusion, increases EET bioavailability, and improves cognitive performance in a bilateral MMI model of MID. These findings identify the astrocytic CYP2C11-EET pathway as a promising therapeutic target for vascular cognitive impairment and dementia.

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