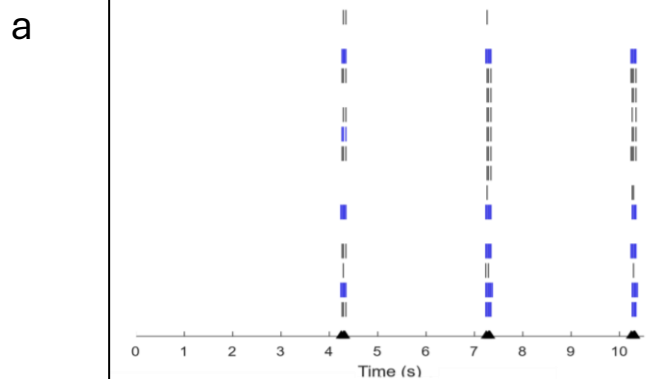
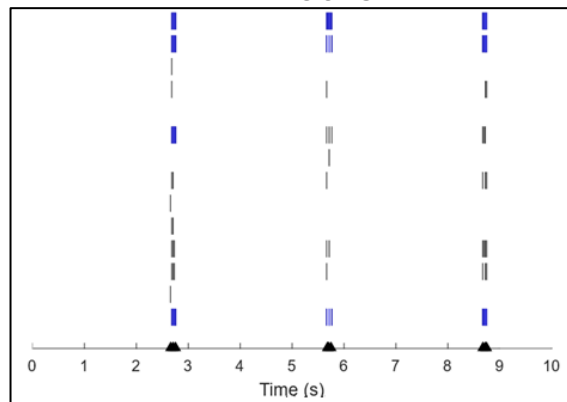


SUPPLEMENTARY FIGURES

Baseline



24 hours



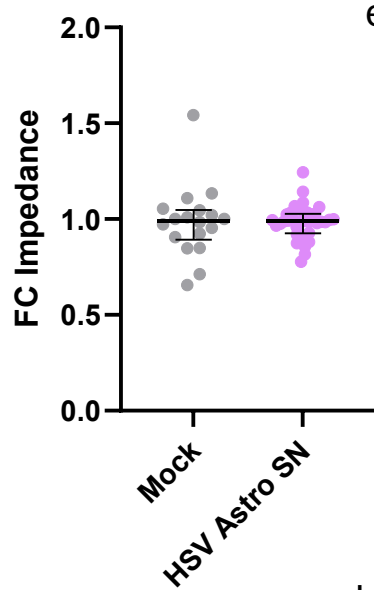
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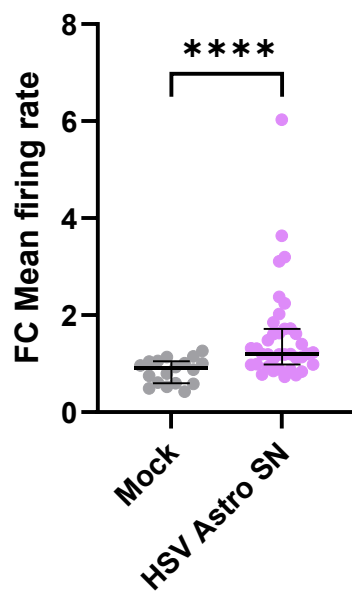
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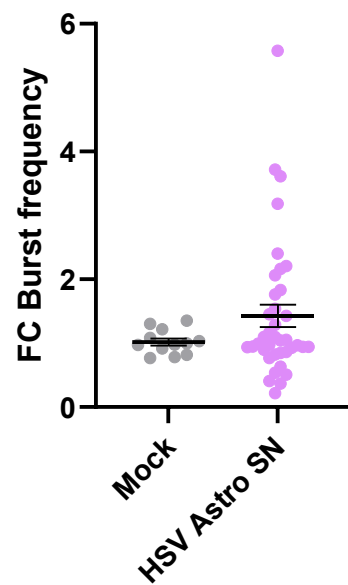
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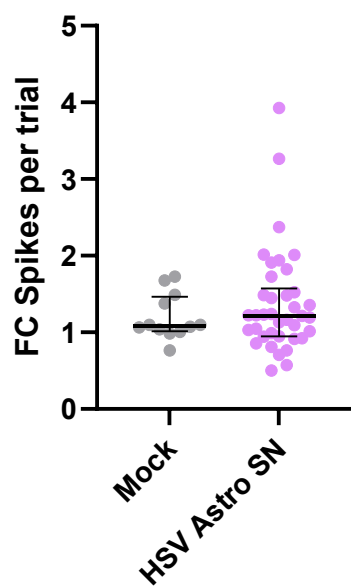
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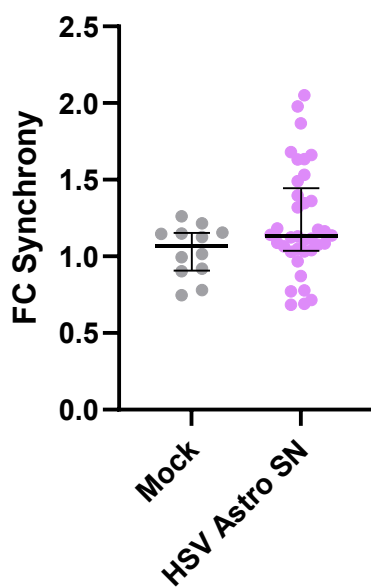
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g



h



i

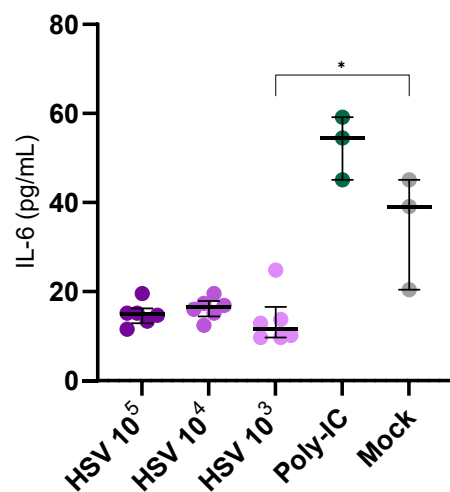


Figure S1 *Exposure to inactivated & filtrated supernatant from infected astrocytes modestly increases evoked firing rate but not other network parameters.* Although GFAP-positive cells were absent from this model of HSV-1-induced hyperexcitability, astrocytes represent a major source of inflammatory cytokines *in vivo*. Astrocyte-derived inflammatory signalling was therefore evaluated as a possible partial or complete mediator of the increased activity observed. Thus, hiPSC-CNs were subsequently exposed to inactivated and filtered supernatants derived from human astrocyte cultures 24 hours post-infection with HSV-1. At 24 hours of exposure, evoked responses were assessed. a) Representative raster plots from 10 seconds of baseline evoked activity (stimulation times at black arrows) and after 24 hours of continuous supernatant exposure for astrocyte-treated hiPSC-CNs. b) Representative single electrode traces and c) waveforms are also shown for baseline and 24-hour astrocyte supernatant treated. d) After 24 hours of treatment, there was no significant change in impedance, indicating no overt cell death. e) Supernatant-treated hiPSC-CNs showed a significant increase in mean firing rate compared with mock-infected controls; f-g) but no significant change was observed in burst frequency (f), spikes per stimulation trial (g) or h) Synchrony. Together, these data indicate that infected astrocyte supernatant was sufficient to modestly increase firing rate but did not induce a broader increase in quantity of synchrony of network activity. All group differences were tested with Mann-Whitney U-test. i) IL-6 secretion in astrocytes infected with HSV-1 at varying doses of virus was not elevated compared to mock infected and Poly:IC stimulated control. Kruskal-Wallis test with Dunn's post-hoc test was used. Data are shown as median $\pm$ IQR.

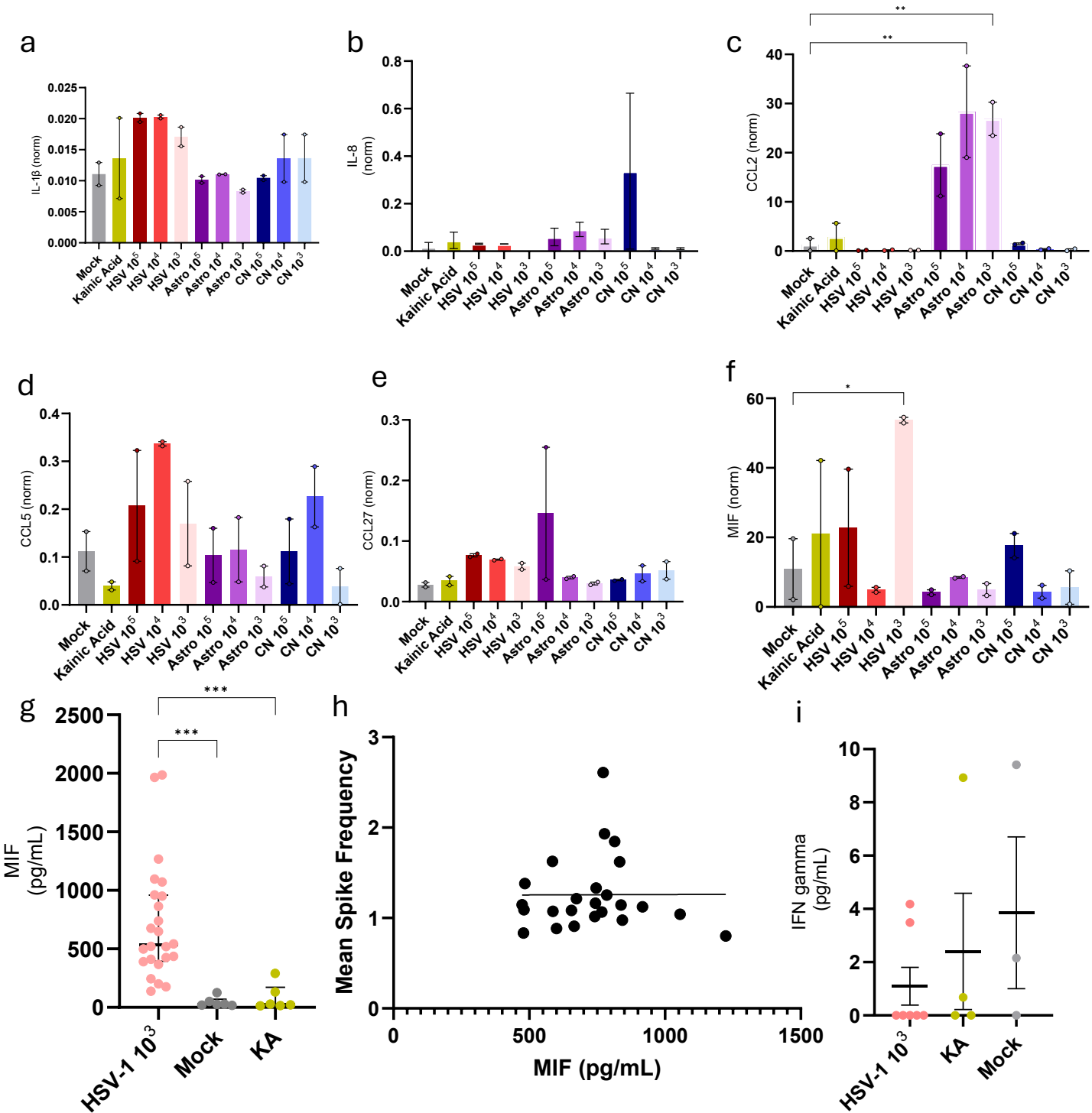
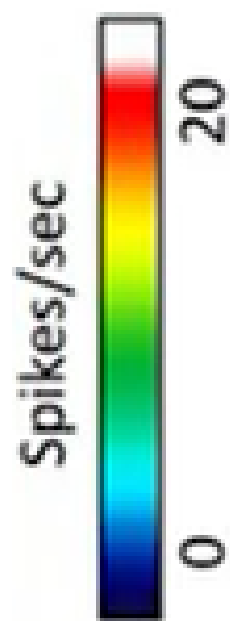
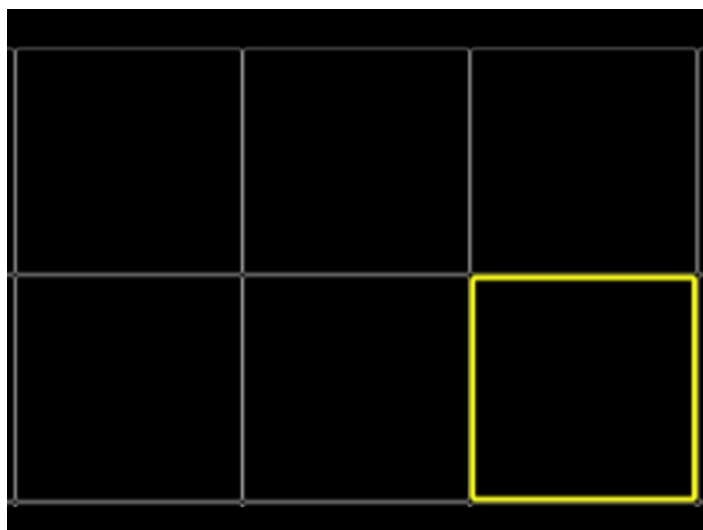
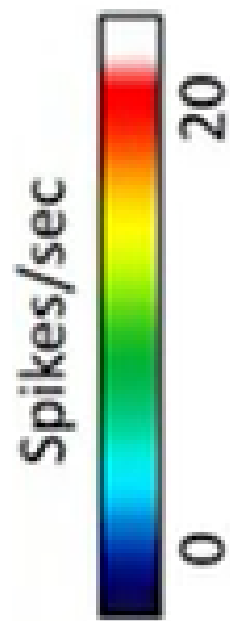
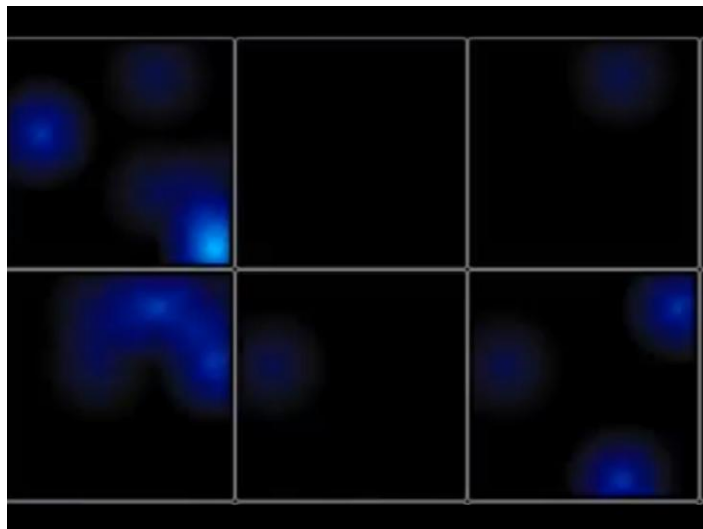


Figure S2: Luminex analysis from infected supernatants and supernatants from neurons and astrocytes at 72 hours post-infection or from neurons after treatment with conditioned neuronal medium. Several cytokines aside from IL-6 were identified from our patient cohort as being associated with seizure during HSE (IL-8, CCL2, CCL5, CCL27). Additionally, there is evidence in the literature for several cytokines being associated with seizure, including IL-1 β . Therefore, we used Luminex to screen supernatants from infected neurons, or from neurons exposed to conditioned medium from infected cells and subsequently showing increased activity at 72 hours. a) IL-1B and b) IL-8 secretion were not significantly different between conditions. c) CCL2 was not secreted by infected neurons but showed significantly elevated secretion by astrocytes. d) CCL5 and e) CCL27 secretion were not significantly different between conditions. f) MIF secretion was significantly elevated at 72 hours post-infection with 2.5×10^3 PFU. Mock infected = Vero supernatant. Measurements are shown as mean $\pm$ SEM dot plot. One-way ANOVA with Dunnett's correction for multiple comparisons with control was used. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. 2 replicates per condition and controls. g) As in Luminex study, elevated MIF secretion was seen in infected neurons on MEA using ELISA. Kruskal-Wallis with Dunn's correction for multiple comparisons with control was used. 24 replicates for MIF, 6 replicates for KA and mock-infected controls. h) However, MIF concentration was not significantly correlated to in vitro neuronal electrophysiological activity ($R^2 = 1.643e-005$, $p = 0.9850$). i) IFN γ secretion from infected control neurons was not significantly different to mock infected or KA treated neurons. Kruskal-Wallis with Dunn's post-hoc correction was used. j) IL-1b secretion by human primary astrocytes at 24 hours post-infection or poly:IC stimulation was elevated compared to mock-infected control. N = 6 wells for HSV, 3 for controls. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

S1



S2



Supplementary Video S1. Movie of spike activity of six baseline wells at 6 weeks of differentiation created with AxIS Navigator software (Axion Biosystems). Spike rate was calculated every 1s. Intensity scale is given at right.

Supplementary Video S2. Movie of spike activity of six wells containing neurons at 20 hours-post infection created with AxIS Navigator software (Axion Biosystems). Spike rate was calculated every 1s. Intensity scale is given at right.

Supplementary Methods

Primary astrocyte culture

Primary astrocytes isolated from humans were obtained from ScienCell Research Laboratories (ScienCell #1800). Astrocytes were first expanded in a Poly-L-Lysine (ScienCell #0403) coated T-75 flask and maintained in Human Astrocyte Medium (ScienCell #1801). 24 hours before use, cells were seeded onto Poly-L-Lysine coated 24 well cell culture plates at a density of 2.5×10^5 cells per well.

Supplementary Methods Table 1: Antibodies

Antibodies	Supplier	Catalogue Number
Anti-beta III Tubulin	Abcam	CAT #ab78078; RRID:AB_2256751
Anti-GFAP	Thermo Fisher	CAT #41-9892-82; RRID:AB_2573656
Anti-GFAP	DAKO	CAT #GA52461-2
Anti-PAX6	Abcam	CAT #ab5790; RRID:AB_305110
Anti-NeuN	Abcam	CAT #ab223994; RRID:AB_2892735
Anti-NMDAR	Abcam	CAT #ab109182
Anti-GABA-B-R	Thermo Fisher	CAT # MA5-52869
Anti-synaptophysin	Abcam	CAT #ab52636
Donkey anti-mouse AF488	Abcam	CAT #AB50105
Donkey anti-rabbit AF647	Thermo Fisher	CAT #A31573

Supplementary Methods Table 2: RT-qPCR primer/probe assays

Gene	Probe & Primer Sequence	Supplier	Catalogue Number
18S	Probe: /56- FAM/TGCTCAATC/ZEN/TCGG GTGGCTGAA/3IABkFQ/ Primer 1: GGACATCTAAGGGCATCAC AG Primer 2: GAGACTCTGGCATGCTAACT AG	IDT	CAT #Hs.PT.39a.22214856
RPLP0	Probe: /56- FAM/CCCTGTCTT/ZEN/CCCT GGGCATCAC/3IABkFQ/ Primer 1: TCGTCTTTAAACCCTGCGTC Primer 2: TGTCTGCTCCCACAATGAAA C	IDT	CAT #Hs.PT.39a.22214824

GRIN1	Probe: /56- FAM/TCTGGATGG/ZEN/CGTT GGGCTTGT/3IABkFQ/ Primer 1: GTGGATGGCTAACTAGGAT GG Primer 2: CTCCTGGAAGATTCAGCTCA A	IDT	CAT #Hs.PT.58.39141804
GABBR1	Probe: /56- FAM/TCCAGAGTC/ZEN/GAA GTGAAGACCTCAGT/3IABkF Q/ Primer 1: ATCTCAATTCCAGCCTCCTT C Primer 2: CCACACTCCACAACCCTAC	IDT	CAT #Hs.PT.58.15219724
SLC32A1	Probe: /56- FAM/CTACCCTAC/ZEN/GCC ATCCTGCACG/3IABkFQ/ Primer 1: GCGAAGATGATGAGAAACA ACC Primer 2: GTGACCAACGCCATCCA	IDT	CAT #Hs.PT.58.4211177
KCNQ2	Probe: /56- FAM/CGTGCTGTC/ZEN/TGTG TTTTCCACCATC/3IABkFQ/ Primer 1: GTCACGATTTCAGGATGTA GA Primer 2: GTTCATCTACCACGCCTACG	IDT	CAT #Hs.PT.58.38991963
SLC17A7	Probe: /56- FAM/AGGGAGTGC/ZEN/TAA ACTTCGTGAGGG/3IABkFQ/ Primer 1: ACTGGCATAGACGTGAAGA AG Primer 2: GGAGCGCAAGTACATCGAG	IDT	CAT #Hs.PT.58.22392732
HSV- Polymerase	Probe: /56-FAM/CC GCC GAA CTG AGC AGA CAC CCG CGC /36-TAMSp/ Primer 1: CAT CAC CGA CCC GGA GAG GGA C Primer 2: GGG CCA GGC GCT TGT TGG TGT A	IDT	Custom