Human neural organoids beading it: neurons in 3D culture show injury following mpox virus infection

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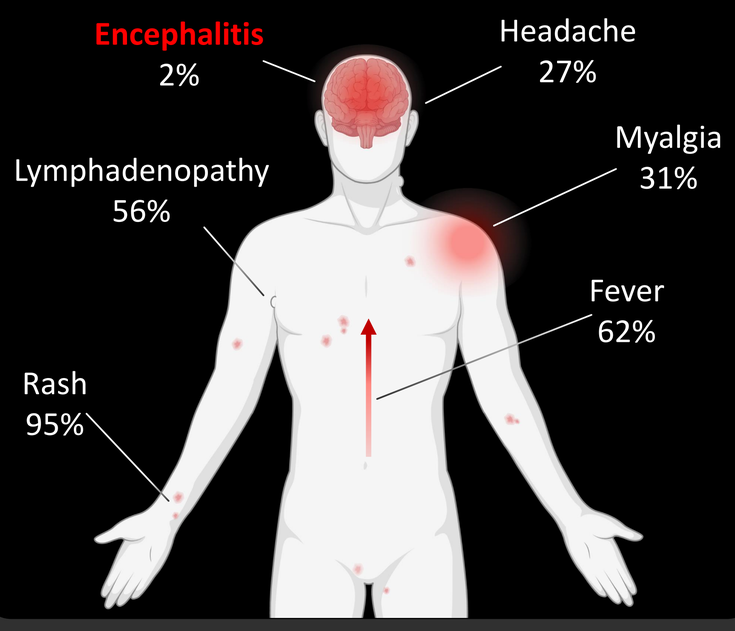
BACKGROUND

- Since May 2022, 113 countries have reported cases of mpox virus (MPXV) infection – the largest ever recorded outbreak outside of Africa.
- Specific symptoms and severe complications reported during the current outbreak, include:



1. MPXV infects human neural organoids

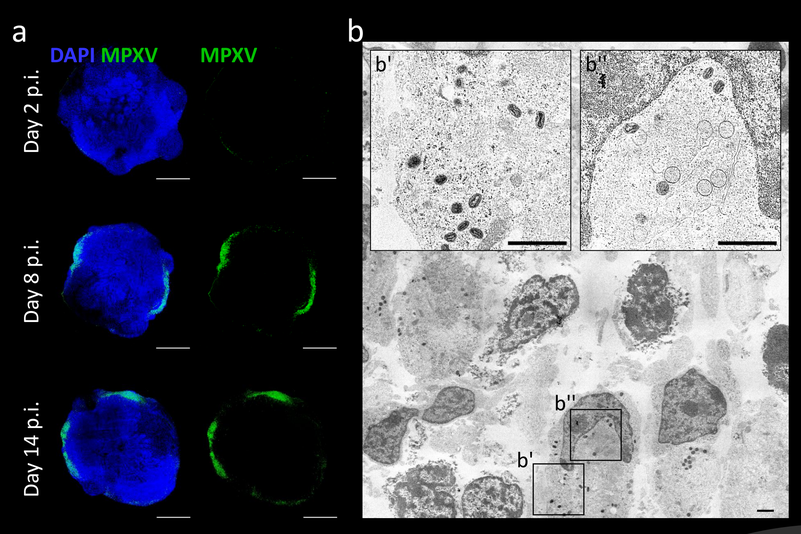


Figure 1: MPXV productively infects human neural organoids. (a) Representative micrographs of 70-95 days old organoids infected with a clade IIb lineage MPXV isolate at a rate of 0.1 $TCID_{50}$/cell. Infection dynamics were followed by immunofluorescence imaging from 2 to 14 days post-infection. Scale bar: 1000 µm. (b) Representative transmission electron microscopy images showing intracellular mature virions grouped in proximity of the cell's membrane (b') and a viral factory (b''). Scale bar: 1 µm.

2. Multiple cell types are targeted by MPXV

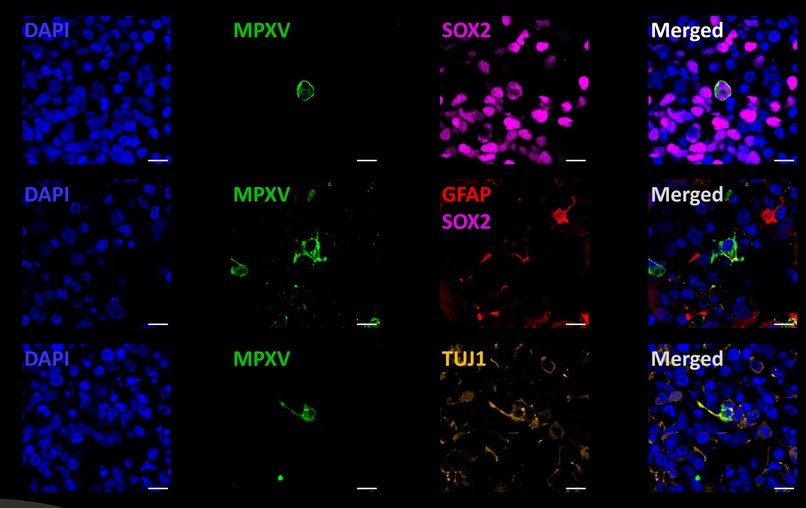
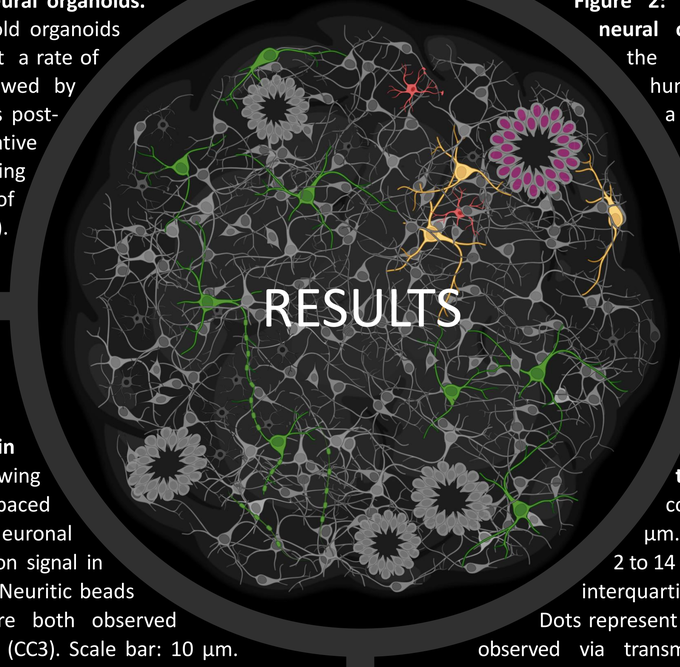
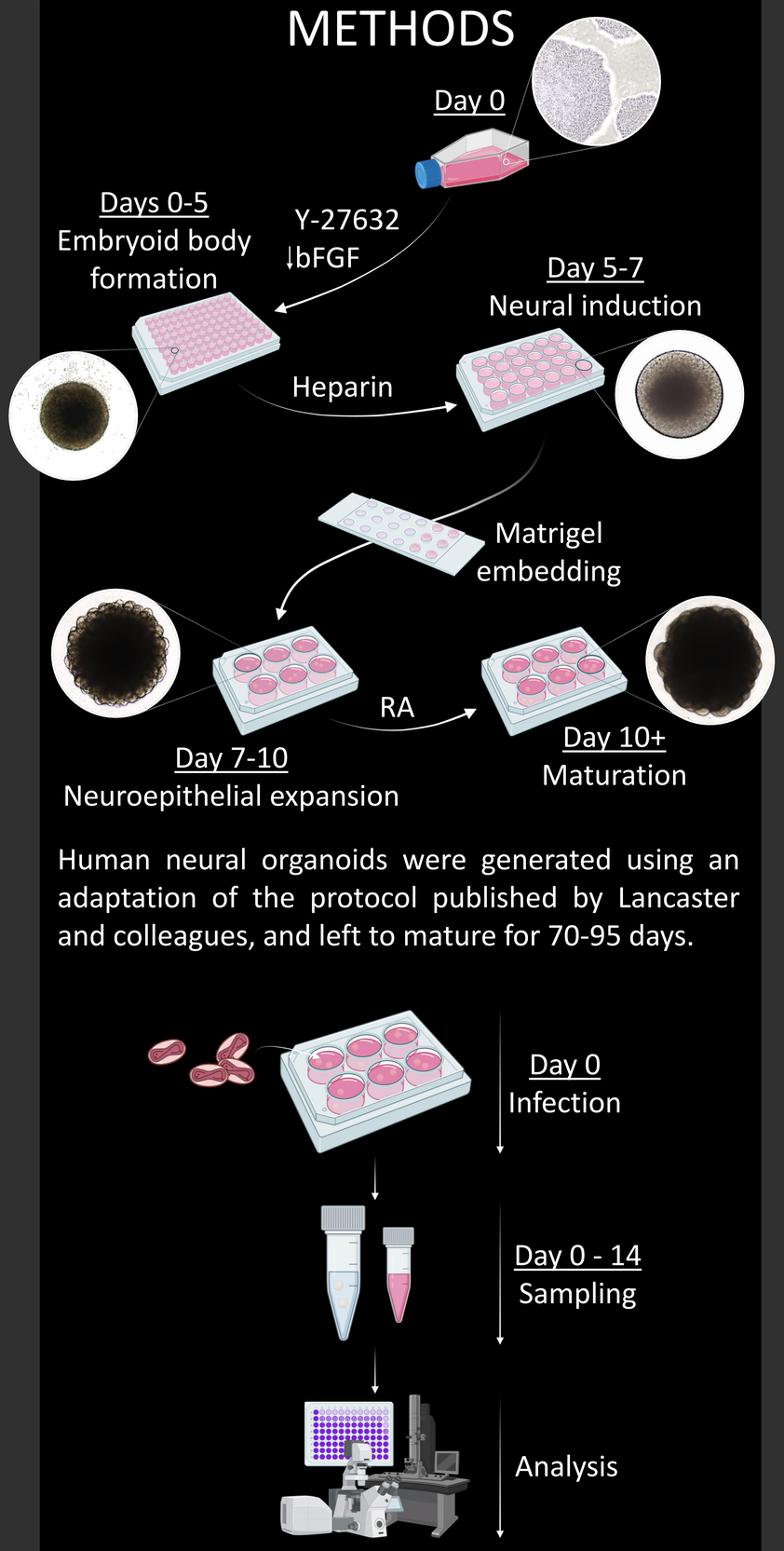


Figure 2: MPXV has a broad cellular tropism in human neural organoids. Representative micrographs illustrating the target cells of MPXV in 70-95 days old human neural organoids 10 days post-infection with a clade IIb lineage isolate at an MOI of 0.1 $TCID_{50}$/cell. SOX2-positive neural progenitor cells (top), GFAP-positive but SOX2-negative astrocytes (middle), and TUJ1-positive neurons (bottom) were observed being susceptible to viral infection. Scale bar: 10 µm .



METHODS

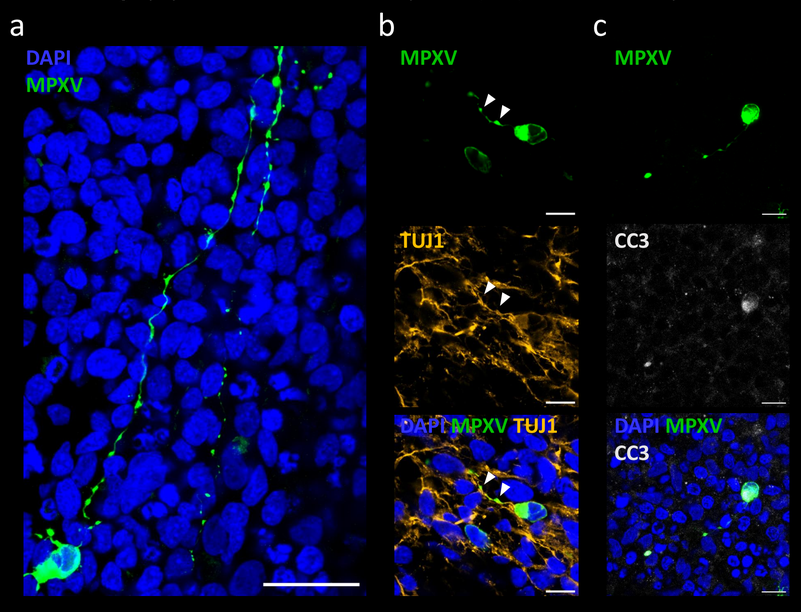


Human neural organoids were generated using an adaptation of the protocol published by Lancaster and colleagues, and left to mature for 70-95 days.

Organoids at 70–95 days of age were infected with MPXV at an MOI of 0.1 $TCID_{50}$/cell. Over a period of 14 days, samples were taken in two-day intervals and analyzed to assess virus replication, MPXV cell tropism and neuropathological tissue changes.

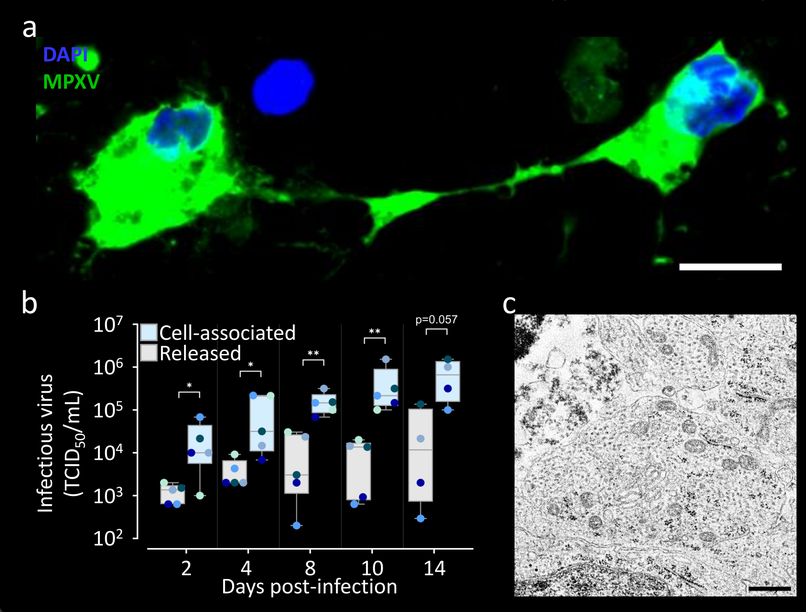
4. MPXV causes neuronal injury

Figure 4: MPXV causes beading and injury in human neural organoids. (a) Micrograph showing viral antigen accumulating within regularly spaced beads on filaments. Scale bar: 25 µm. (b) Neuronal marker TUJ1 was observed co-localizing with virion signal in both filaments and beads. Scale bar: 10 µm. (c) Neuritic beads and cell somata of MPXV-infected cells were both observed accumulating apoptotic marker cleaved caspase-3 (CC3). Scale bar: 10 µm.

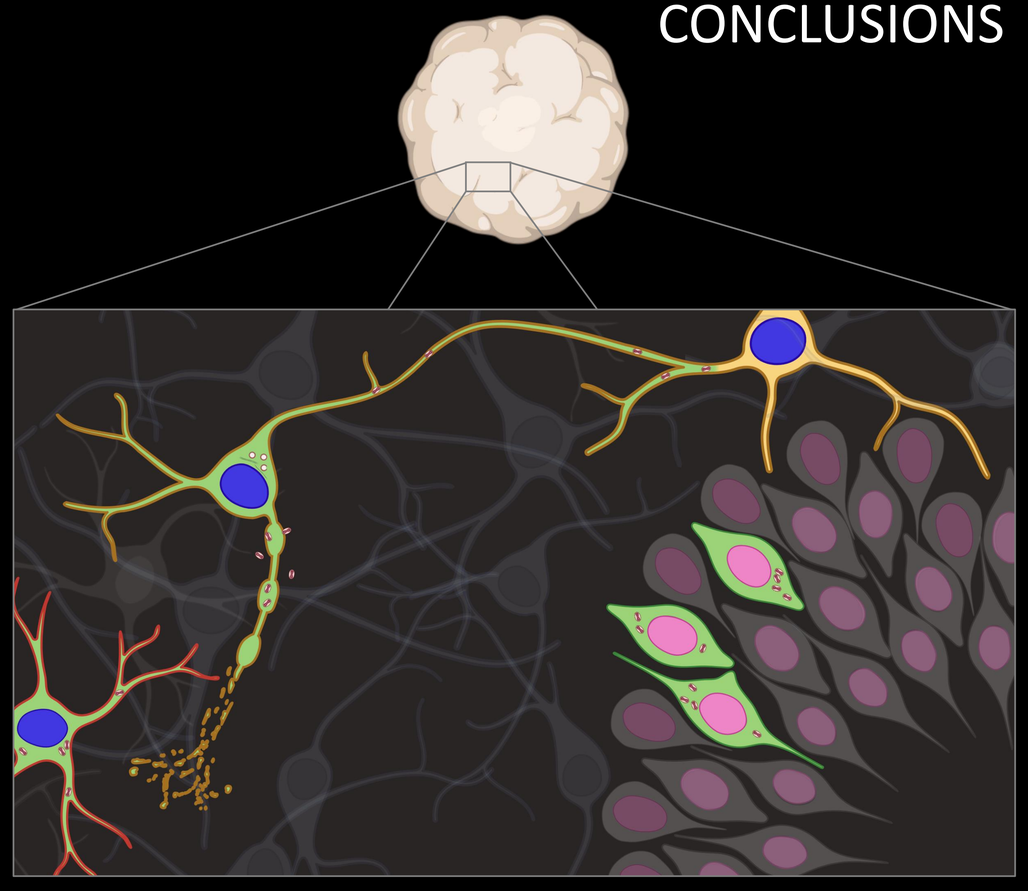


3. MPXV spreads from cell to cell

Figure 3: MPXV transmits preferentially from cell to cell in neural organoids. (a) MPXV-harboring cells connected by antigen-carrying filaments. Scale bar: 10 µm. (b) Cell-associated and released infectious MPXV 2 to 14 days post-infection. Boxplots indicate median value and interquartile ranges, whiskers show lowest and highest values. Dots represent distinct batches (n = 4-5). (c) Virion-bearing neurites observed via transmission electron microscopy. Scale bar: 0.5 µm.



CONCLUSIONS



Human neural tissue, modelled in a complex 3D environment, is highly susceptible to infection with the contemporary clade IIb MPXV. Within human neural organoid cells, viral replication factories are successfully established, allowing replication of MPXV in several cell lineages. Viral antigen localizes not only to cell somata, but also to filaments of variable nature. We propose that MPXV preferentially spreads from cell-to-cell, exploiting not only previously described mechanisms, but also through axonal transport. We hypothesize the observed serial filament swellings represent sites of virus egress and cell-to-cell transmission, eventually leading to neuronal injury. Our findings constitute the basis for further exploration of the neurobiology of orthopoxviruses.

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