Cross-Species Comparison of Viral Infection Responses in Human and Rodent Complex Neuronal Cultures

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Overview

We compared human iPSC-derived neuroimmune cultures (Neucyte Inc.) and complex primary neuronal cultures (in house preparation based on *Goshi et al, 2020*)[1] to evaluate cross-species responses to infection with attenuated Venezuelan equine encephalitis virus (VEEV) strain TC-83. Co-cultures (containing neurons and astrocytes) and tri-cultures (containing neurons, astrocytes, and microglia) from both species supported robust viral replication and showed similar functional impairments, with reduced neural activity and burst frequency by 48h post inoculation. Transcriptomic analysis revealed many conserved responses across species, including increased neuroinflammatory signaling and reduced synaptic gene expression. Additionally, microglia containing tri-cultures showed additional immune-related gene activation, highlighting the importance of microglia in effectively modeling viral infection. Treatment with EIDD-1931 (active form of the oral antiviral drug molnupiravir) reduced viral titers and restored neural activity in both human iPSC and primary rat cultures, highlighted the value of animal-based models for validating human-relevant microphysiological systems.

Complex Cultures

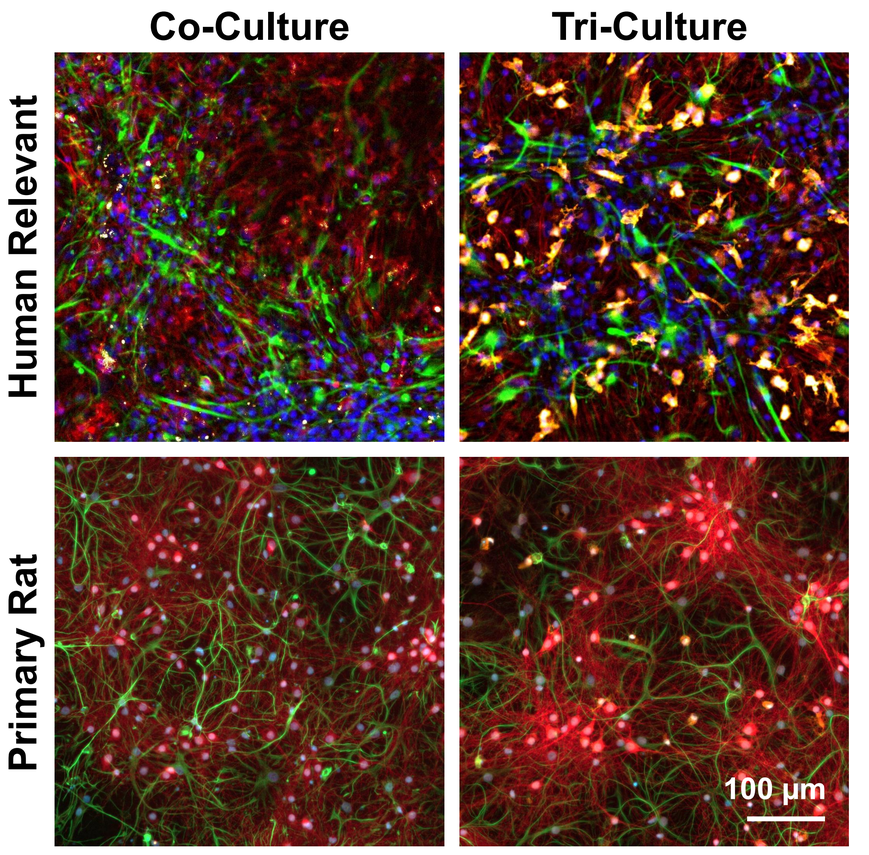


Figure 1: Representative fluorescence images of human iPSC-derived (NeuCyte Inc.) and primary rat cultures containing excitatory/inhibitory neurons, astrocytes, and microglia (tri-culture only). The cultures were immunostained for the three cell types of interest: Neurons – NF-H (red); Astrocytes – GFAP (green); Microglia – Iba1 (Orange); General nuclear stain – DAPI (Blue).

Functional Response to Viral Infection

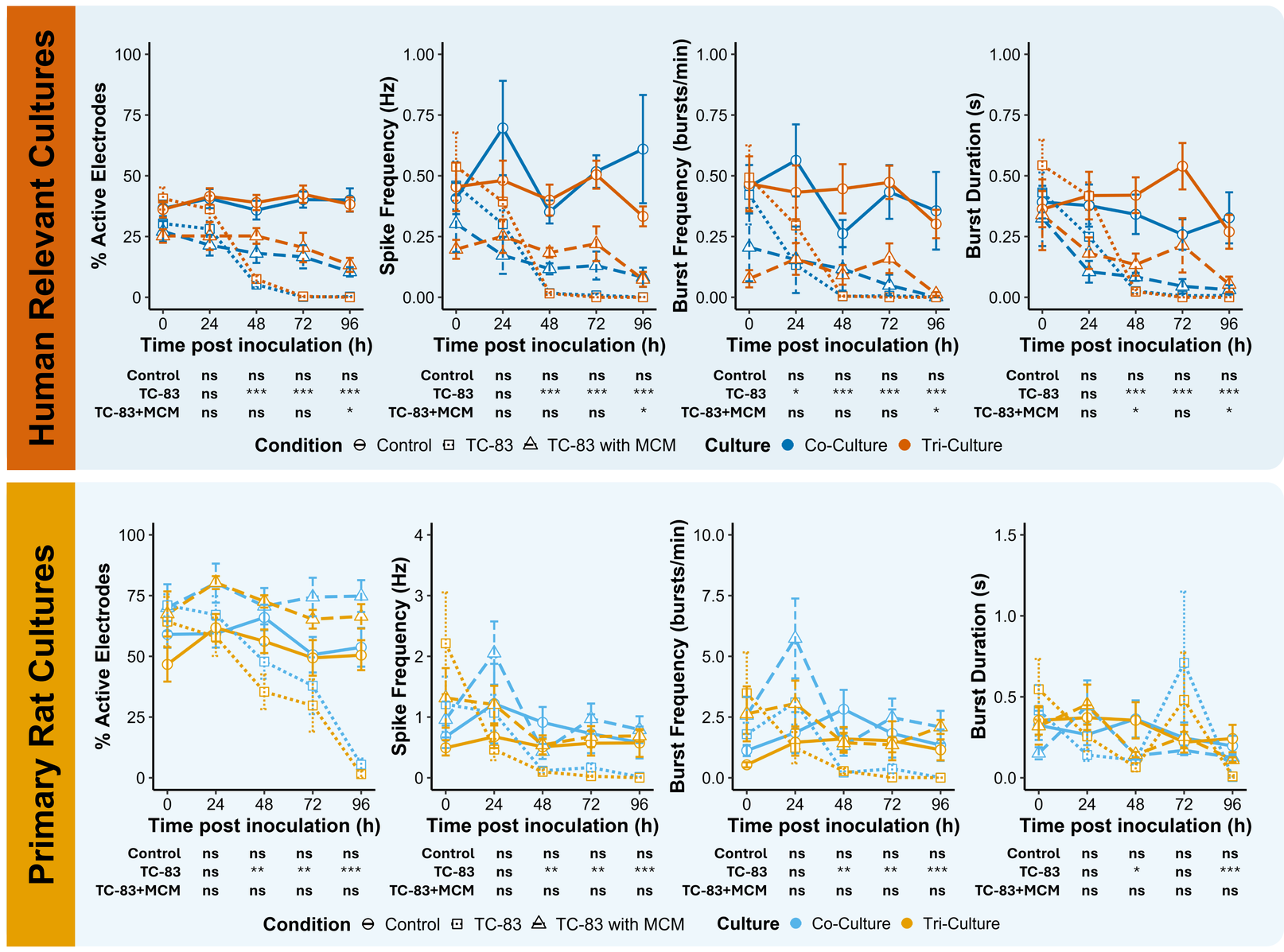


Figure 2 (above): Changes in electrophysiological features following inoculation with TC-83 (MOI 0.01) and treatment with 10 µM EIDD-1931 1h post inoculation (TC-83 + MCM). For all features there was no significant 3-way interaction (culture type, treatment, and time), however all features showed a significant interaction between treatment and time post inoculation as determined by the ANOVA-type statistic ($p < 0.001$ for all electrophysiology features across both species).[2] As there was no significant effect of culture type (tri- vs. co-culture), post-hoc tests were collapsed across culture type. Post hoc paired Wilcoxon signed-rank tests were used to compare each timepoint to baseline for each condition with p-values adjusted using the Holm-Bonferroni method and reported in the table below each panel. Data is shown as mean ± SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. n = 6-16 based on condition from at least two independent seedings.

EIDD-1931 Reduces Viral Load

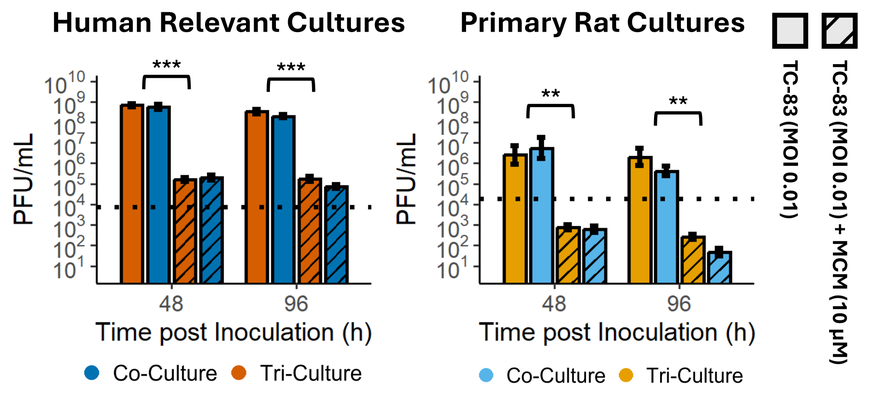
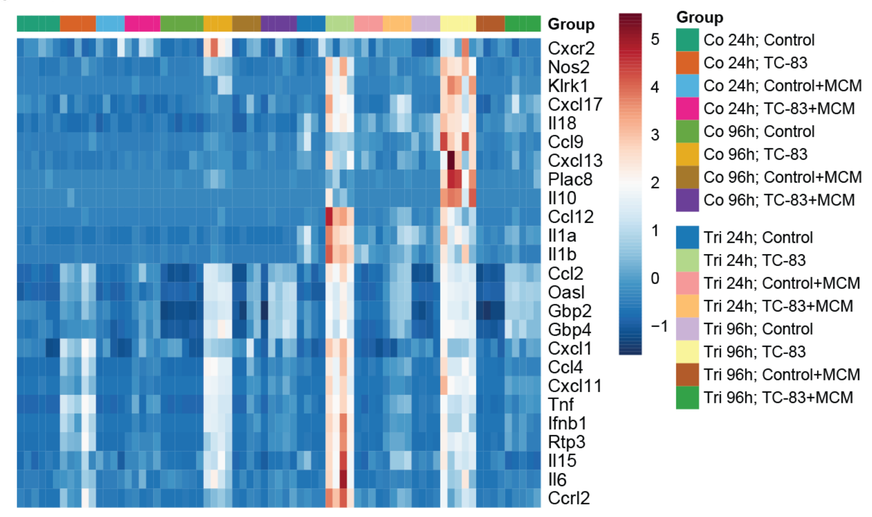


Figure 4 (above): Infectious viral titers measured in culture supernatants at 48h and 96h post inoculation (MOI 0.01) in human relevant (left) and primary rat (right) co- and tri-cultures. The addition of 10 µM EIDD-1931 (MCM) one hour post inoculation significantly reduced viral titers as compared to positive controls across all conditions (main effect of treatment, ANOVA-type statistic). Data is shown as mean ± SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. n = 3-4 based on condition from at least two independent seedings.

Figure 5 (below): Z-scored heat map of representative inflammatory genes upregulated following TC-83 inoculation (MOI 0.01) with or without EIDD-1931 treatment (10 µM added 1h post inoculation) in primary rat cultures at 24h or 96h post inoculation. Color-coded columns indicate groups of technical replicates.



Transcriptomic Profiling

Human Relevant Cultures

Primary Rat Cultures

Figure 3: Upregulation of innate immune and antiviral genes in TC-83 infected human-relevant and primary rat complex neuronal cultures. GO enrichment analysis of upregulated DEGs in human and rat co- and tri-cultures at 24h and 96h post inoculation (MOI 0.01); dot size represents the transcript count and color indicates adjusted p-value **(top)**. Z-scored heat map of representative inflammatory genes in human and rat co- and tri-cultures at 24h and 96h post inoculation; color-coded columns indicate groups of technical replicates from at least two individual seedings **(bottom)**.

Conclusions

- Infection with TC-83 lead to a significant reduction in neural activity in both primary rat and human iPSC-derived complex cultures, which could be rescued by treatment with the active form of the anti-viral drug molnupiravir (EIDD-1931).
- While the presence of microglia within the cultures did not significantly alter their functional response to infection, it did dramatically impact the transcriptomic profile, better recapitulating the neuroinflammatory response to viral infection.
- Parallel human-relevant and animal-based MPS enable researchers to correlate readouts from animal-based MPS to *in vivo* studies, and better predict how human-relevant MPS will translate to clinical outcomes

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References: [1]Goshi et al. J. Neuroinfl. (2020). [2]Brunner et al (1997)