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CME Continuing Medical Education

TOPICS IN FETAL MEDICINE

"Look What the Mouse Brought In: Congenital LCM Virus Infection"

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*The speaker has no conflicts of interest to report.

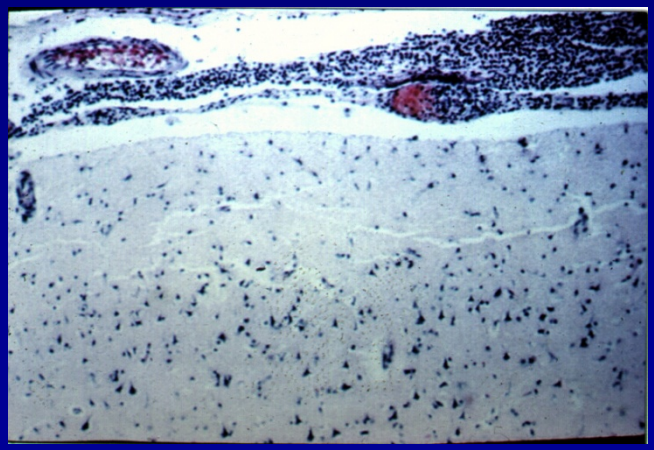
I have nothing to disclose

Discovery of LCMV

1933: A woman in St. Louis became ill with meningo-encephalitis

- fever
- altered mental status
- nuchal rigidity

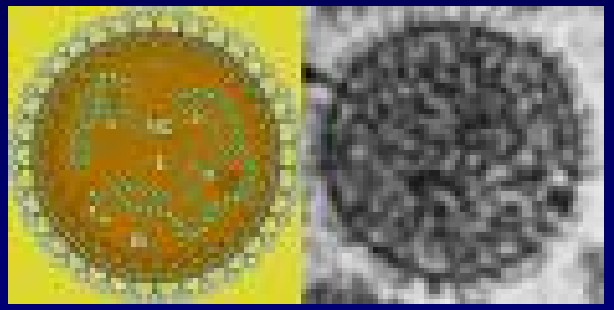
Injection of the virus into experimental animals produced an intense infiltration of lymphocytes into the meninges and choroid plexus.



"Lymphocytic choriomeningitis virus" (LCMV)

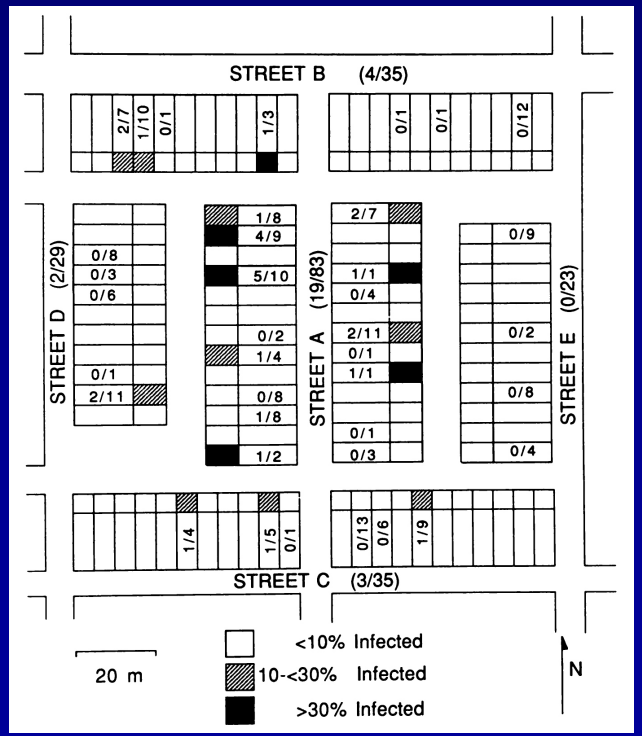
Lymphocytic Choriomeningitis Virus

- An arenavirus
- Enveloped
- RNA-containing
- Indigenous to wild mice (*Mus musculus*)
    - Harbor the virus for life
    - Vertically transmit the virus to succeeding generations

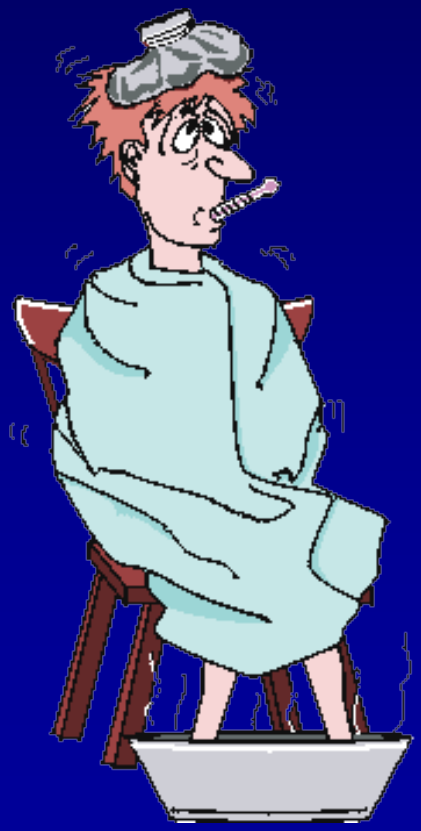


LCMV

- prevalent pathogen
- a common cause of
  aseptic meningitis
- great geographic range
- nine percent of house mice
in urban Baltimore infected
- significant clustering



LCMV is a prominent human pathogen, transmitted by contact with infected fomites.



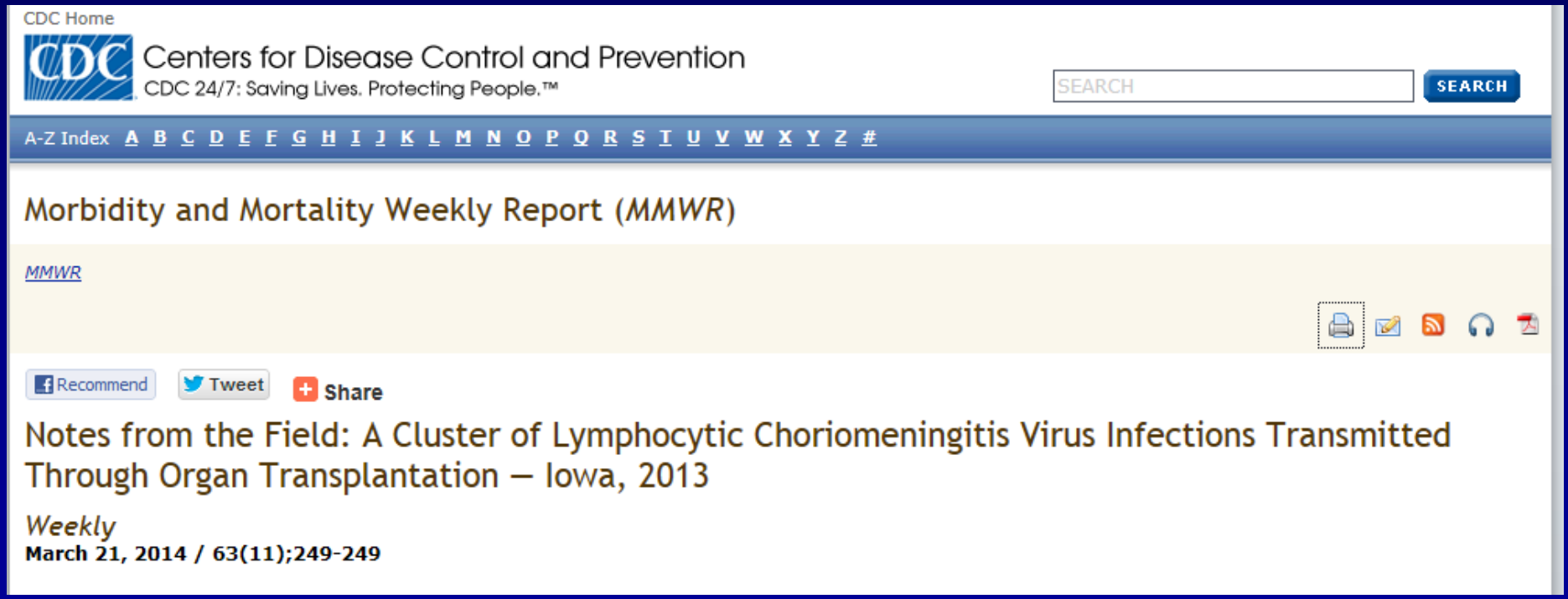
Five percent of humans in America possess antibodies to LCMV.

LCMV typically produces only a mild “flu-like” illness with two phases:

1. Fever and malaise
2. Aseptic meningitis

One-third of adult patients are asymptomatic.

LCMV can be transmitted through organ transplantation



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Morbidity and Mortality Weekly Report (*MMWR*)

MMWR

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Notes from the Field: A Cluster of Lymphocytic Choriomeningitis Virus Infections Transmitted Through Organ Transplantation — Iowa, 2013

Weekly

March 21, 2014 / 63(11);249-249

LCMV can be transmitted vertically

1950s: First reports from Europe of congenital LCMV infection

1993: First reports of congenital LCMV infection in the US

LCMV can cross the placenta:

- targets the fetal brain
- induces substantial neurological damage

My first patient with congenital LCMV

Microcephalic
Blind
Spastic quadriparesis
Mental retardation
Seizures

Prospective study examining the presentation and outcome of congenital LCMV infection.

1. What are the presenting clinical signs?
2. What are the radiographic findings?
3. What are the long-term outcomes?

We identified n=20 newborns with congenital LCMV infection and followed them clinically for 6 months – 11 years.

Bonthius et al., Ann Neurol, 2007

Most mothers of affected babies had a known exposure to mice in their urban apartments or rural out-buildings.

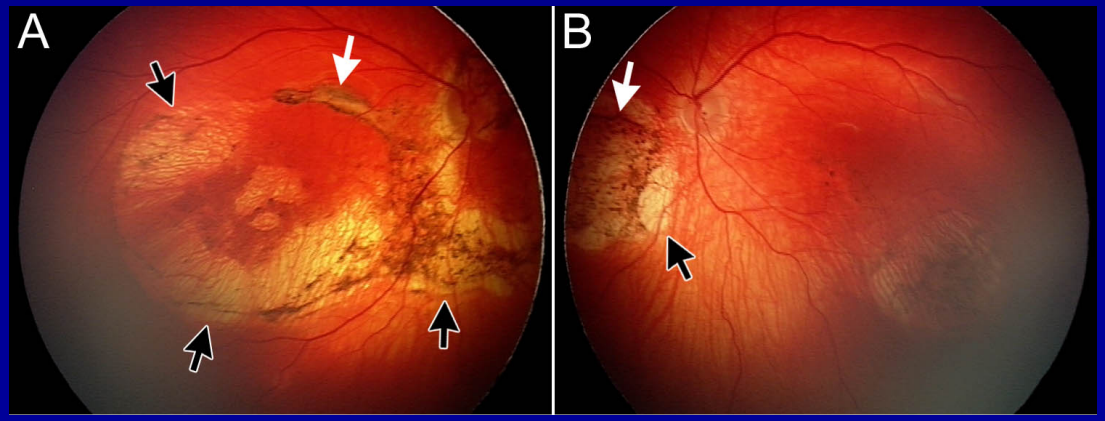
Rural	11/20
Urban	7/20
Suburban	2/20

Known exposure to wild mice	12/20
Known exposure to a sick pet hamster	1/20

Febrile “flu-like” illness during pregnancy	12/20
No illness during pregnancy	8/20

Presenting signs of congenital LCMV infection In the newborn

Chorioretinitis	20/20	100%
Microcephaly	13/20	65%
Hydrocephalus	4/20	20%
Seizures	4/20	20%
Jitteriness	3/20	15%



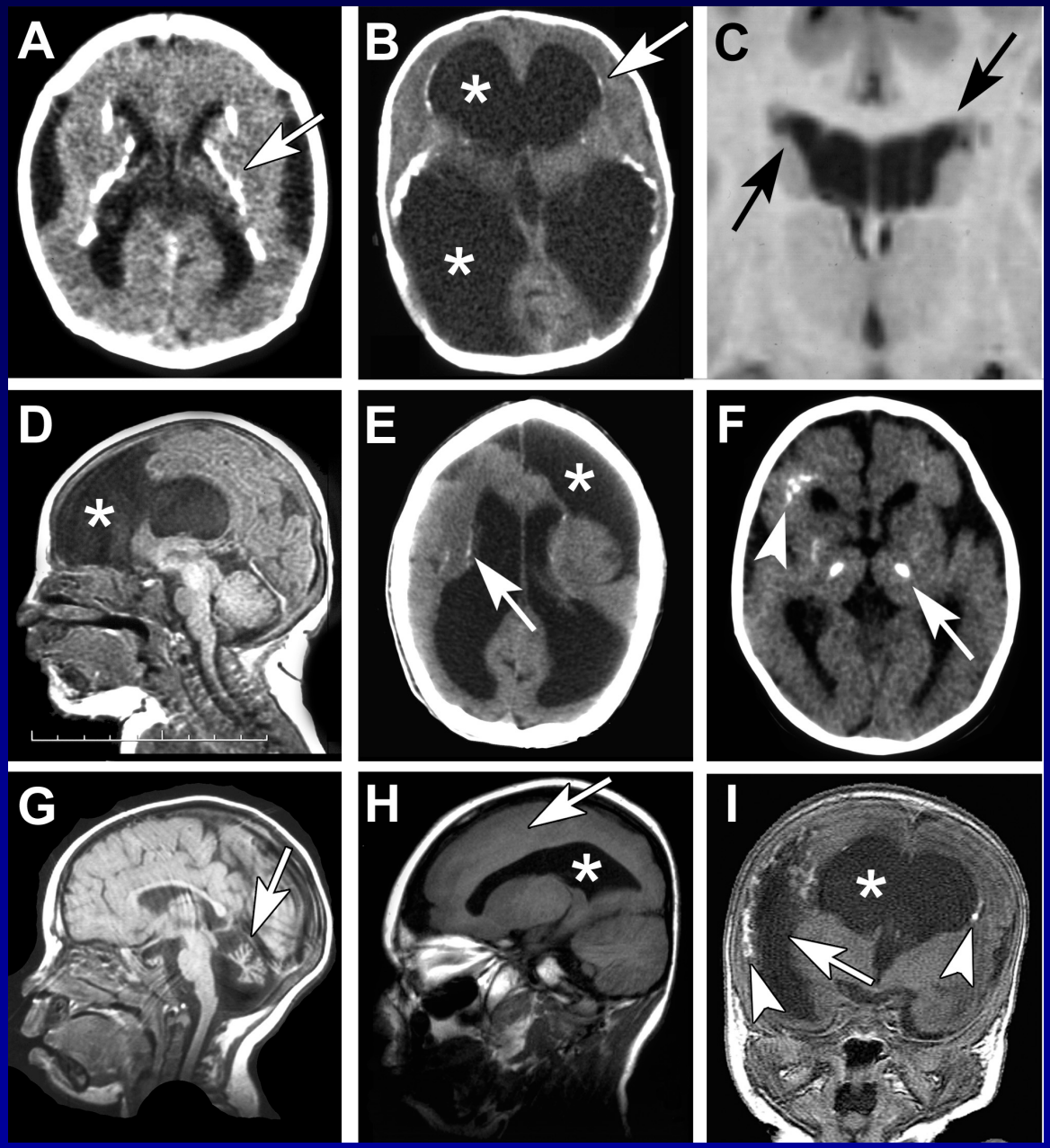
Clinical signs in congenital LCMV infection are largely confined to the nervous system.

Appropriate for gestational age	14/20	70%
Small for gestational age	6/20	30%
Large for gestational age	0/20	0%

Signs of nervous system dysfunction	20/20	100%
Signs outside the nervous system (rash)	1/20	5%

No hepatosplenomegaly, bone marrow suppression, cardiac defects, limb dysplasia, vesicular rash.

Neuroimaging of LCMV reveals a variety of pathologic findings.



(A) Microencephaly with periventricular calcifications
(B) Hydrocephalus
(C) Perivent cysts
(D) Porencephaly
(G) Cerebellar Hypoplasia
(H) Lissencephaly

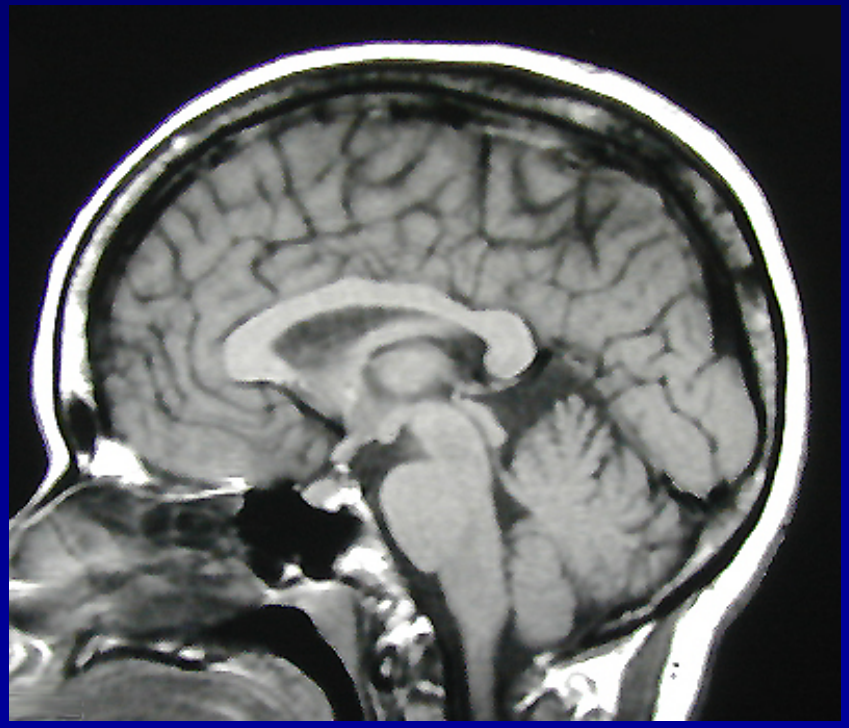
The long-term outcomes of congenital LCMV infection were diverse.

Chorioretinitis and vision loss	20/20
Cognitive deficits	20/20
mild	5/20
severe	10/20
profound	5/20
Spastic quadriparesis	14/20
Epilepsy	15/20
Ataxia	4/20
Hearing impairment	3/20
Regression	0/20

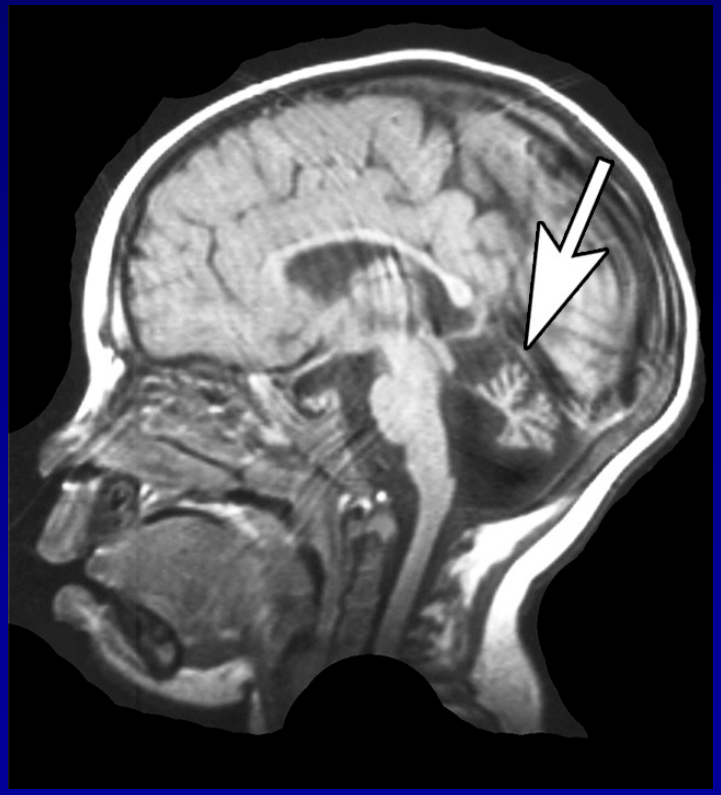
LCMV-induced Cerebellar Hypoplasia

Presentation: jitteriness in the newborn with LCMV.
Long-term outcome: ataxia and mild cognitive deficits.

Normal



LCMV



Congenital LCMV should be suspected in newborns who:

- have a suspected congenital infection that is not CMV or toxoplasmosis
- especially if there are no systemic signs outside the CNS
- especially if there was prenatal exposure to wild mice

How to diagnose congenital LCMV?

Important to test in the newborn period.

Method	Advantage	Disadvantage
1. Virologic (culture)	High specificity	Low sensitivity
2. Molecular (PCR)	Fast & accurate	Viral clearance may precede test
3. Serologic		
A. CF	Widely available	Low sensitivity
B. Imm Flu Ab	Moderate sensitivity & specificity	Moderate sensitivity & specificity
C. ELISA	Detects IgM & IgG with high sensitivity	Available only at CDC

Differential diagnosis of congenital LCMV

Diagnosis	Clues to differentiation from LCMV
Toxoplasmosis	Diffuse calcifications, jaundice, rash
CMV	Hearing deficits, hepatosplenomegaly
Rubella	Heart disease, IUGR, cataracts, purpura
Herpes	Vesicular rash, hepatic dysfunction
Syphilis	Lymphadenopathy, rhinitis, periostitis
Aicardi-Goutieres	Progressive course, mutations of TREX1 and RNASEH2 genes
Tuberous Sclerosis	Ash leaf spots, affected family members, cardiac rhabdomyomas

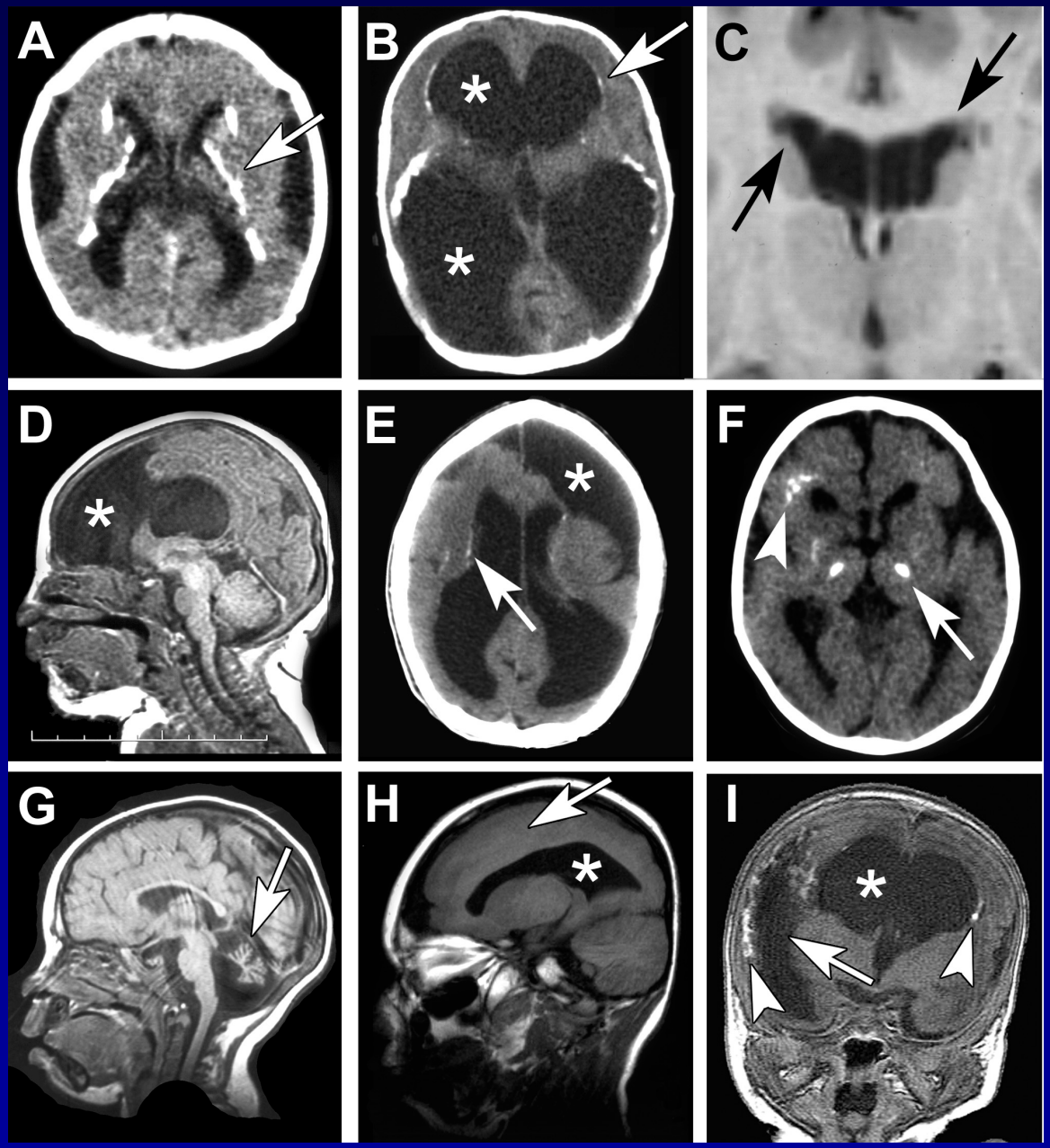
Prevention of LCMV

No vaccine exists to prevent LCMV.

However, measures can be taken:

1. Minimize exposure to potentially infected rodents
   - eliminate cohabitation with mice
   - avoid contact with pet rodents
   - caution by workers in animal care facilities
2. All animal colonies should be tested periodically for LCMV.
3. Be aware of risks of transmission via organ donation

Neuroimaging of LCMV reveals a variety of pathologic findings.



(A) Microencephaly with periventricular calcifications
(B) Hydrocephalus
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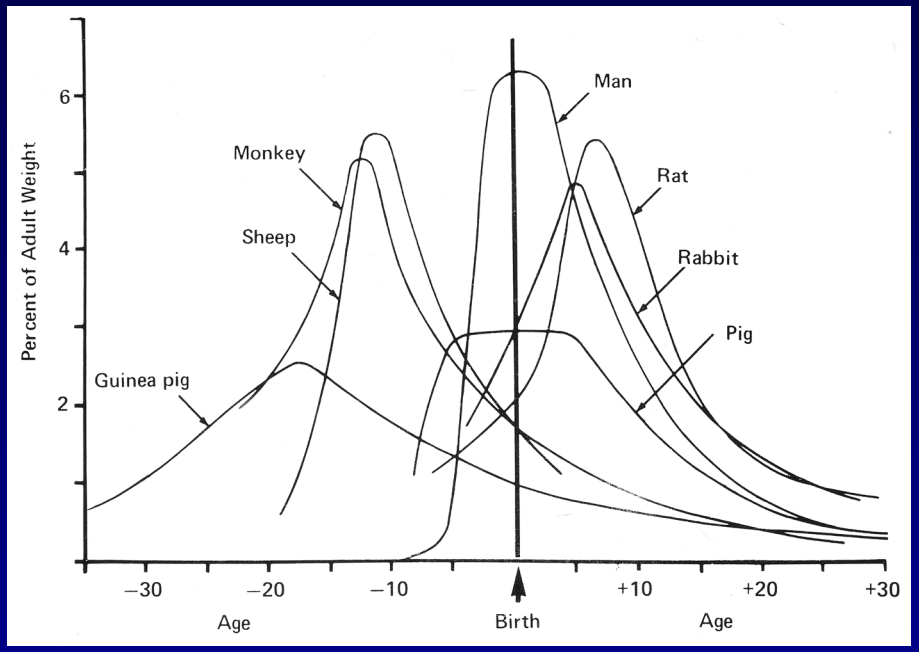
(G) Cerebellar Hypoplasia
(H) Lissencephaly

Hypothesis:

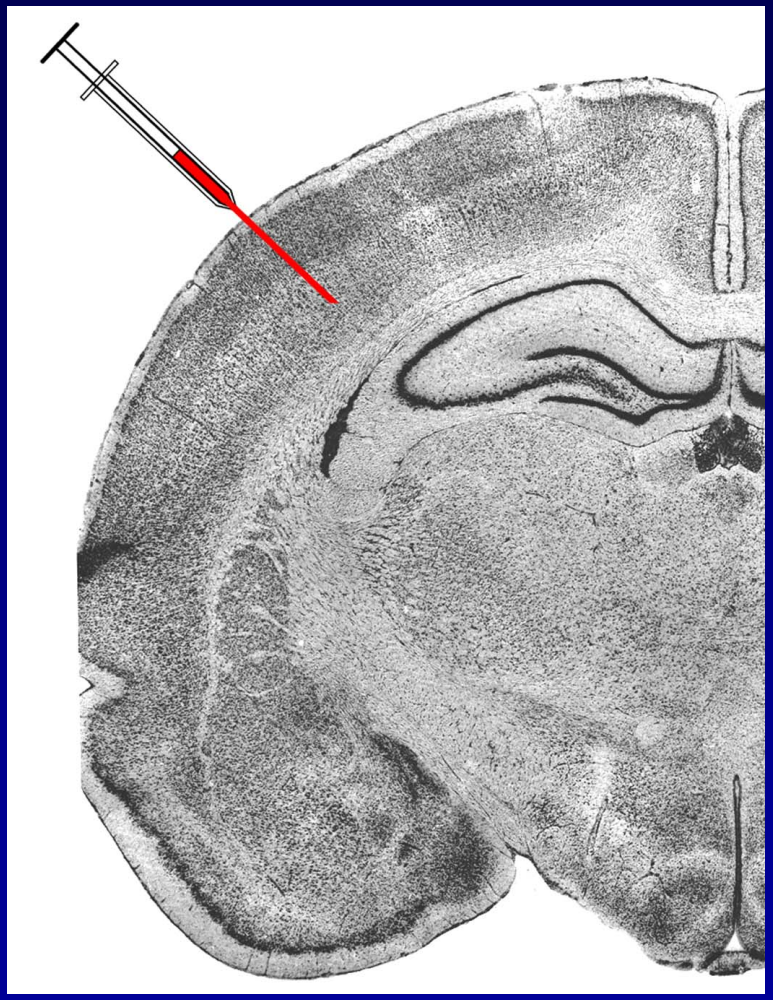
The variability in pathology among children with congenital LCMV infection is due to differences in *gestational age* at the time of infection.

Neonatal Rat Inoculated With LCMV



- Rat brains are immature at birth, relative to humans
- Exposure of neonatal rats to teratogens simulates human fetal exposure
- A useful model system of human congenital LCMV infection

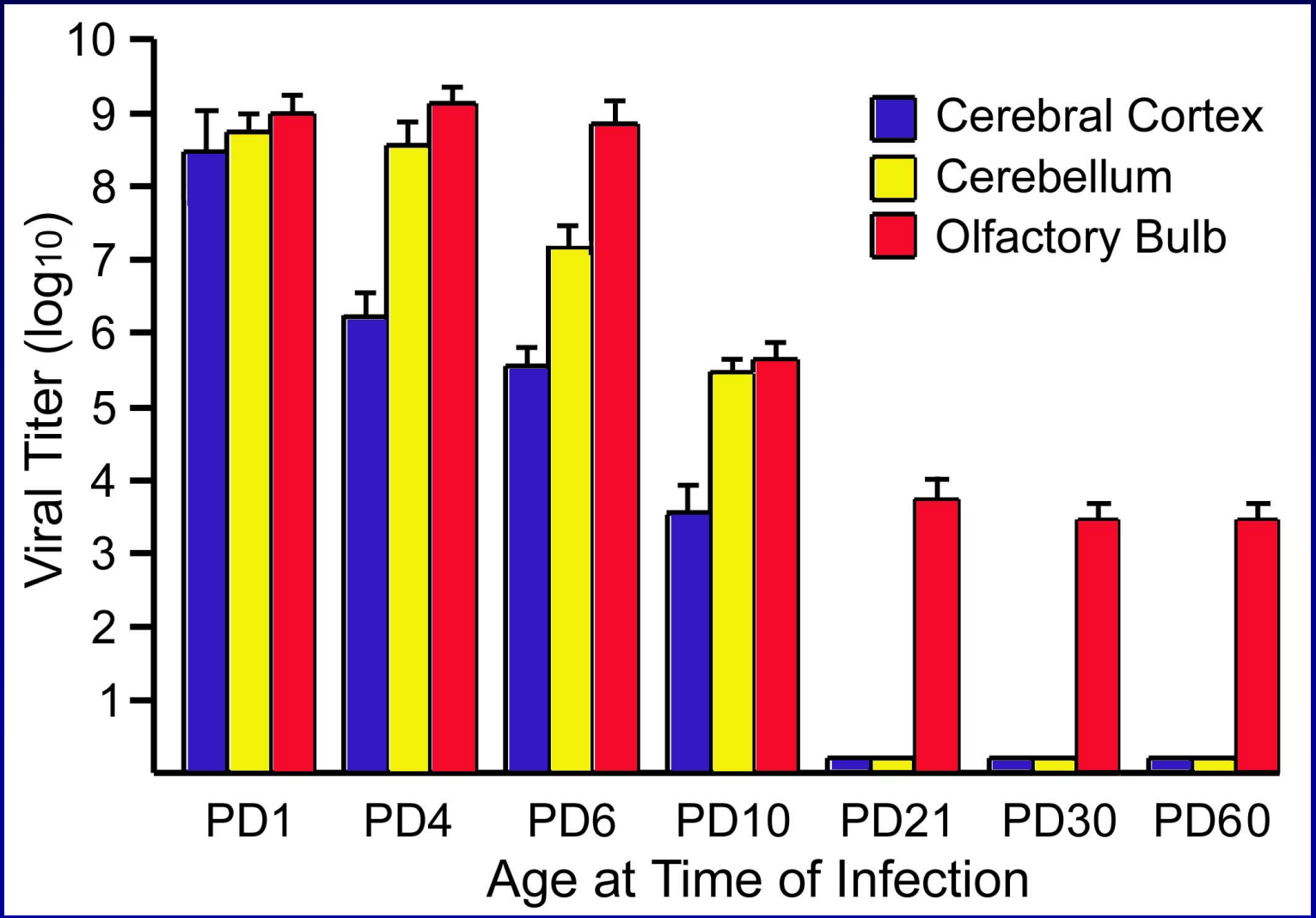
Rat model system of congenital LCMV infection.



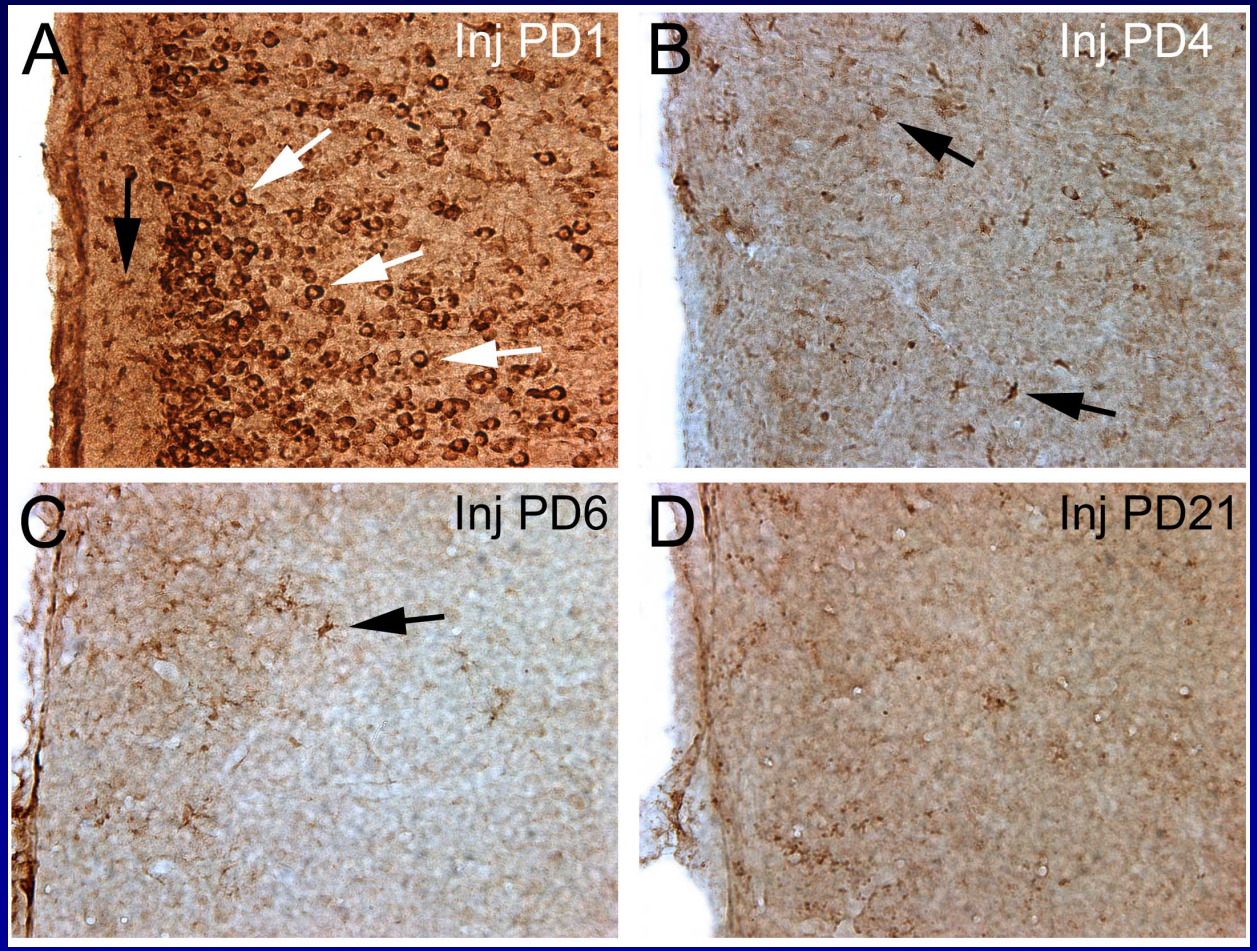
Rat pups injected with 1000 pfu of LCMV at a variety of ages:

PD 1, 4, 6, 10, 21, 30, 60

Viral titer at the peak of infection depends strongly on the age of inoculation.



Cerebral cortex: Cellular targets of infection depend on the age of the host.



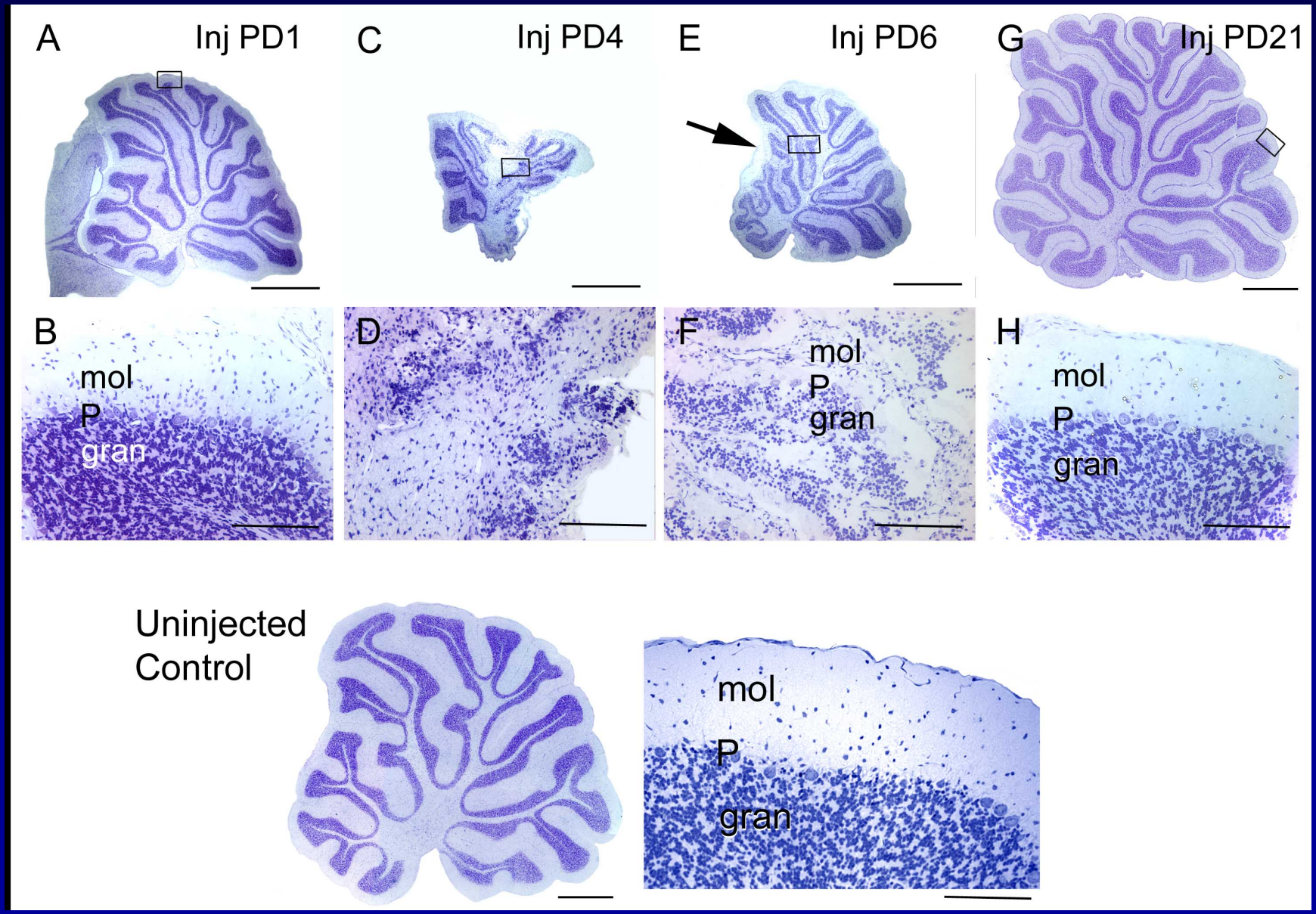
PD1: Widespread infection of neurons and astrocytes

B. PD4: Infection restricted to astrocytes

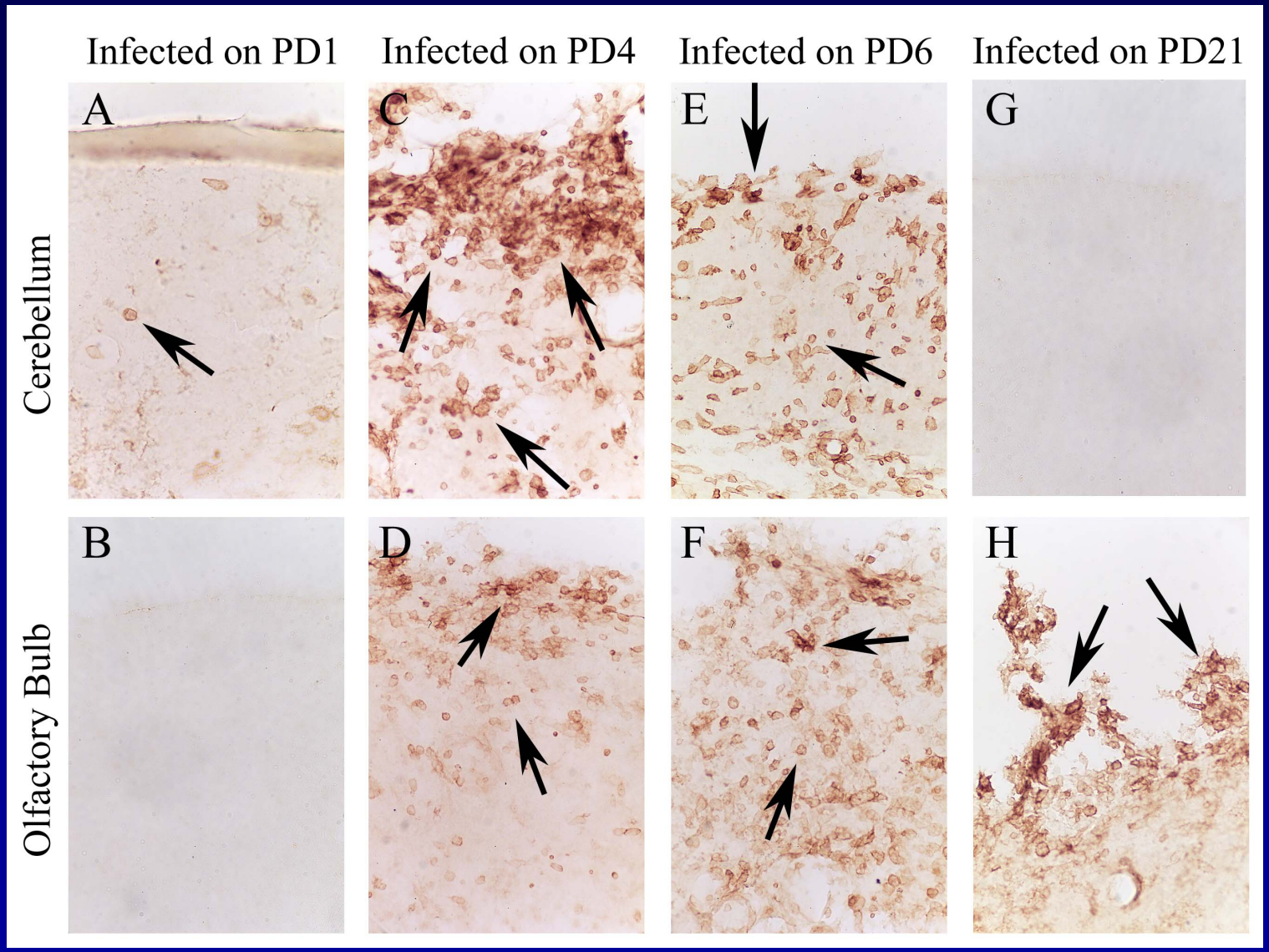
C. PD6: Infection of fewer astrocytes

D. PD21: Infection of no neurons or astrocytes

Cerebellar pathology depends on the age of the host at the time of infection.

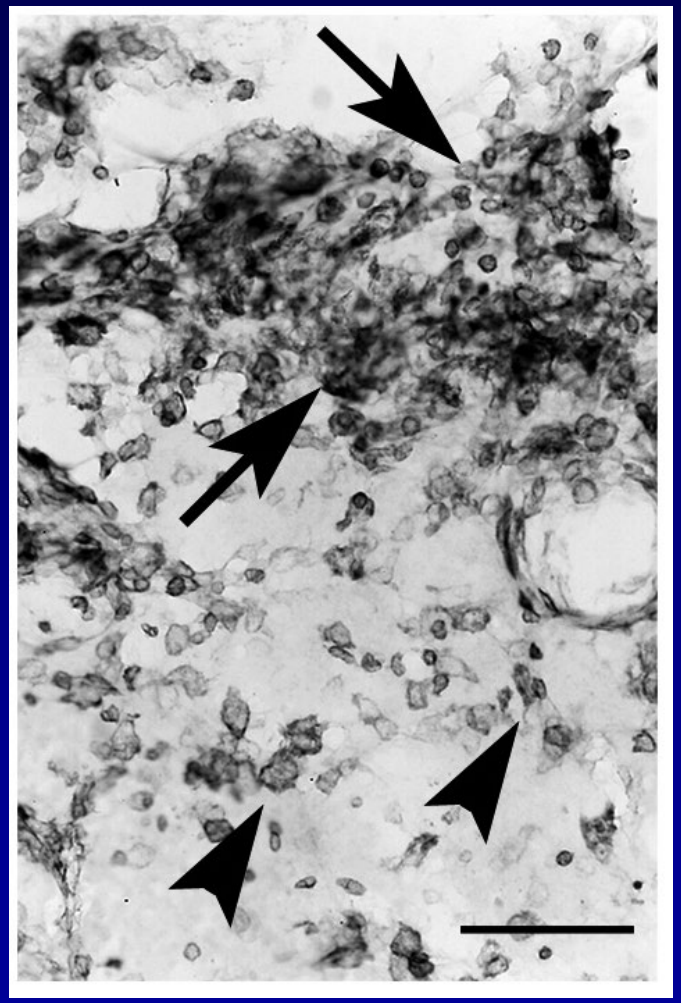


Immune response to the viral infection depends on the age of the animal at the time of infection.



Pathology in humans	Pathology in rat model	Infection day
Microencephaly	Microencephaly	PD1-PD6
Cerebellar hypoplasia	Cerebellar hypoplasia	PD1
Encephalomalacia	Encephalomalacia (cerebellum)	PD4-PD6
Porencephalic cyst	Porencephalic cyst (olfactory bulb)	PD10-PD60
Periventricular calcifications	Periventricular infection	PD1-PD10
Neuronal migration disturbance	Neuronal migration disturbance (cerebellum)	PD4-PD6

LCMV infection induces a robust infiltration of T-lymphocytes into the cerebellum.



Immunostained for CD8 antigen

What role do T-lymphocytes play in the cerebellar pathology of LCMV?

Are they responsible for the

- *destructive lesions?*
- *hypoplasia?*
- *disturbed neuronal migration?*

Is the pathology in congenital LCMV infection immune-mediated or is it virus-mediated?

To determine the importance of T-lymphocytes, we compared wild type rats with athymic (nude) rats.

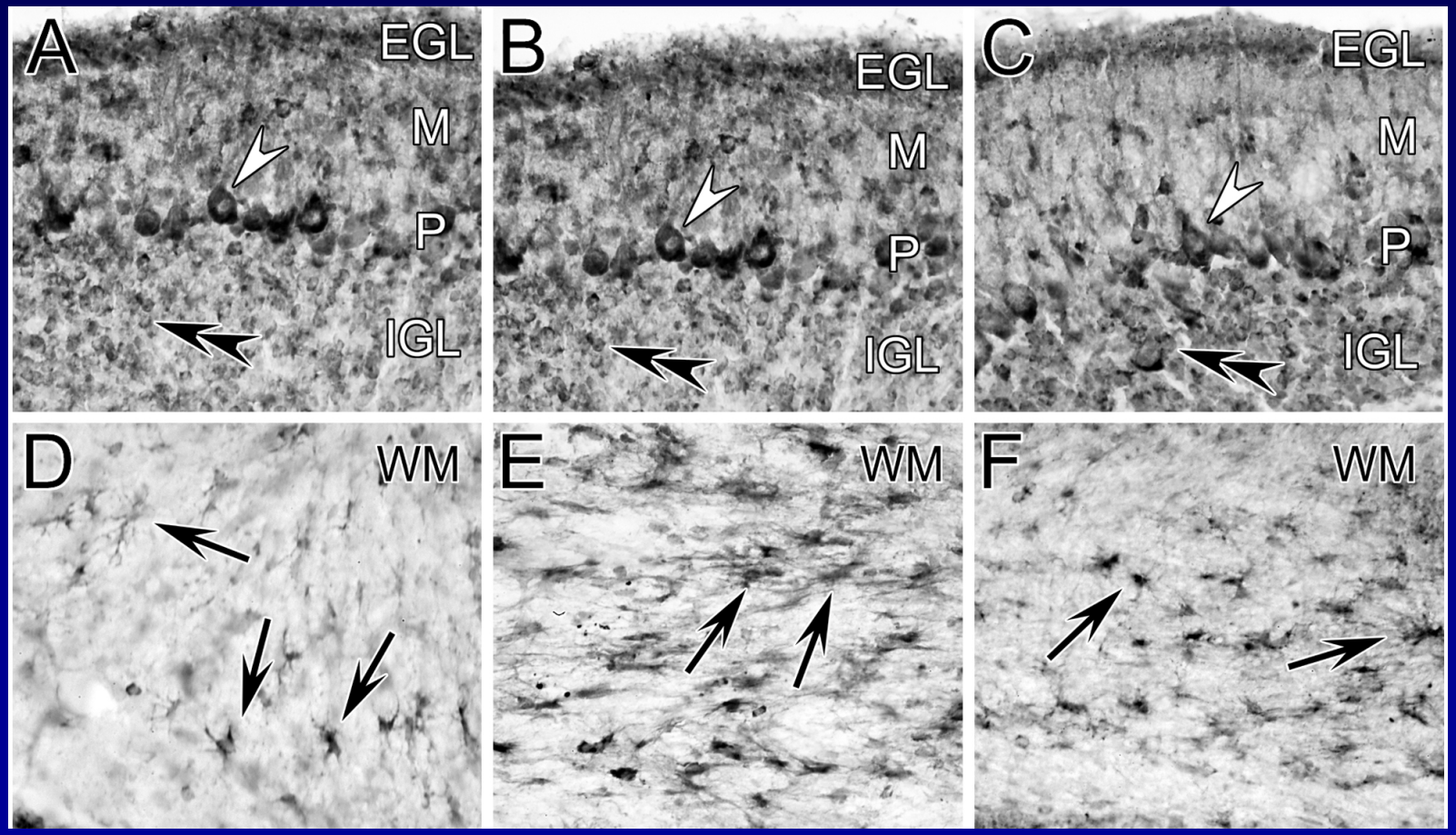
Wild Type Rat

(Intact immune system)

Athymic (Nude) Rat

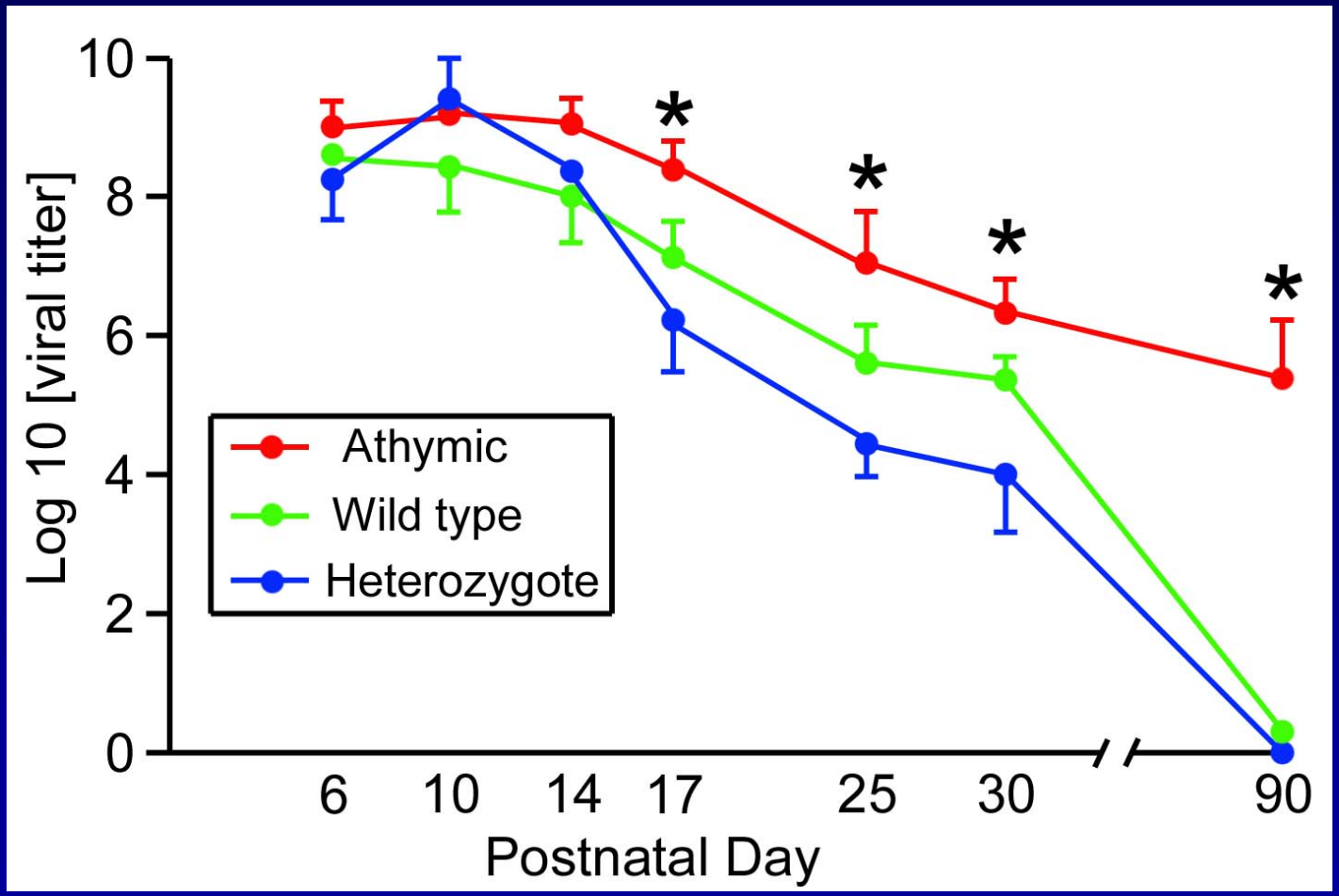
(Lacks T-lymphocytes)

The cellular targets of LCMV infection are identical in the three genotypes.

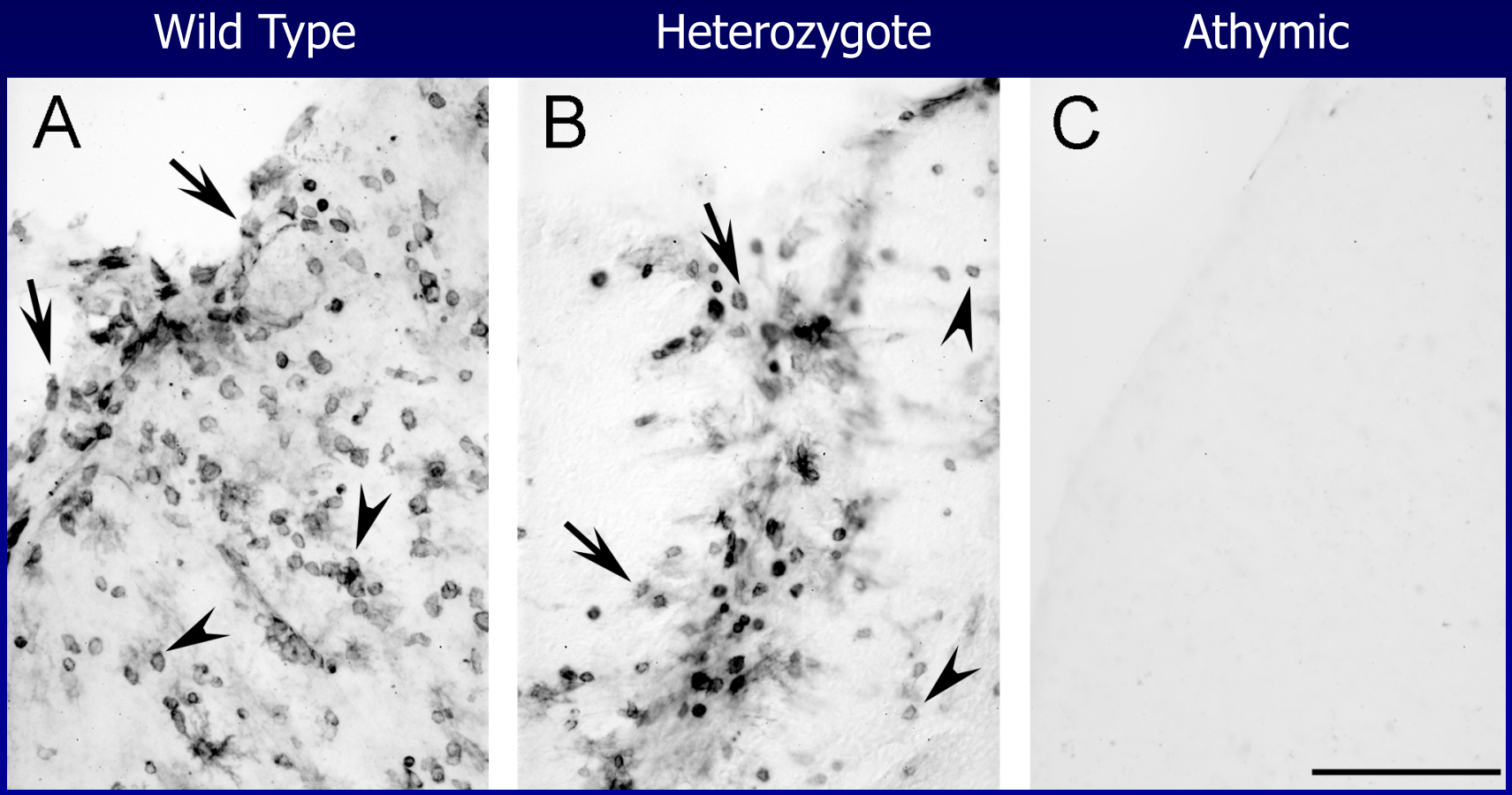


Anti-LCMV Immunostain

Viral clearance from the cerebellum is delayed in rats lacking T-lymphocytes.

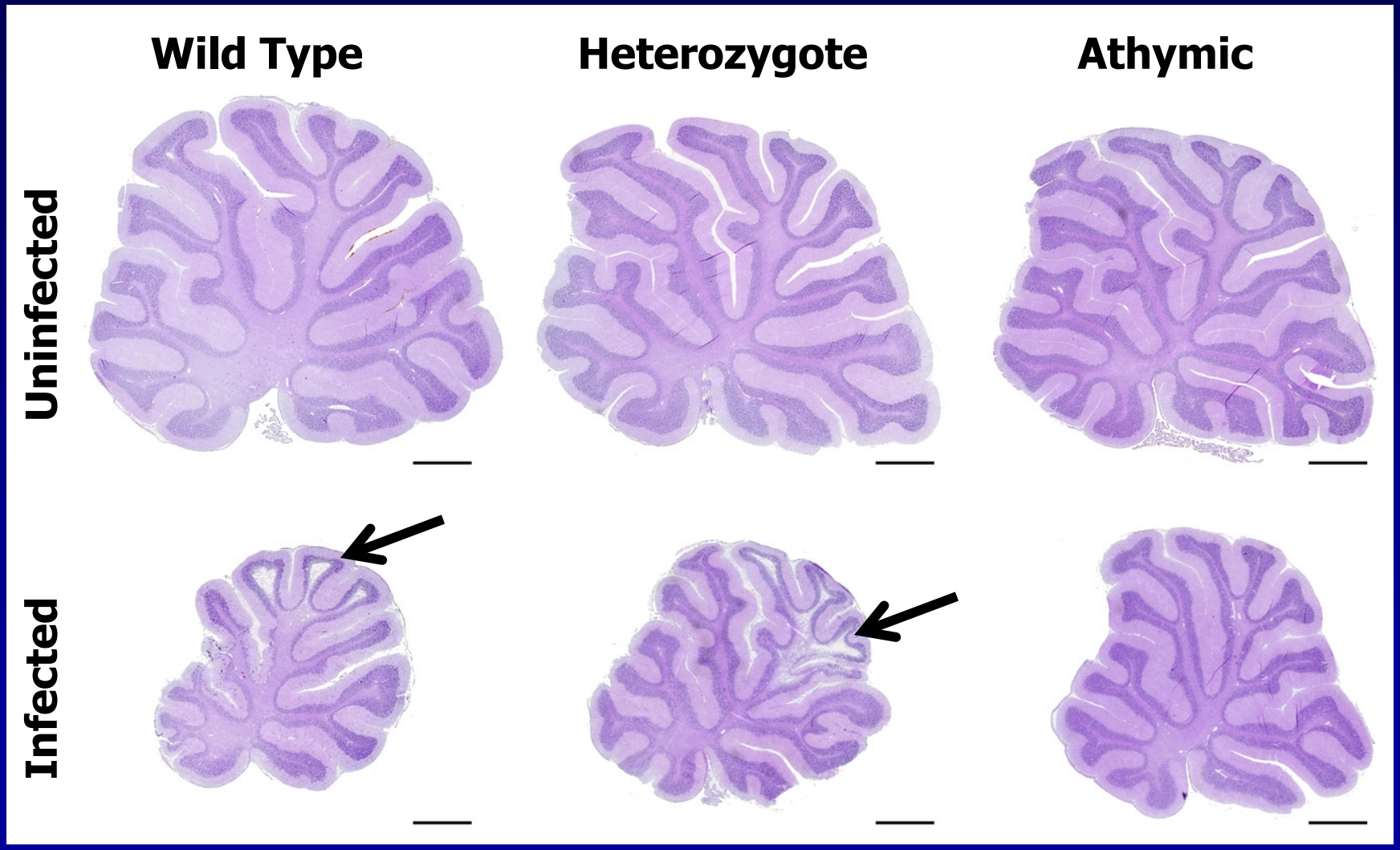


In immunocompetent rats, LCMV induces a robust lymphocytic infiltration.

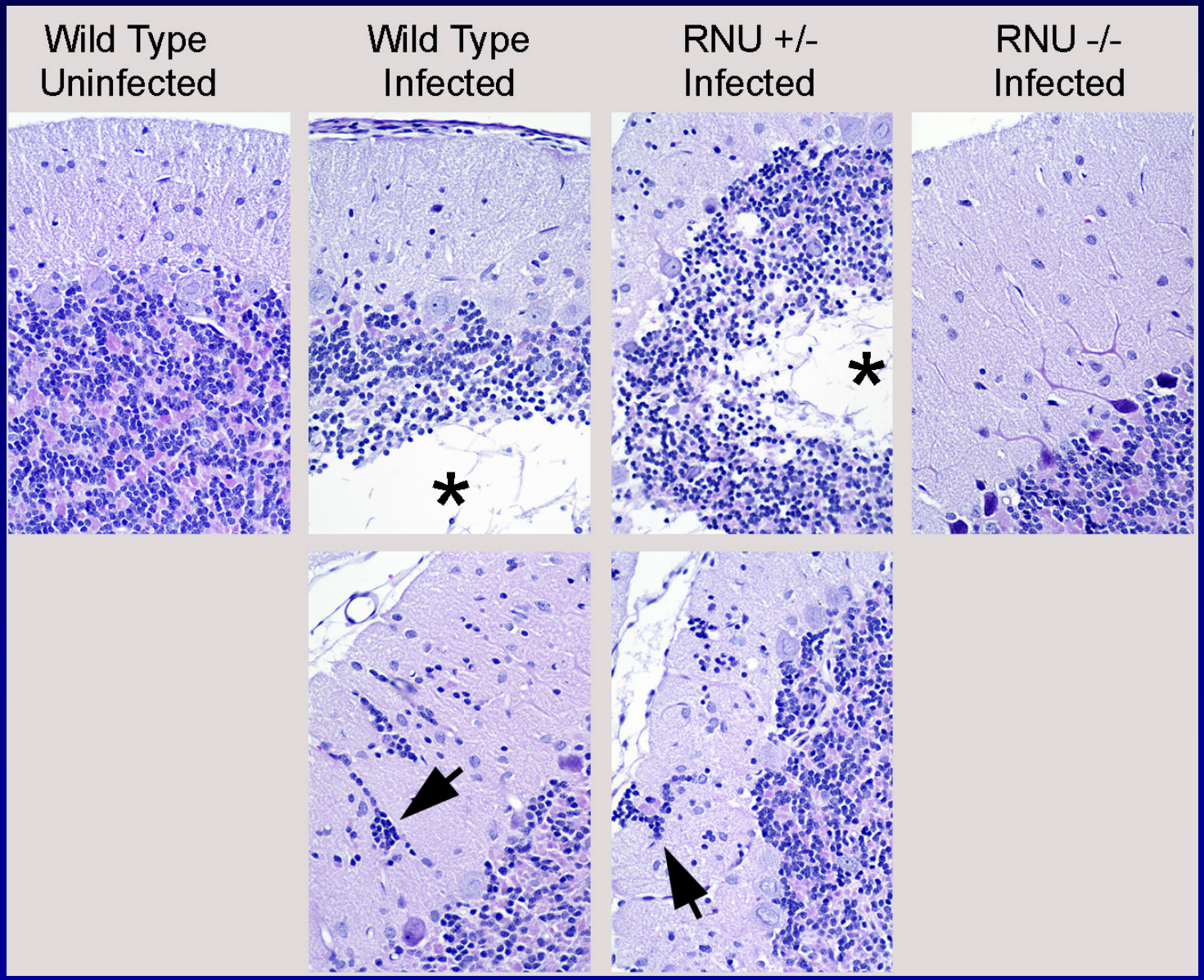


Anti-CD8α Immunostain

In the athymic rats, LCMV infection induces cerebellar hypoplasia, *but no destructive lesions*.



Neuronal migration disturbances are present in infected wild type and heterozygote rats, *but not in athymic rats*.



Adoptive Transfer of T-lymphocytes: *will the tissue destruction and neuronal migration defects be restored in nude rats that receive LCMV-sensitive T-lymphocytes?*

Inject adult heterozygote rats (rnu+/-) with LCMV

Wait 7—10 days

Spleen

rnu-/- pups

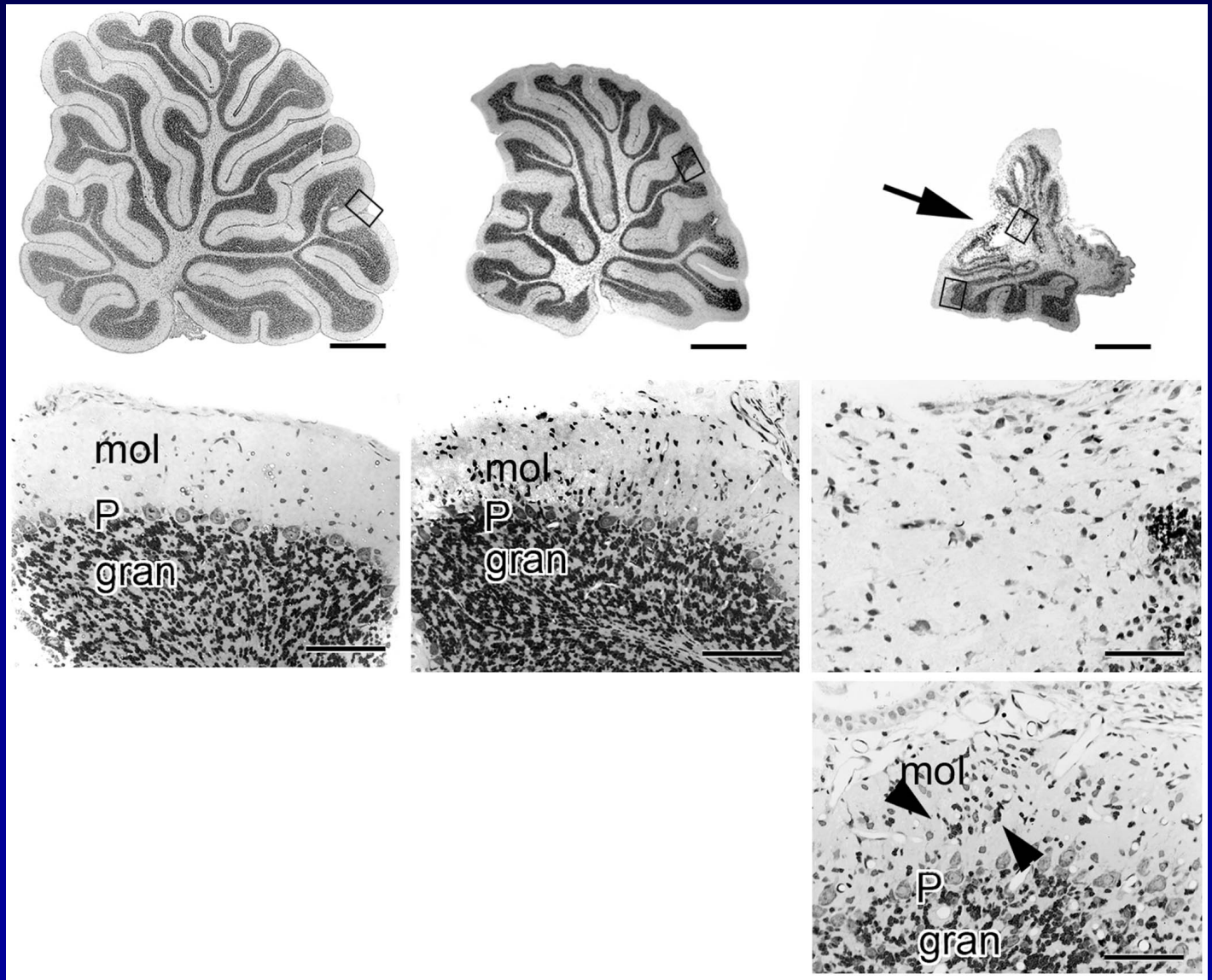
Inject splenocytes into athymic pups that were infected 1 day earlier with LCMV

Splenocytes

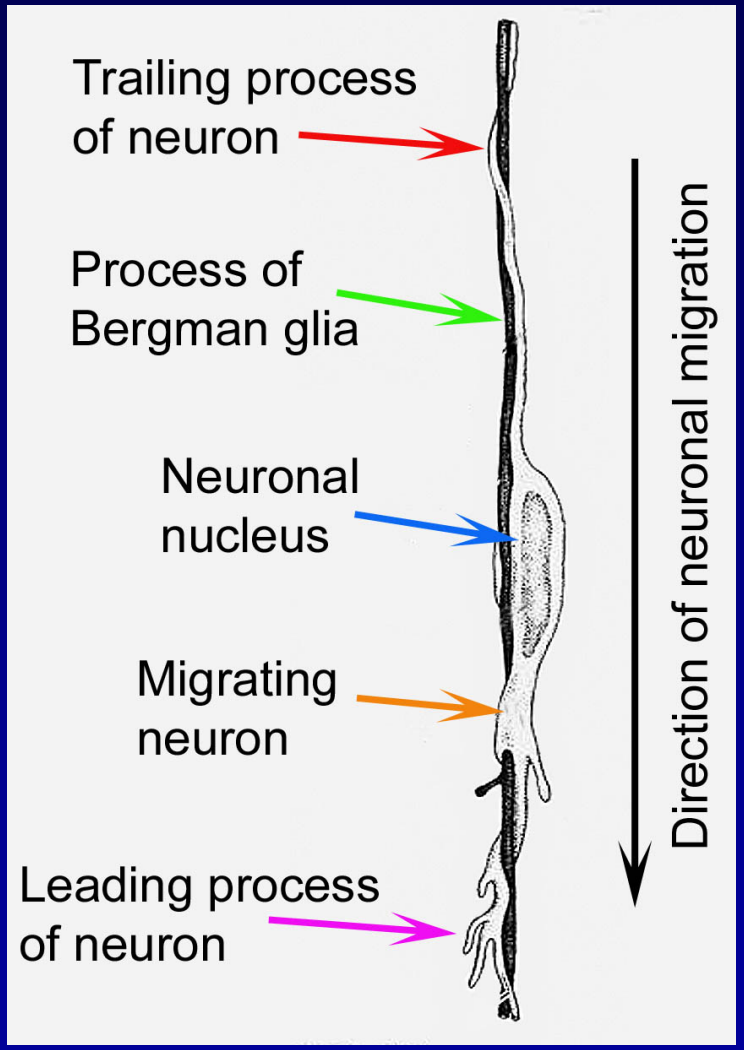
Athymic
Uninfected

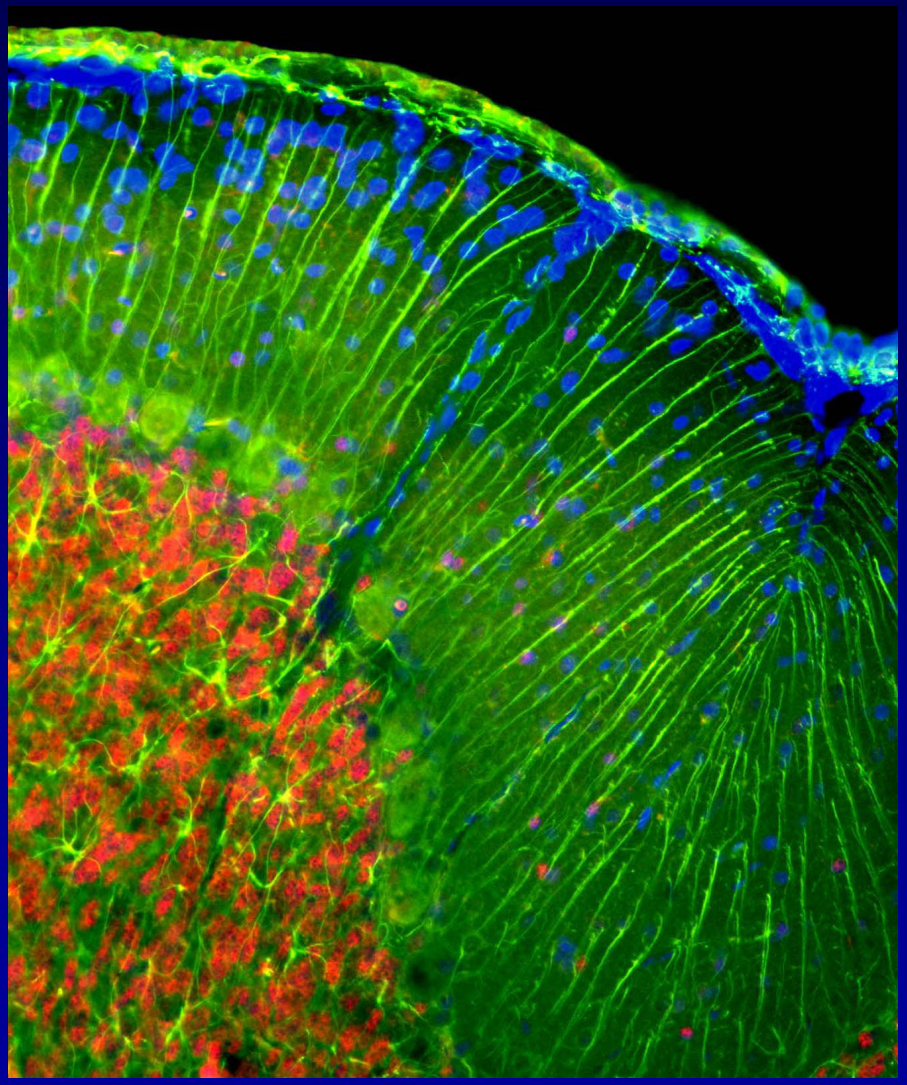
Athymic
Infected

Athymic
Infected +
T-cell adoptive transfer



Bergmann glia: the scaffolding on which granule neurons depend for guidance during migration to the granule cell layer.





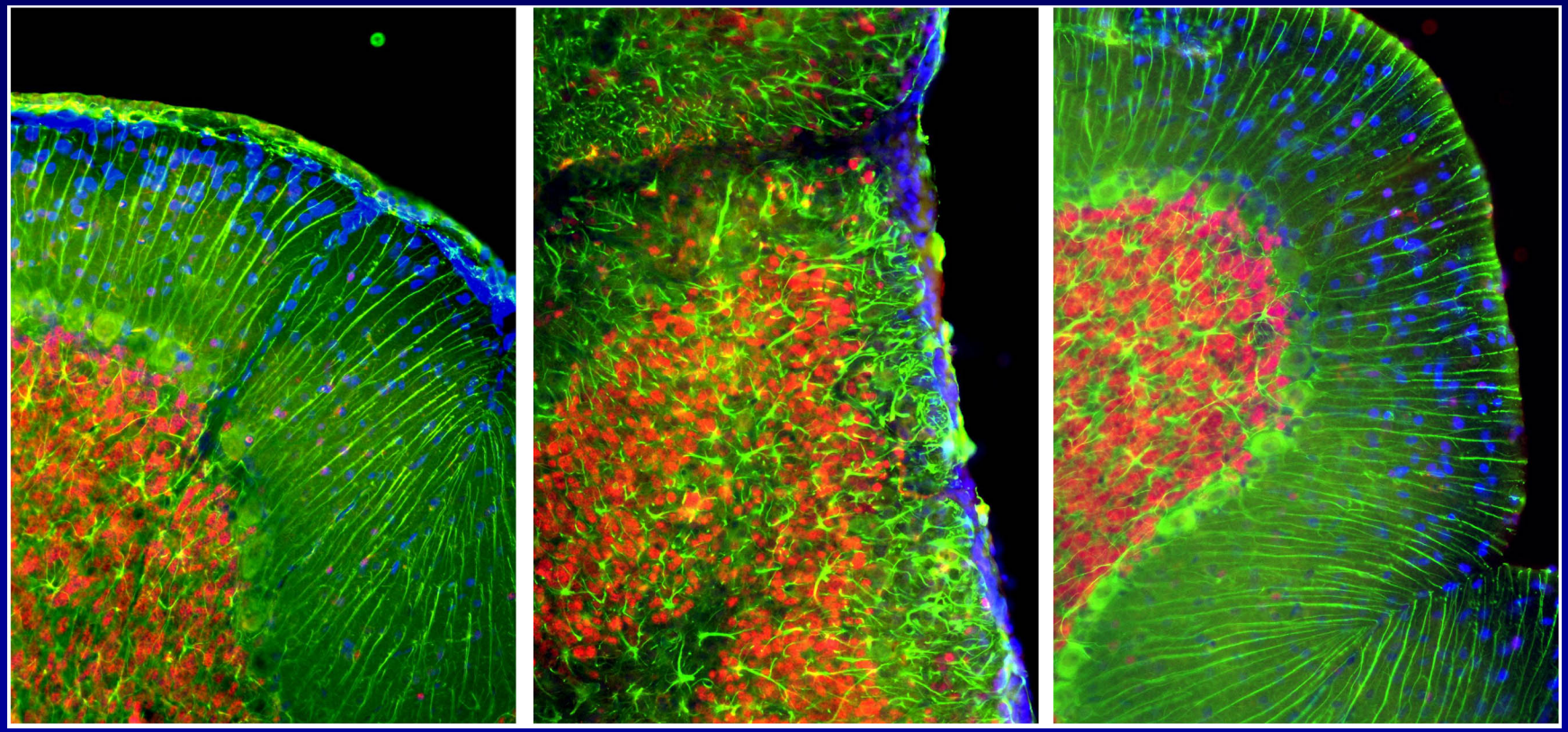
Immunostained for NeuN, GFAP, and DAPi

LCMV infection alters the structure of Bergmann glia in wild type rats, *but not in athymic rats*.

Wild type uninfected

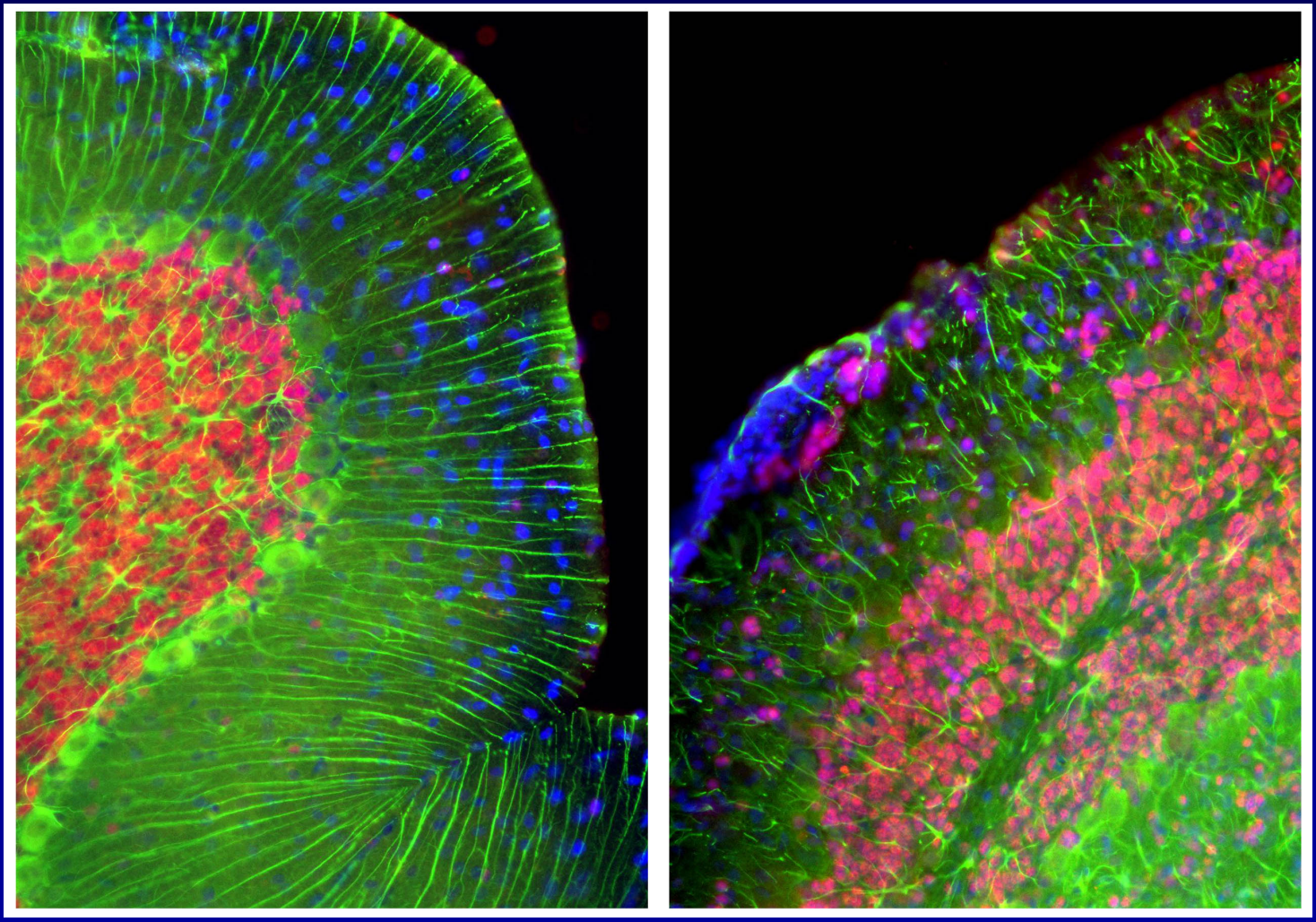
Wild type LCMV-infected

Athymic LCMV-infected



Athymic
LCMV infected

Athymic
LCMV infected +
T-cell adoptive transfer



Summary and Conclusions

- LCMV is a prevalent pathogen, indigenous to wild mice.
- LCMV is a common cause of aseptic meningitis in human adults.
- LCMV can cross the placenta and induce substantial neurological damage in the fetus.
- Chorioretinitis and periventricular calcifications are the most common neuropathological changes.
- However, there is a wide range of effects and outcomes in children prenatally infected with LCMV.

Summary and Conclusions (continued)

- All of the effects of LCMV observed in humans with congenital LCMV infection can be replicated in the rat model by infecting the rat pups at different ages.
- Infiltrating lymphocytes are responsible for the destructive lesions and neuronal migration defects, but not the hypoplasia.
- LCMV infection disturbs neuronal migration by corrupting the structure and arrangement of Bergman glia cells.

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Q&A and Wrap-Up

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