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Therapy and prevention of long-term cognitive impairment following neonatal HSV infection

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Advancing Science Series
Herpes Cure Advocacy/HVTN
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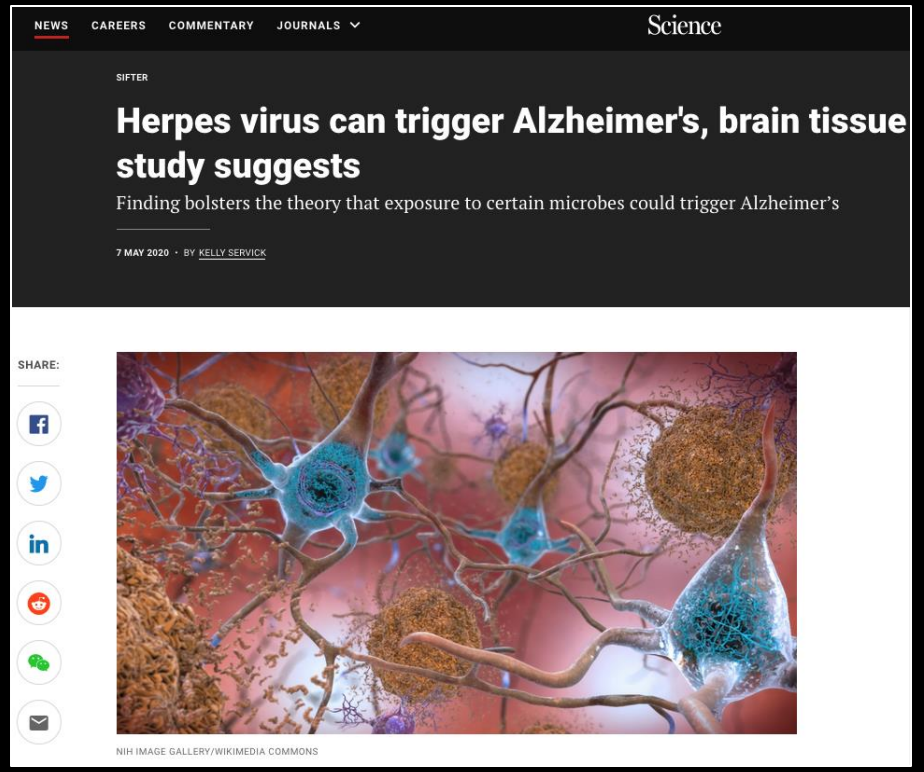
(54) Title: COMPOSITIONS AND METHODS FOR PREVENTING OR AMELIORATING NEONATAL HSV INFECTION

David A. Leib, Margaret Ackerman, Iara Backes, Chaya Patel (Dartmouth) and Tony Moody (Duke).

Patent WO2020077119A1 “Compositions and Methods for Preventing or Ameliorating nHSV Infection” filed 4/16/2020.

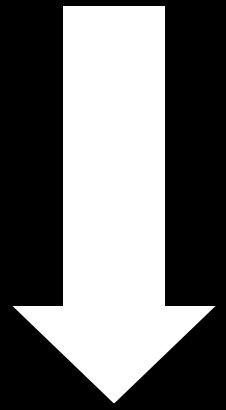
HSV epidemiology and disease

- HSV-1 ~80% seroprevalent in older adults, HSV-2 ~25%
- Cold sores, genital sores, blindness, encephalitis
- Especially devastating in neonates
- Trigger for neurodegeneration?
- Nucleoside analogs cannot treat latency
- No vaccine



HSV Pathogenesis

PRIMARY INFECTION IN MUCOSAE



HOST

IMMUNE CONTROL

VIRUS

IMMUNE MODULATION

LATENCY IN PNS/CNS

HSV Pathogenesis

PRIMARY INFECTION IN MUCOSAE

HOST

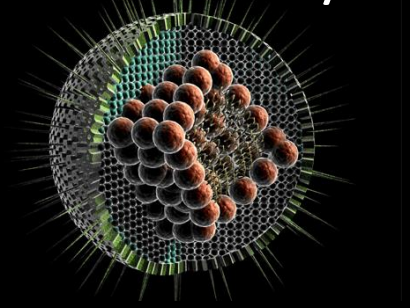
IMMUNE CONTROL

VIRUS

IMMUNE MODULATION

STIMULI

LATENCY IN PNS/CNS



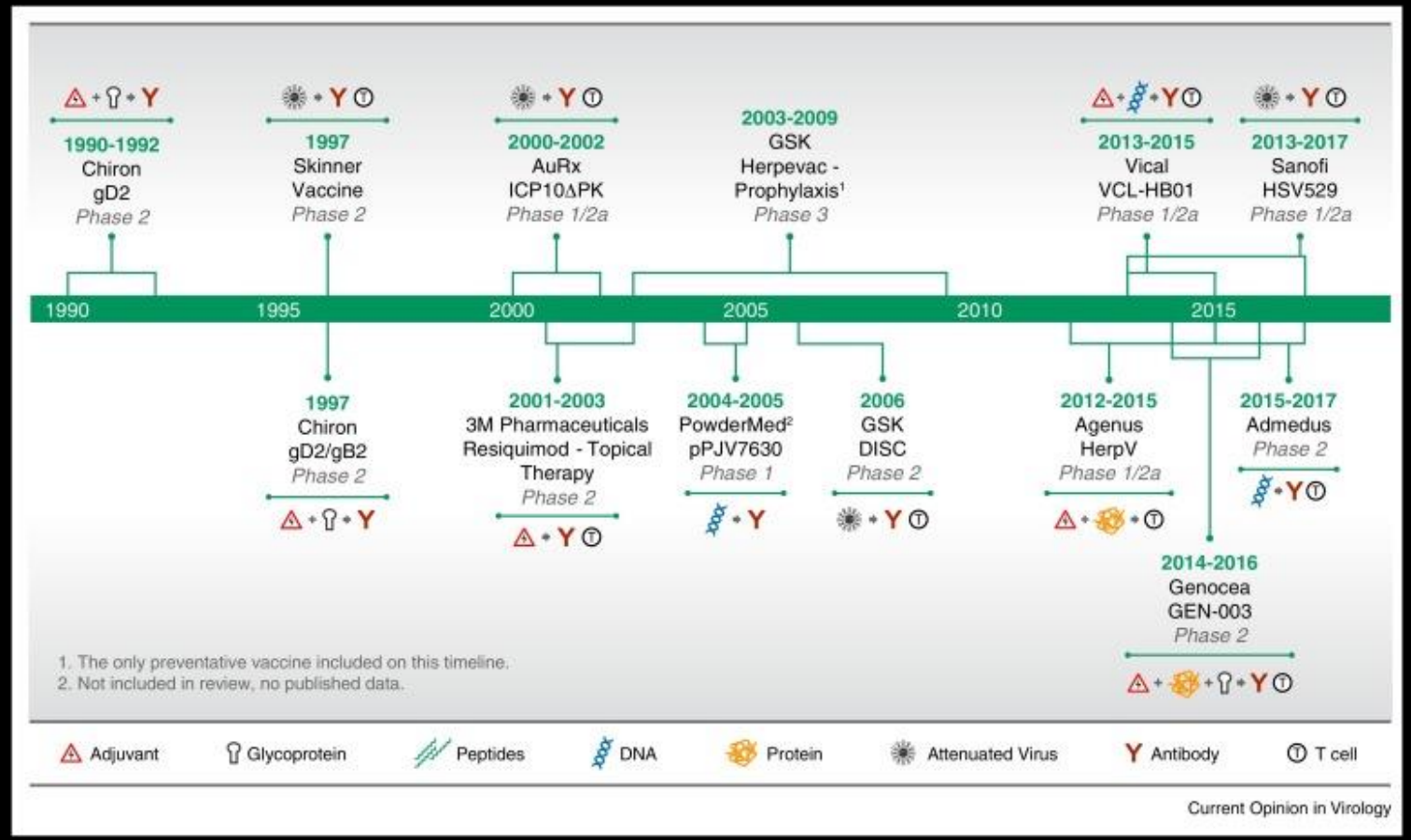
IMMUNE CONTROL

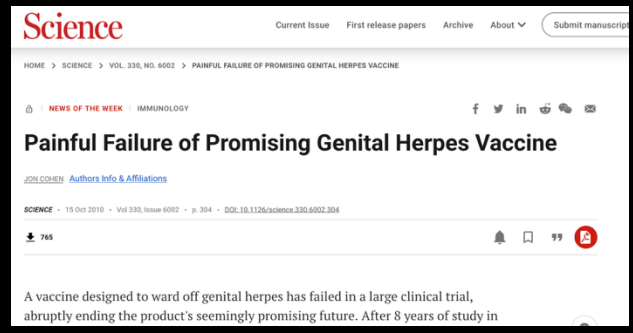
IMMUNE MODULATION

REACTIVATION/SHEDDING

+/- Symptoms

HSV and vaccination





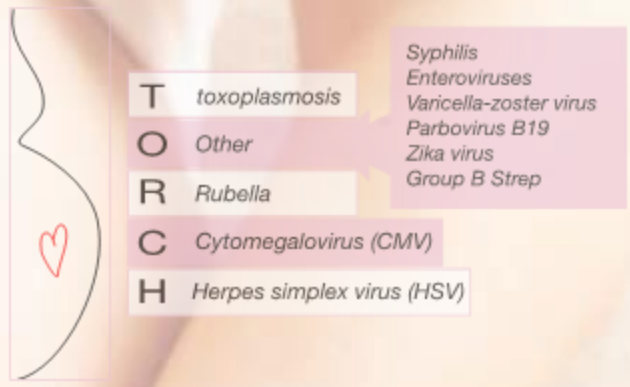
HSV vaccination – difficulties/opportunities

- Lifelong latency with asymptomatic shedding
- Highly prevalent, many vaccinees already seropositive
- Immune determinants incompletely defined
- Powerful immunomodulation by HSV
- Requires lifelong protection at mucosal (genital) surfaces
- nHSV only requires protection in a narrow time frame

Neonatal HSV (nHSV)

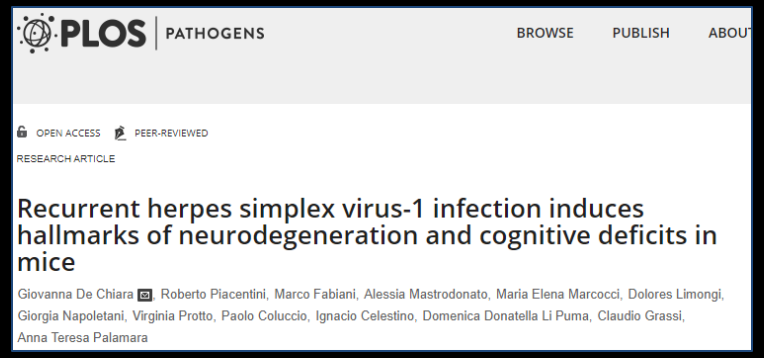
- *"Neonatal herpes is a devastating disease…….the doctor must explain to the mother that there is a high likelihood that her baby might die, or an equally high likelihood of permanent brain damage".*

  David Kimberlin M.D., The Lancet, March 2017.



- nHSV can be local or disseminated, ~1:3,000 live births.
- Disseminated disease can occur with or without skin lesions.
- Early symptoms frequently non-specific, similar to bacterial sepsis.
- ACV treatment requires clinical suspicion and often delayed.
- Even with ACV treatment, high risk of neurological sequelae remains.
- 2 monh postpartum is window of susceptibility – protection window for vaccines/therapeutics?

HSV and other brain infections are associated with neurodegeneration


PLOS PATHOGENS

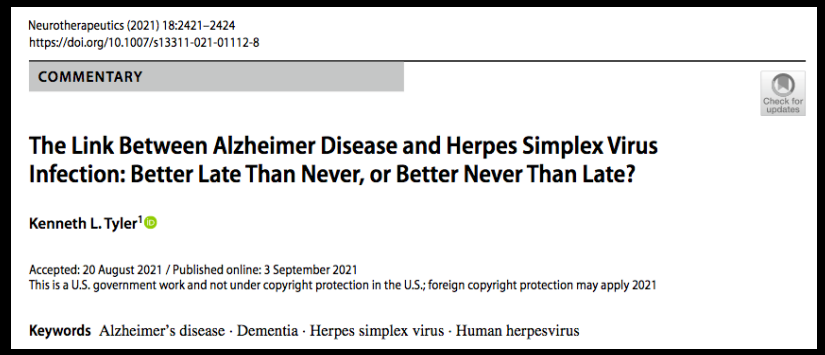
BROWSE PUBLISH ABOU

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice

Giovanna De Chiara, Roberto Piacentini, Marco Fabiani, Alessia Mastrodonato, Maria Elena Marcocci, Dolores Limongi, Giorgia Napoletani, Virginia Protto, Paolo Coluccio, Ignacio Celestino, Domenica Donatella Li Puma, Claudio Grassi, Anna Teresa Palamara


Neurotherapeutics (2021) 18:2421–2424
https://doi.org/10.1007/s13311-021-01112-8

COMMENTARY

The Link Between Alzheimer Disease and Herpes Simplex Virus Infection: Better Late Than Never, or Better Never Than Late?

Kenneth L. Tyler[1]

Accepted: 20 August 2021 / Published online: 3 September 2021
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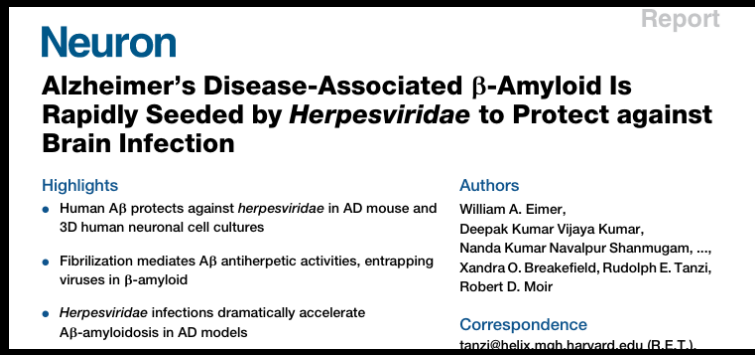
Keywords Alzheimer's disease · Dementia · Herpes simplex virus · Human herpesvirus


Neuron

Report

Alzheimer's Disease-Associated β-Amyloid Is Rapidly Seeded by *Herpesviridae* to Protect against Brain Infection

Highlights

- Human Aβ protects against *herpesviridae* in AD mouse and 3D human neuronal cell cultures
- Fibrilization mediates Aβ antiherpetic activities, entrapping viruses in β-amyloid
- *Herpesviridae* infections dramatically accelerate Aβ-amyloidosis in AD models

Authors

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tanzi@helix.mgh.harvard.edu (R.E.T.)

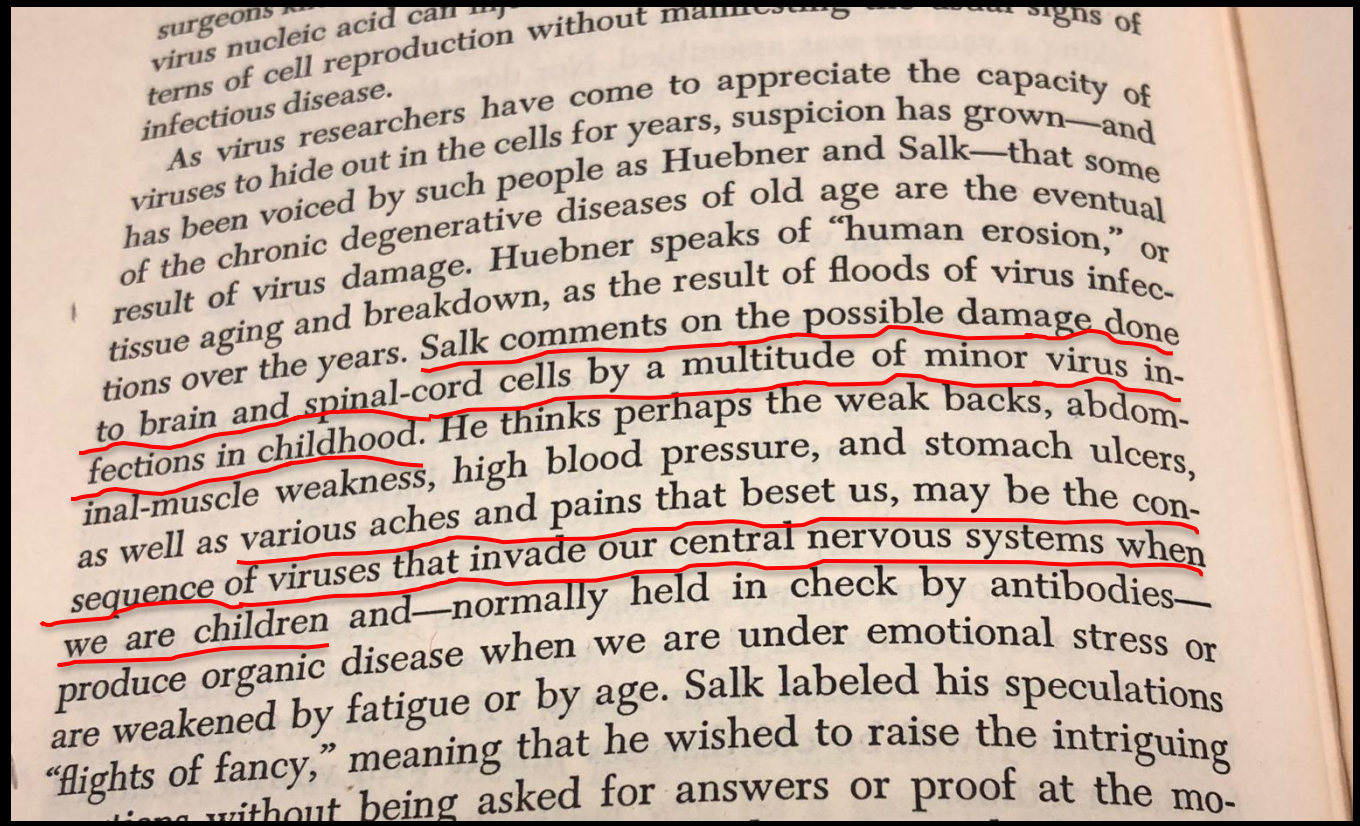
What are the neurological consequences of neonatal HSV?

- The developing neonatal nervous system is exquisitely sensitive to perturbation.
- Asymptomatic nHSV infections could cause significant neurological damage over a lifetime.
- 1:3,000 likely an underestimate given ubiquitous asymptomatic HSV shedding.
- New data suggest neonatal exposure >1%.
- Could clinically inapparent nHSV contribute to cognitive deficits/neurodegeneration?

HSV and other brain infections are associated with neurodegeneration

What are the neurological consequences of neonatal HSV?

- Virus Hunters (Greer Williams, 1959) quotes Jonas Salk:



surgeons
virus nucleic acid can
terns of cell reproduction without manifesting
signs of
infectious disease.

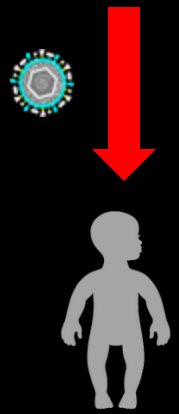
As virus researchers have come to appreciate the capacity of viruses to hide out in the cells for years, suspicion has grown—and has been voiced by such people as Huebner and Salk—that some of the chronic degenerative diseases of old age are the eventual result of virus damage. Huebner speaks of "human erosion," or tissue aging and breakdown, as the result of floods of virus infections over the years. Salk comments on the possible damage done to brain and spinal-cord cells by a multitude of minor virus infections in childhood. He thinks perhaps the weak backs, abdominal-muscle weakness, high blood pressure, and stomach ulcers, as well as various aches and pains that beset us, may be the consequence of viruses that invade our central nervous systems when we are children and—normally held in check by antibodies—produce organic disease when we are under emotional stress or are weakened by fatigue or by age. Salk labeled his speculations "flights of fancy," meaning that he wished to raise the intriguing
without being asked for answers or proof at the mo-

Transmission patterns of neonatal HSV

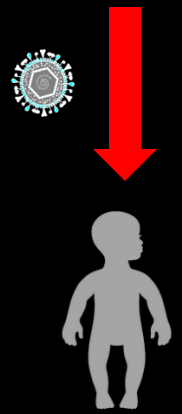
HSV acquisition >28 weeks

Pre-partum reactivation

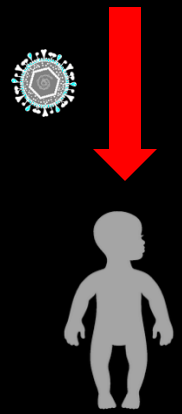
Postnatal transmission



85% of cases



<1% of cases



10-15% of cases

Transmission patterns of neonatal HSV

HSV acquisition >28 weeks

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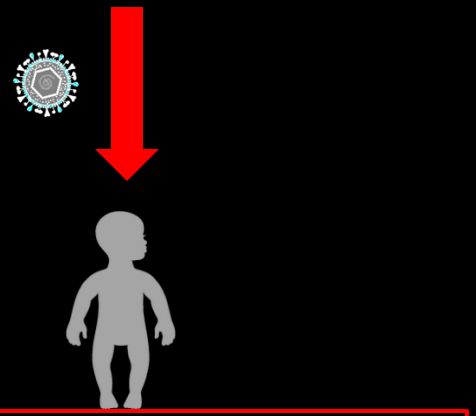


10-15% of cases
Offspring of seronegative moms

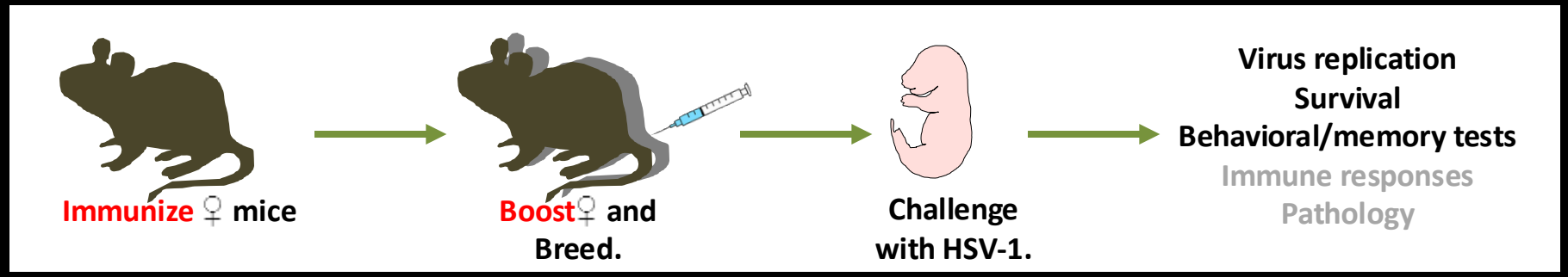
Suggests a role for maternal immunity in protecting neonate.

Maternal immunity: The Concept

- Maternal Ab is key for prevention of several perinatal and congenital infections.
- Maternal IgG crosses the placenta using FcR^{n}.
- Clinical evidence suggests maternal Ab protects newborns from nHSV, yet clinical translation is lacking.
- The short window (~2 months) needed for protection against nHSV provides optimism for intervention.

Can we harness maternal immunity to prevent nHSV?

- Can maternally-derived Ab protect offspring?
  - Vaccine-induced Ab (active)
  - AAV vector-expressed Ab (passive vectored)
- Experimental outline:

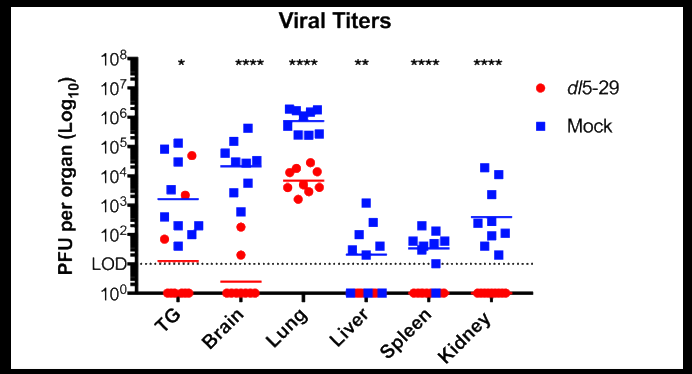


Backes et al: J Exp Med Dec 2022

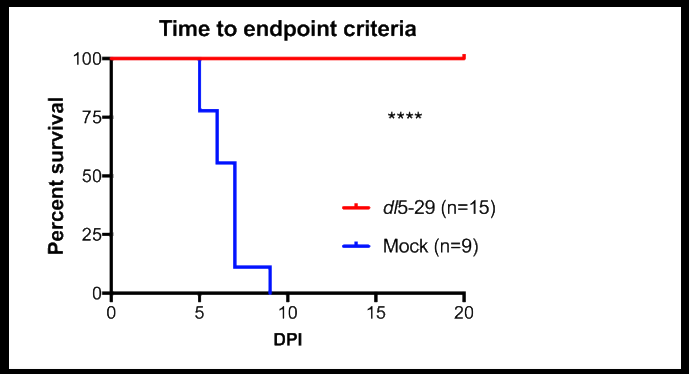
Maternal immunization protects neonatal mice

- Maternal immunization:
  - Live replication-defective (*dl*5-29, Knipe)
  - Subunit vaccine (gC,gD,gE, Friedman)

- Intranasally challenge pups with 1,000pfu wild type HSV:



*dl*5-29



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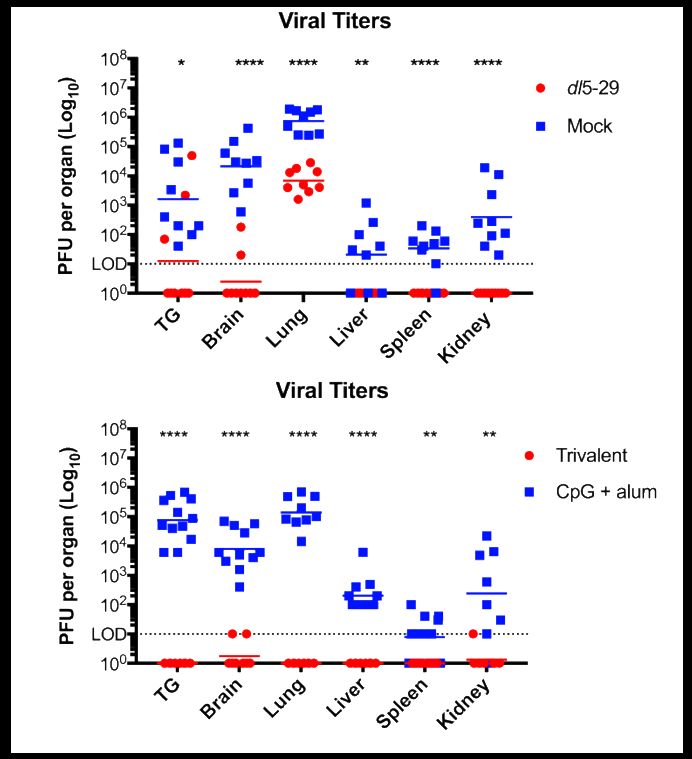
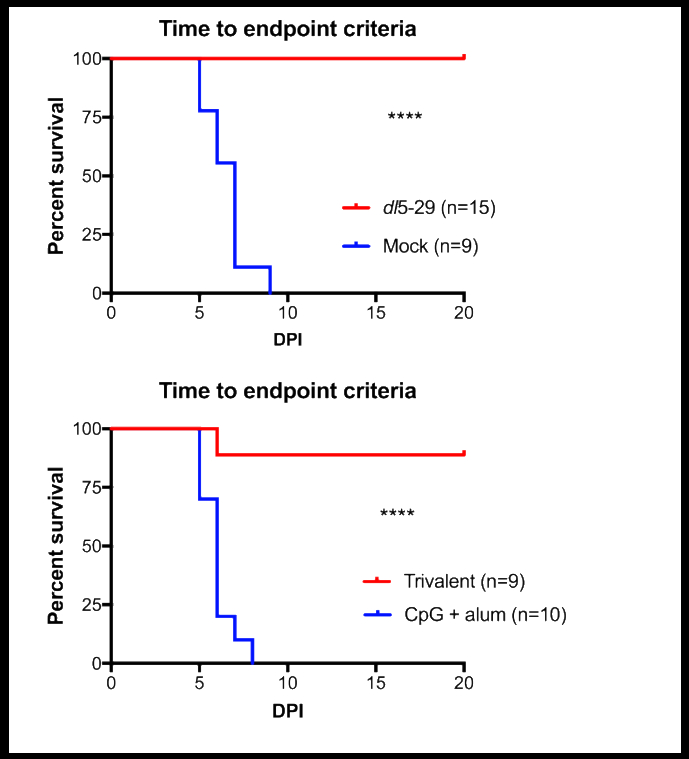


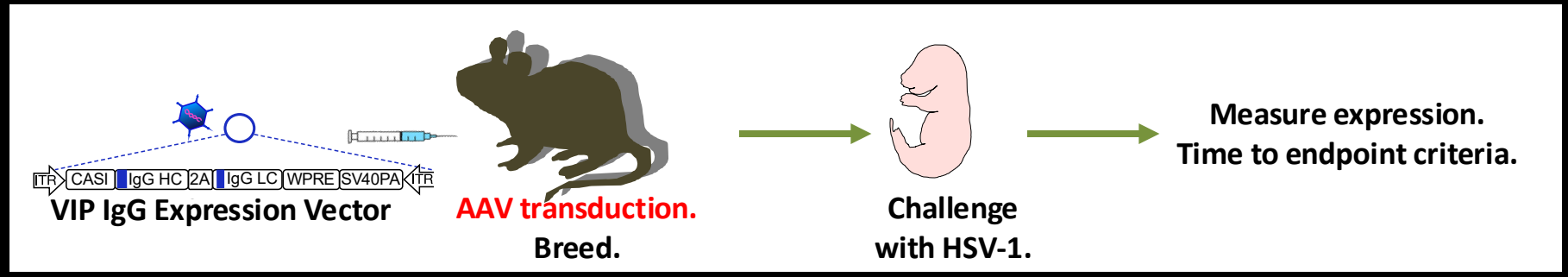
dl5-29

gC/gD/gE

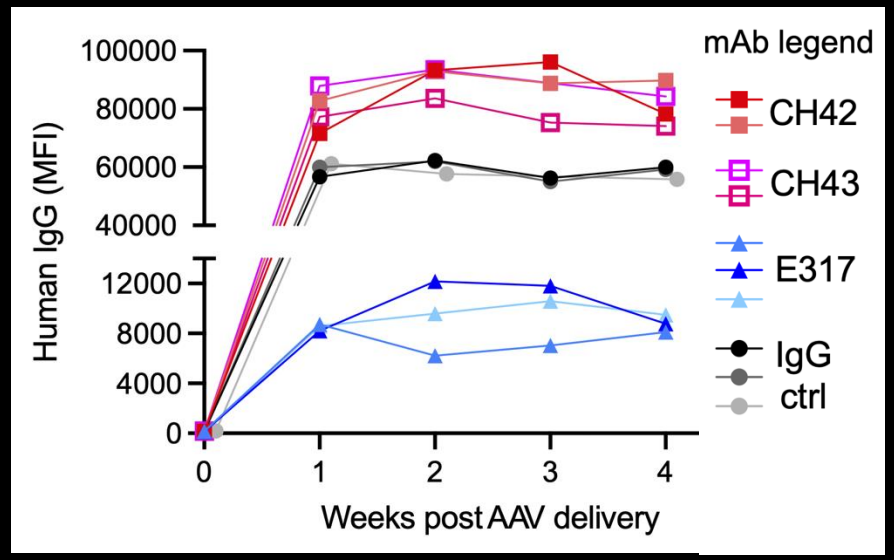


AAV-vectored expression persists and protects

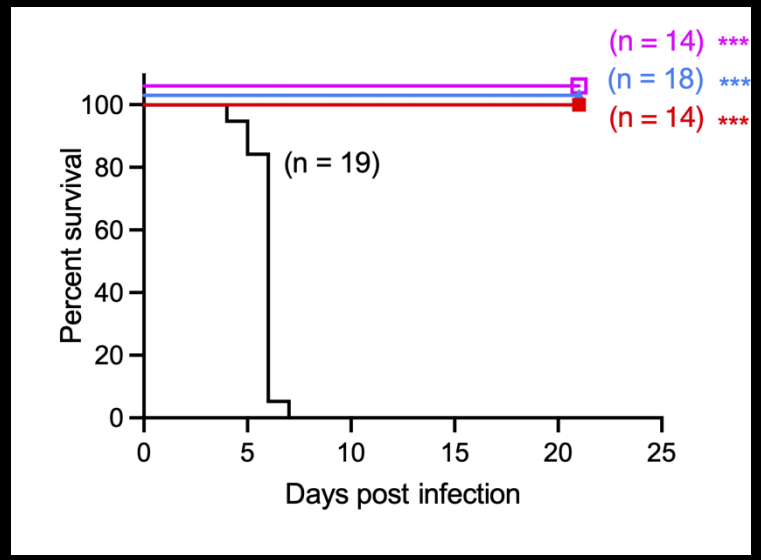
- AAV vectored expression of 3 HSV gD-specific mAbs:



Persistence of IgG expression



Survival following HSV challenge



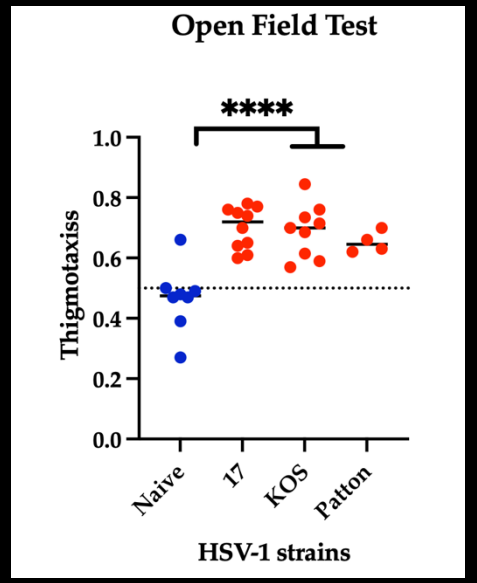
- Vectored Ab expression is defined, persistent, and prevents mortality.

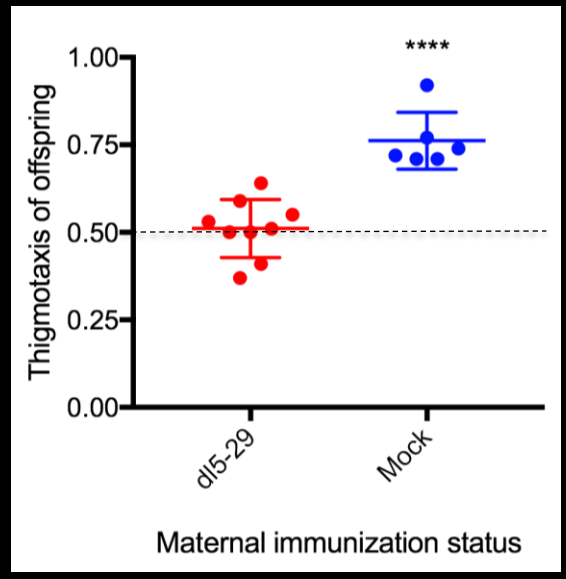
What are the neurological consequences of nHSV?

- "Psychovirology".

- Infection of neonatal mice HSV (Patel et al, Sci Trans Med 2019):

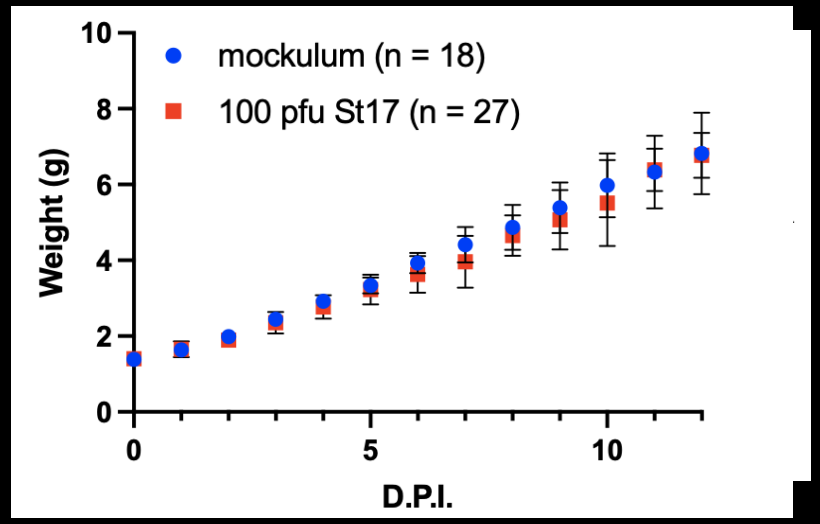
    - Test for anxiety-like behavior (thigmotaxis) in OFT.
    - Elevated anxiety-like behavior following HSV infection.
    - *dl*5-29 maternal immunization prevents elevation of anxiety in pups.



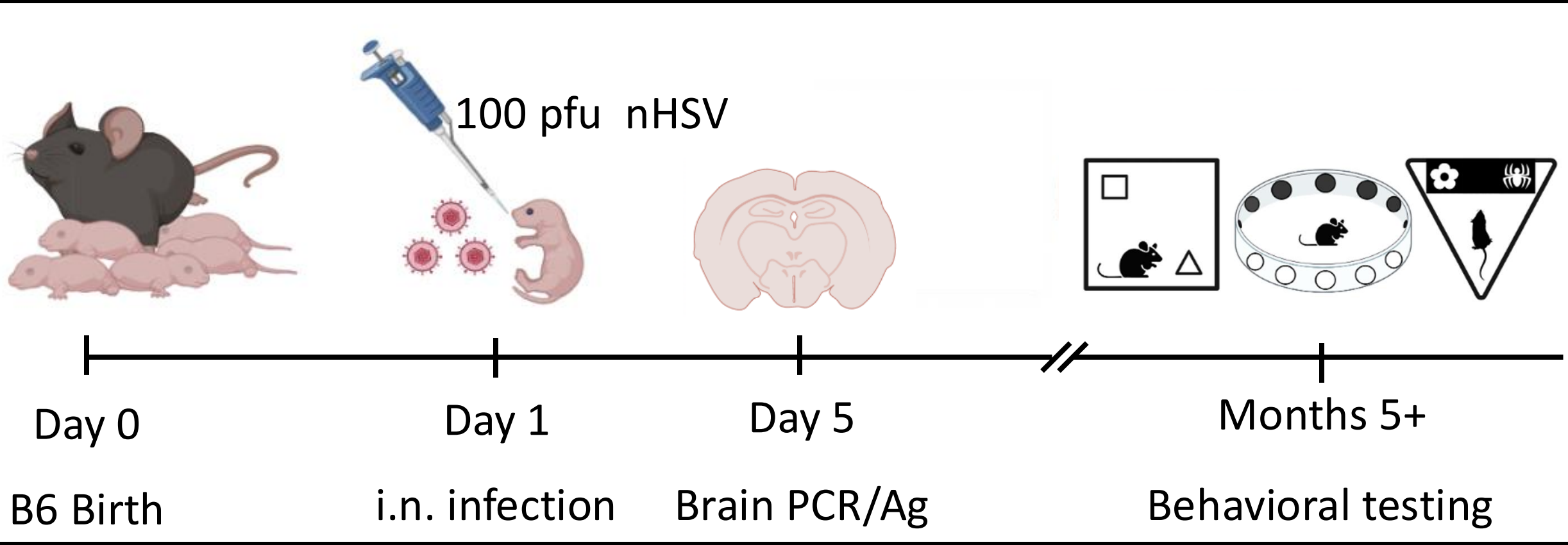


What are the neurological consequences of nHSV?

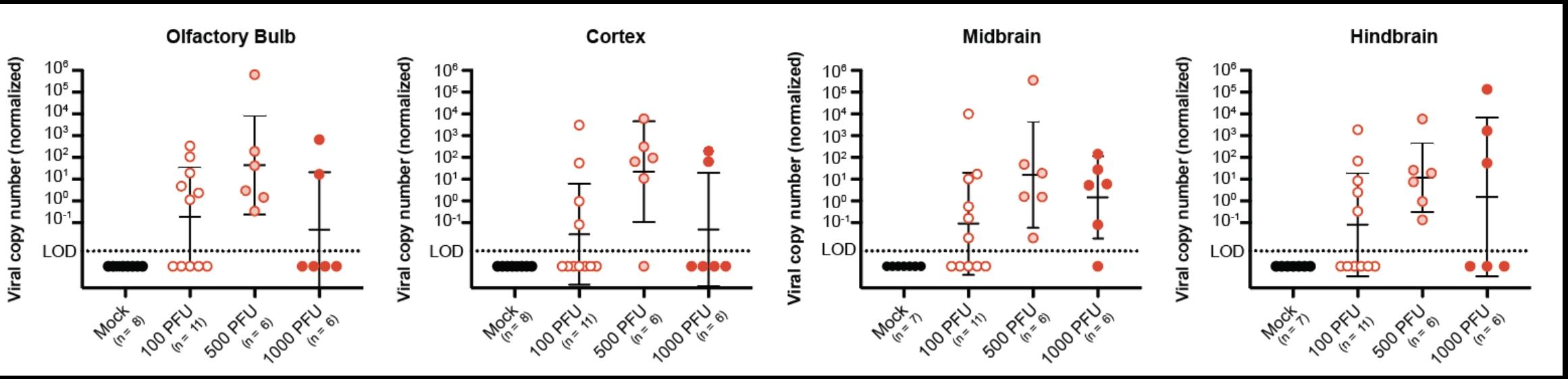
- Survivors of nHSV often show lifelong neurological and cognitive deficits.
- Can we measure other cognitive outcomes in our nHSV model after asymptomatic infection?
- Developed a low-dose nHSV infection model (100pfu i.n.):
  - 90% survival
  - Pups gain weight normally.

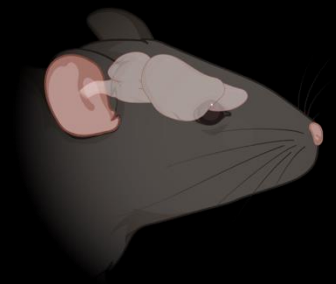


Mouse model of asymptomatic nHSV



Establishing a low-dose nHSV infection model: Virus detection by PCR in CNS.

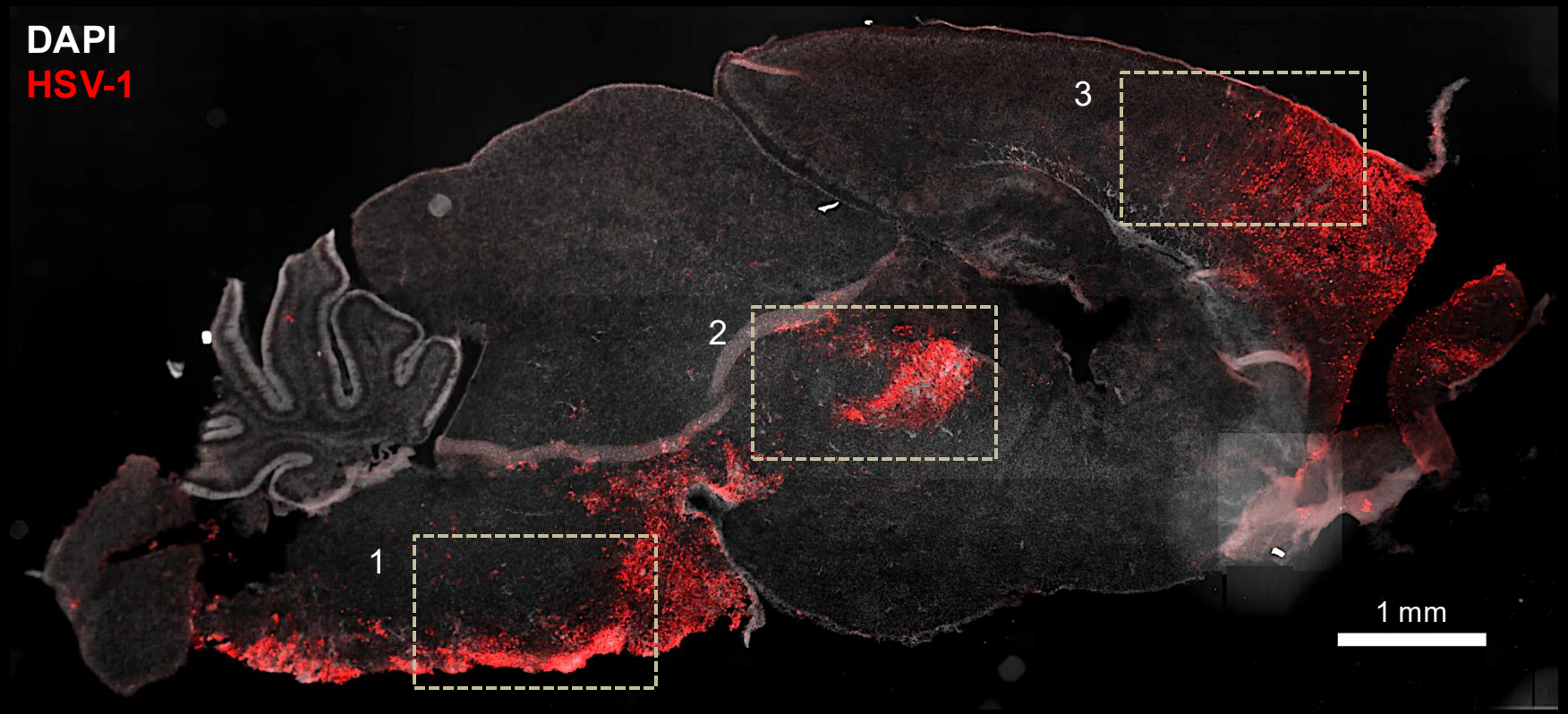


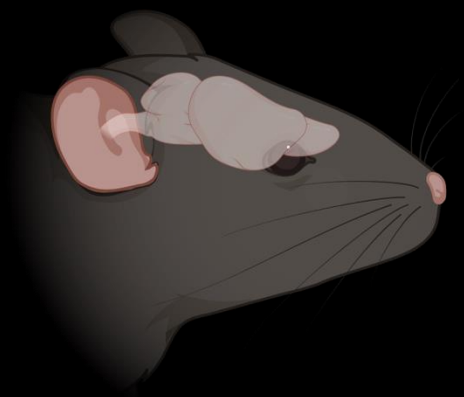


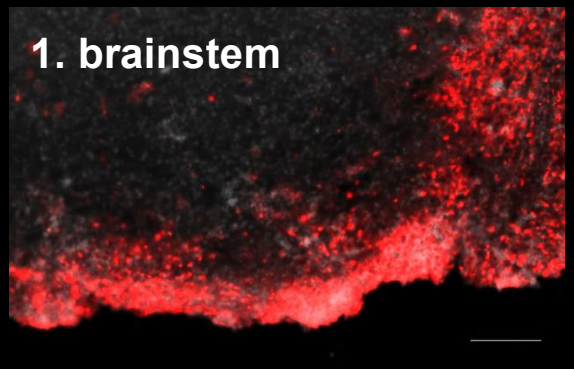
- Viral DNA detectable at 100pfu inoculum by PCR in CNS at 5dpi.

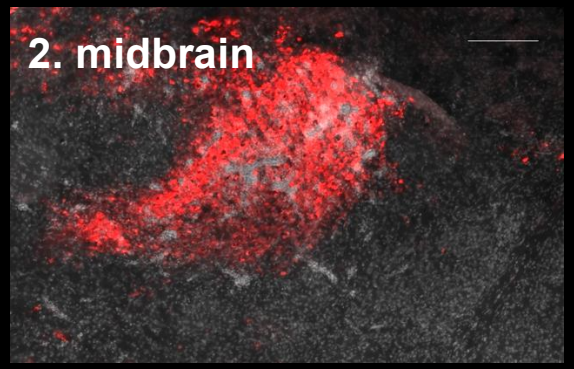
Dutton et al., PLoS Pathogens, 2025

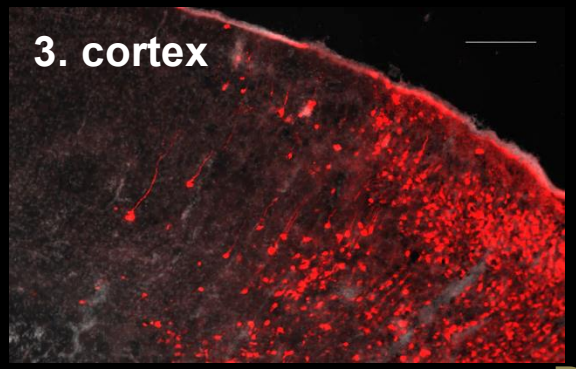
Establishing a low-dose nHSV infection model: 1000pfu i.n. infection





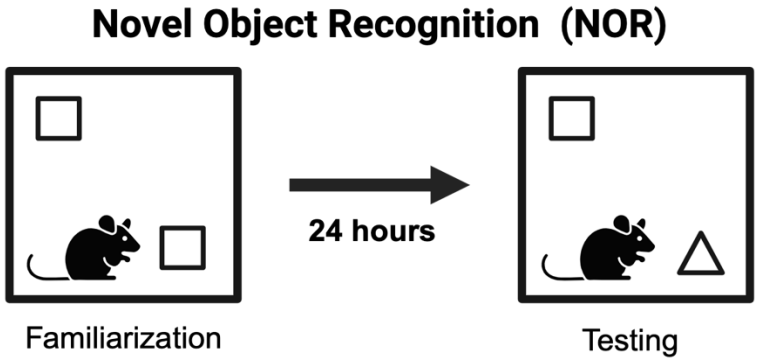






Dutton et al., PLoS Pathogens, 2025

Assessing novel object recognition following nHSV infection

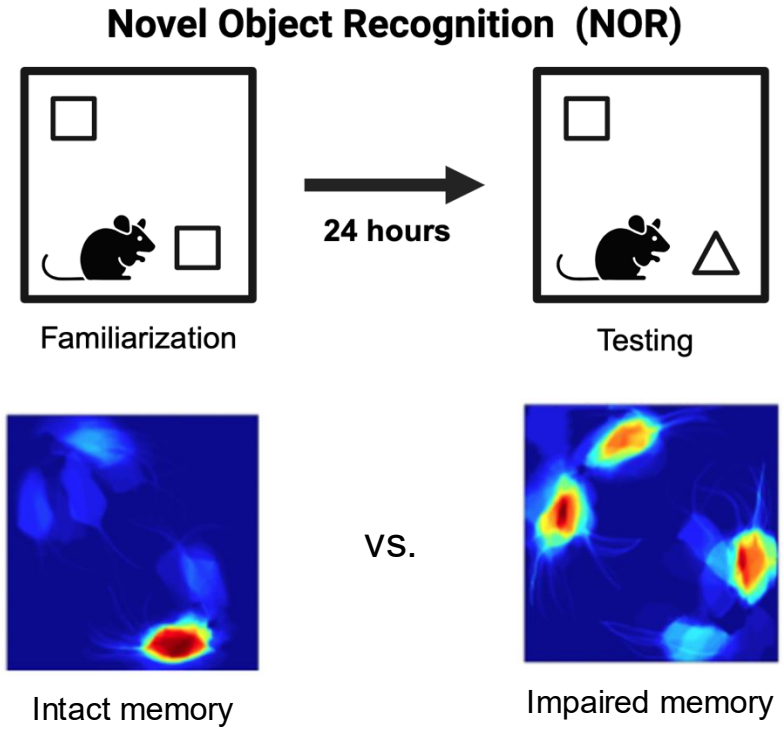


Memory, cognition

5 months post infection

Hippocampus, frontal cortex

Assessing novel object recognition following nHSV infection



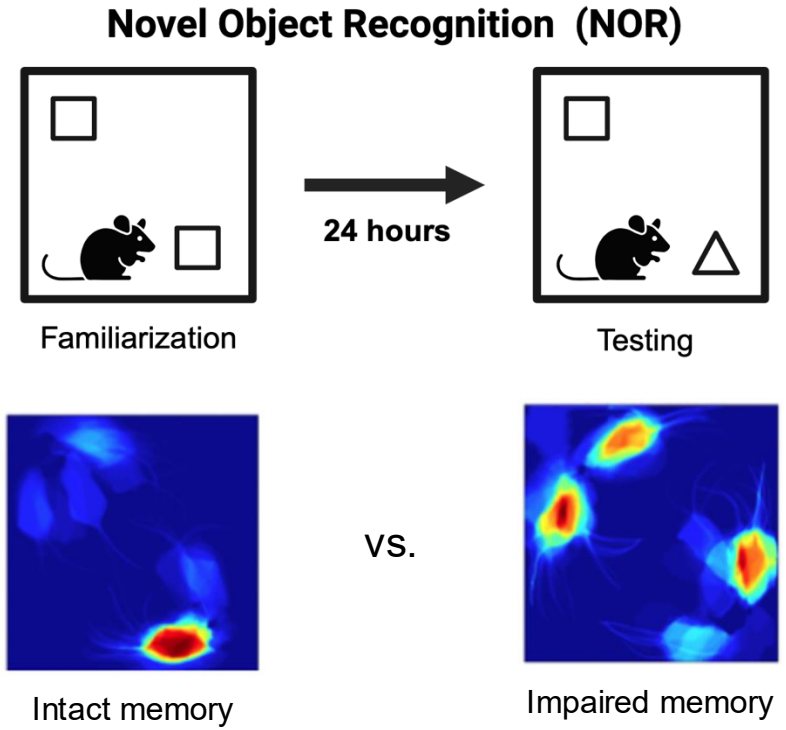
Memory, cognition

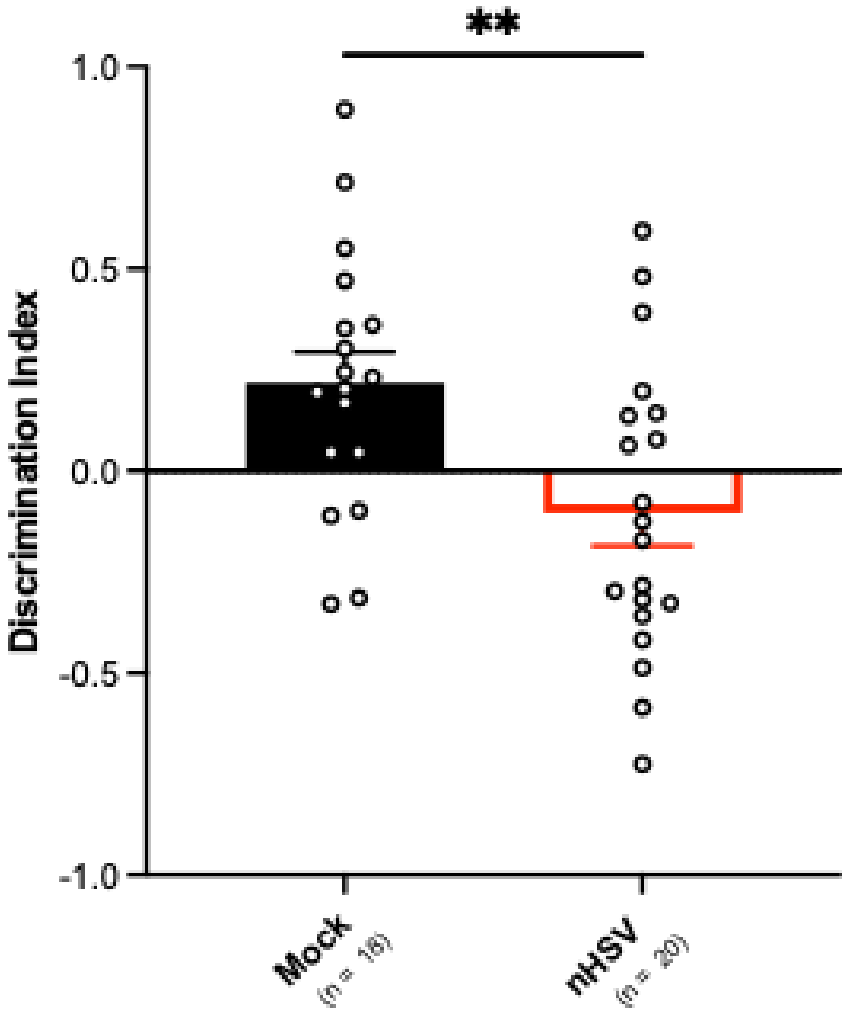
5 months post infection

Hippocampus, frontal cortex

Assessing novel object recognition following nHSV infection

$$Discrimination\ index = \frac{Time_{novel} - Time_{familiar}}{Time_{total}}$$





Memory, cognition

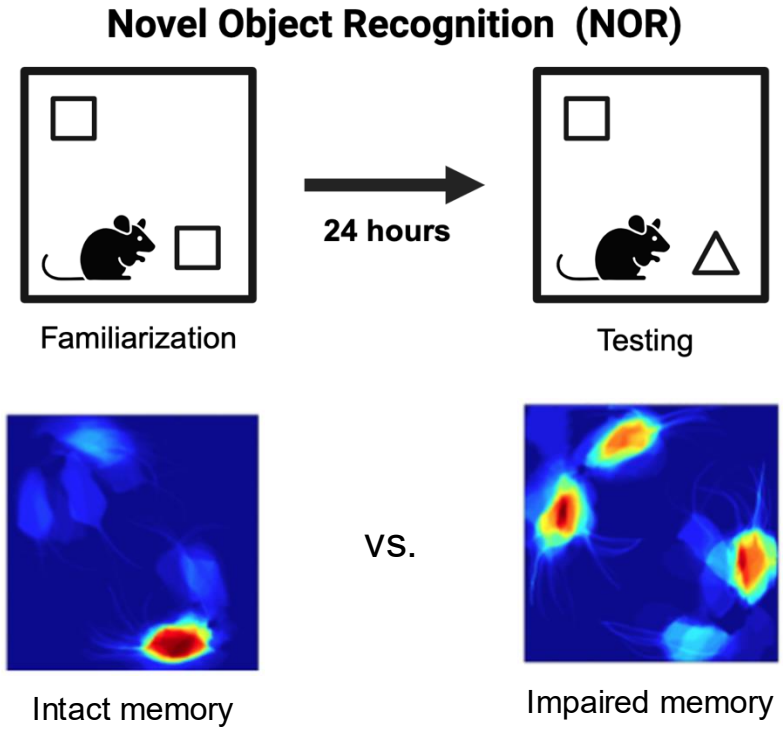
5 months post infection

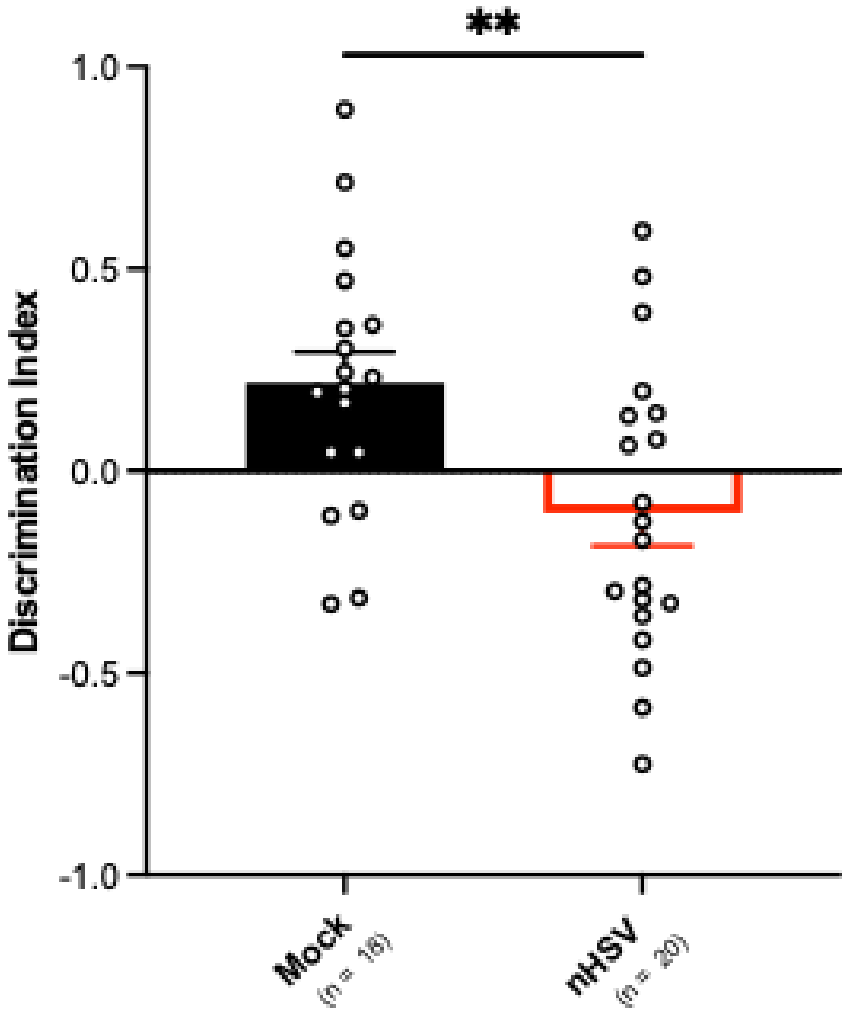
Hippocampus, frontal cortex

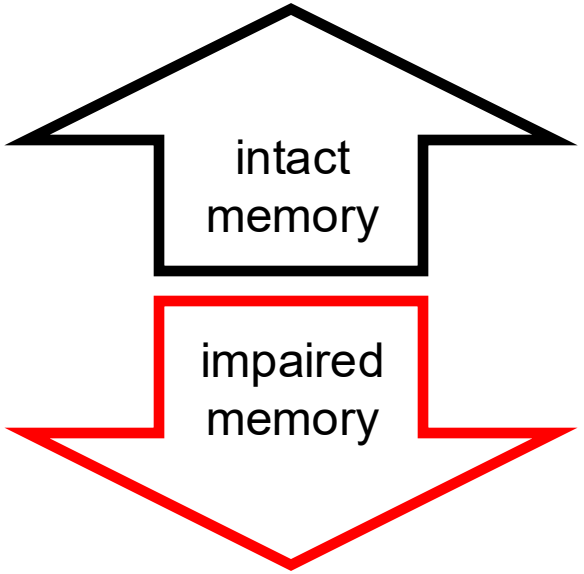
Dutton et al., PLoS Pathogens, 2025

Assessing novel object recognition following nHSV infection

$$Discrimination\ index = \frac{Time_{novel} - Time_{familiar}}{Time_{total}}$$







Memory, cognition

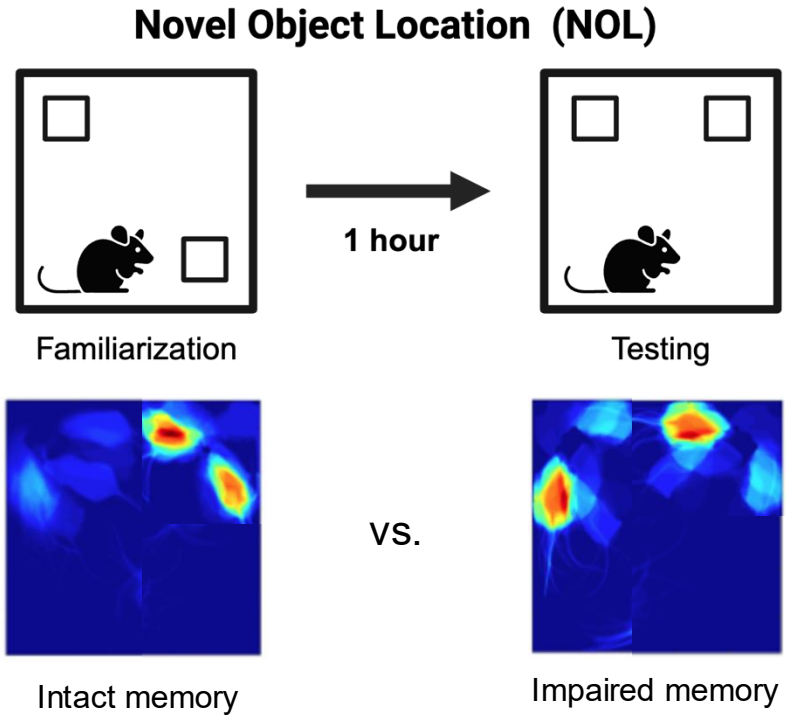
5 months post infection

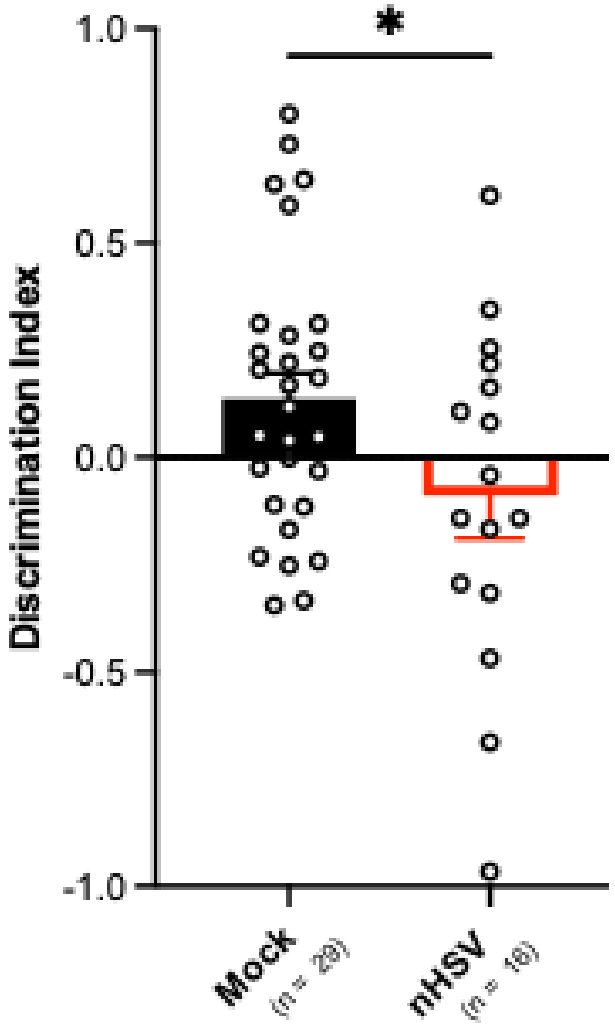
Hippocampus, frontal cortex

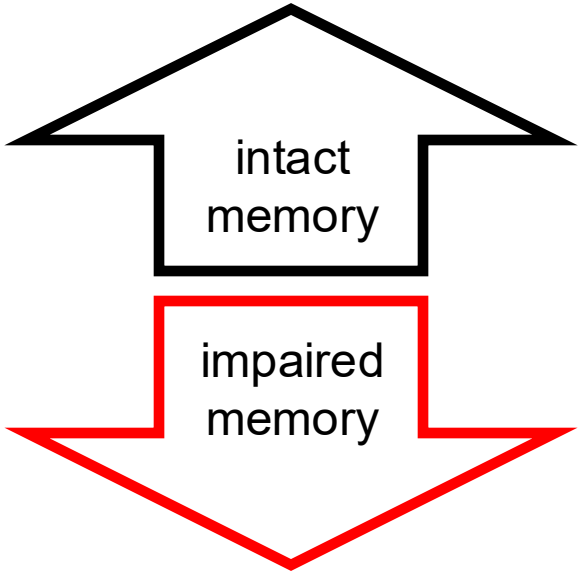
Dutton et al., PLoS Pathogens, 2025

Assessing novel location discrimination following nHSV infection

$$Discrimination\ index = \frac{Time_{novel} - Time_{familiar}}{Time_{total}}$$





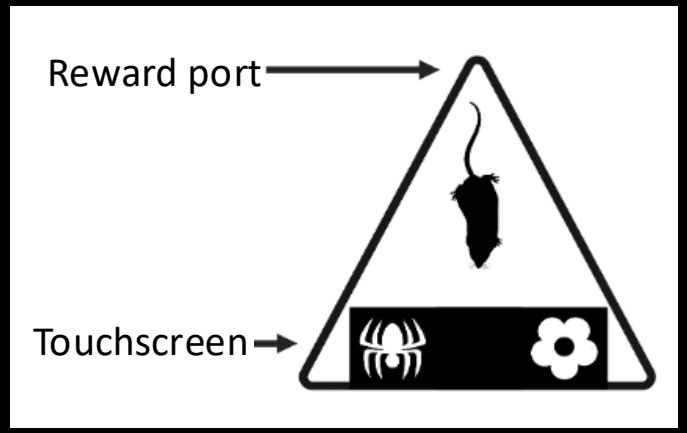


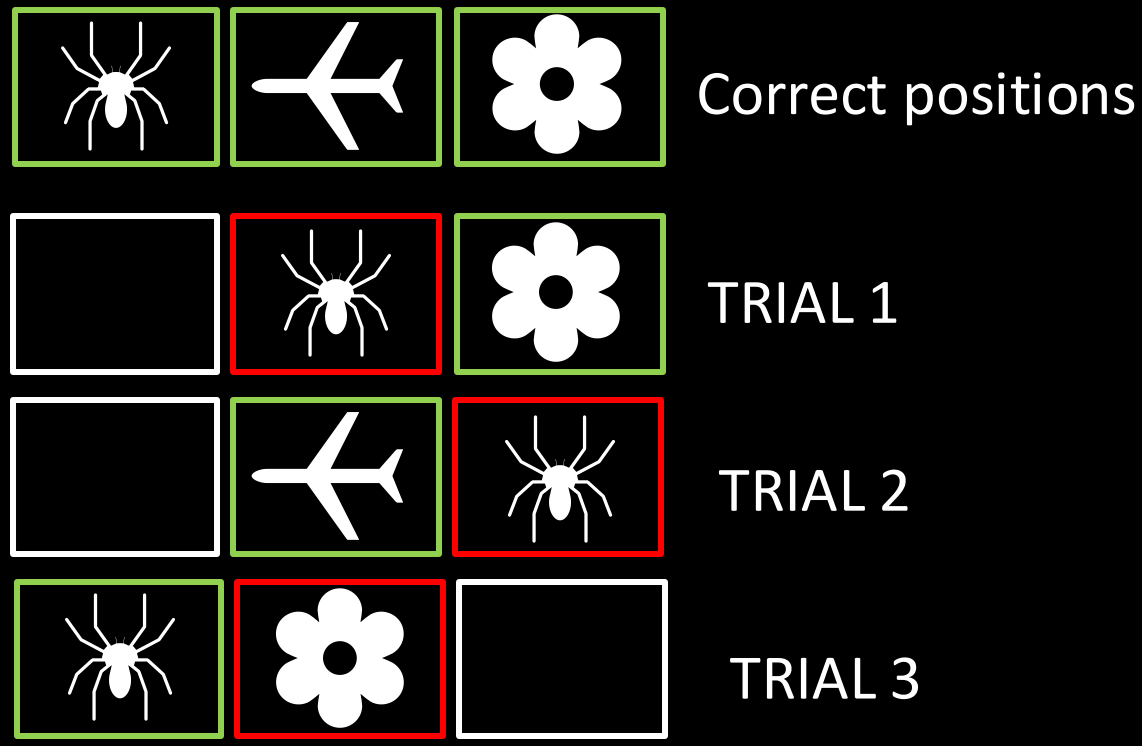
Spatial memory
5 months post infection
Hippocampus

Dutton et al., PLoS Pathogens, 2025

What are the neurological consequences of nHSV?

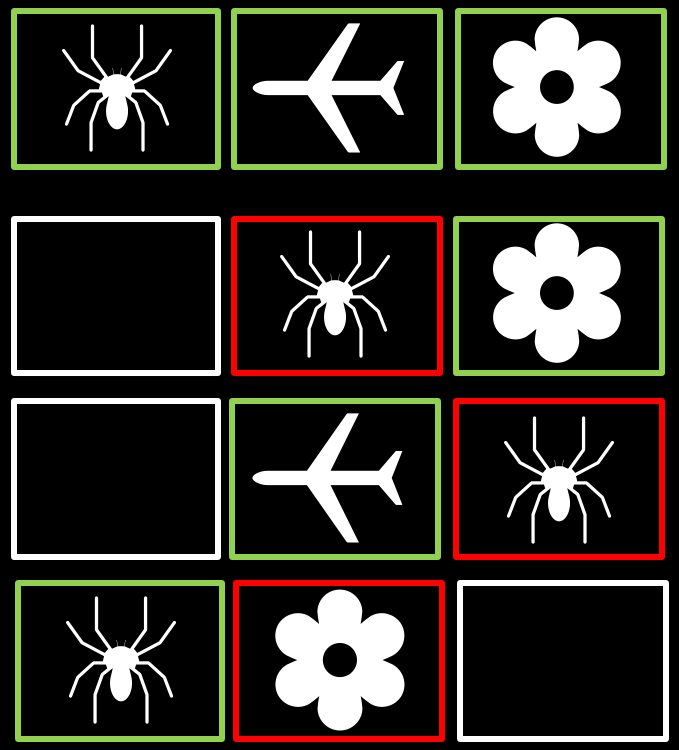
- Different paired associates learning (dPAL).
- Same test given to humans for early AD – hippocampal learning.
- Train mice to use touchscreens to perform memory tests.
- 2nd generation Bussey-Saksida Touch Screen Chambers -- to measure dPAL:

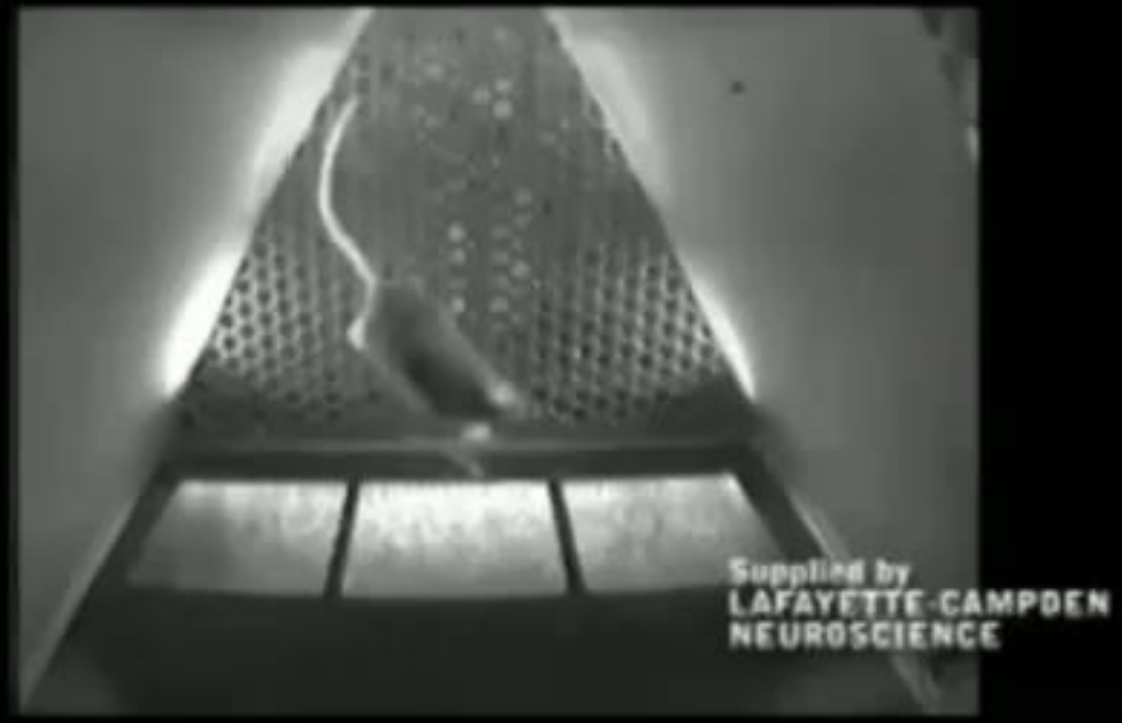




What are the neurological consequences of nHSV?

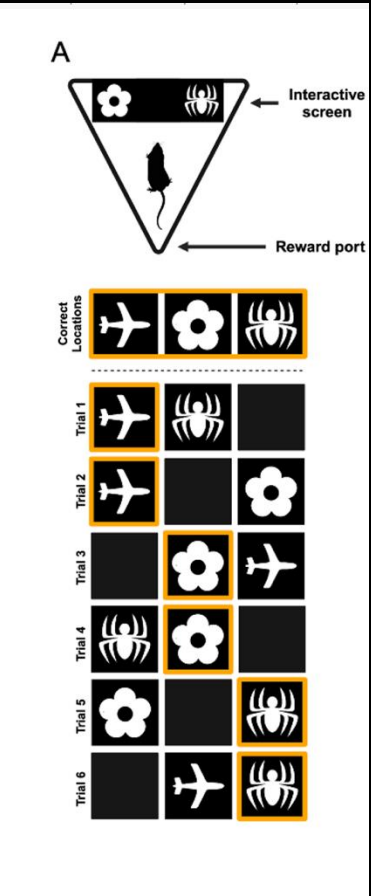
- Different paired associates learning (dPAL).
- Same test given to humans for early AD – hippocampal learning.
- Train mice to use touchscreens to perform memory tests.
- Bussey boxes, a semi automated method to measure dPAL:



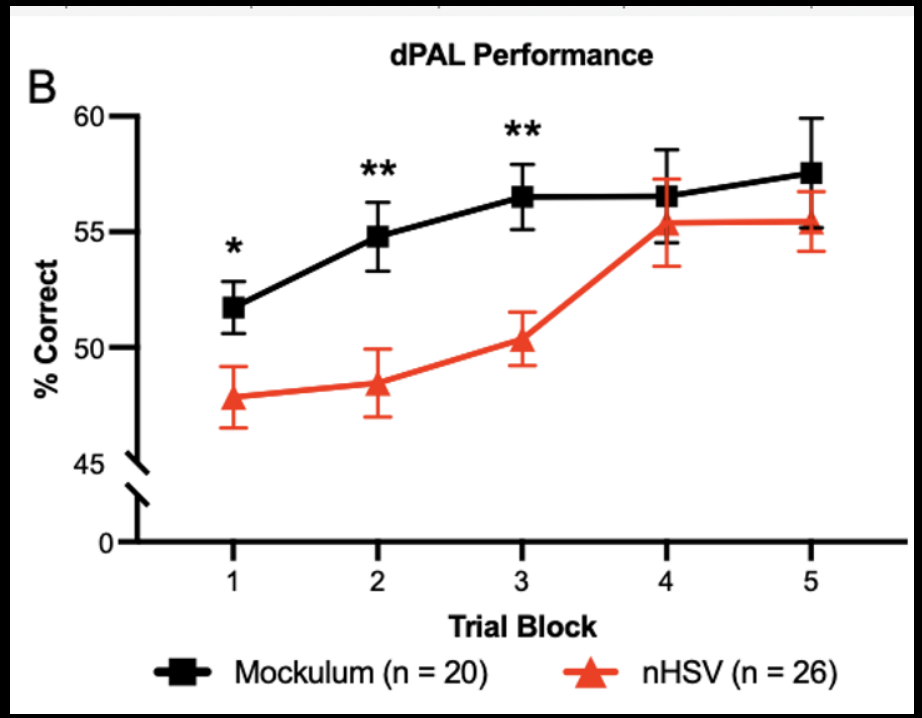


What are the neurological consequences of nHSV?

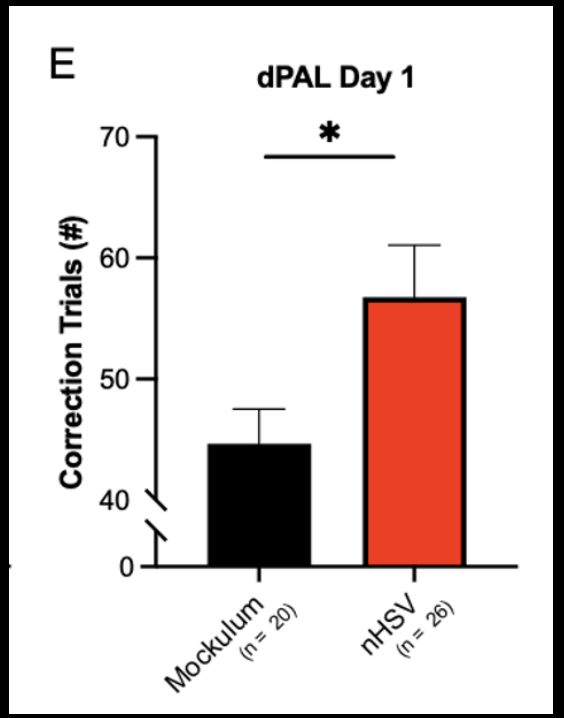
Bussey box dPAL trials



Percentage of trials completed correctly



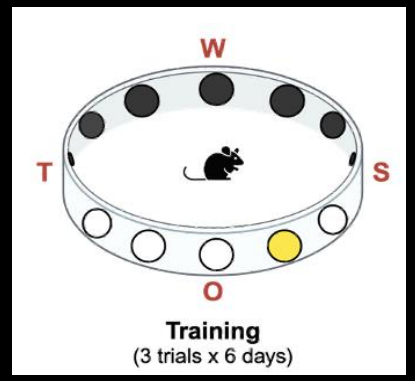
Number of correction trials

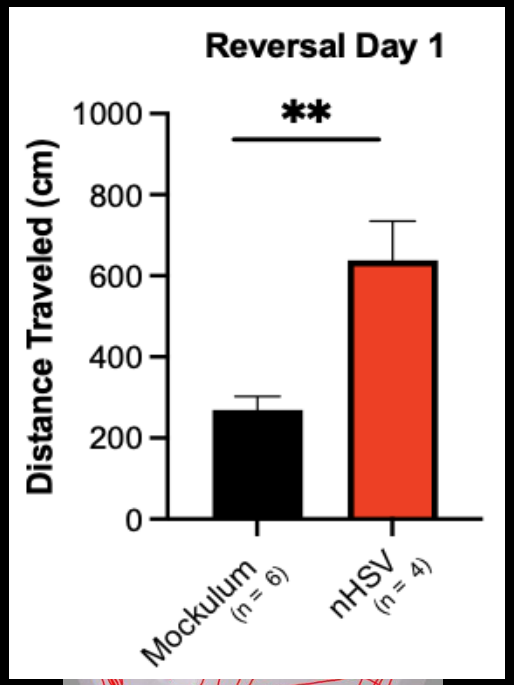


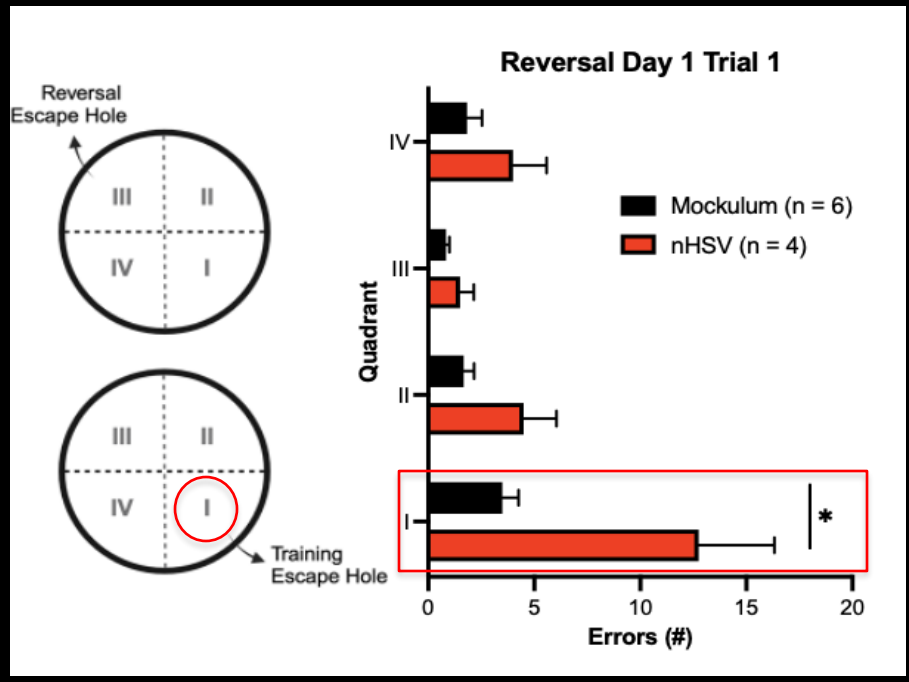
- Low dose nHSV-infected mice made significantly more errors than controls on dPAL.
- Consistent with the idea of loss of hippocampal learning and short-term memory.

What are the neurological consequences of nHSV?

- Modified Barnes Maze, testing for prefrontal cortex attention and task switching.
- Train mice for 4 days to find single exit hole to home cage from a bright arena.
- 2 days off, then 2 days of reinforcement, reverse the exit hole to opposite side of the arena.
- Measure how efficiently mice can find the reversed exit hole, and where errors are made:

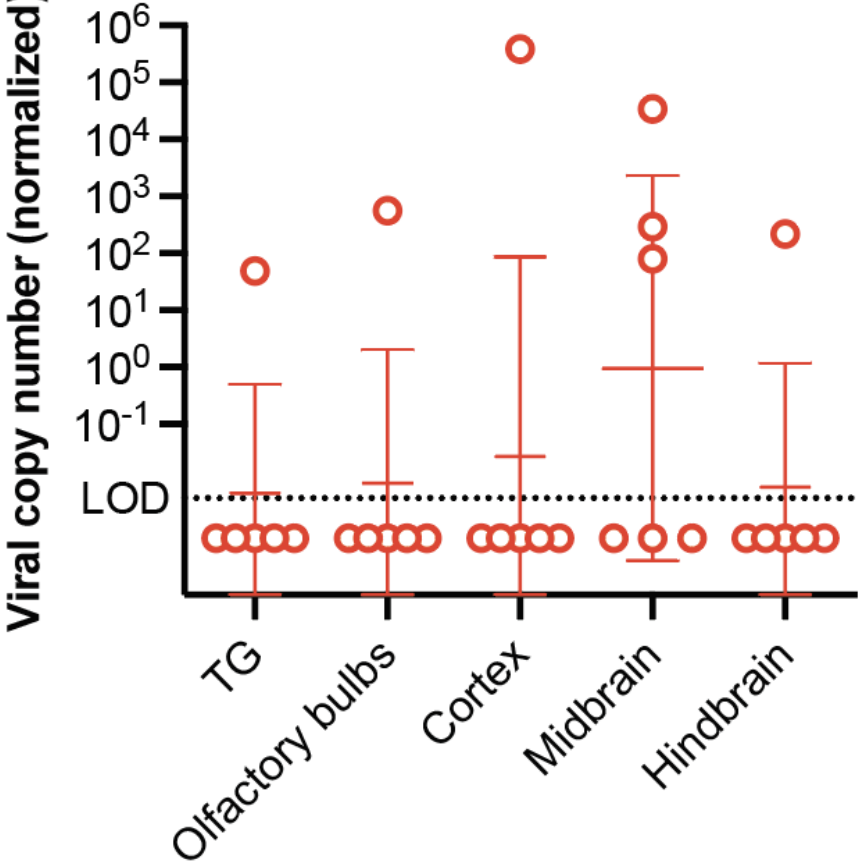


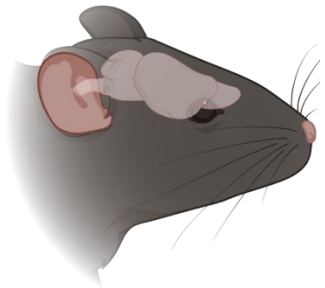




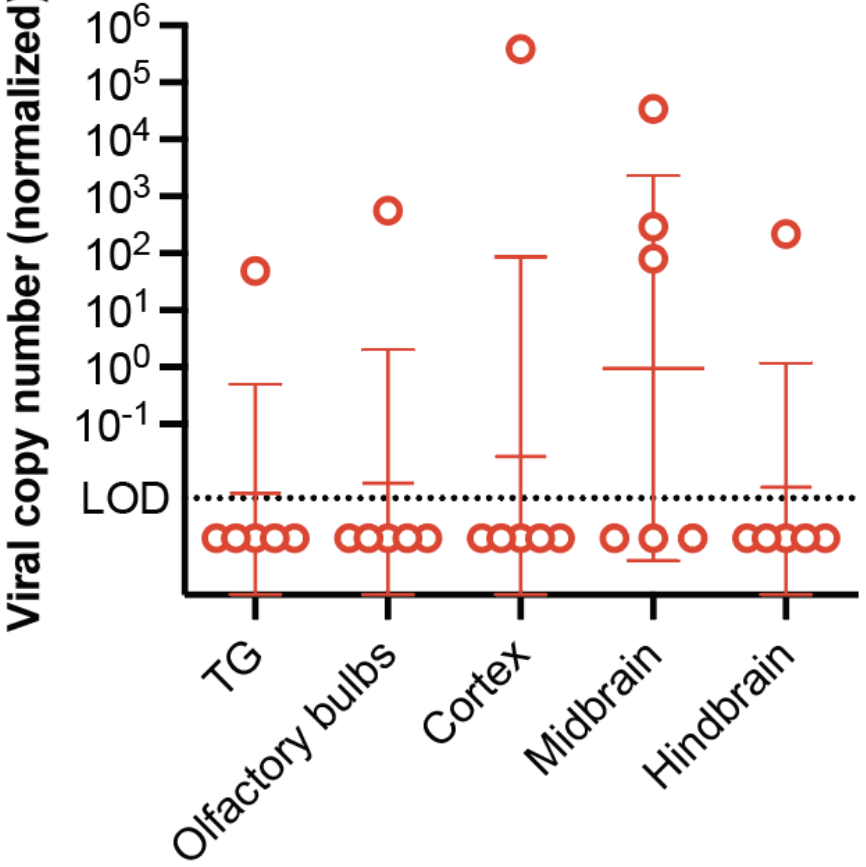
- nHSV causes loss of ability to task switch through prefrontal cortex.
- “Perseveration”.

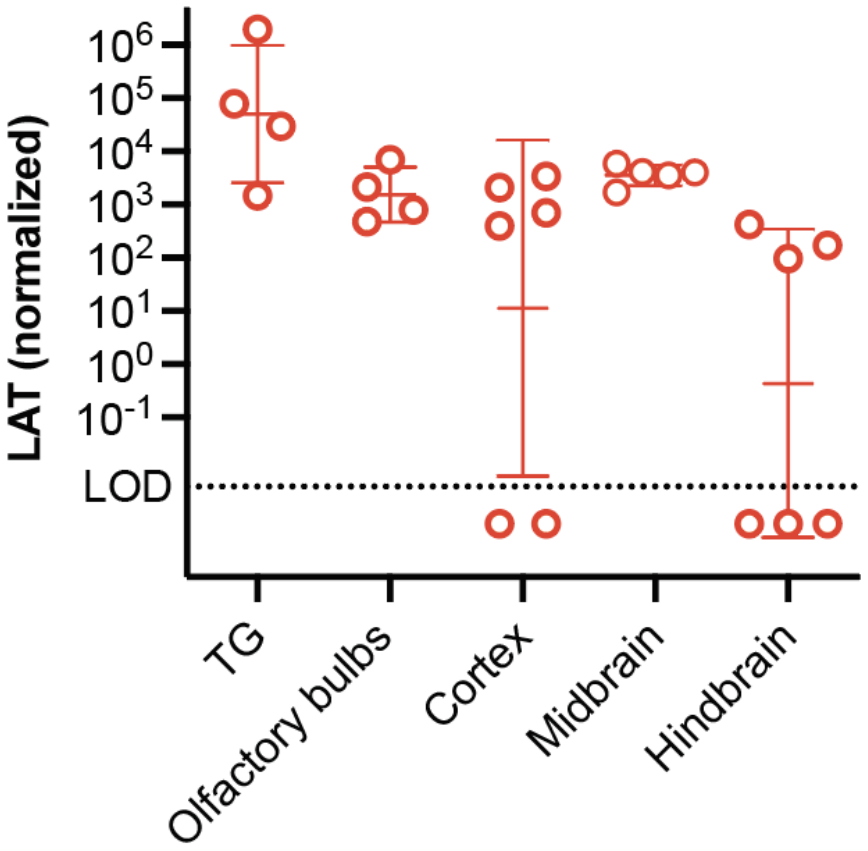
Quantifying HSV persistence in the CNS following low-dose nHSV infection

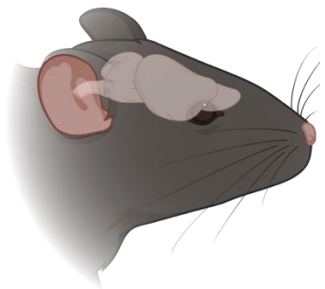




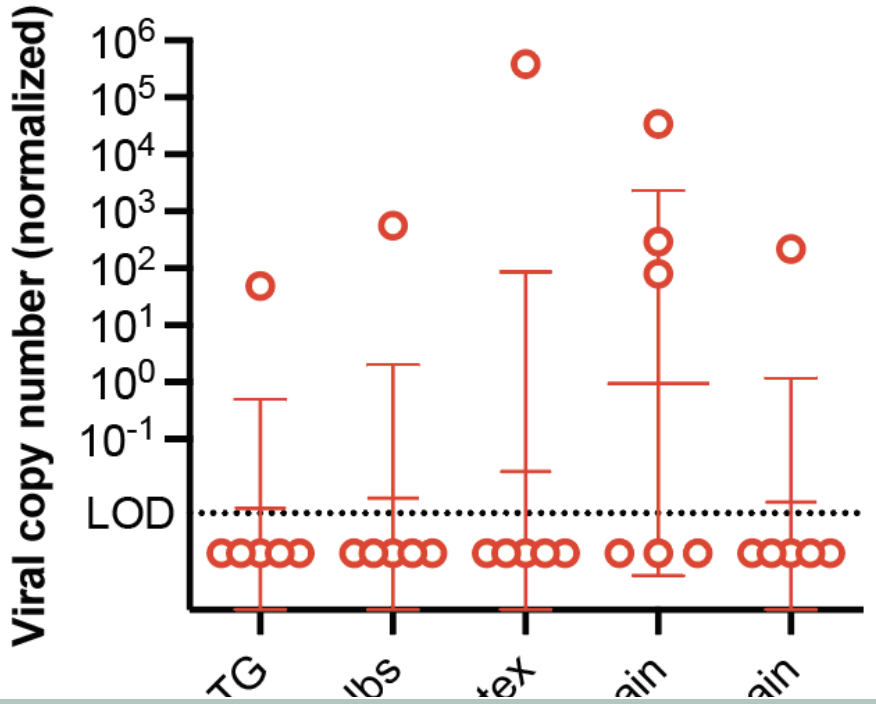
Quantifying HSV persistence in the CNS following low-dose nHSV infection

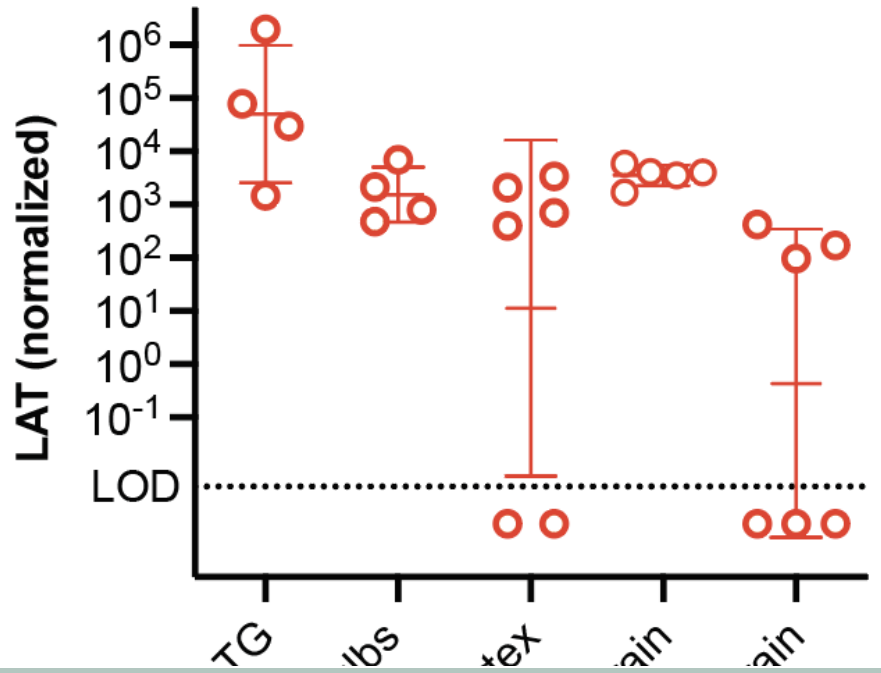






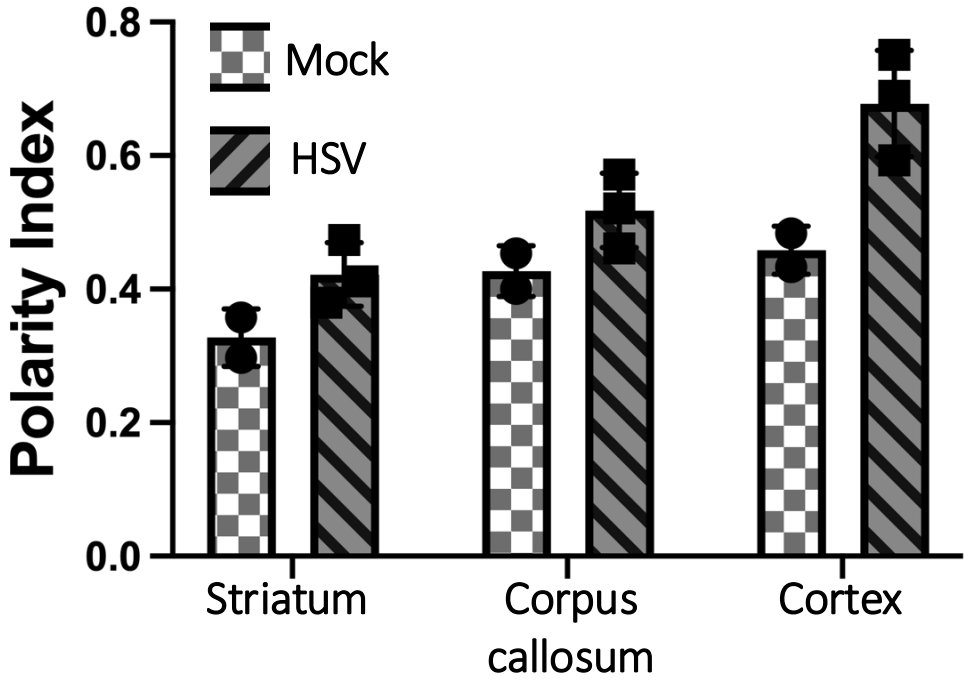
Quantifying HSV persistence in the CNS following low-dose nHSV infection





Low-dose nHSV infection leads to persistent CNS infection with viral genome and latency associated transcript (LAT) present in the trigeminal ganglia and CNS at 6 mpi.

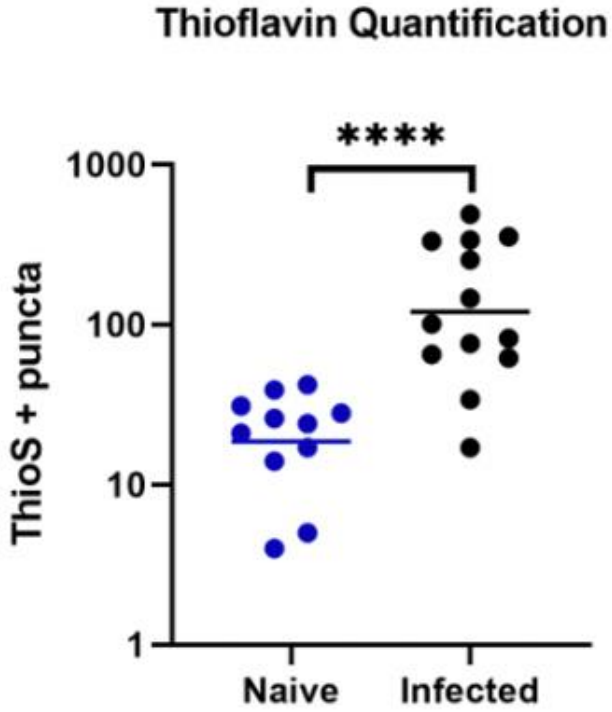
Quantifying HSV-mediated damage in the CNS



- 6 months postinfection, Nile red staining of brains.
- Examines cholesterol content in white matter.
- Increased polarity = decrease in cholesterol = myelin disruption.

- **Evidence of increased myelin disruption in the infected brains, especially in the cortex**

Quantifying HSV-mediated damage in the CNS



- 6 months postinfection, thioflavin S staining of brains.
- Stains accumulated misfolded proteins.
- Shows increased Thioflavin S staining in hippocampus.

- **Thios S staining may indicate neurodegenerative processes/accumulation of pathogenic proteins.**

Summary and conclusions

- We have developed a model of subclinical HSV infection that results in cognitive decline.
- Infection results in cognitive deficits that are associated with disparate parts of the brain.
- Deficits include:
  - hippocampal associated learning and memory
  - prefrontal cortex attention & perseveration
  - spatial and short-term memory
  - demyelination and misfolded proteins in CNS.

- Maternal immunity/immunization can prevent behavioral sequelae.
- Replication-defective virus (dl5-29) does not induce behavioral sequelae.
- Ongoing work to elucidate presence of misfolded proteins/neurodegenerative markers.
- Examining the roles of acute inflammation and reactivation as sources of CNS damage.
- Intermittent antiviral therapy?

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University of Colorado

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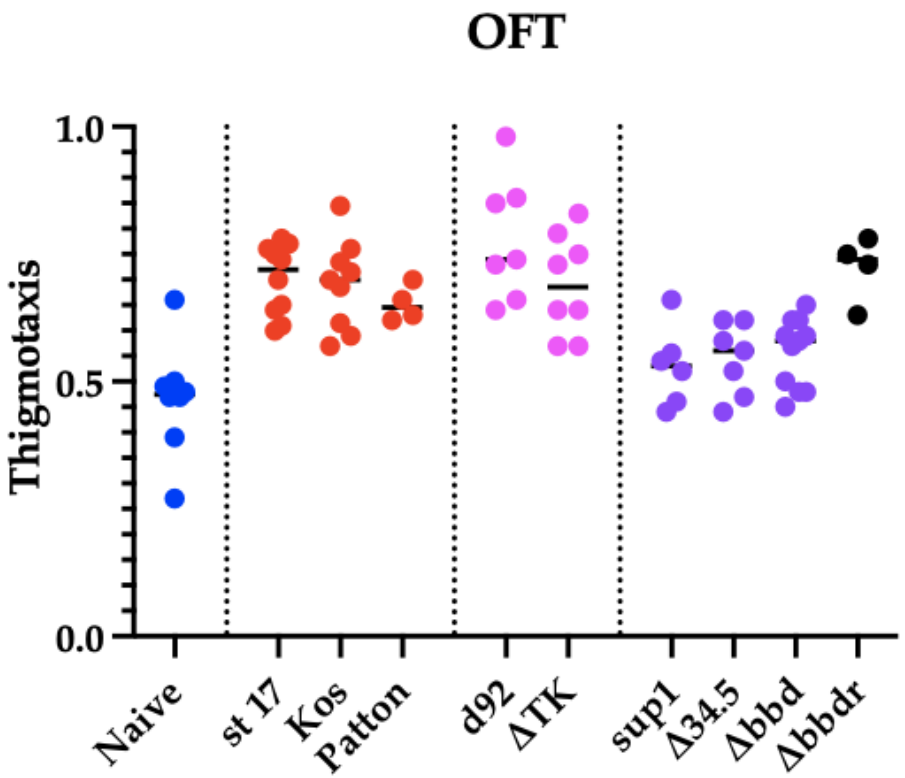
Mechanism: Mutant Virus Screen



with Chaya Patel