



National Institute of  
Neurological Disorders  
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NIH Counter**ACT**  
Program

# Status Epilepticus after Benzodiazepines: Seizures and Improving Long-term Outcomes

February 28 - March 1, 2023



## Therapeutic approaches for treating organophosphate-induced status epilepticus co-morbidities based on changes in calcium homeostasis

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# Status Epilepticus after Benzodiazepines: Seizures and Improving Long-term Outcomes



## Disclaimer

This certifies that the views expressed in this presentation are those of the author and do not reflect the official policy of NIH.

## Disclosure

This certifies that I, Laxmikant Deshpande, have no financial relationship that is relevant to the subject matter of the presentation.

# Organophosphate Compounds

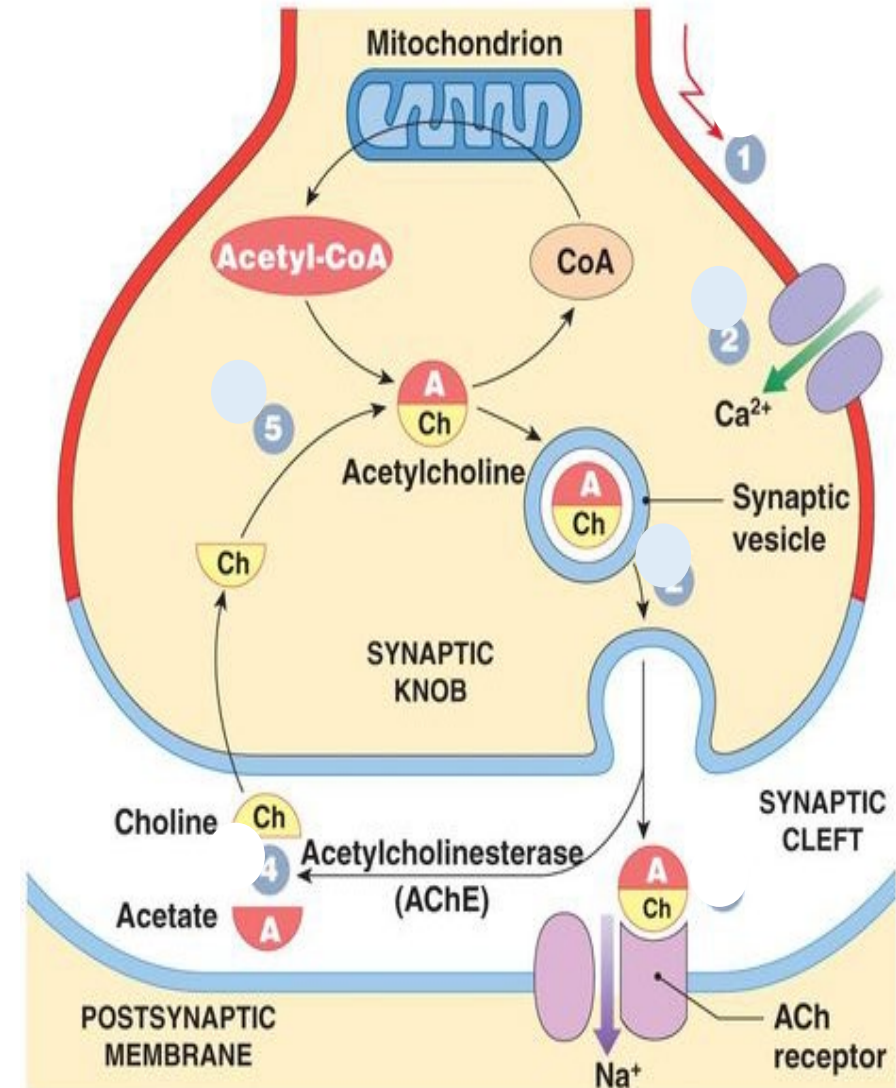
OP compounds have industrial, agricultural, household, and medicinal uses

Commonly used in pesticides, industrial solvents, pharmaceuticals, and chemical-warfare nerve agents (CWNA)

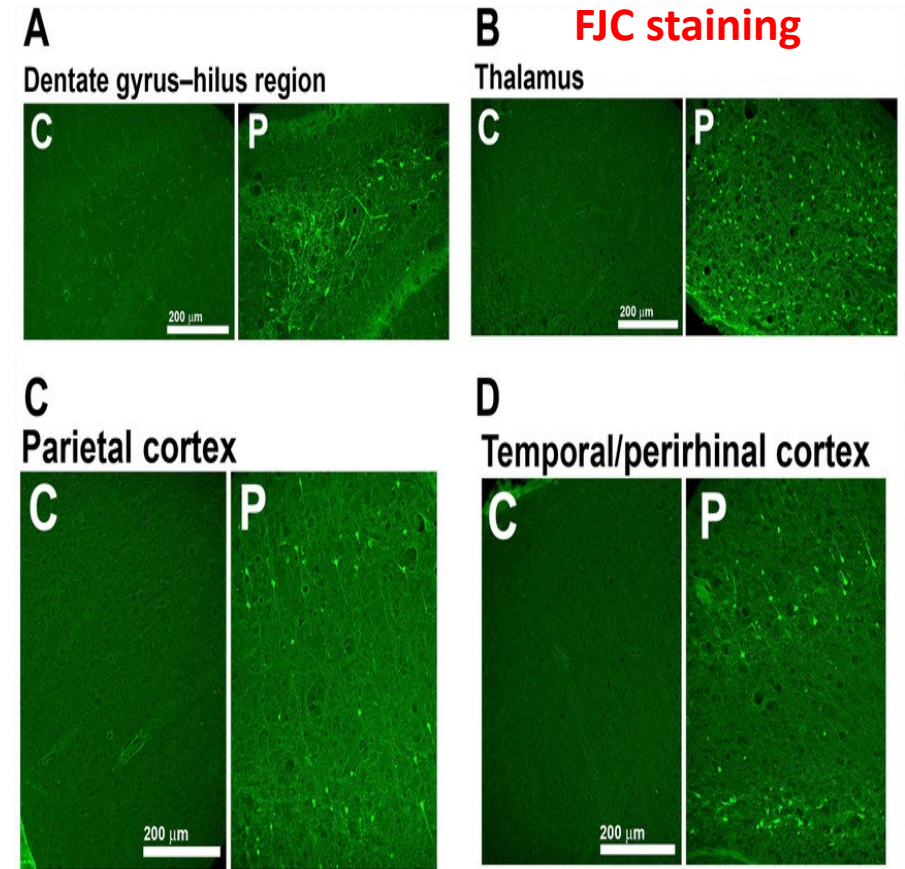
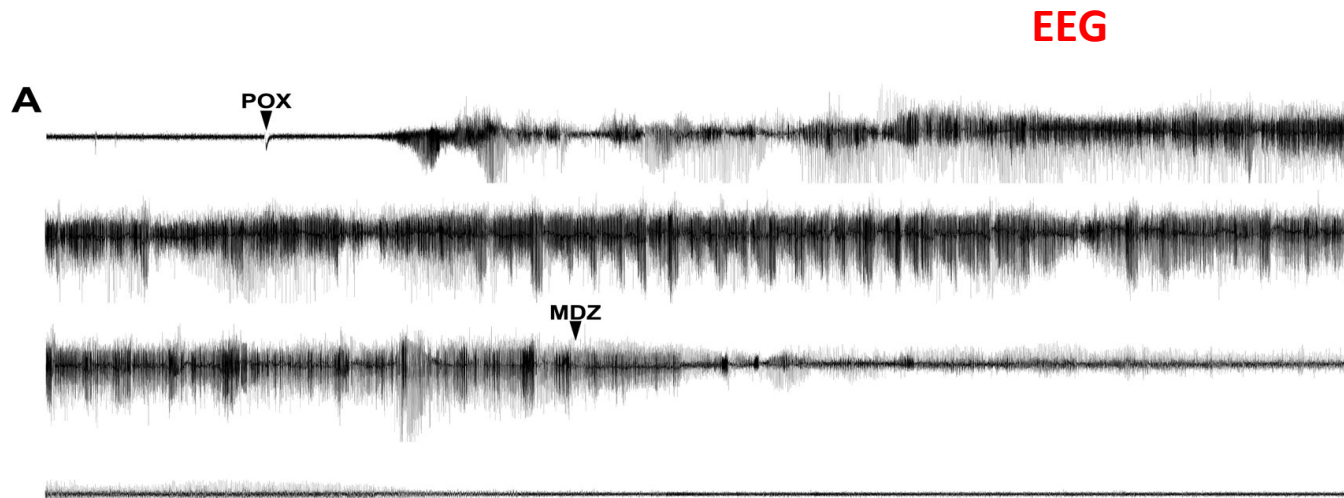
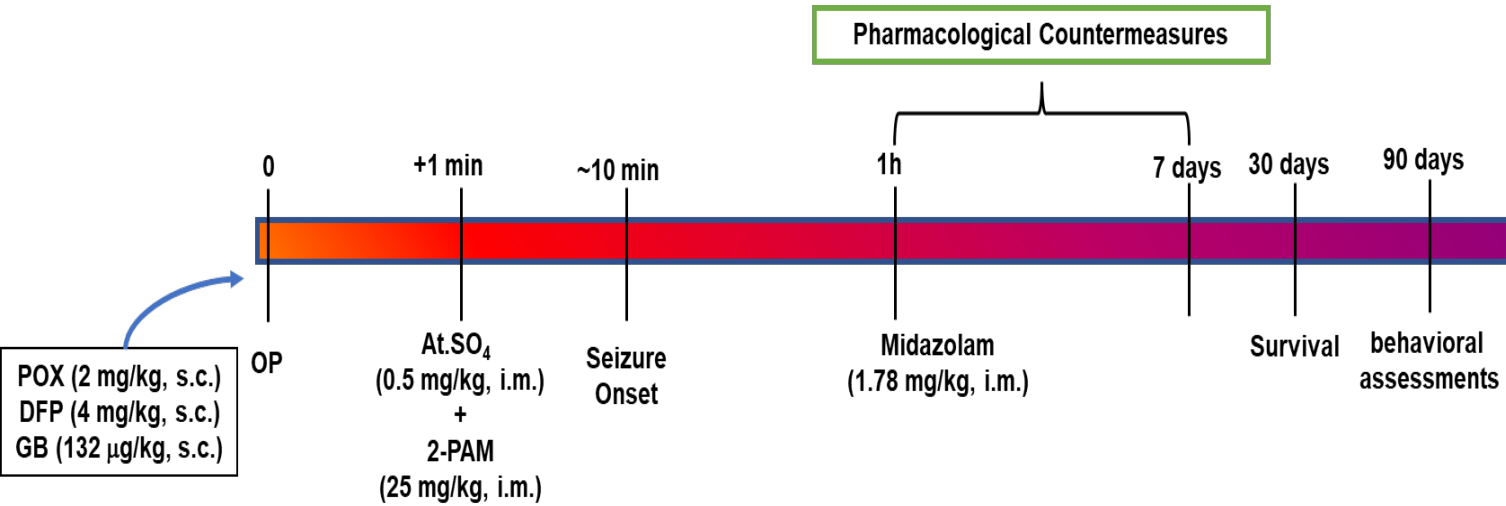
High-dose exposure is extremely toxic and rapid death occurs in the absence of emergency care (Atropine, 2-PAM, Midazolam: *SOC*)

Acute exposure symptoms include SLUDS, seizures, cardiac and respiratory effects

Survival despite *SOC* treatment is associated with long-term neurological morbidities including mood and memory dysfunction and recurrent seizures

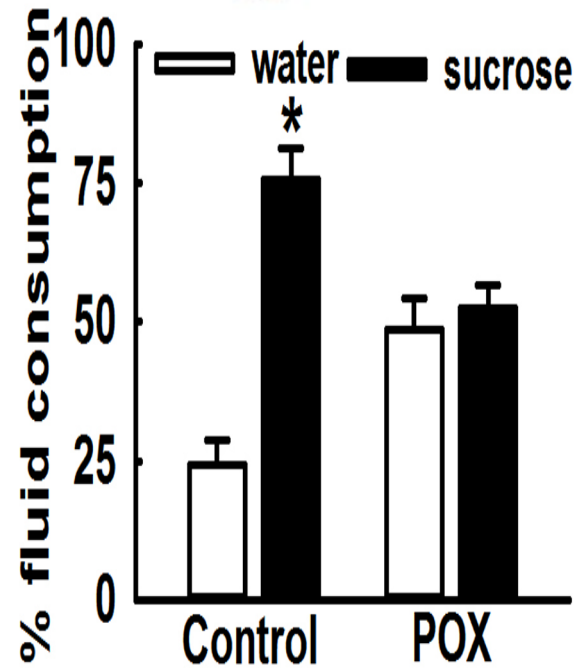
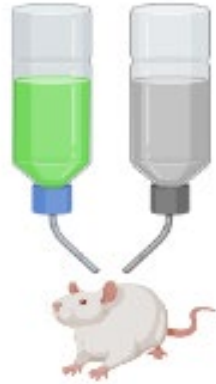


# Rat Model of OP-SE

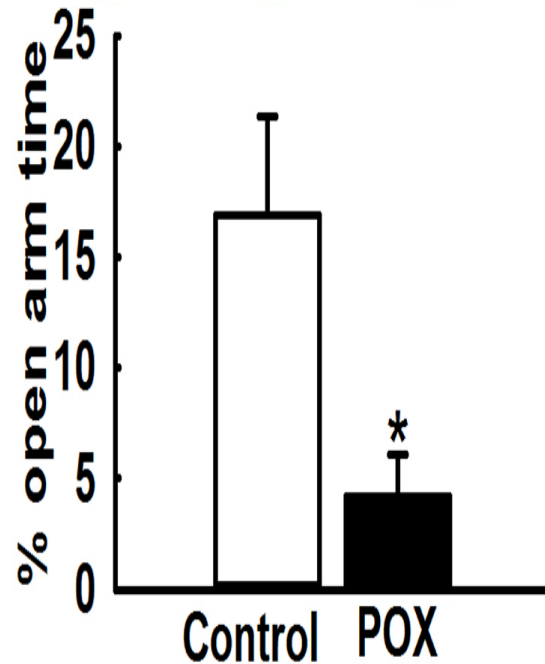
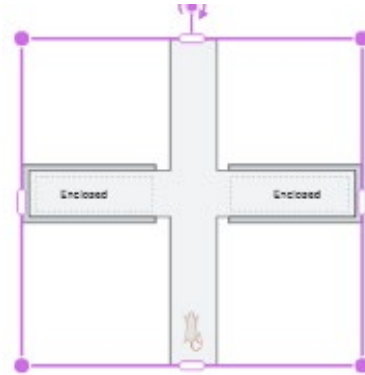


# Long-term morbidities following POX SE

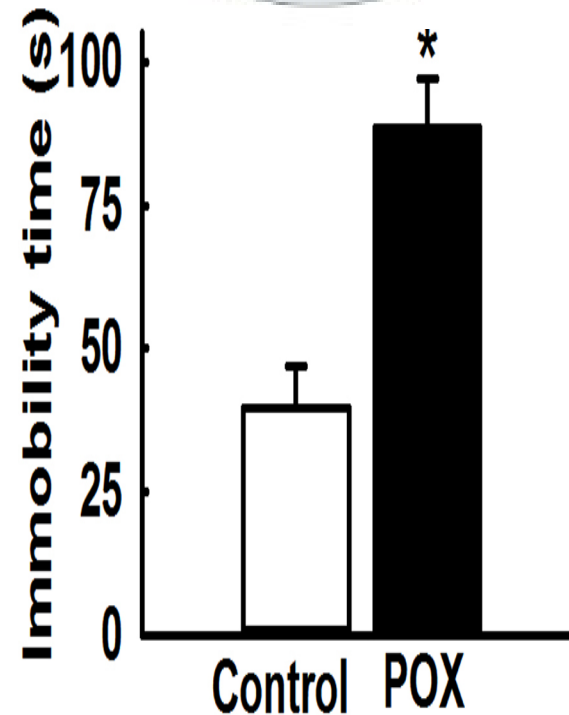
**SPT**  
(anhedonia)



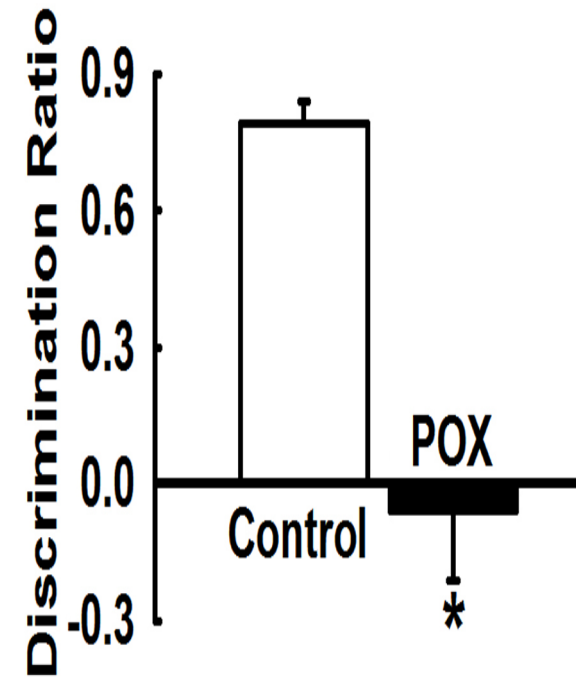
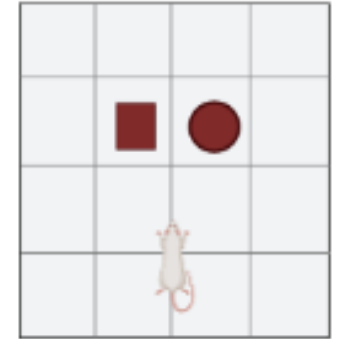
**EPM**  
(anxiety)



**FST**  
(despair)

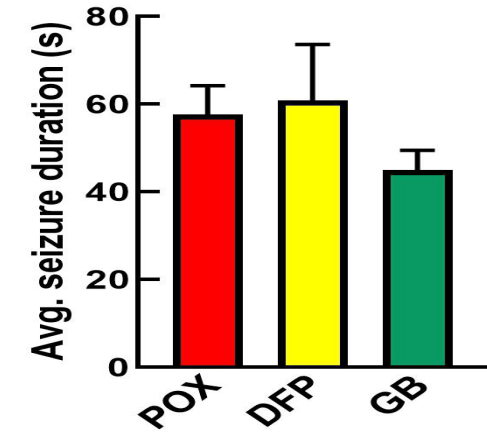
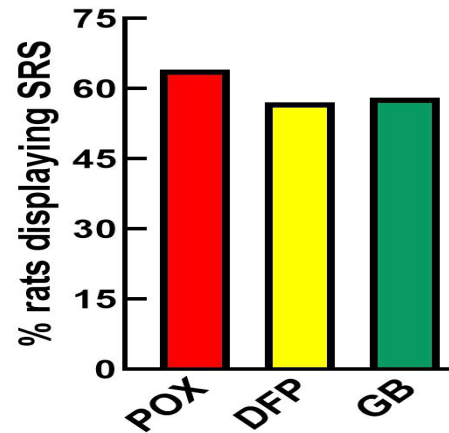
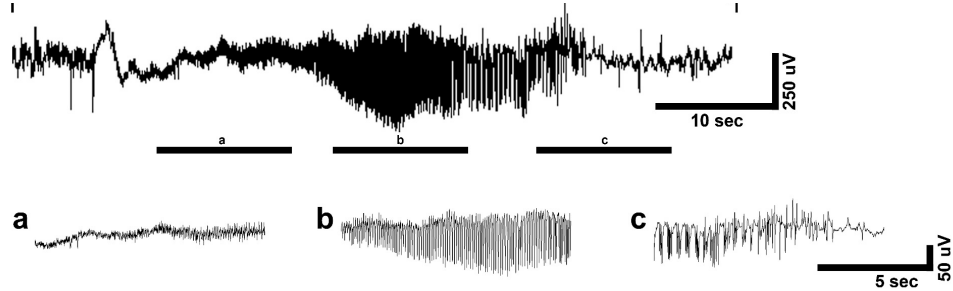


**NOR**  
(memory)

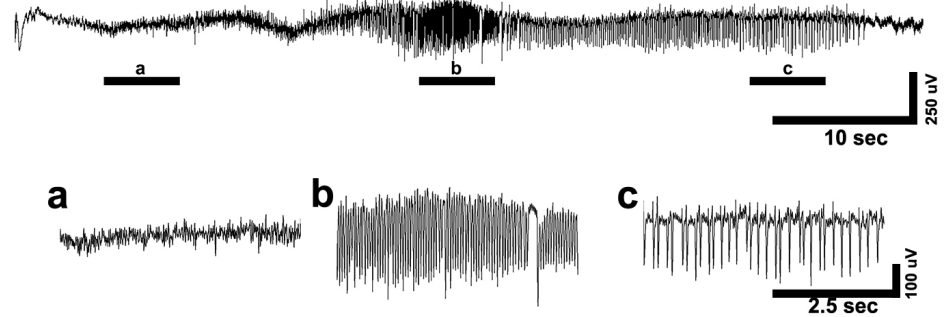


# Chronic SRS following OP SE

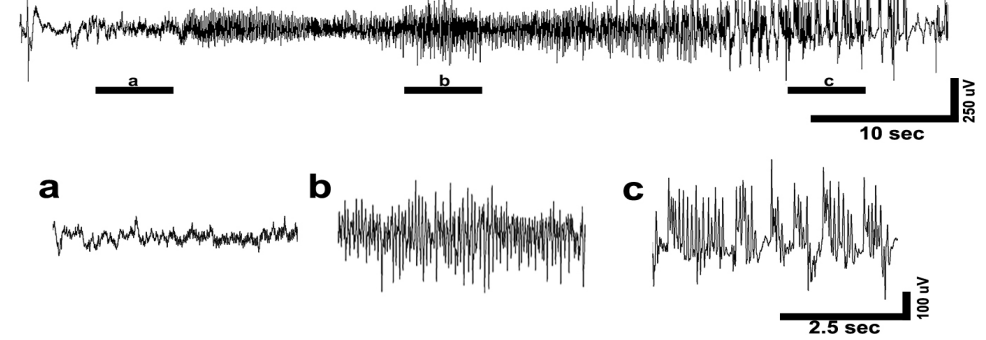
POX



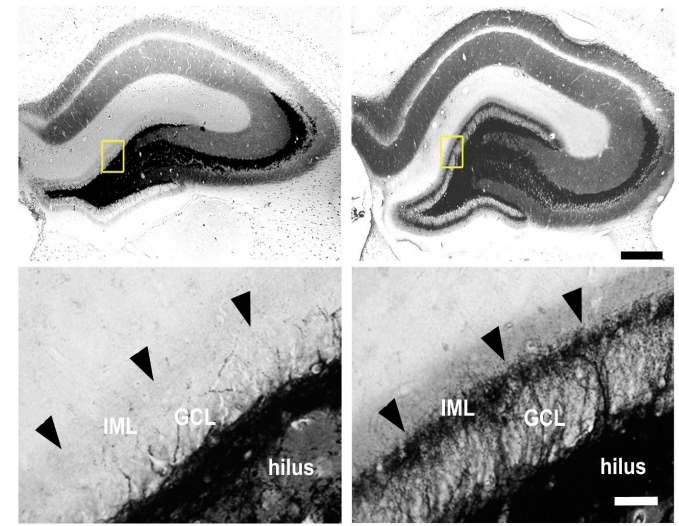
DFP



GB



(Mossy Fiber Sprouting)  
CONTROL PARAOXON



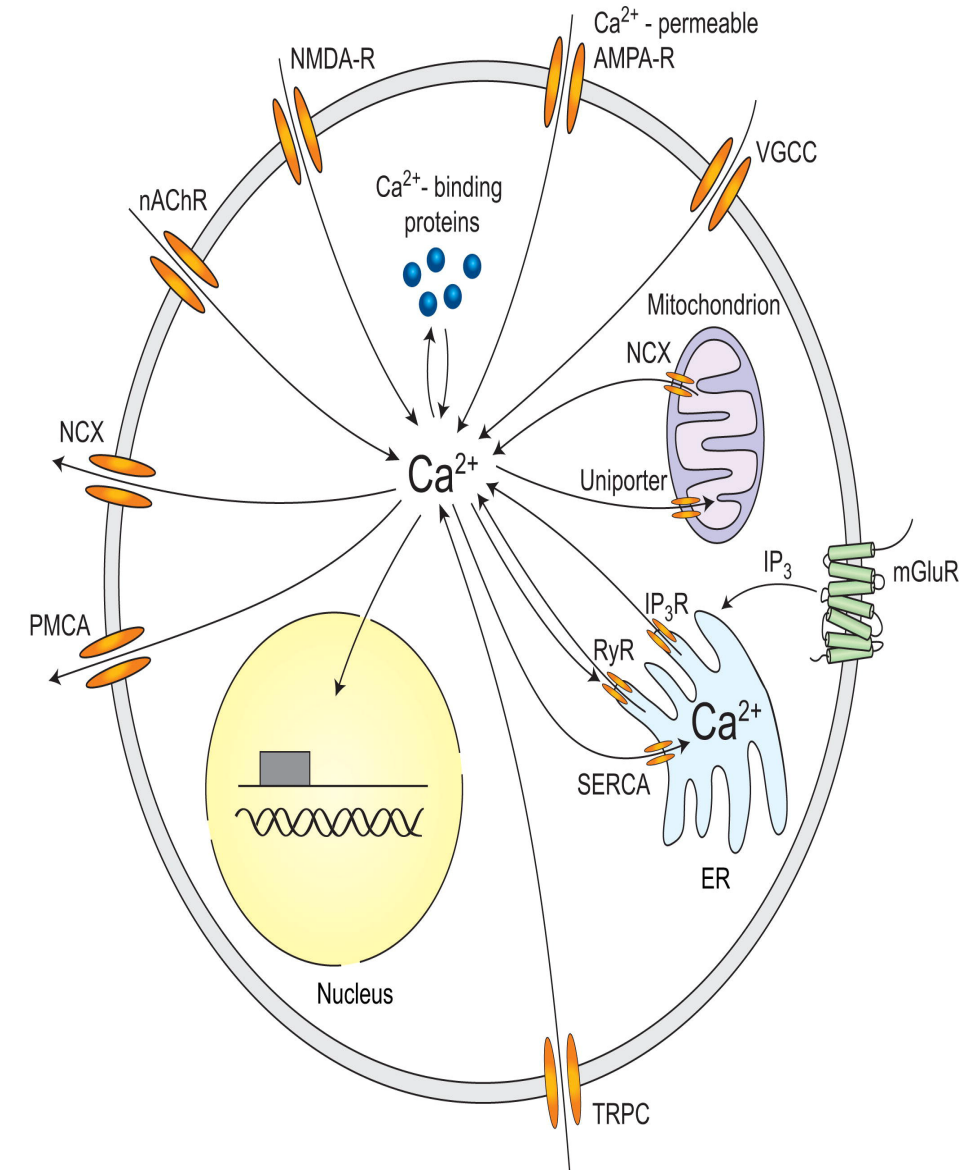
Around 60-65% of rats develop SRS following OP-SE

# Neuronal Calcium Homeostasis

Calcium ions charge carriers across the membrane, a ubiquitous second messenger

Governs many cellular functions, excitability, differentiation, exocytosis, synaptic activity

Ca<sup>2+</sup> homeostasis is careful coordination between entry, exit, uptake and buffering mechanisms



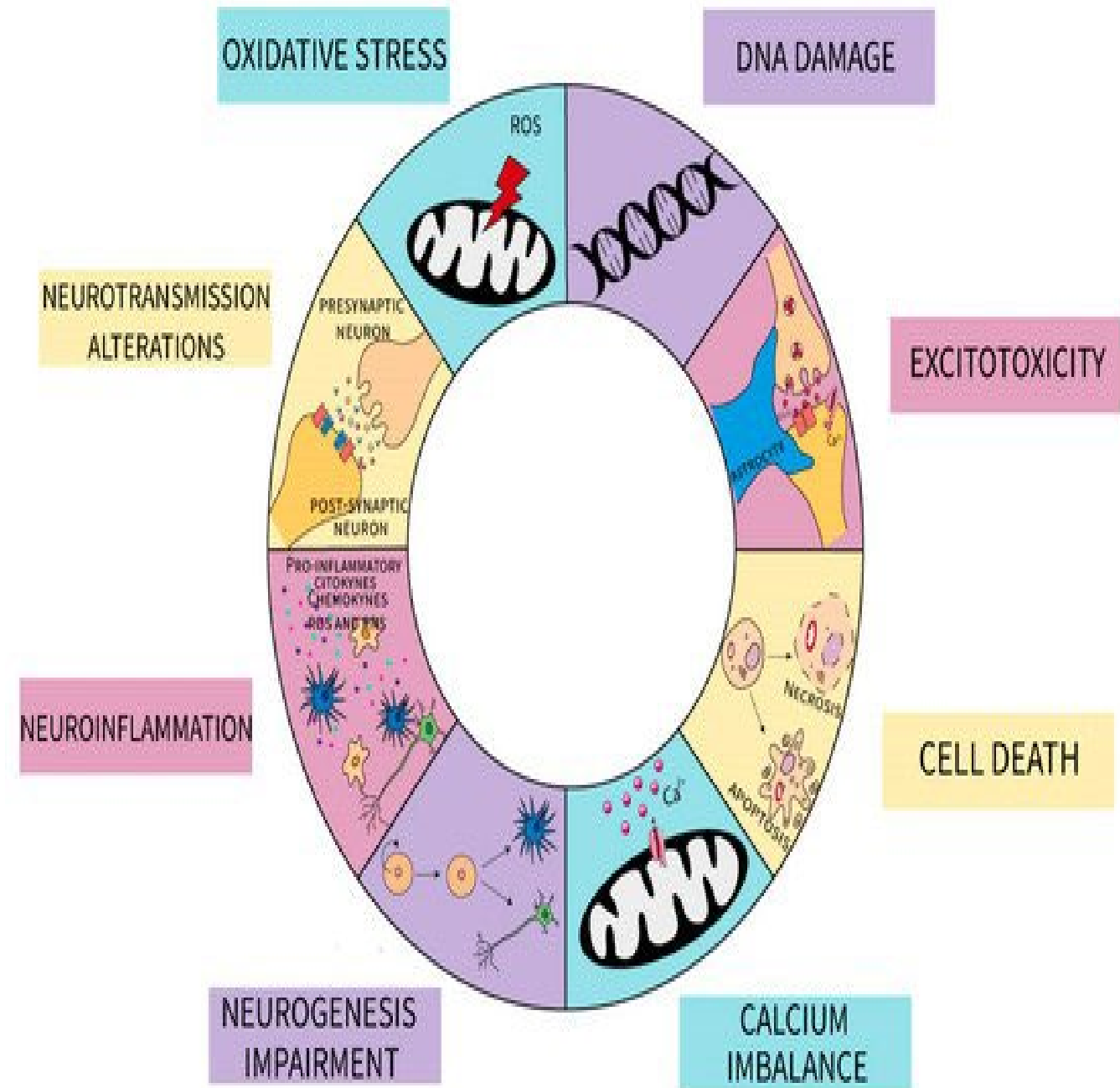
# Calcium Toxicity

Prolonged  $\text{Ca}^{2+}$  elevations produce acute neuronal injuries

Sustained  $\text{Ca}^{2+}$  elevations trigger activate degradative pathways

Affects the expression of genes in synaptic plasticity mechanisms

Long-term neurological morbidities such as acquired epilepsy, depression, PD, AD





# Ca<sup>2+</sup> Dynamics following SE

Initial insult, Instantaneous    Acute seizures, Hours    Epileptogenesis, Months, years    Epilepsy, Persistent

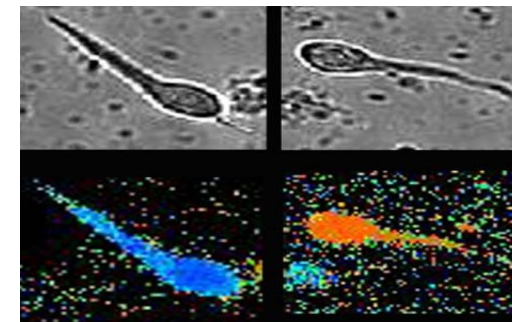
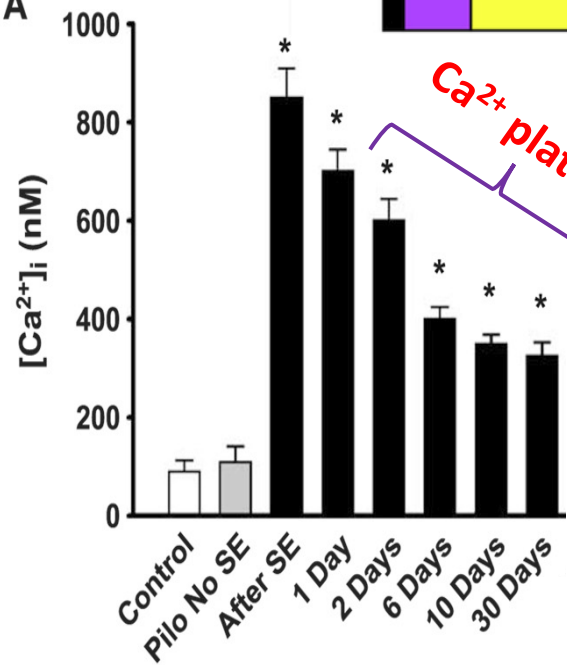
Neuronal calcium levels rise after SE

Calcium levels start to recover over the next few days

Does not recover completely: "Ca<sup>2+</sup> Plateau"

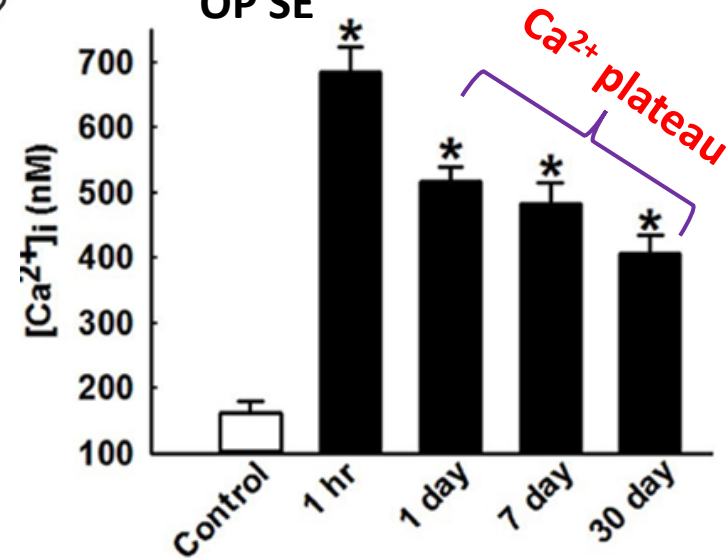
Ca<sup>2+</sup> Plateau also seen after OP-SE (DFP, POX models)

A Pilo SE

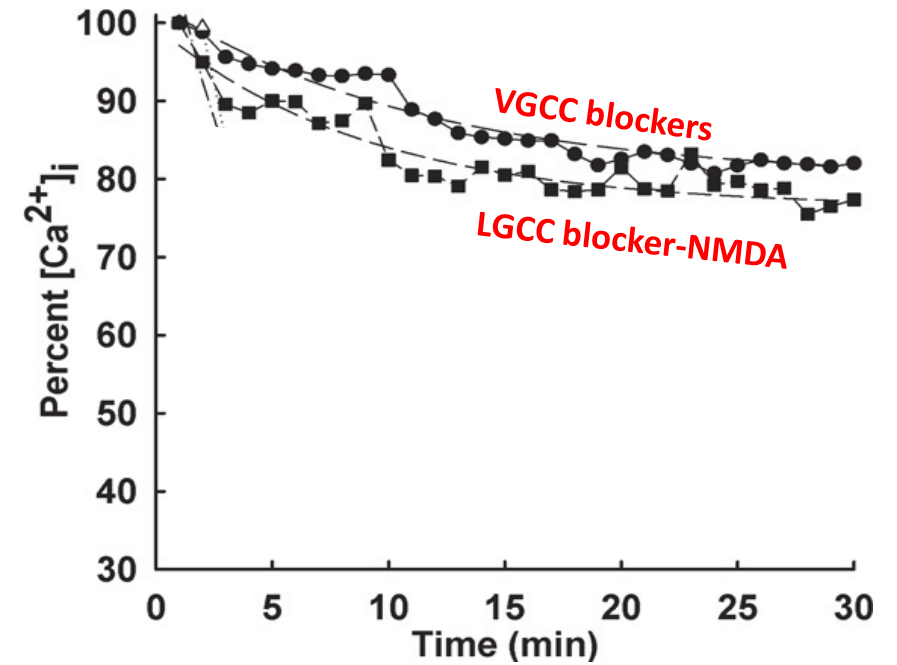
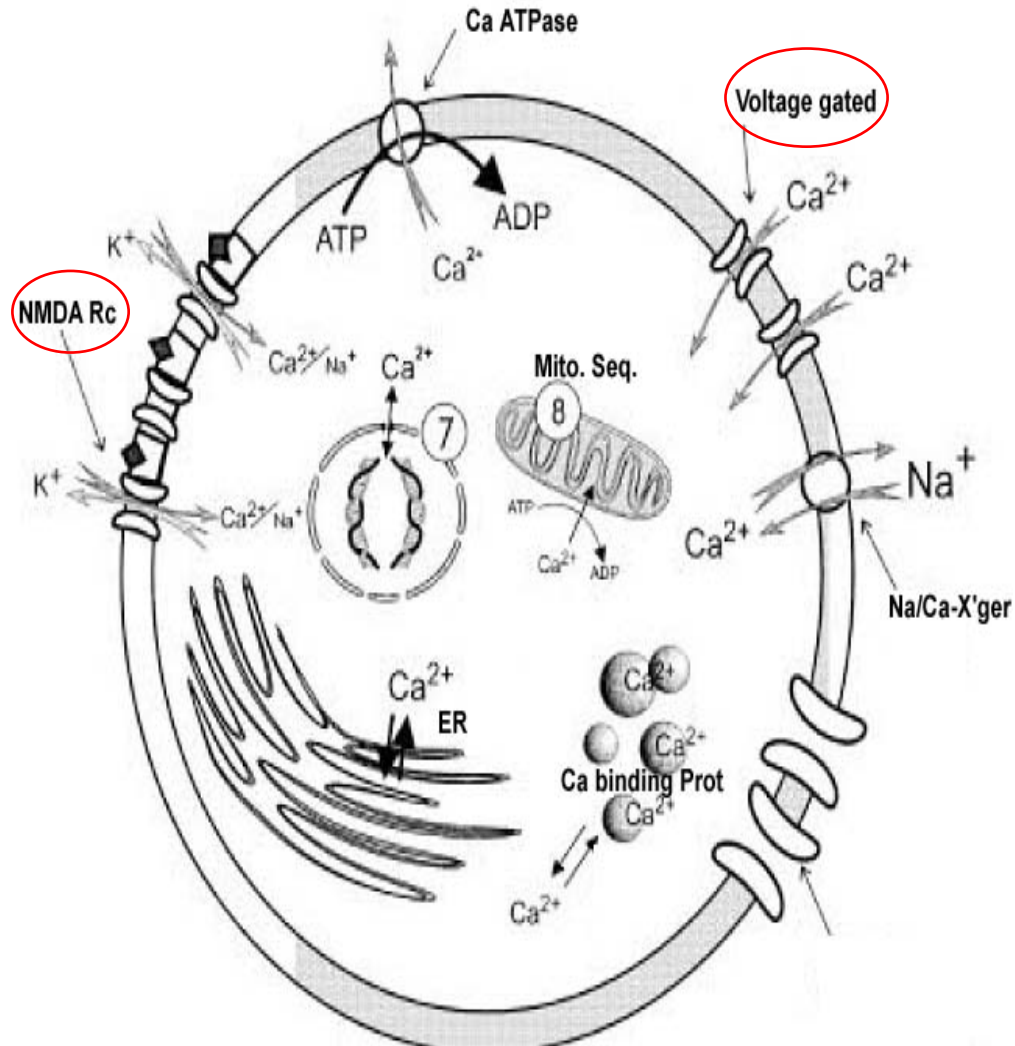


CA1 Fura-2 ratiometric

OP SE



# Calcium entry blockers do not lower OP-SE $\text{Ca}^{2+}$ elevations



(Nifedipine, MK-801, DNQX,  $\text{Gd}^{3+}$ )

# Effect of NMDAR antagonism on SE Calcium

## Intervention with MK-801

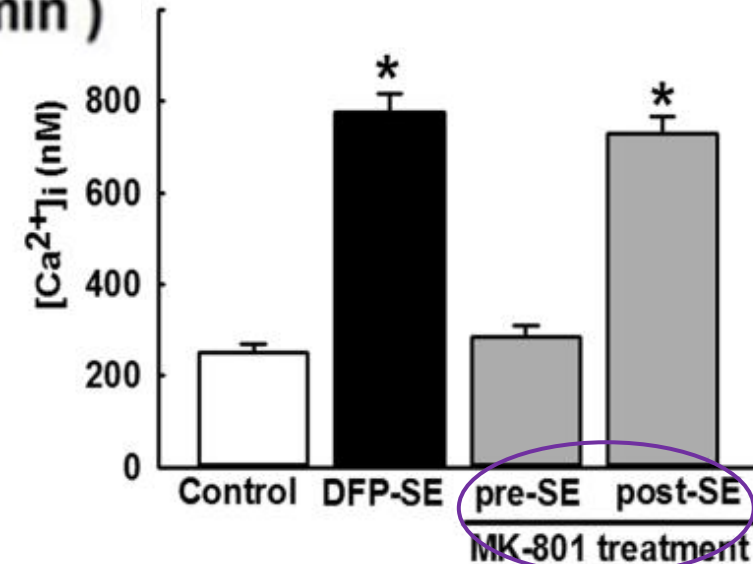
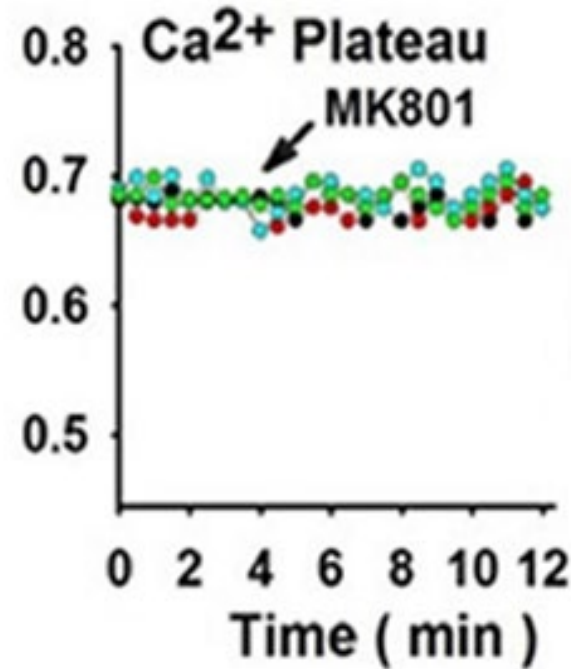
Before SE: blocks  $\text{Ca}^{2+}$  increases

After SE: no effect on  $\text{Ca}^{2+}$  elevations

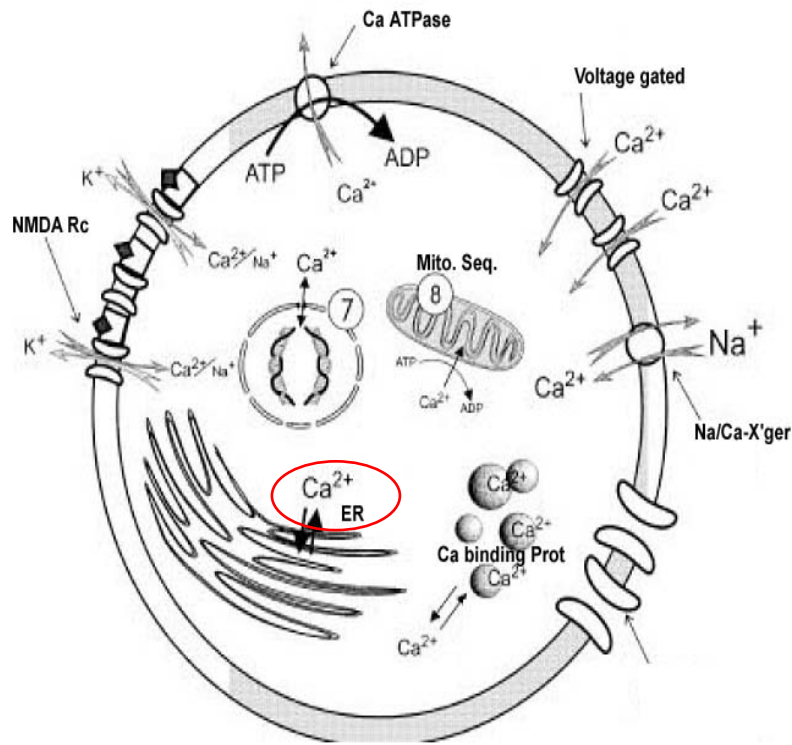
Prophylaxis works, treatment doesn't

Induction but not the maintenance of  $\text{Ca}^{2+}$  plateau dependent on NMDAR

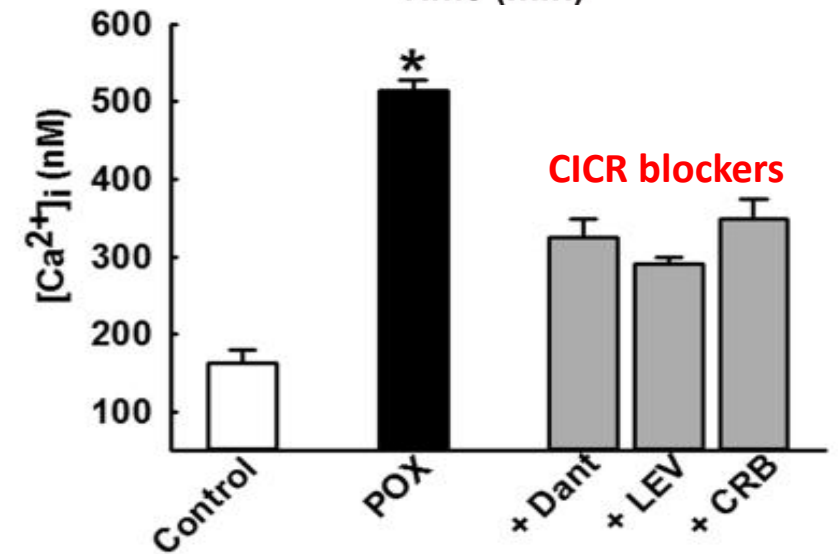
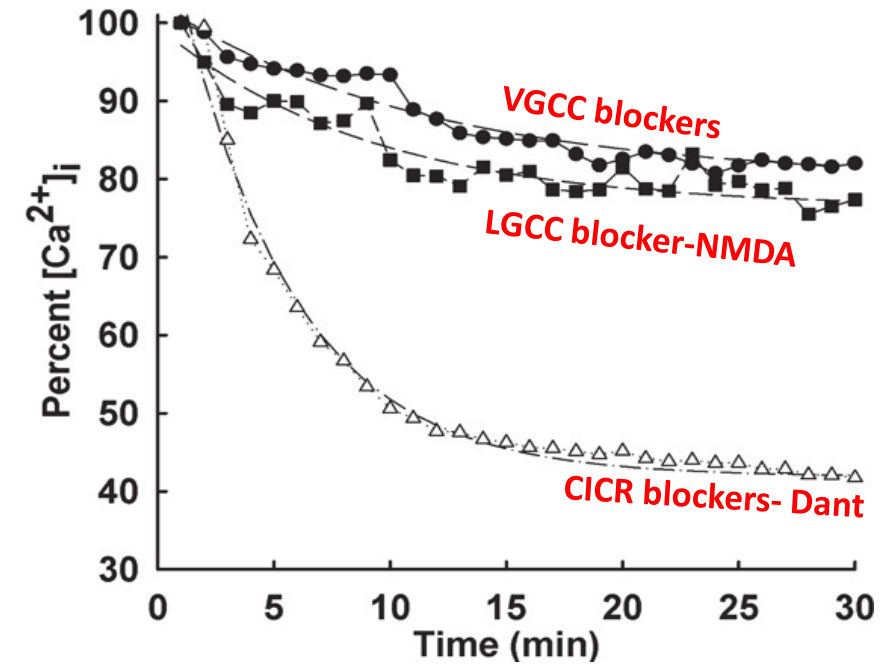
What maintains  $\text{Ca}^{2+}$  plateau after SE?



# Ca<sup>2+</sup> Plateau is Maintained by Intracellular Ca<sup>2+</sup> release



Treatment with blockers of intracellular Ca<sup>2+</sup> release lowers elevated Ca<sup>2+</sup> levels (Dantrolene, Levetiracetam, Carisbamate)

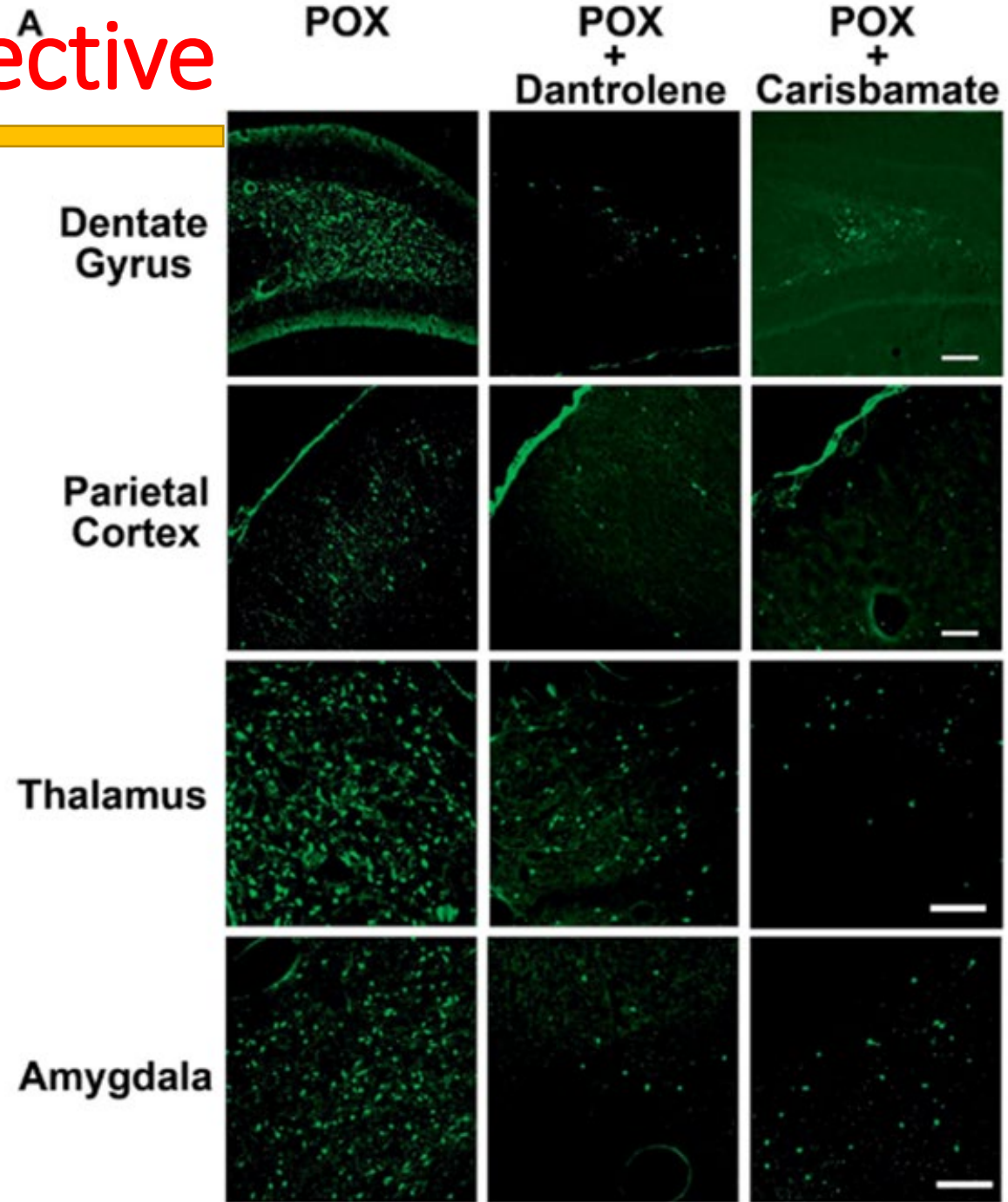
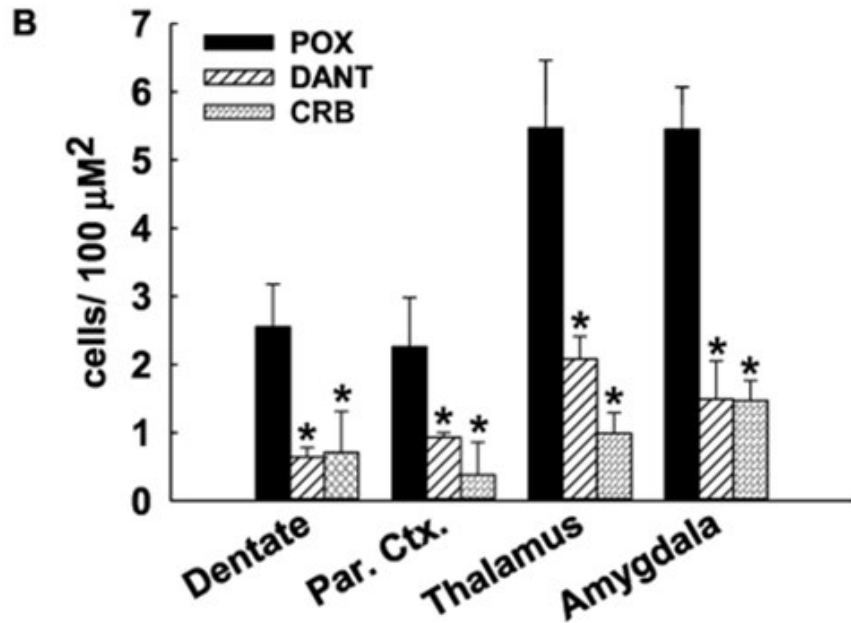


# CICR inhibitors are neuroprotective

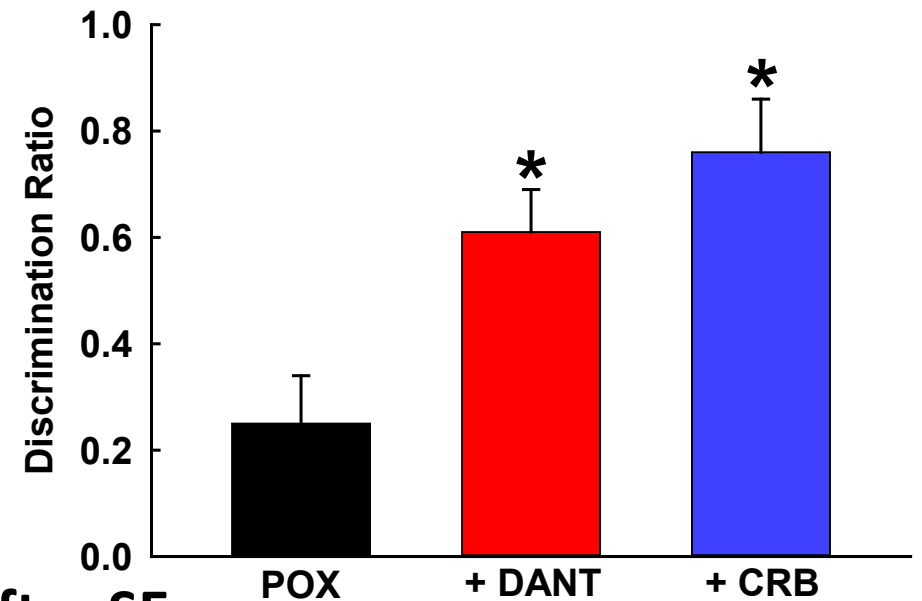
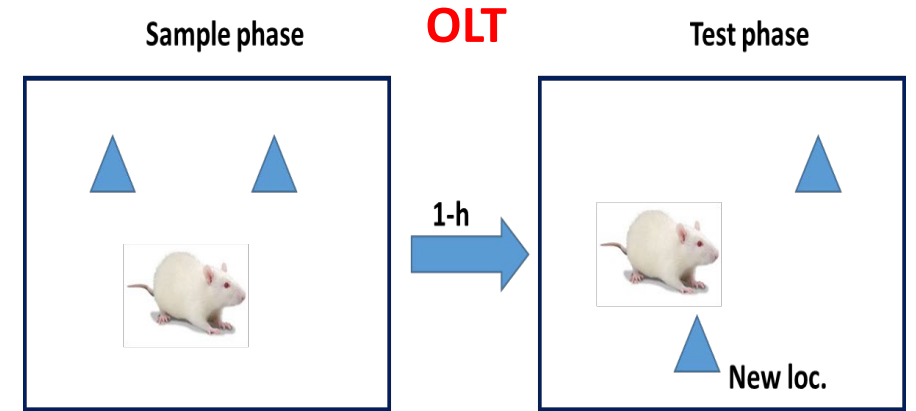
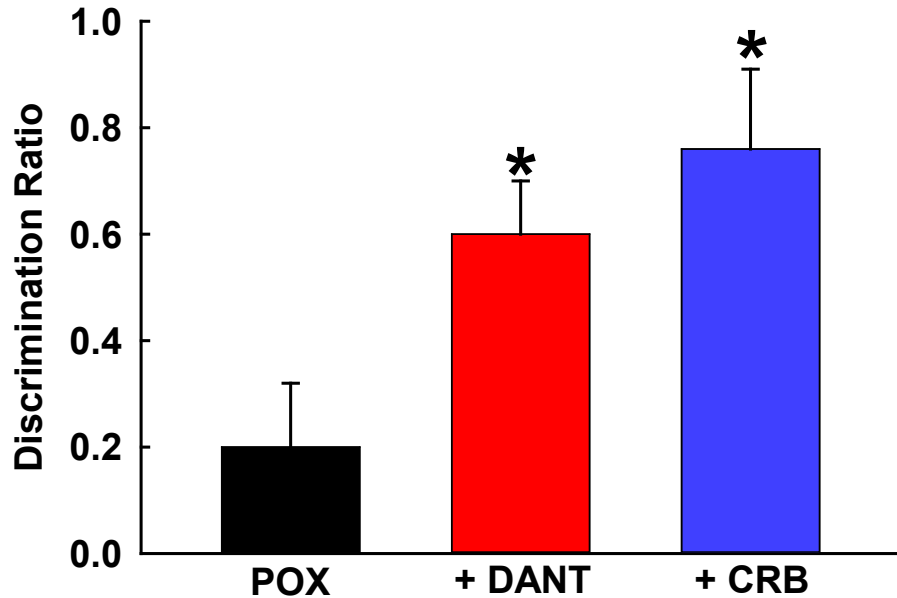
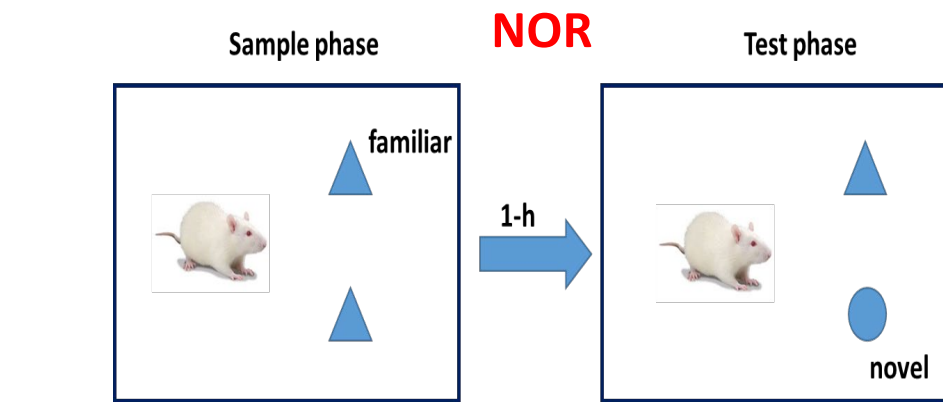
Extensive neuronal injury throughout the brain following OP SE

Treatment with CICR blockers: dantrolene or carisbamate 1-h after OP-SE

Decreased FJC staining in the presence of CICR blockers



# RyR/ IP3R inhibition improved long-term OP-SE cognitive outcomes

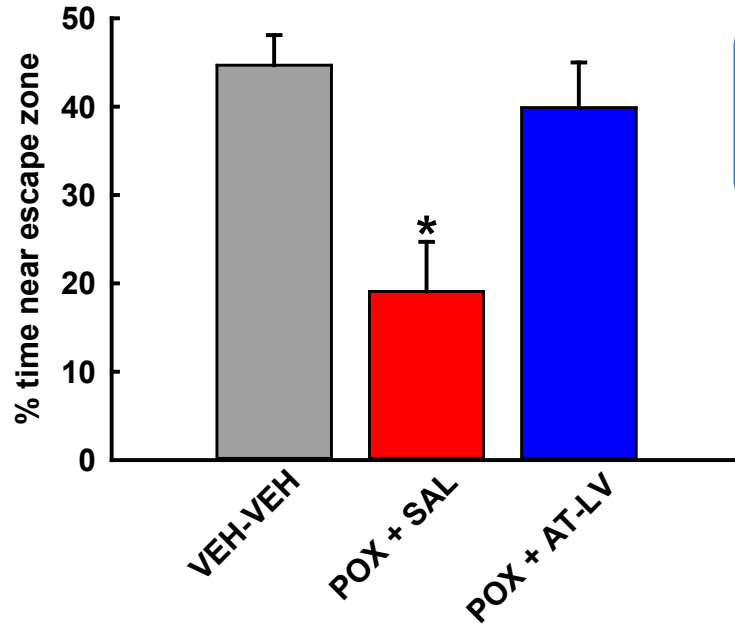
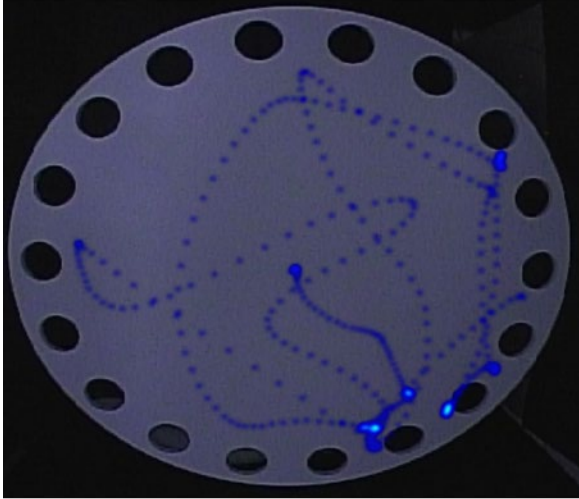


**DANT (10 mg/kg) or CRB (90 mg/kg) *b.i.d.* for 7-days after SE**

**Memory assessments at 3-months post OP-SE**

# RyR/ IP3 inhibition improved cognitive outcomes on the BMT

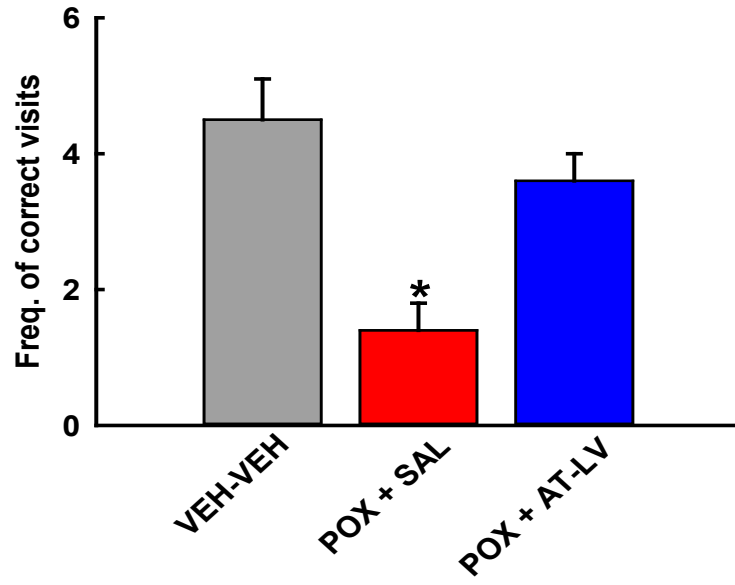
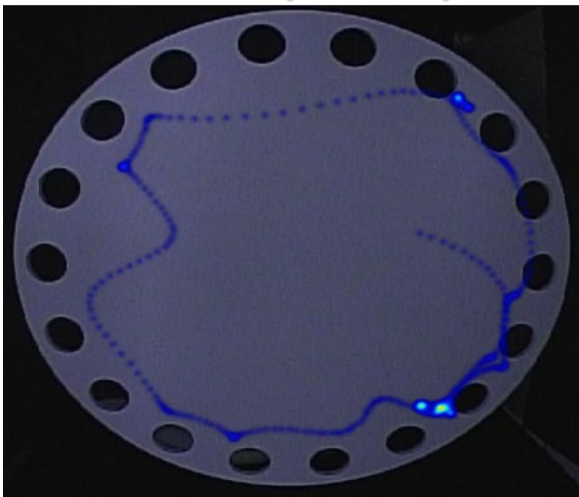
POX + SAL



AT + LV combination improved long-term memory outcomes after POX SE

AT: atenolol to improve cardiac function  
LV: levetiracetam as RyR/ IP3 inhibitor

POX + (AT+LV)



AT (5 mg/kg) + LV (50 mg/kg), i.m.,  
b.i.d., 7-days, beginning 1-h post OP-SE

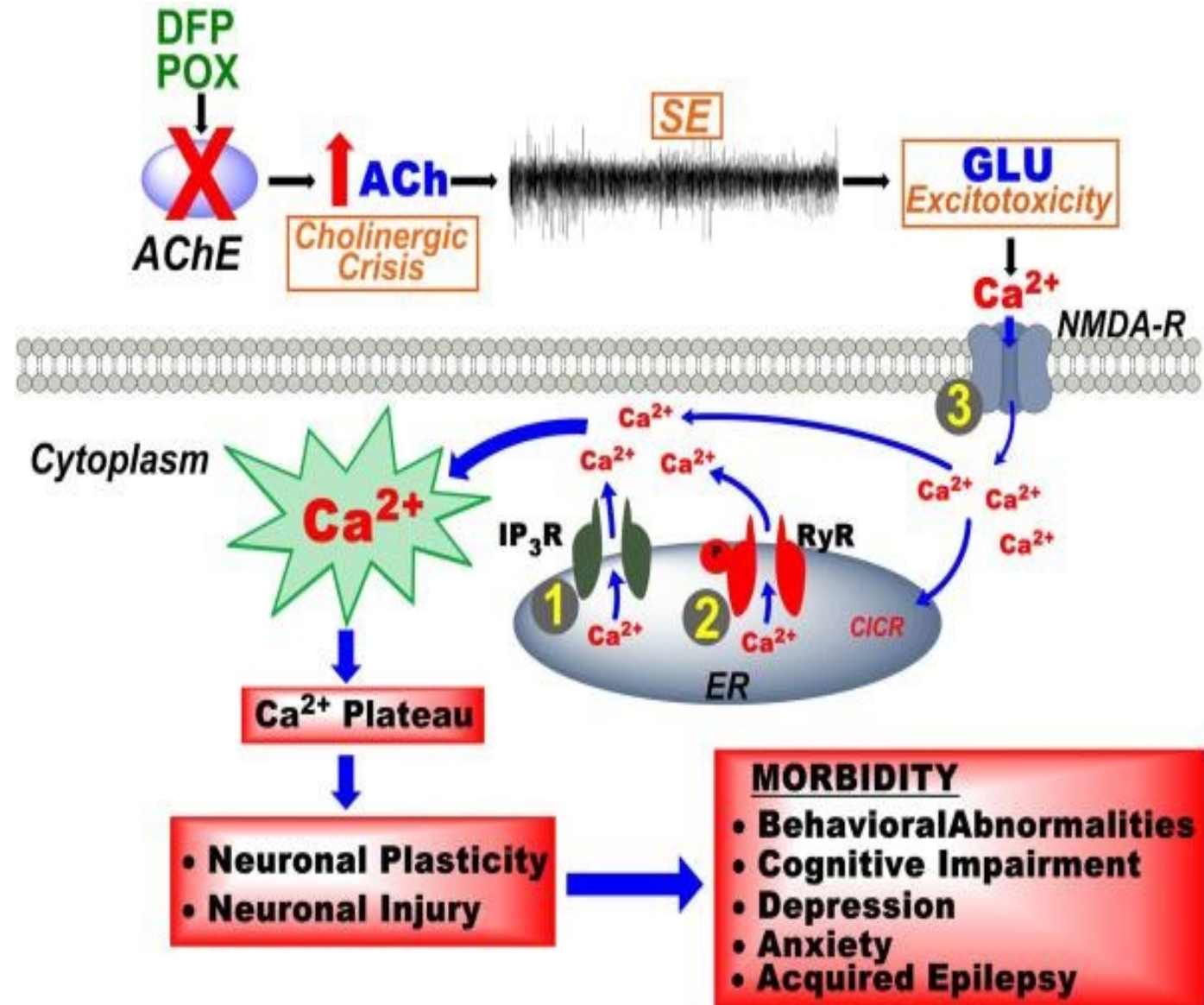
# Conclusions and Working Hypothesis

Neuronal  $\text{Ca}^{2+}$  increases after SE and stays elevated for weeks

Sustained ER  $\text{Ca}^{2+}$  release maintains the  $\text{Ca}^{2+}$  Plateau

CICR underlies the expression of OP SE morbidities

Blocking CICR after SE lowers elevated  $\text{Ca}^{2+}$  and improves behavioral outcomes





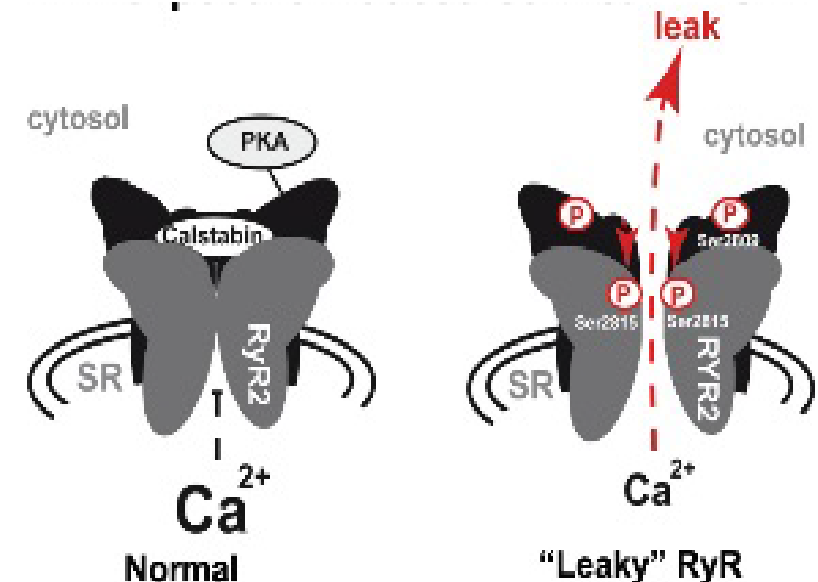
# Current Studies

Ongoing studies addressing SRS outcomes following treatment with CICR inhibitors in OP-SE

Proof-of-Concept studies indicate that RyR/ IP3 antagonism lowers OP-SE Ca<sup>2+</sup> elevations, is neuroprotective, and improves neurological outcomes after OP-SE onset.

What are molecular mechanisms responsible for the sustained release of Ca<sup>2+</sup> from ER?

- Low Dose DFP model
- “Leaky” RyR
- Post-translational modification in RyR
  - Increased pRyR2
  - Decreased Calstabin2
- Dendritic remodeling



# Challenges and Gaps

## Challenges with currently identified therapies

- **Dantrolene: Muscle relaxant**
- **Levetiracetam: Reports of aggression, combativeness, personality changes**
- **Carisbamate: Drug development on-hold, focused on LGS**

**We have a good target in RyR/ CICR but need to identify better Rx candidates**

# Conclusions

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OP-SE produces sustained neuronal  $\text{Ca}^{2+}$  elevations

Intracellular  $\text{Ca}^{2+}$  release is a dominant mechanism for this “ $\text{Ca}^{2+}$  Plateau”

$\text{Ca}^{2+}$  dysregulations participate in neuronal injury and chronic morbidities

RyR/  $\text{IP}_3\text{R}$  are attractive therapeutic targets for lowering OP-SE toxicities

# Acknowledgments

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- **Dr. Robert DeLorenzo**
- **Dr. Robert Blair**
- **Elisa Hawkins**
- **Dr. Ana Ribeiro**
- **Dr. Kristin Phillips**
- **Dr. Dawn Carter**

**CounterACT award number  
U01NS105058**



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