

Developing a Neurological Assessment Protocol Safe for Implementation in High- and Maximum-containment Facilities

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Introduction to Sequelae

Sequelae: *noun*

“a condition that is the consequence of previous disease or injury”

- Viral infections are a common cause of sequelae
 - Alphaviruses, flaviviruses, arenaviruses, herpesviruses, filoviruses
- Sequelae affect the quality of life of survivors, often regardless of age, gender, and economic status
- Treatment and management of these long-term problems is expensive
- **The study of sequelae requires interdisciplinary techniques**

What's our goal?

- **Combine** techniques of neuroscience, virology, and experimental pathology to solve a complex, biological problem related to viral pathogenesis and survivor quality of life
- **Perform** neuroscience techniques in the BSL-3 and BSL-4 labs in rodent models for the first time
- **Establish** new models to study the direct impact of infection on CNS functions that can be applied to other infectious disease

SHIRPA/General Health Assessment

- **SHIRPA: S**mithKline Beecham, **H**arwell, **I**mperial College, **R**oyal London Hospital, **p**henotype **a**ssessment
- General screen that evaluates an animal's behavior
- Three stages
 - Primary: evaluates gait, motor control, coordination, and muscle tone, scaled for quantitative analyses
 - Secondary: locomotor activity, food/water intake, balance, startle reflex, prepulse inhibition, and histology and biochemistry, no scale required for quantitative analyses
 - Tertiary: anxiety, learning, memory, nerve conduction

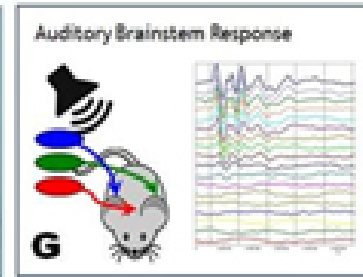
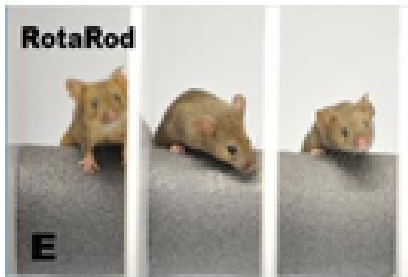
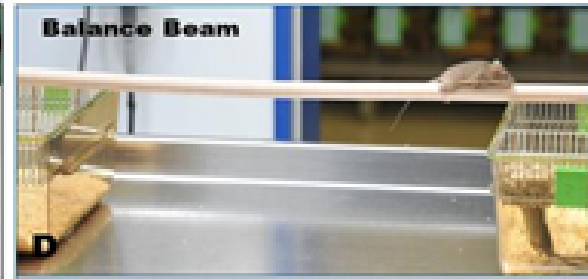
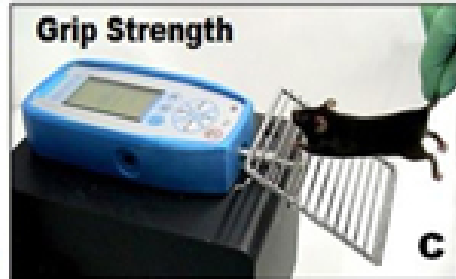
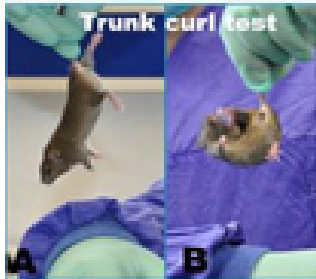
Primary Screen



<http://cdn.elifesciences.org/elifesciences/articles/08352/pdf/elifesciences08352.pdf>

http://www.hopkinsmedicine.org/mcp/PHENOCORE/CoursePDFs/2012/Lab_Man_Binder_41p.pdf

http://www.klinikum.uni-muenchen.de/Deutsches-Schwindelzentrum-IFB-LMU/en/Research_projects/Full_research_projects/Project-TRFS/index.html



	0	1	2 (normal)	3
Body Tone	Flaccid	Allows depression to the floor	Allows some flattening	Hunches back to completely resist compression
Trunk Curl	No response or hindlimb clasp	<90degrees	>90degrees	Climbs up tail
Righting	Does not right itself	Struggles to right itself	Rights itself	Hyperactive response
Whisker Response	No response	Difficult to elicit a response	Normal response	Hyperactive response
Ear Twitch	No response	Difficult to elicit response	An obvious response	Hyper repetitive response (continual movement of ears)
Palpebral Reflex	No response	Slow blink	Quick blink	Hyper repetitive blinking
Forelimb Place	Leg stays where placed	Slow or incomplete return	Promptly returns let to normal position	Hyperactive response
Clicker	No response	Difficult to elicit response	Immediate response	Abnormal response (seizures, hyperactive escape)

What's wrong with SHIRPA?

- Lots of animal handling involved
 - Increased risk of bite or scratch while performing tests, as some things may agitate the animal

What's wrong with SHIRPA?

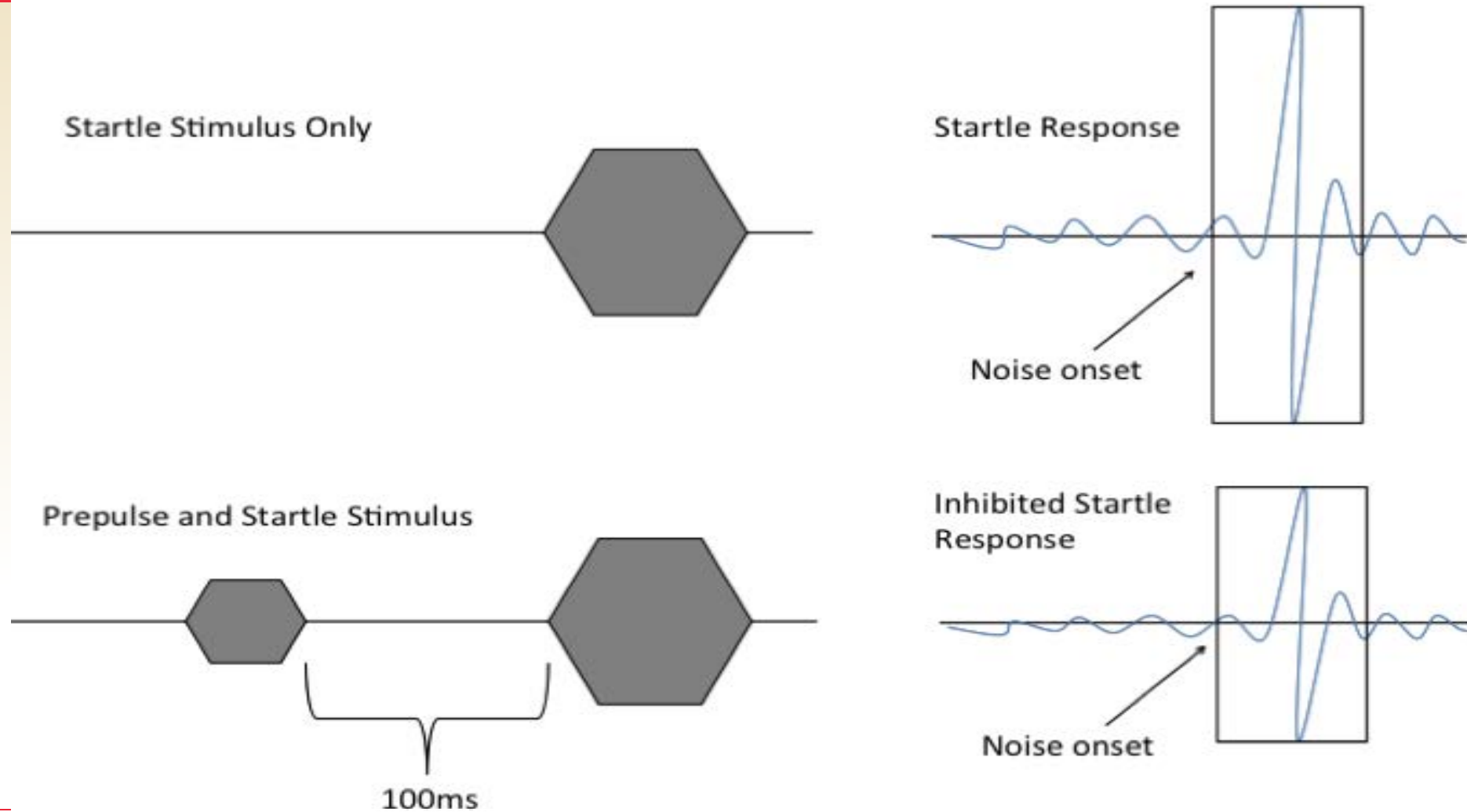
- Lots of animal handling involved
 - Increased risk of bite or scratch while performing tests, as some things may agitate the animal
- Solution?
 - Wearing leather gloves on top of facility-specific PPE allows users to safely handle animals while still accurately performing the general health assessment



Acoustic Startle and Prepulse Inhibition

- **Startle response**
 - Involuntary motor response elicited by an unexpected sound stimulus
- **Prepulse inhibition**
 - Extension of the startle response
 - Occupation of the acoustic pathway to lead to decreased motor response

Acoustic Startle and Prepulse Inhibition

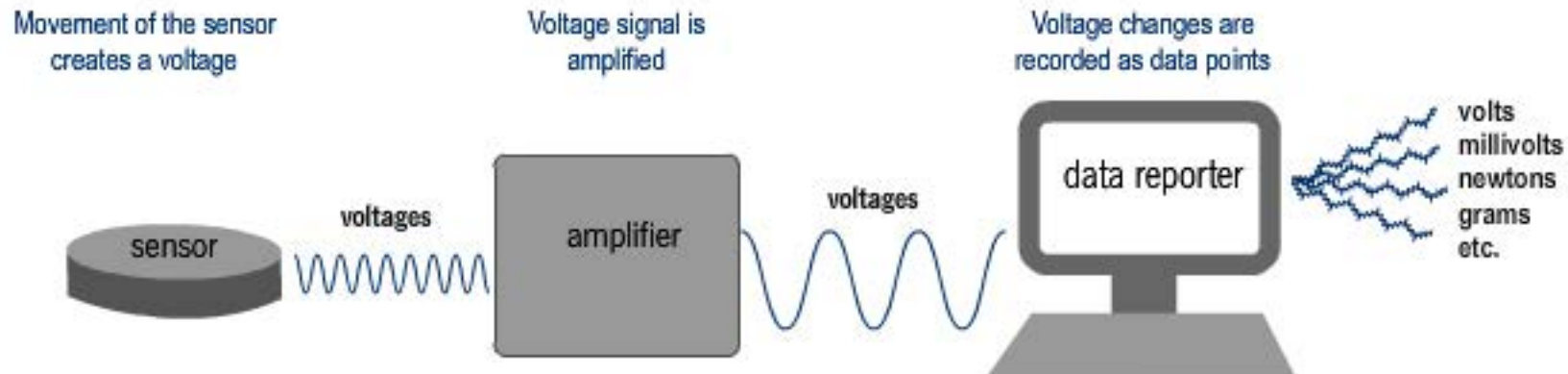


What do deficits in these paradigms mean?

- Increased startle response commonly associated with post-traumatic stress disorder
- Decreased startle response indicative of depression, bipolar disorder, overactive pro-inflammatory response, and/or habituation to sound stimulus
 - Habituation to the startle response is typically considered normal over prolonged exposure
 - Decreased habituation is seen in Parkinson's patients and other neurodegenerative diseases
- Ablated startle response indicative of hearing loss
- Decreased percent inhibition is characteristic of schizophrenia
 - Also seen in attention-deficit disorders

How are these tests performed?

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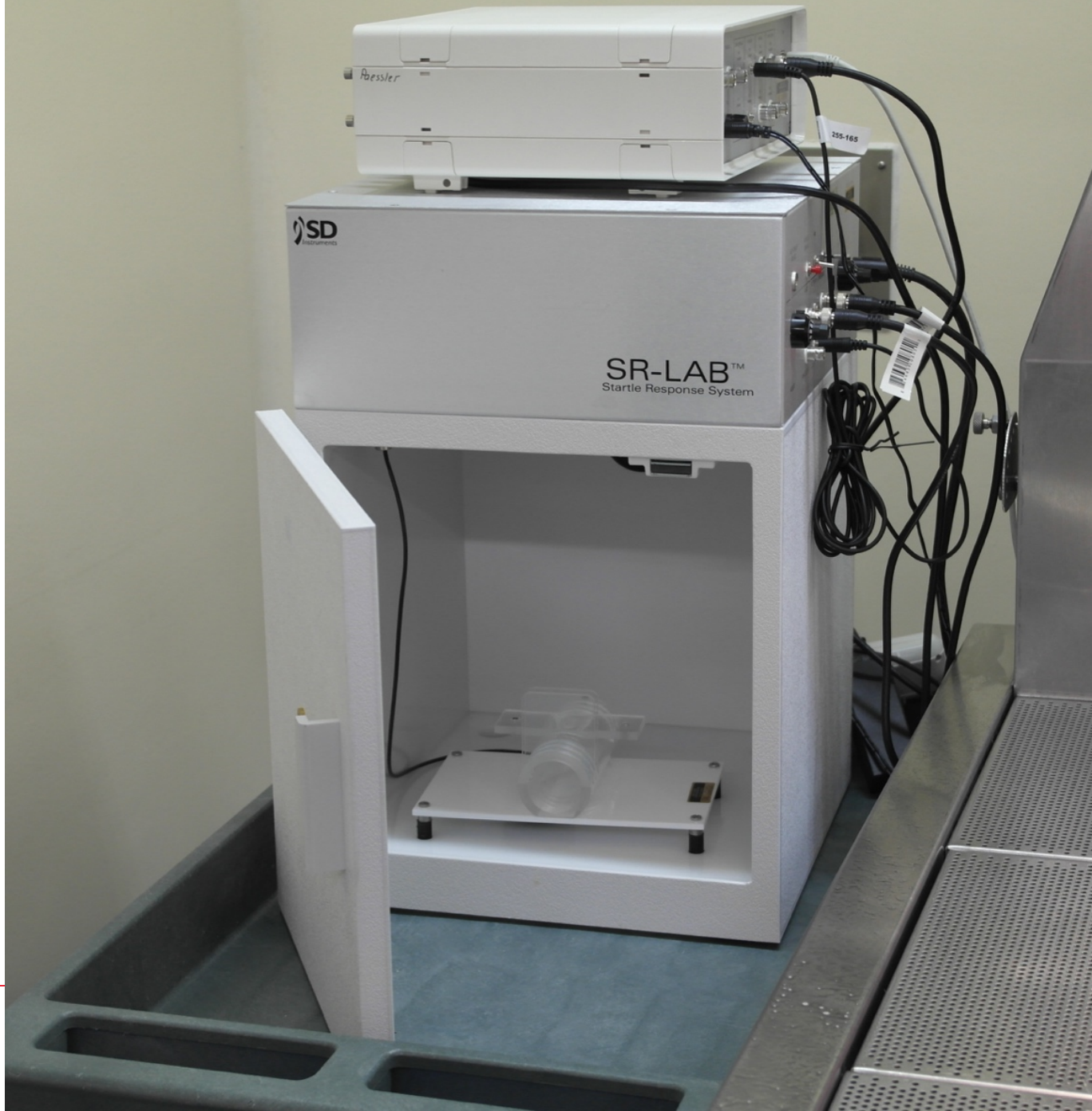


MedAssociates Startle Package



SR Lab for Acoustic Startle and Prepulse Inhibition





PPE Requirements

- BSL2
 - Facility requirement but **N95 replaces face mask and leather gloves**
- BSL3
 - Facility requirement but **PAPR is necessary and leather gloves**
- BSL4
 - Facility requirement plus **leather gloves**

How do we apply this?

Neurological Sequelae of Viruses

- Ebola
 - Vision and hearing deficits, arthralgia
- Lassa
 - Hearing loss
- Cytomegalovirus
 - Hearing loss
- Flaviviruses and **alphaviruses**
 - Epilepsy, paralysis, hallucinations, cognitive disorders, behavioral changes

Alphaviruses

- ssRNA viruses
- Mosquito transmission
- Cause arthralgic or **encephalitic** disease
 - Venezuelan, western, and eastern encephalitis viruses
- Onset of febrile symptoms 2-10 days post infection

Alphaviruses

- Infection can be asymptomatic and undetected
- Mortality rate is low
 - Mortality rate in hospitalized patients can exceed 75%
 - Death usually due to encephalitis
- **Many survivors develop long-term neurological complications**
- Study requires BSL-3 laboratory
 - VEEV and EEEV are select agents
- **No licensed vaccines available for humans**
 - **Annual vaccination requirement for horses**

Alphavirus: Human Case Data

	VEEV	WEEV	EEEV
% Survivors with Neurological Sequelae	4-14	15-30	50-90
Associated Sequelae	Somnolence, confusion, seizures, photophobia, coma, intellectual disability, emotional instability	Confusion, visual disturbances, photophobia, seizures, somnolence, coma, intellectual disability, emotional instability , spastic paresis, psychosis	Seizures, paralysis , intellectual disability
Mortality (%)	1	3-7	50-75

Case Study: Female, Age 22

- Patient's family had her evaluated by a psychiatrist for severe mood swings, increasing confusion, paranoia, and decreased ability to perform daily tasks.
 - They believed she was emotionally stressed:
 - **Her horse died**, she was fired from her job, and she had broken her engagement
 - Psychiatrist diagnosed her with **schizophrenia**, recommended permanent institutionalization
- Institutionalized for 18 months, no improvements with medication
- Condition deteriorated
 - Self-mutilating, mute, unwilling to eat

Case Study: Female, Age 22

- Second clinician determined that her horse died of acute WEE after receiving the information
 - Led to hypothesis that she might have had WEEV-induced encephalitis
- Laboratory results confirmed WEEV infection
- Behavioral symptoms onset coincided with onset of encephalitis
- Moved to a different hospital ward for alternative treatment approach
 - Discharged once she began speaking again
- Her overall condition did not improve

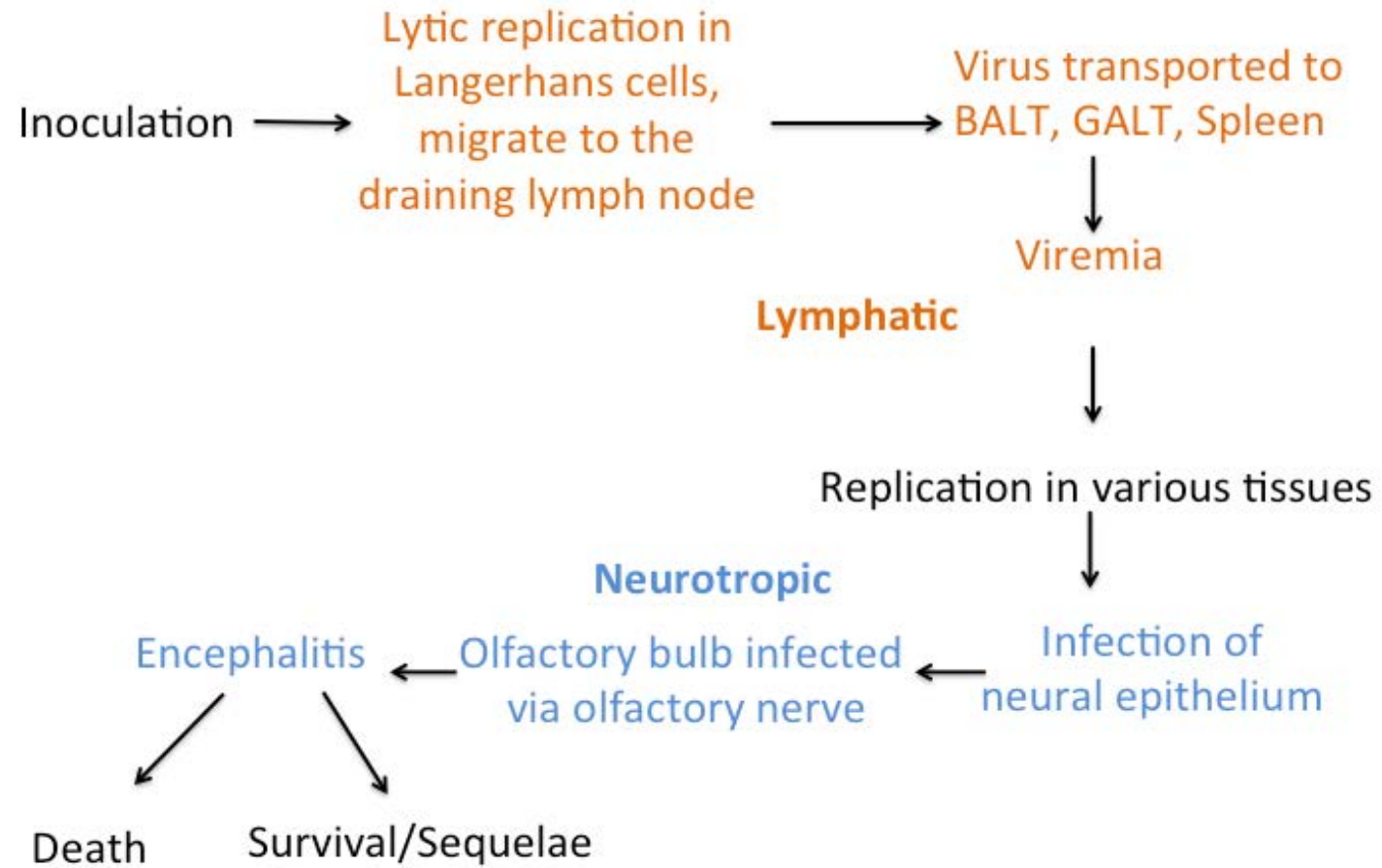
VEEV

- **VEEV** to model sequelae of alphaviruses
 - Highest number of reported human infections
 - Lowest mortality rate in humans
 - 4-14% of survivors develop sequelae
 - Sequelae is consistent for all encephalitic alphaviruses
 - Important veterinary pathogen
 - Pathogenesis in horses is similar to humans
 - Have well-established murine models
- Requires BSL-3 to study

TC-83

- **TC-83** is attenuated VEEV
 - Allow studies in BSL-2
 - Provides a tool for vaccination
 - Genetically modified virus with luciferase allows us to visualize location of infection without euthanization
 - Allows establishment of nonlethal brain infection similar to what has been reported for VEEV infection in human brains

Animal Model of Infection



Baseline SHIRPA, evaluation of sensory functions, and acoustic startle/prepulse inhibition on WT C57BL/6 mice



Intranasal infection with low dose TC-83 or PBS



**Monitor for signs of disease
Mock, Asymptomatic, Symptomatic groups**



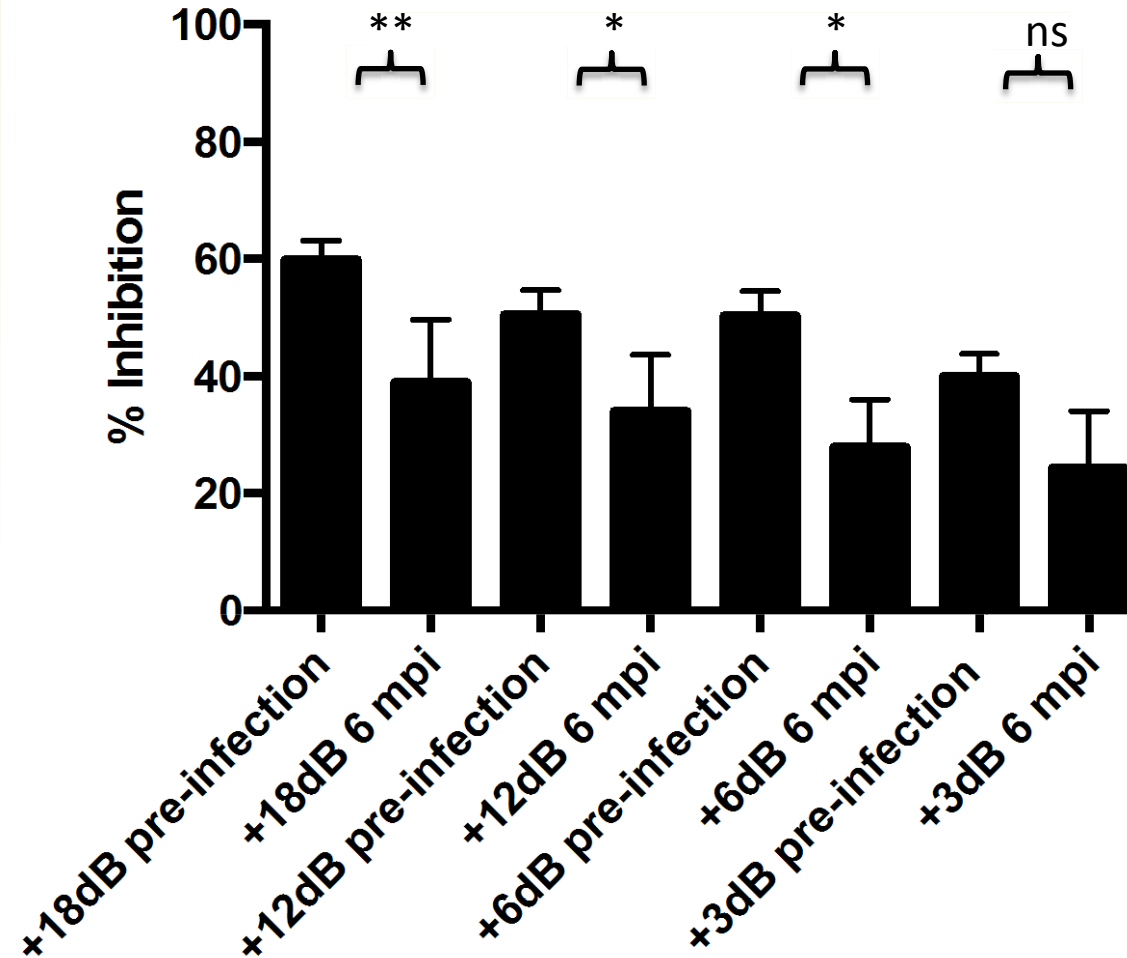
SHIRPA, ASR, PPI follow up for 6 mpi



Evaluate the histopathology of the brain

Decreased PPI persists 6mpi in symptomatic infected animals

** p<0.01
*p<0.05
NS: not significant



Summary

- Infection with TC-83 results in 20% of animals symptomatic
 - These animals seroconvert
- All symptomatic animals have altered PPI
 - Reduction in PPI beginning at 1mpi, continues to 6mpi
 - Consistent with schizophrenic-like phenotypes in previous cases

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