

Investigating the Effects of Neuroinflammation on Synaptic Integrity in Human and Mouse Brain Slice Cultures

Lewis W. Taylor^{(1,2)*}, Max Knops^(1,3), Calum Bonthron^(1,2), Soraya Meftah^(1,2), Danilo Negro^(1,2), James H. Catterson^(1,2), Imran Liaquat⁽⁴⁾, Sam A. Booker^(1,3), Paul M. Brennan^(1,4,5), **Claire S. Durrant**^(1,2)

⁽¹⁾ The Institute for Neuroscience and Cardiovascular Research, The University of Edinburgh, Edinburgh, UK
⁽²⁾ UK Dementia Research Institute, The University of Edinburgh, Edinburgh, UK
⁽³⁾ Simons Initiative for the Developing Brain, The University of Edinburgh, Edinburgh, UK
⁽⁴⁾ Department of Clinical Neuroscience, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, UK
⁽⁵⁾ Cancer Research UK Brain Tumour Centre of Excellence, CRUK Edinburgh Centre, The University of Edinburgh, Edinburgh, UK

1. Introduction

1. Neuroinflammation contributes to the progression of multiple neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and Multiple Sclerosis¹.
2. The effects of neuroinflammatory pathways on synaptic and neuronal structure and function, the mechanisms by which these effects occur and whether they can be reversed pharmacologically remain to be fully investigated.
3. We treated mouse (MBSCs) and human (HBSCs) brain slice cultures with lipopolysaccharide (LPS), a toll-like receptor 4 agonist, to investigate the effects of a pro-inflammatory state on synaptic protein integrity.

2. Key Questions

- Does LPS lower excitatory and inhibitory synaptic proteins to differential extents in neonatal mouse brain tissue?
- Does LPS stimulate neuroinflammation and lower synaptic protein integrity in living human brain tissue?

3. LPS Lowers Excitatory Presynaptic Synaptic Protein Levels in Neonatal Mouse Brain Slice Cultures

5. Conclusions

14 Days of LPS Exposure in Brain Slice Cultures (BSCs):

- Reduces excitatory presynaptic protein (VGluT-1) levels in neonatal mouse BSCs.
- Induces a robust, but transient, neuroinflammatory response and a strong trend towards reduced VGluT-1 protein levels in human BSCs.

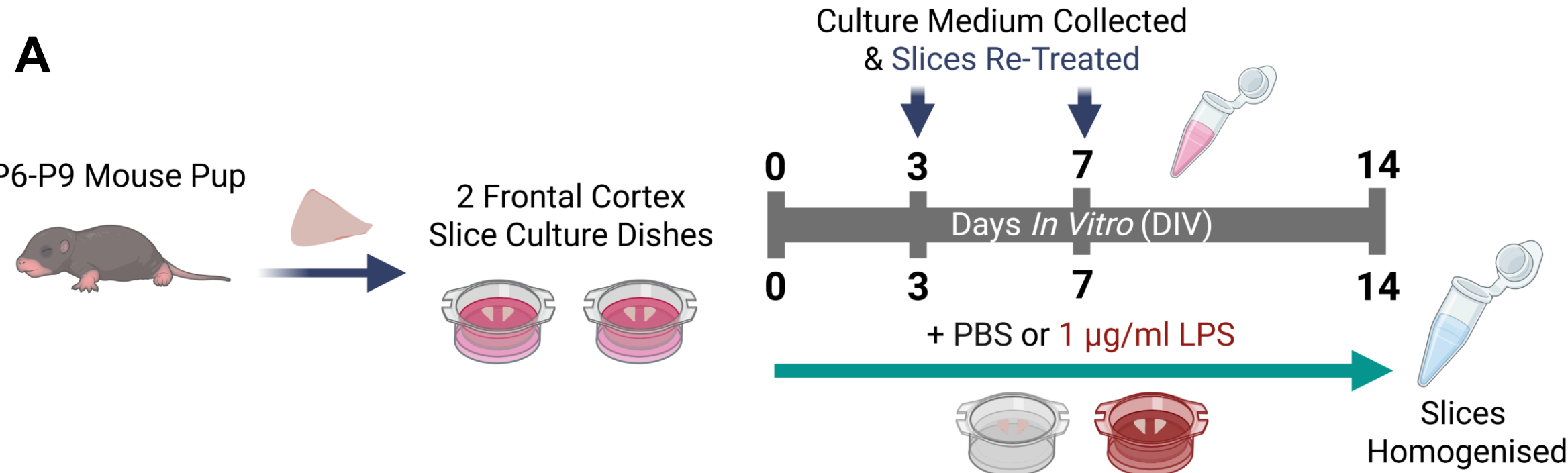
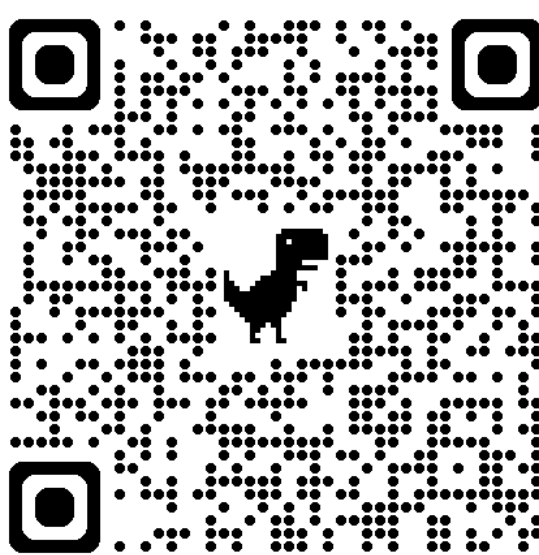
6. Future Directions

- Identify mechanistic targets, using proteomic analysis, associated with LPS-induced synaptic protein loss in human and mouse brain slice cultures.
- Determine whether LPS induces functional impairments in human cortical pyramidal neurons.

7. References

1. The role of neuroinflammation in neurodegenerative diseases: current understanding and future therapeutic targets. **Adamu, A, et al.** s.l. : *Frontiers in Aging Neuroscience*, 2024, Vol. 16. DOI=10.3389/fnagi.2024.1347987.

8. Google Scholar

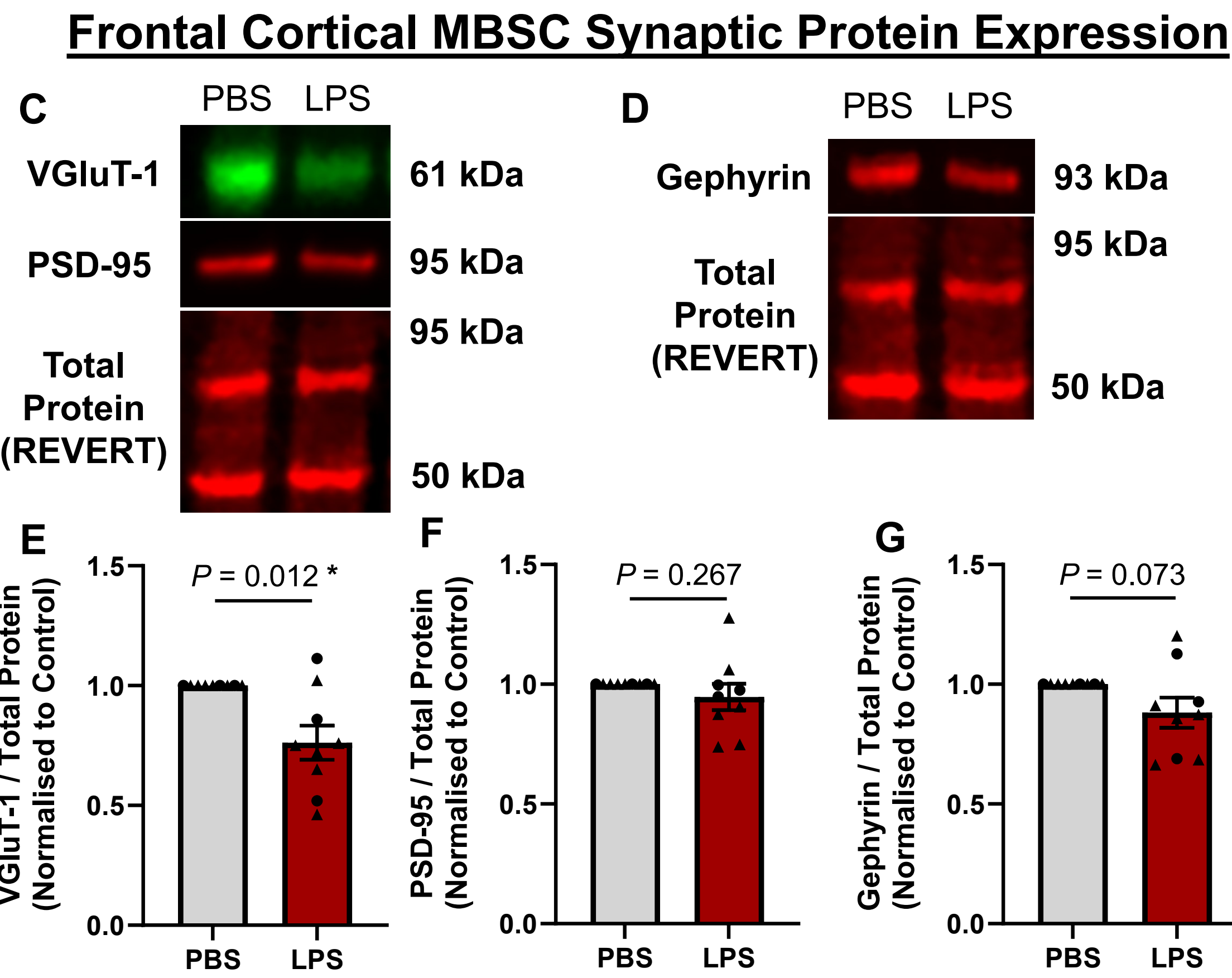
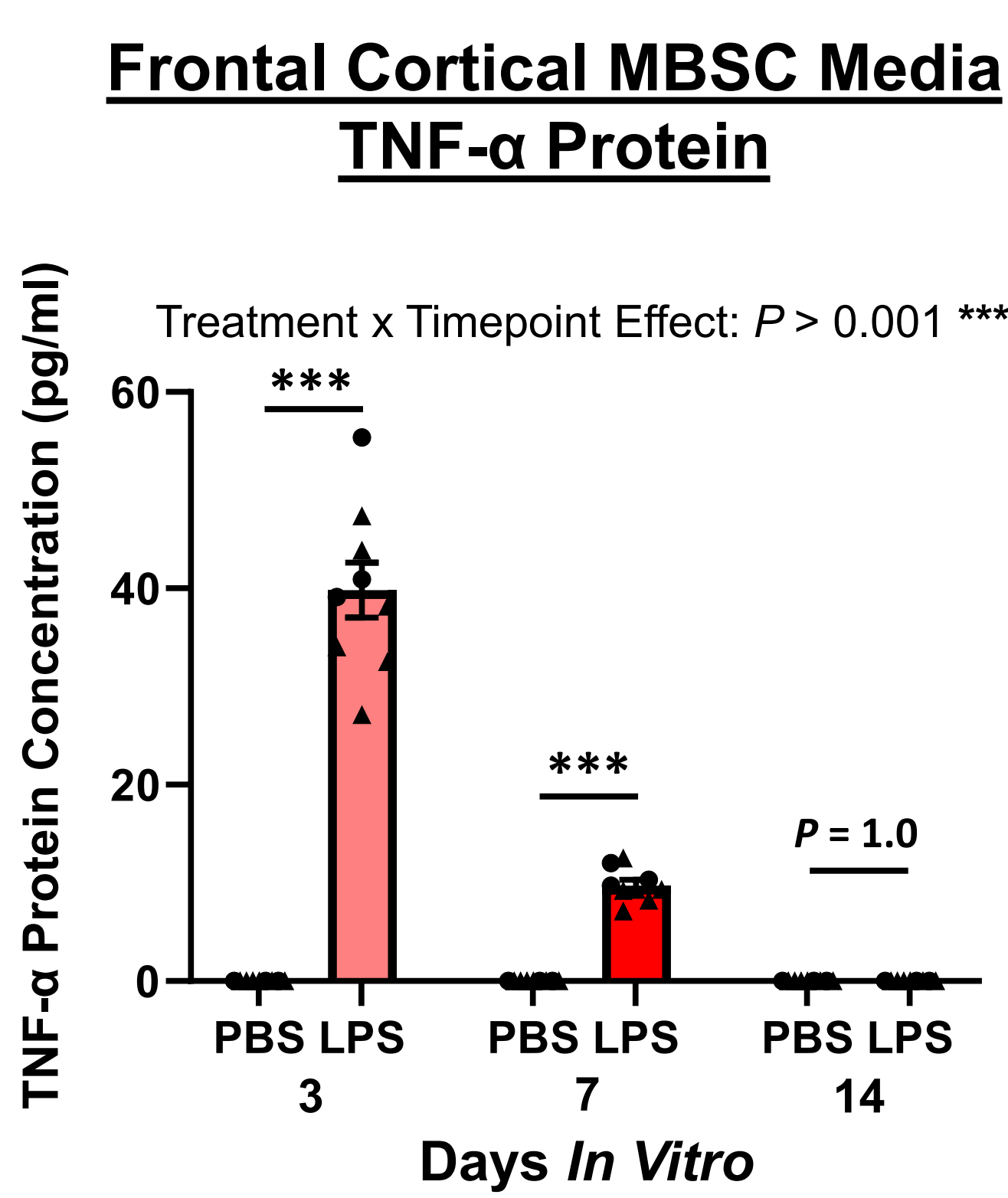


(A) 350 µm thick MBSCs were generated from 6-9 day-old (P6-9) wild-type (WT) mouse pups. 2 dishes of frontal cortical slices (2 slices per dish), treated with PBS control or 1 µg/ml LPS from 0-14 days *in vitro* (DIV), were prepared from each animal. The LPS-induced inflammatory response was confirmed by quantifying TNF-α protein levels, using enzyme-linked immunosorbent assay, in slice culture medium samples. N = 9 animals; triangles = males, circles = females. *** = $P < 0.001$.

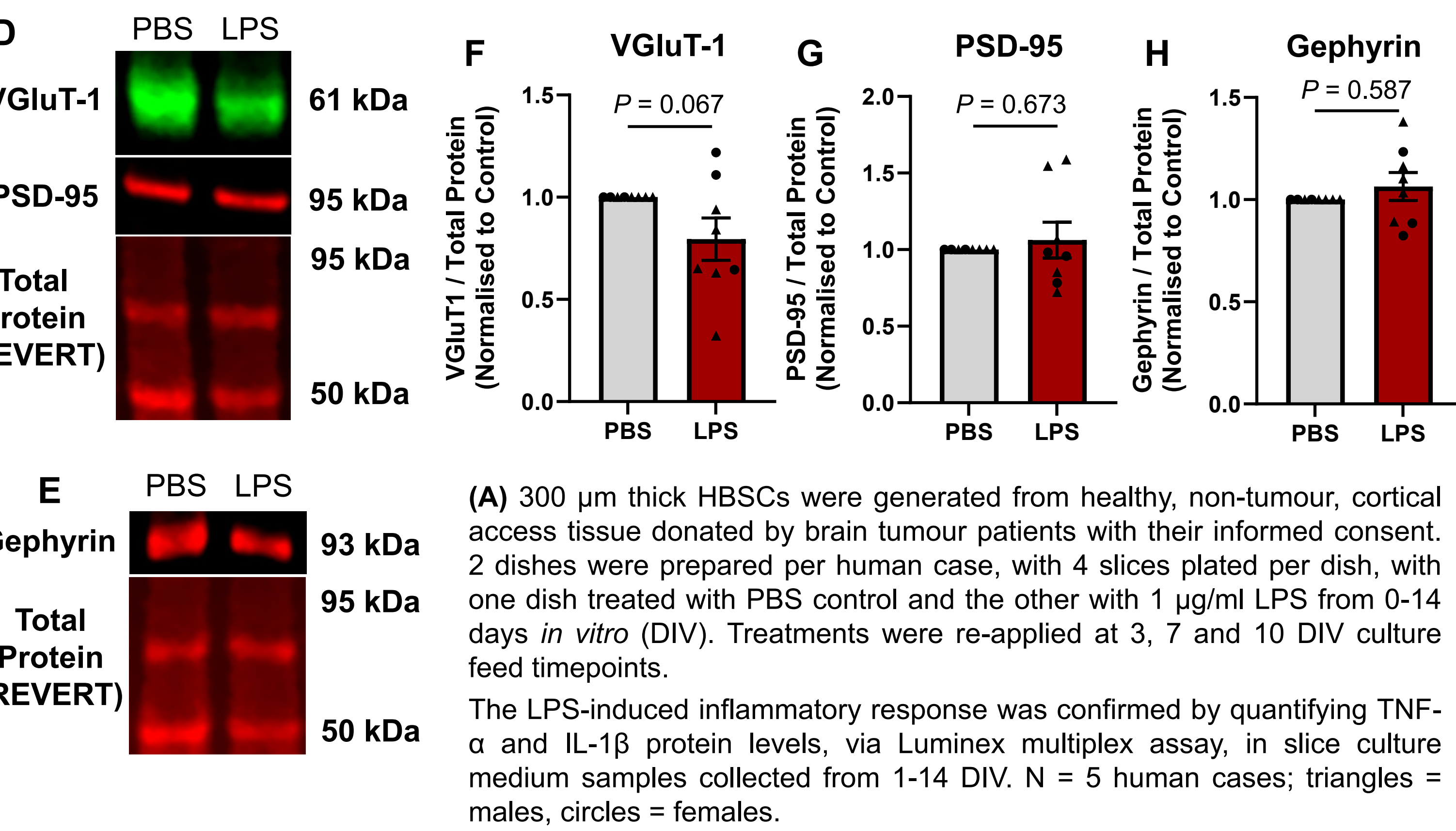
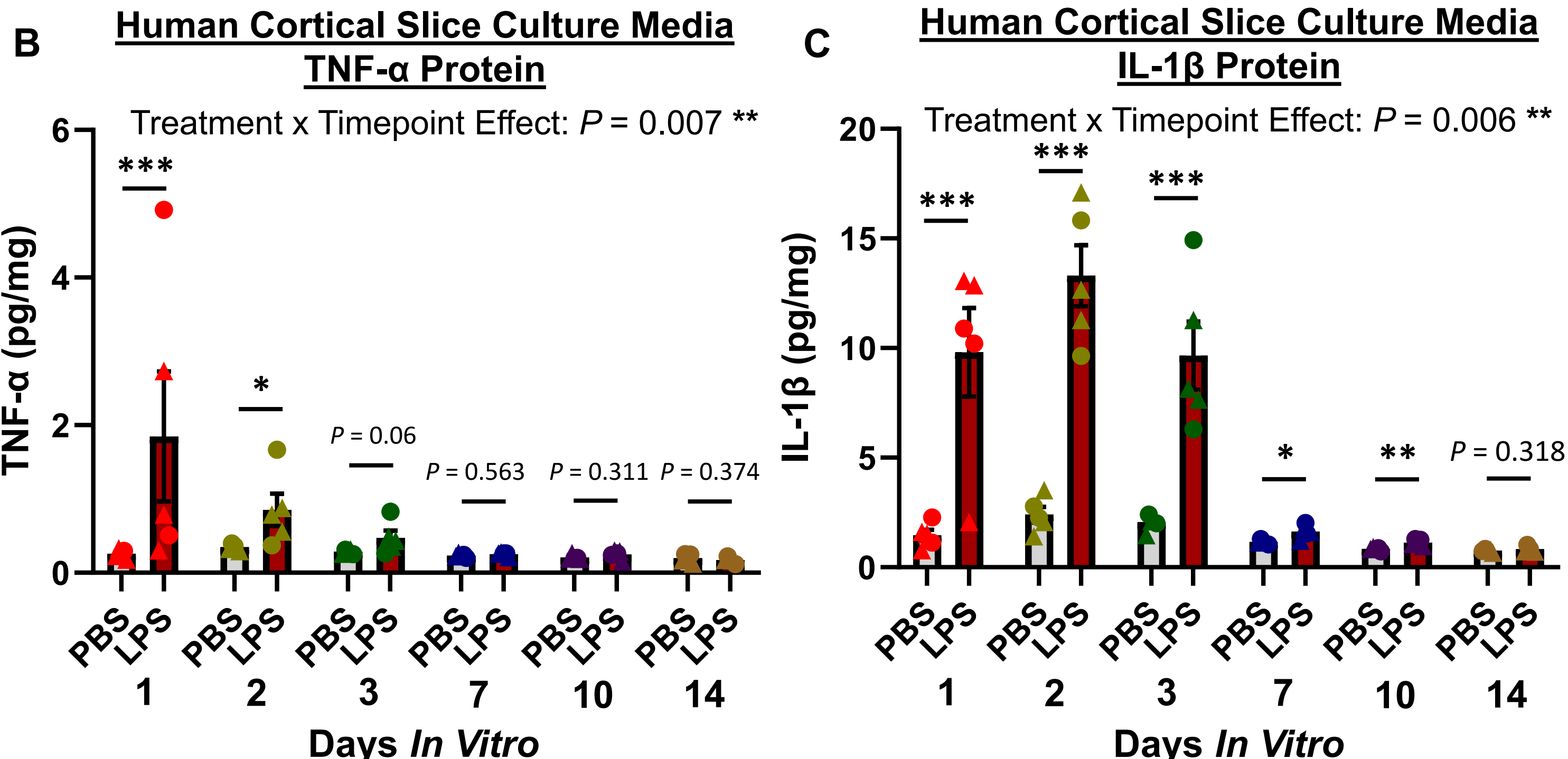
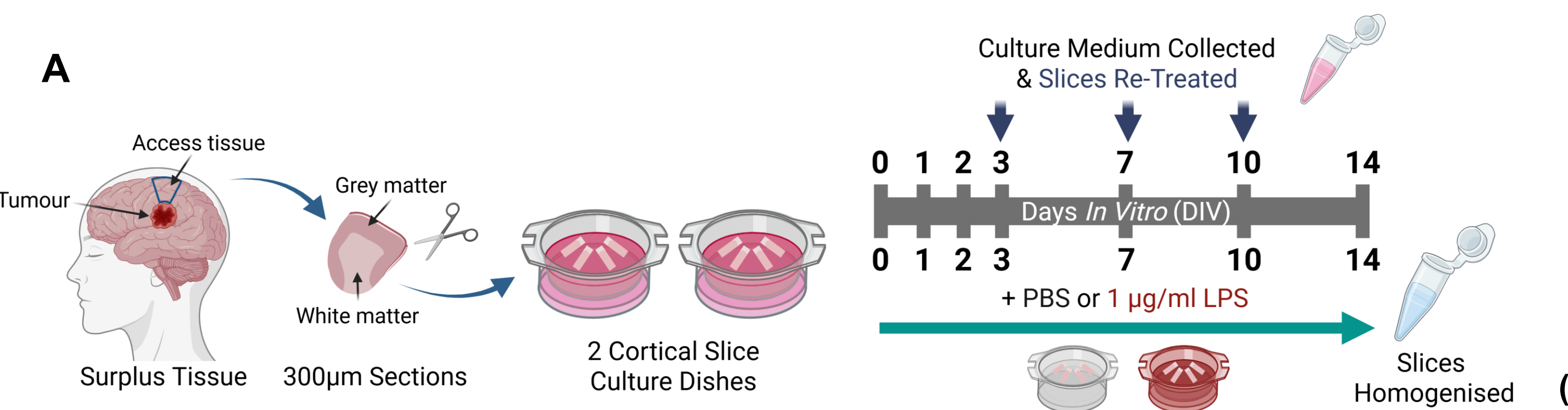
In MBSCs, increased culture medium TNF-α protein concentrations were observed following 3 and 7, but not 14, days of LPS treatment (B).

Western blot was used to quantify excitatory (C, E, F) and inhibitory (D, G) synaptic proteins, which were normalised to total protein, in 14 day treated frontal cortical MBSCs. N = 9 animals; triangles = males, circles = females.

14 days of LPS treatment significantly lowered VGluT-1 (E), but not PSD-95 (F) or Gephyrin (G), synaptic protein levels.



4. LPS Induces Neuroinflammation and Trend Reductions in Excitatory Presynaptic Protein Levels in Human Brain Slice Cultures



(A) 300 µm thick HBSCs were generated from healthy, non-tumour, cortical access tissue donated by brain tumour patients with their informed consent. 2 dishes were prepared per human case, with 4 slices plated per dish, with one dish treated with PBS control and the other with 1 µg/ml LPS from 0-14 days *in vitro* (DIV). Treatments were re-applied at 3, 7 and 10 DIV culture feed timepoints.

The LPS-induced inflammatory response was confirmed by quantifying TNF-α and IL-1β protein levels, via Luminex multiplex assay, in slice culture medium samples collected from 1-14 DIV. N = 5 human cases; triangles = males, circles = females.

14 days of LPS treatment induced strong trend reductions in VGluT-1 (F), but did not significantly alter PSD-95 (G) or gephyrin (H) protein expression.