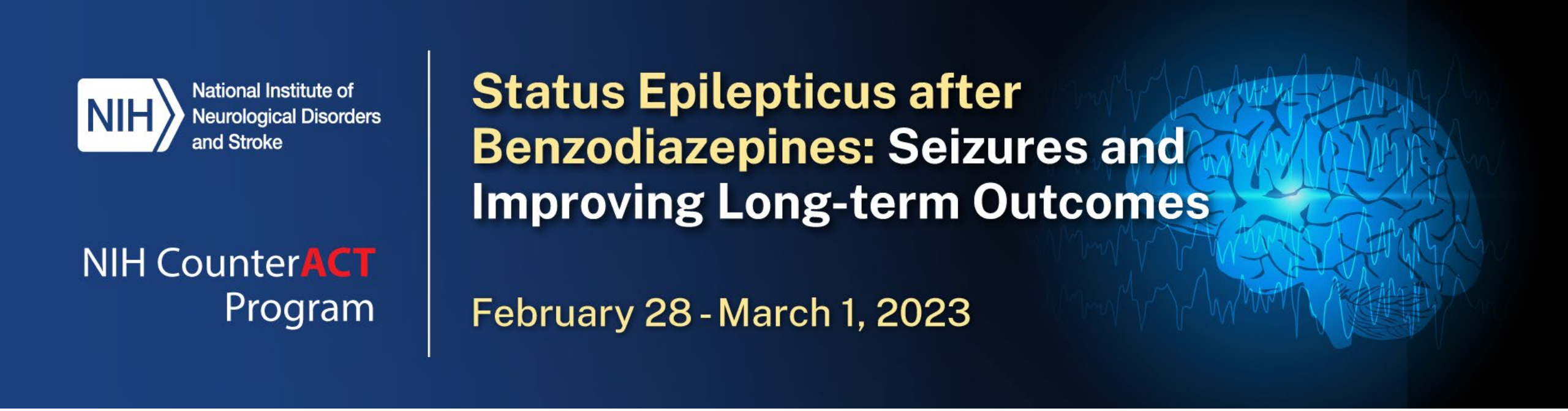
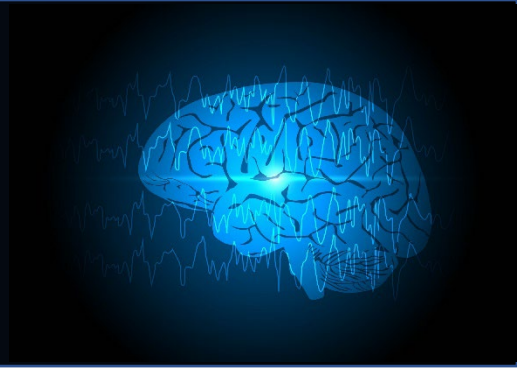


The Superior Neuroprotective Efficacy of Antiglutamatergic Therapy as First-Line Treatment Against Nerve Agent-Induced Status Epilepticus

Maria F. Braga, DDS, PhD,
Professor of Anatomy, Physiology and Genetics,

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



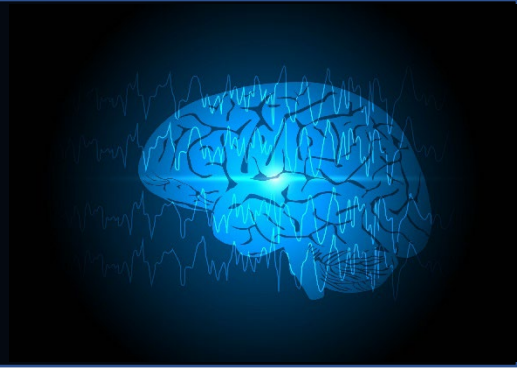
Disclaimer

This certifies that the views expressed in this presentation are those of the author and do not reflect the official policy or position of NIH, the Uniformed Services University of the Health Sciences, the Department of the Defense, or the United States government.

Disclosure

This certifies that I, Maria F. Braga, have no financial relationship that is relevant to the subject matter of the presentation.

Clinical manifestations of acute exposure to nerve agents in Humans



Acute Intoxication:

Miosis, bronchospasm, bronchorrhea (increased respiratory secretions), involuntary fasciculations of skeletal muscles leading to flaccid paralysis, and, in severe intoxication cases, status epilepticus (SE), central apnea and death.

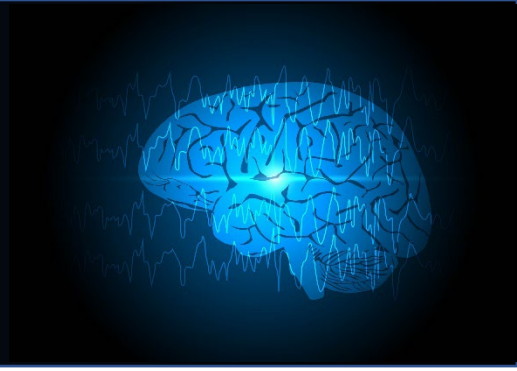
CNS-related acute effects include anxiety, restlessness, confusion, ataxia, tremors, **seizures, status epilepticus (SE)**, and central cardiorespiratory paralysis.

Long-term deficits:

Surviving victims of nerve agent exposures develop **neurological and psychiatric illnesses** that last for decades after the attack:

Mood disorders, specifically anxiety and depression, significant EEG alterations, some of which were consistent with recurring electrical seizures, attention deficits, memory impairments.

The Limitations of Benzodiazepines



We have been utilizing benzodiazepines as a first-line treatment for SE of any etiology for a long time. The first benzodiazepine, chlordiazepoxide (Librium), was synthesized in 1955 by Leo Sternbach.

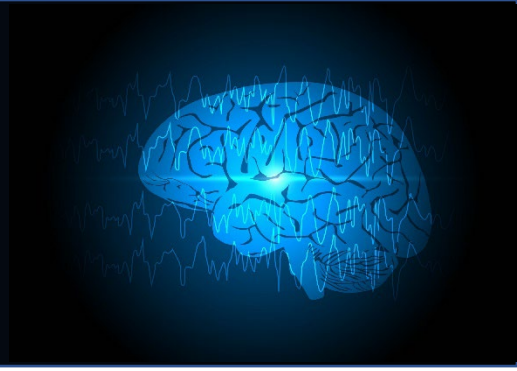
FDA has approved diazepam and, recently, midazolam for the treatment of SE induced by nerve agents.

However, clinical data derived from the treatment of SE of any etiology have indicated that **diazepam and midazolam control seizures only temporarily.**

Their antiseizure **efficacy is reduced as the latency of treatment from the onset of SE increases**, and their neuroprotective efficacy is limited or absent.

There is a need for the development of a new and safe anticonvulsant and neuroprotective therapy that is effective even when administered with a significant delay after nerve agent exposure.

Our Hypothesis



Exposure to a Nerve Agent

↓

Cholinergic Hyperstimulation
(Initiates Seizure Activity)

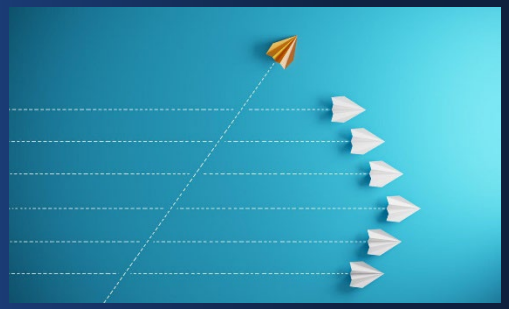
↓

Glutamatergic Hyperactivity
(Intensifies and Sustains Seizures)

↓

Brain Damage

Antiglutamatergic Agents can be effective against seizures induced by Cholinergic Hyperstimulation

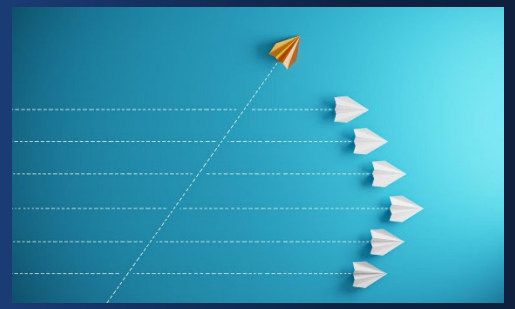


Paradigm shift

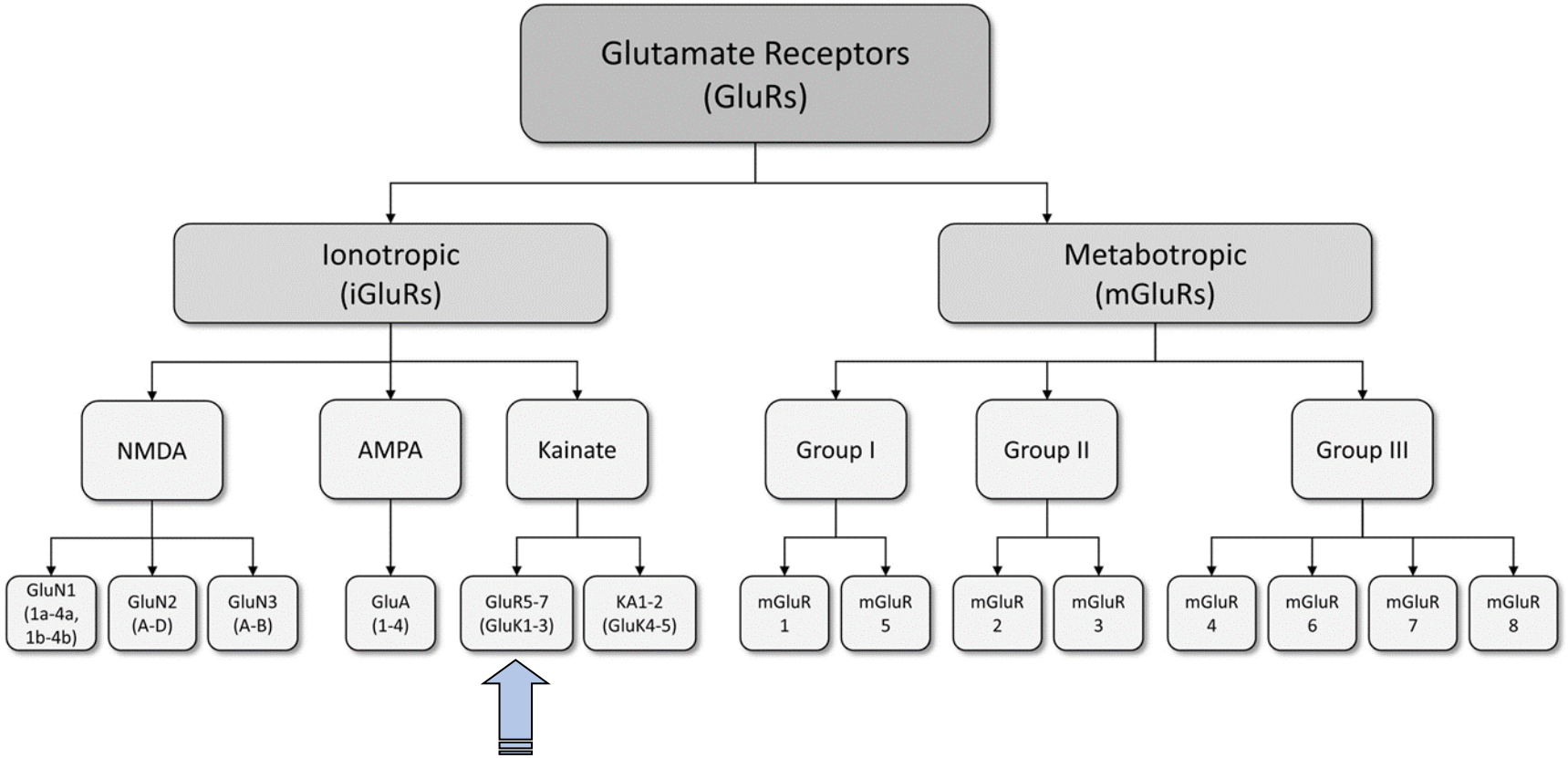
A fundamental change in the current therapeutic approach:

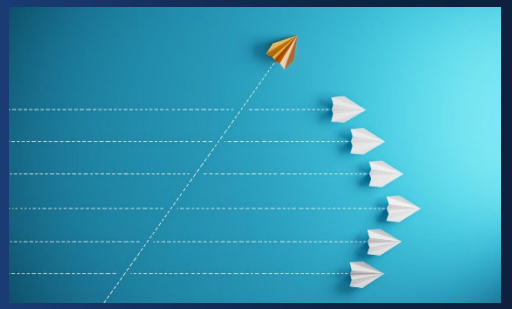
Instead of facilitating **GABAergic Inhibitory Transmission** to reduce the generalized hyperexcitability that causes seizures and SE after nerve agent exposure

Reduce the activity of the **Glutamatergic Excitatory Transmission** with Antiglutamatergic Agents that can be much more effective against nerve agent-induced hyperexcitability that causes seizures and SE

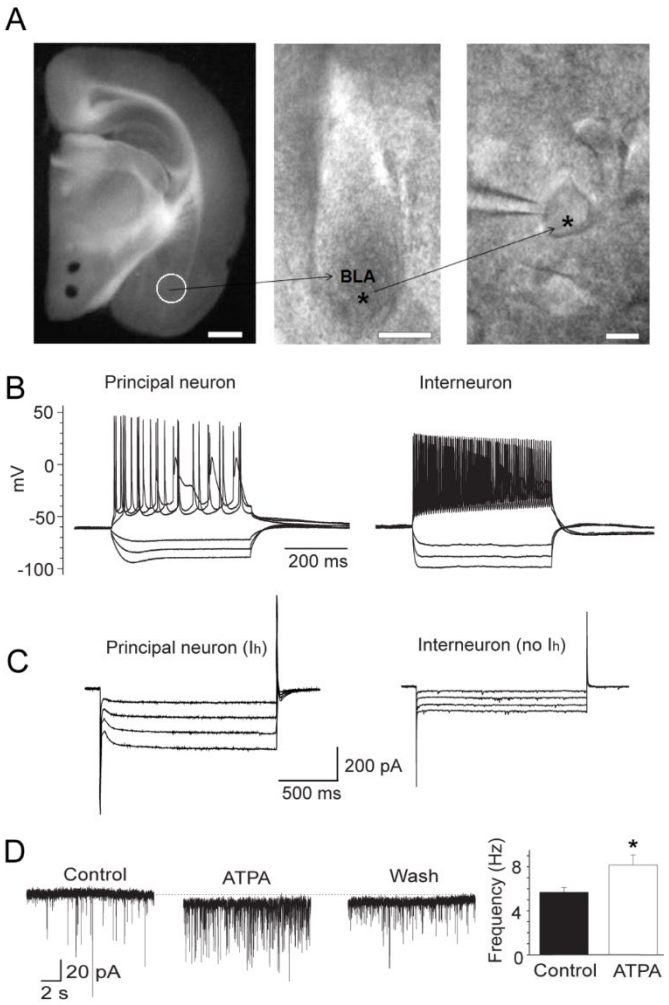


Gluk1 Kainate Receptors as a mechanism regulating neuronal excitability in the Basolateral Amygdala





Gluk1 Kainate Receptors as a mechanism regulating neuronal excitability in the Basolateral Amygdala



GluK1Rs are present on presynaptic terminals of principal neurons where they **facilitate the release of glutamate release**.

Activation of GluK1 Kainate Receptors generates epileptic activity in the amygdala and in the hippocampus.

The amygdala and the hippocampus play a critical role in the generation and spread of seizures in the brain.

GluK1 receptor antagonists prevent the initiation and block the expression of limbic seizures induced by intense electrical stimulation, or by administration of the muscarinic agonist pilocarpine.

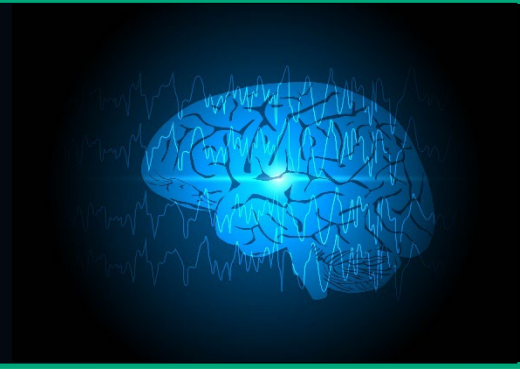
The risk of a terrorist attack in the United States involving **chemical agents** has created new challenges for many departments and agencies across the federal government.

Within the **Department of Health and Human Services (HHS)**, the **NIH** is taking a leadership role in pursuing the development of new and improved medical countermeasures designed to treat the conditions caused by chemical threat agents.

The CounterACT program is a translational research program supporting translational, and clinical research aimed at the discovery and identification of better therapeutic medical countermeasures against chemical threat agents.

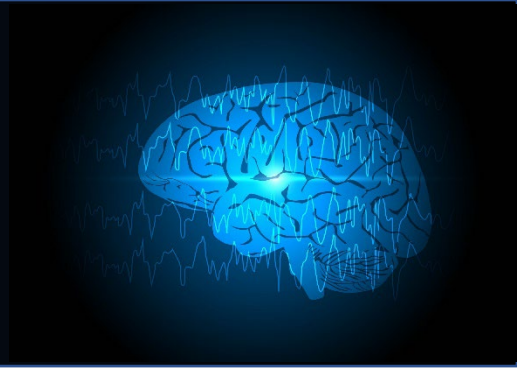
NIH National Institute of Neurological Disorders and Stroke

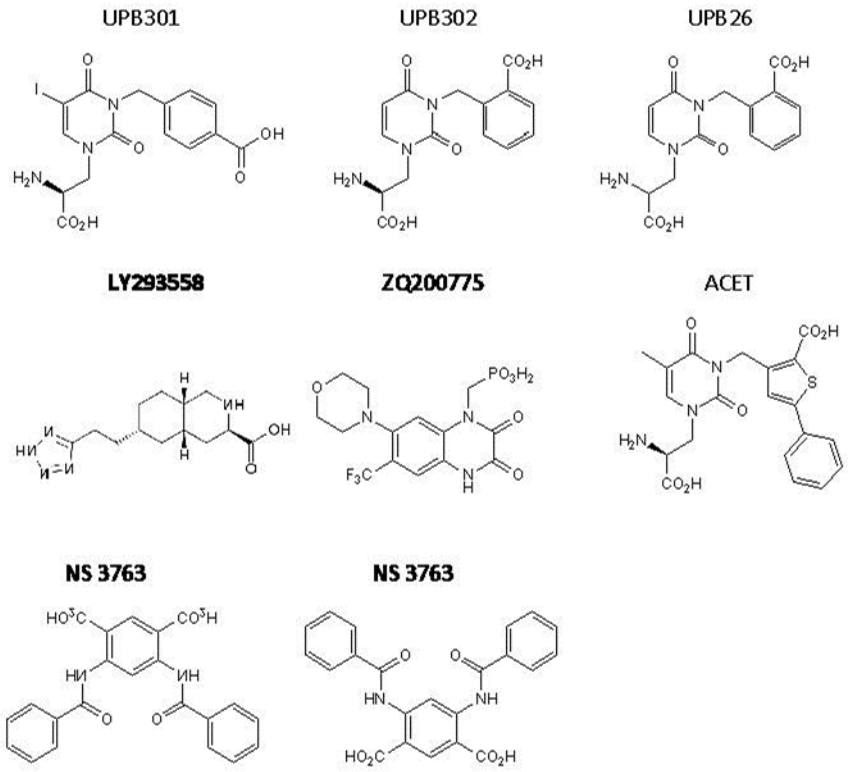
Central Hypothesis



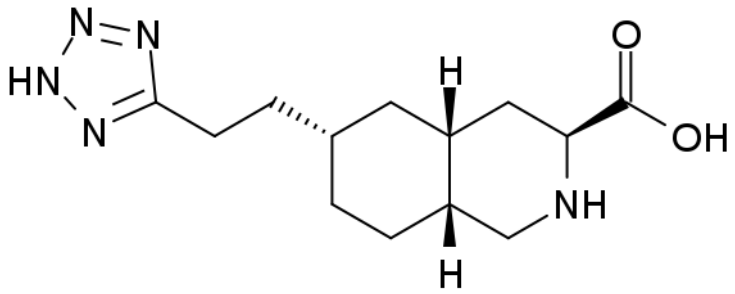
GluK1KR antagonists are effective in preventing the initiation of seizures by nerve agents, and in terminating already ongoing seizure activity and SE.

Gluk1 Kainate Receptor Antagonists





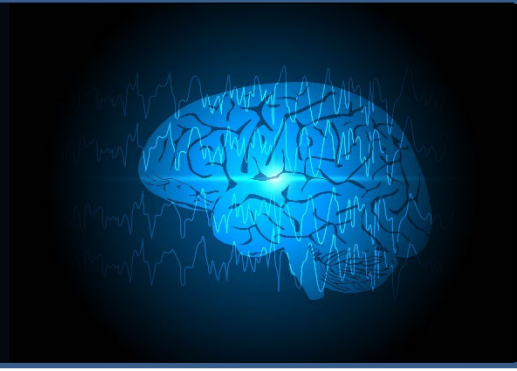
LY293558



LY293558 had undergone clinical trials and has been found efficacious in models of pain, including acute migraine (Gilron et al., 2000; Sang et al., 2004).

Well tolerated by patients.

The Hunt for LY293558



Decahydroisoquinolines: novel competitive AMPA/kainate antagonists with neuroprotective effects in global cerebral ischaemia

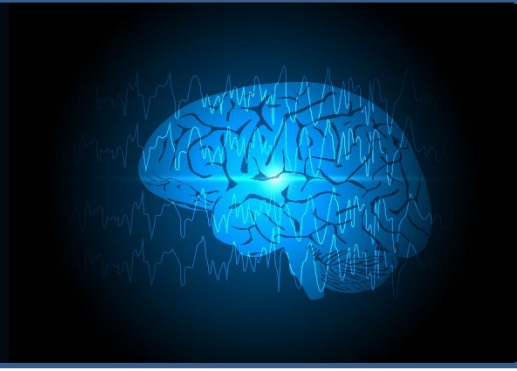
M J O'Neill [1], A Bond, P L Ornstein, M A Ward, C A Hicks, K Hoo, D Bleakman, D Lodge
Affiliations

Affiliation
Lilly Research Centre, Eli Lilly and Co. Ltd., Windlesham, UK. ONEILL_MICHAEL_J@Lilly.com

David Lodge, PhD and **Zuner A Bortolotto, PhD**

Centre for Synaptic Plasticity, School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom.

The Hunt for LY293558



raptor pharmaceutical corp.

Todd C. Zankel, PhD
Vice President and
Chief Scientific Officer
Raptor Pharmaceutical, Inc.
5 Hamilton Landing
Suite 160
Novato, CA 94949

Material Transfer Agreement (MTA)

I'm in business

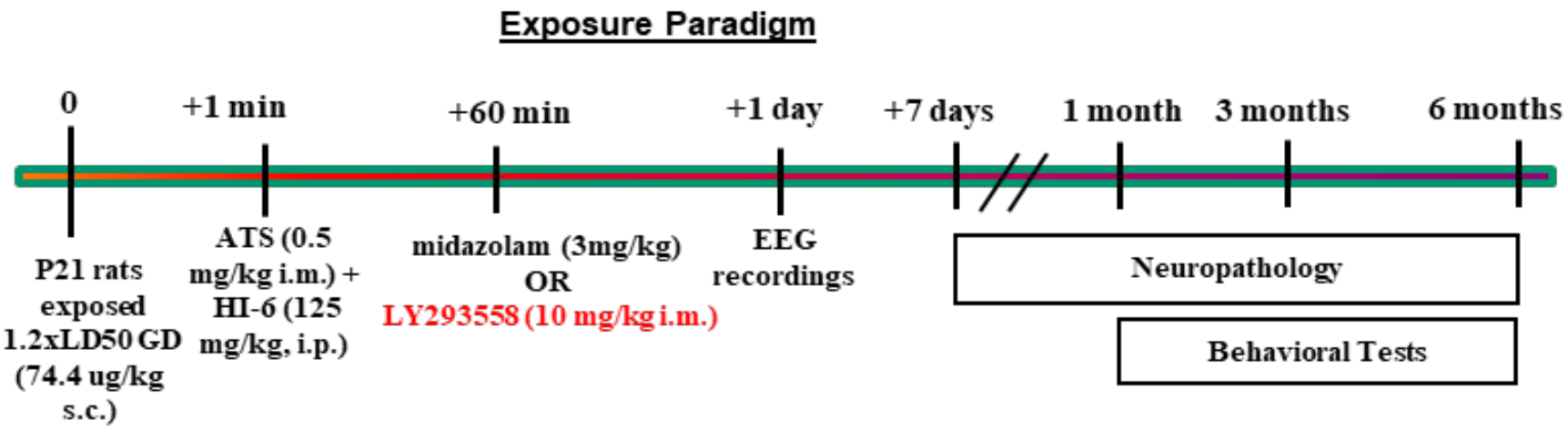
The Animal Model

Partnership with USAMRICD was established:

James P. Apland, PhD
Lucille Lumley, PhD

We utilize an Model of Nerve Agent Exposure that involves no pre-treatment to mimic a real-case scenario of an unexpected attack with a nerve agent.

The Animal Model: Experimental procedures



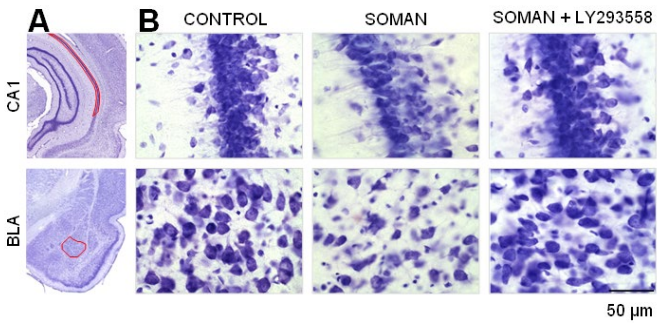
EEG Recordings:
electrode implanted P21 rats were monitored 1 to 2 hours before exposure until 24 hours after anticonvulsant treatment.

Neuropathology:
Neuronal degeneration (FJC)
Neuronal Loss in Basolateral Amygdala (BLA) and CA1 hippocampus subfield
Loss of GABAergic Interneurons in Basolateral Amygdala (BLA) and CA1 hippocampus subfield
Atrophy of Amygdala and Hippocampus (Volumetric quantification at 1, 3 and 6 months)

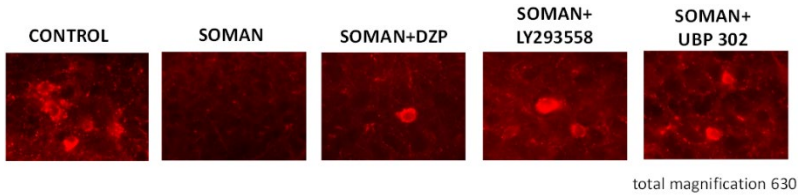
Behavioral Tests:
Acoustic Startle
Open Field

Quantitative Morphology

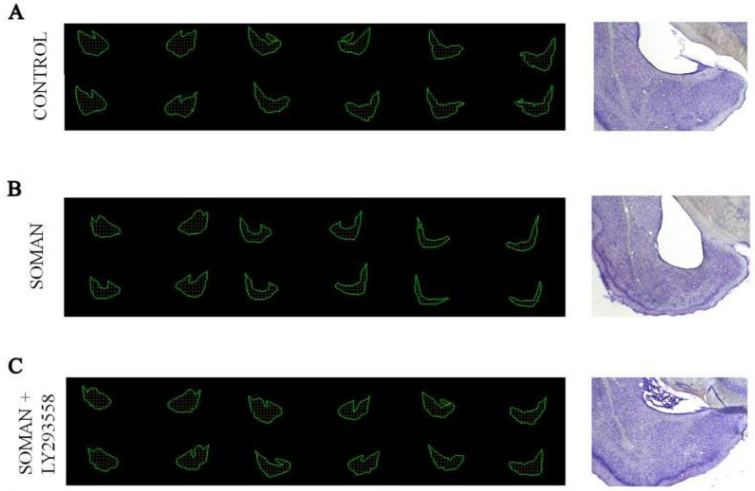
Design-based stereology provides the tools for obtaining accurate, precise quantitative structural data from tissue sections.



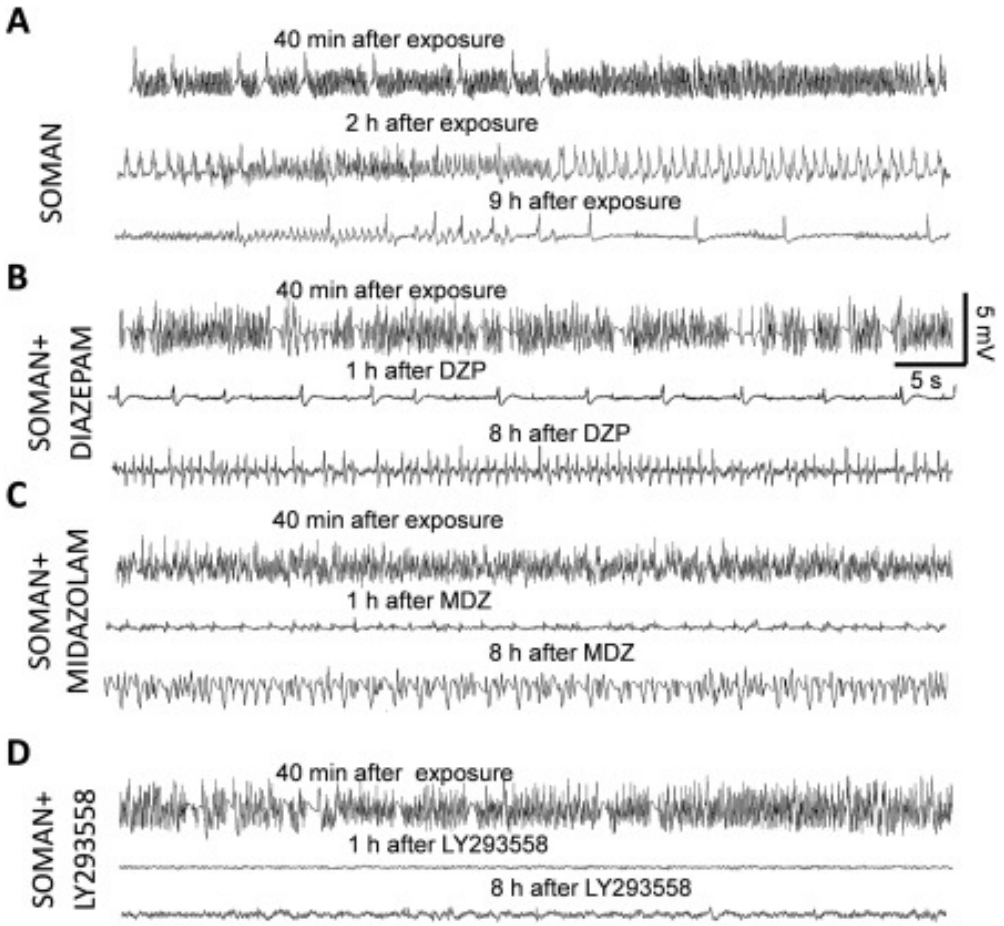
To determine what proportion of the lost neurons are GABAergic interneurons, we combine **Stereological Quantification with GAD67 immunohistochemistry**.

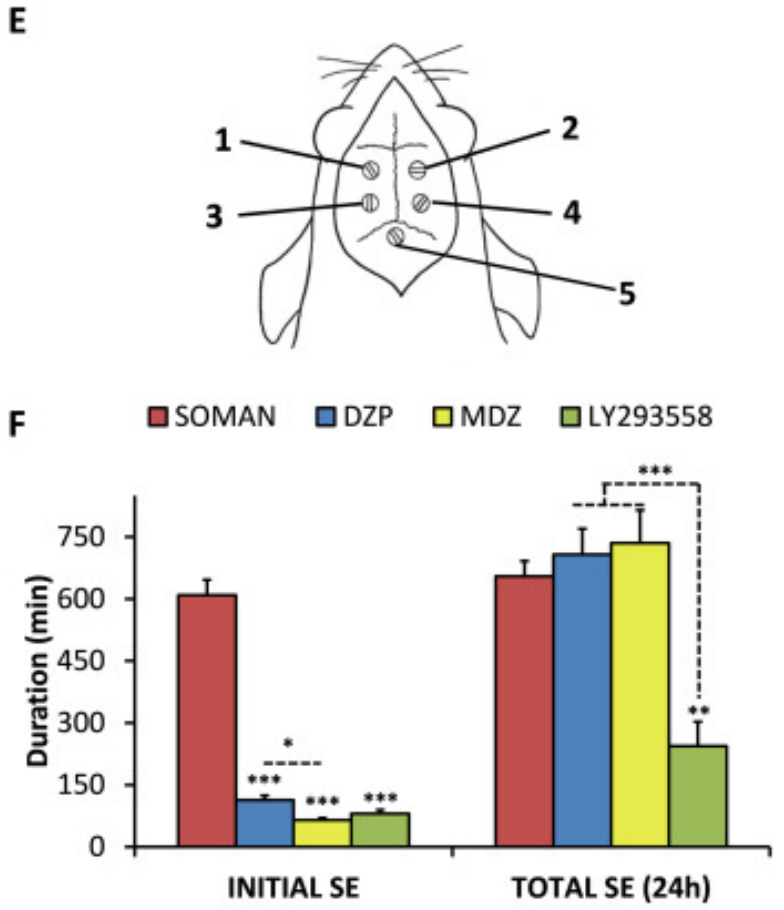


For **Volumetric Analysis**, Nissl-stained sections containing the amygdala and hippocampus are used to estimate their volume based on the Cavalieri principle using StereoInvestigator 9.0.

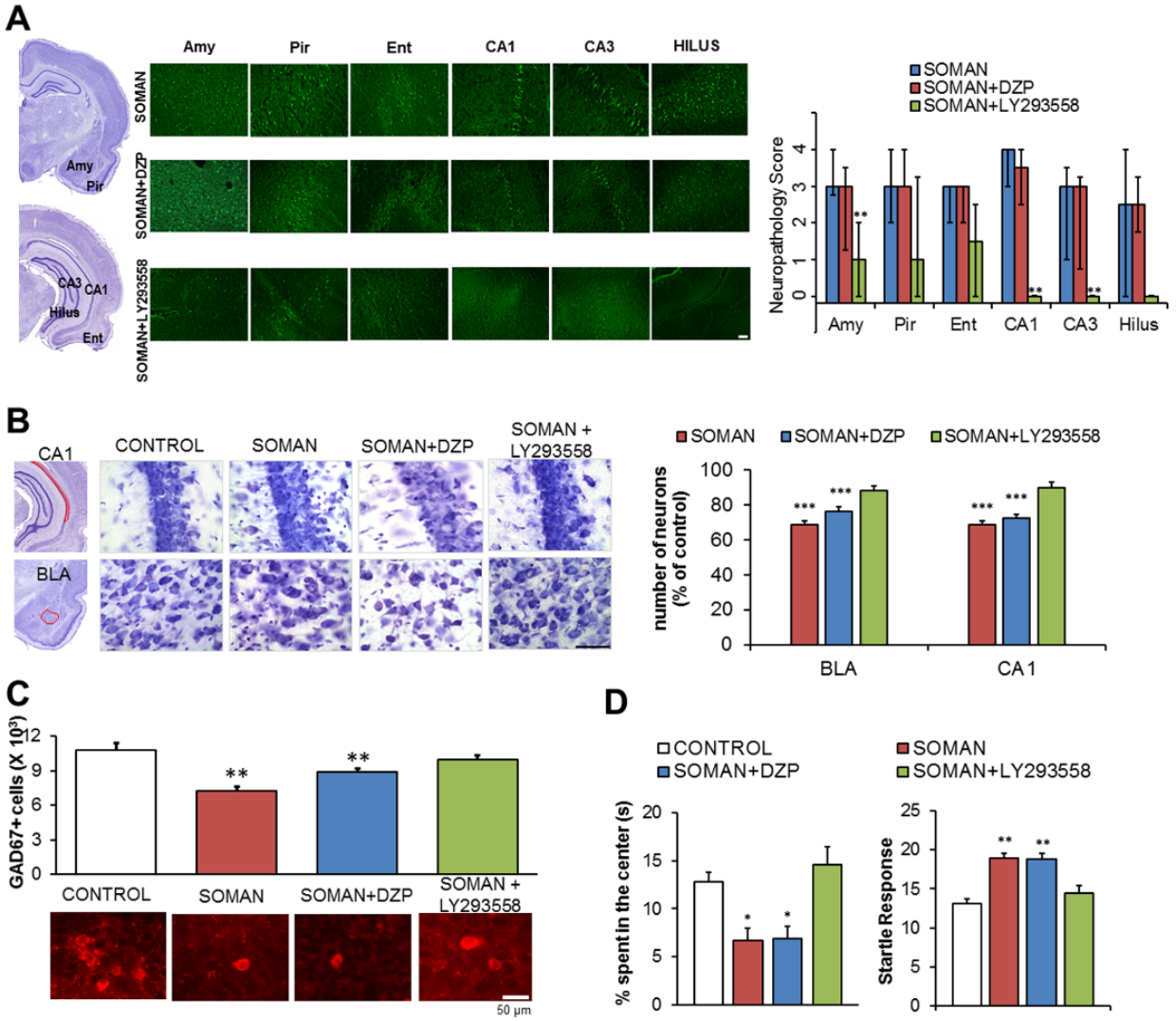


LY293558, Diazepam (DZP), and Midazolam (MDZ) terminate promptly the initial SE induced by Soman, but only LY293558 reduces the total duration of SE within 24 h post-exposure





LY293558 but not diazepam (DZP) protects against neurodegeneration, neuronal loss and behavioral alterations



SRI International

Summary of Study B473-11: Pharmacokinetic Study of LY293558 Following Single Dose Administration to Male Sprague-Dawley Rats

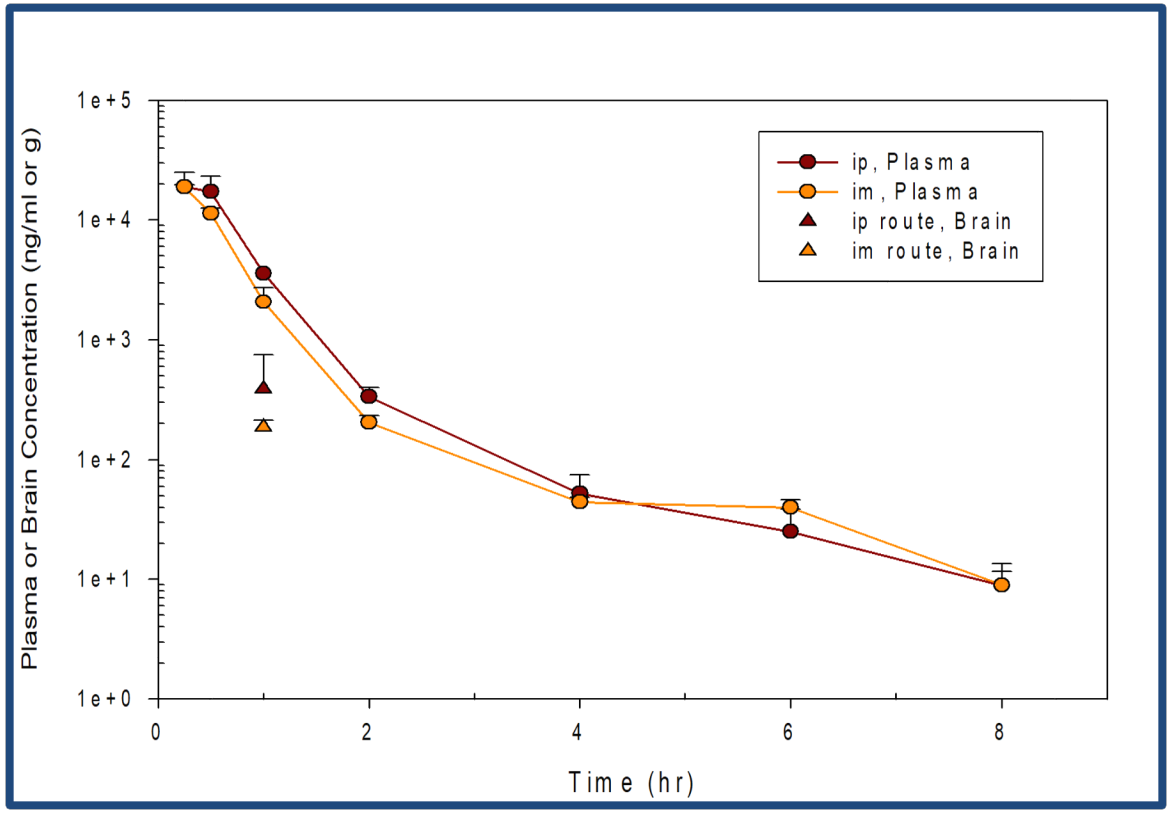
Study Design

Group	Route	Dose Level (mg/kg)	Dose Conc. (mg/ml)	No. of Animals	Blood Sampling Times[a]	Brain Collection Times[a]
1	ip	20	4	9	0.25, 0.5, 1, 2, 4, 6, 8, 24 and 48 hr	1, 8, and 48 hr
2	im	15	25	9	0.25, 0.5, 1, 2, 4, 6, 8, 24 and 48 hr	1, 8, and 48 hr

Species and Strain: Sprague-Dawley rat
Route of Administration: ip or im
Frequency: Single dose on Day 1

[a] Three rats were assigned for each blood/brain sampling time point.
[b] Three blood collection time points were assigned to each rat. Approximately 300 µl blood were drawn at each time point via retro orbital sinus.
[c] Three rats were assigned for each brain collection time. Brains were collected after the third (terminal) blood sampling time point.
[d] Control plasma samples (approximately 1 ml or maximum possible) and control brain samples were individually collected from 3 additional male rats not treated with the test article.

LY293558 is rapidly absorbed after both ip and im administration and results in high plasma concentrations (C_{max} ~19000 ng/ml) in Sprague Dawley rats that are greater than the 5 ng/ml through 8 hr



Plasma and brain concentrations of LY293558 after a single ip (20 mg/kg) or im (15 mg/kg) dose to male Sprague Dawley rats. LY293558 levels were less than the lower limit of quantitation in the brain at 8 and 48 hr and in the plasma at 48 hr. LLOQ = 27.4 ng/g brain; LLOQ = 5 ng/ml plasma.

Conclusions I

LY293558 (Tezampanel), an antagonist of AMPA and GluK1 receptors, is very effective against nerve agent-induced seizures and neuropathology, even when administered at delayed time points after the exposure.

LY293558 is rapidly absorbed after both ip (20 mg/kg) and im (15 mg/kg) administration and results in high plasma concentrations (C_{max} ~19000 ng/ml) in Sprague Dawley rats that are greater than the 5 ng/ml through 8 hr.

The AUC values for both the ip and im routes were similar, indicating that LY293558 is readily absorbed after im administration.

At 1 hr post dose LY293558 was detected in the brains of treated rats, and the brain/plasma ratio was ~0.1.

The plasma half life was less than the 8 hr that our milestone mentioned but it is likely to be significantly longer in larger species, especially humans than in rats.

Next Step: BARDA

The **Biomedical Advanced Research and Development Authority** (BARDA) is a HHS office **responsible for the procurement and development of medical countermeasures**, principally against bioterrorism, including chemical, biological, radiological and nuclear (CBRN) threats, as well as pandemic influenza and emerging diseases.

BARDA reports to the Office of the Assistant Secretary for Preparedness and Response (ASPR) and manages Project BioShield.

Is BARDA interested in the further development of LY293558?

TechWatch Program

BARDA developed the TechWatch program as an opportunity for external organizations to meet with the Federal Government to discuss their **new and innovative medical countermeasure technologies**.

These meetings are intended to provide the Government with the latest information about **emerging technologies** and tools that may guide its strategic and programmatic planning for effective public health emergency response.

In turn, the meetings give organizations the chance to receive guidance from BARDA scientific and contracting staff on possible next steps in **the development of their countermeasure products and how they may work with the Government as part of this process."**

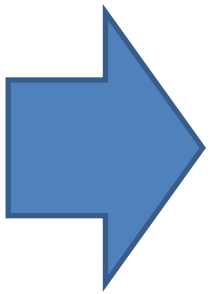
TechWatch Program

Presentation well received and BARDA is interested

The need to partner with Industry

Partnership with Raptor

Todd C. Zankel, PhD
Vice President and
Chief Scientific Officer
Raptor Pharmaceutical, Inc.
5 Hamilton Landing
Suite 160
Novato, CA 94949



Drug License Back to

Lilly

Convincing Lilly to Partner with us

David Bleakman, Ph.D.
Chief Scientific Officer and
Neuroscience New York Site Leader
Neuroscience Research
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Kirk W. Johnson, Ph.D.
Senior Research Advisor –
Pain/Migraine–Neuroscience Discovery
Eli Lilly and Company
Lilly Corporate Center, Indianapolis
IN46285

Set up a tcon amongst BARDA, USUHS (myself), Lilly (Drs. David Bleakman and Kirk Johnson) and the NIH (CounterACT Program representatives) to discuss how BARDA can help us to further develop LY293558 with the objective of eventually getting FDA approval.

Accelerator Corporation

Accelerator Corporation, a **venture capital-backed investment firm**, intends to diligently pursue a transaction with Eli Lilly & Company that will create a new company (**Proniras**) that will have an exclusive license to LY293558, an experimental compound that Proniras intends to develop for the treatment of organophosphate (OP) intoxication.

Accelerator is backed by top tier pharmaceutical companies, including Lilly, as well as traditional venture capital firms.

Noting our well established relationship with Lilly we are confident that **Proniras** should be able to quickly move forward in partnership with **you (USUHS) and BARDA to develop a dialogue with the FDA** and receive guidance on the steps necessary to gain approval for LY293558.

BARDA Award

Our extensive pre-clinical studies demonstrating the effectiveness of LY293558 against nerve agents have led to the formation of a **new company (Proniras)** to further develop LY293558 as a new countermeasure.

On April 25, 2018, it was announced that the Biomedical Advanced Research and Development Authority (BARDA) awarded a $89.5 million contract to Proniras for advanced research and development of LY293558, for the treatment of nerve agent induced-seizures that are refractory to benzodiazepines.

All of the Pre-Clinical data presented to BARDA in order to receive the award was obtained in our labs at USUHS in collaboration with USAMRICD.

In the meanwhile…

Nerve Agent Intoxication in Children

In August 2013, the nerve agent sarin was released during a terrorist attack in Syria which resulted in the death of over 1400 civilians, **426 of which were children** (Dolgin, 2013).

More recently (April 4, 2017) another sarin attack killed 89 people, including **33 children**, and 541 people were injured.

Mass Casualties During Terrorist Attacks that Employ Nerve Agents are Expected to Disproportionately Affect Children

- Greater body surface area-to-body mass ratio,
- Increased skin permeability,
- Faster respiration rate,
- Breathing at a level where nerve agent vapor density would be highest,
- Increased susceptibility to seizures.

(American Academy of Pediatrics, 2000)

Why Target the Glutamatergic System to Terminate Seizures and Protect Against Brain Damage in children?

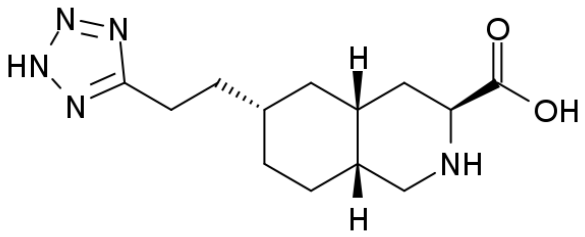
- In the immature brain there is a **high NMDA receptor activity**, which coupled with an augmented glutamatergic tonus due to the OP-induced intoxication leads to an increased Ca^{2+} influx through the NMDA channels - a primary mechanism for **excitotoxicity and cell death**.

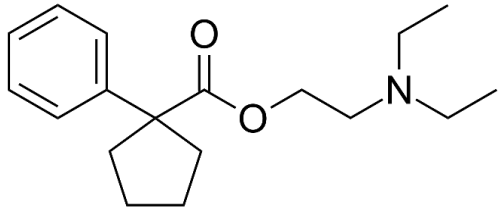
- **The inclusion of an NMDA receptor antagonist in the treatment approach in children is attractive, and highly advantageous in protecting the immature brain from seizure-induced damage.**

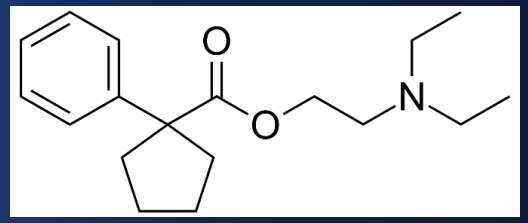
- **We hypothesize that the greatest antiseizure and neuroprotective efficacy will be obtained when LY293558and Caramiphen are administered in combination.**

The New Kid on the block: The Combination Therapy

LY293558 + Caramiphen







Caramiphen

The greatest benefit of utilizing caramiphen as the NMDA receptor antagonist in this combined therapy is its safety record in human usage, including children.

Caramiphen was approved by the FDA in 1973 as an over-the-counter antitussive for human use from the age of two years and above.

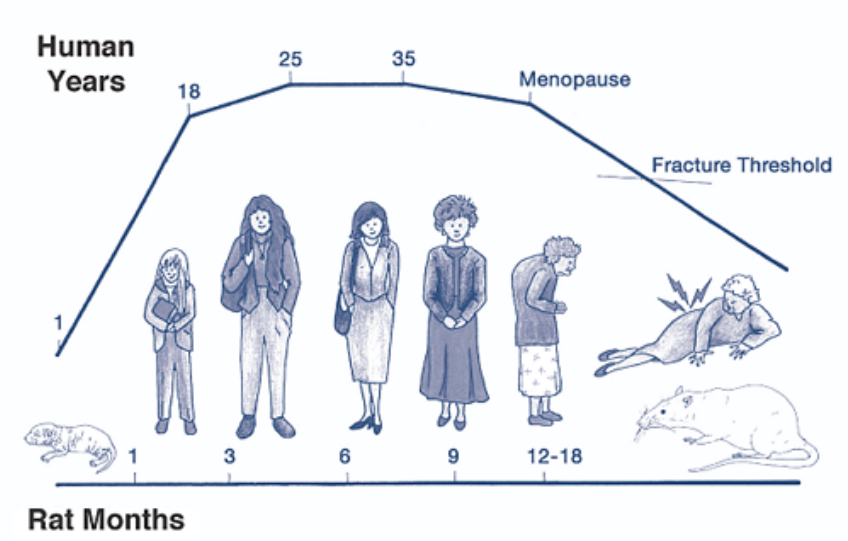
The FDA approval was withdrawn in 1984 due to its lack of efficacy as an antitussive but not because of safety concerns.

LY293558 and Caramiphen

A combination therapy would be very beneficial as a treatment because it would allow the doses of **LY293558 and Caramiphen to be reduced considerably**, thus decreasing the incidence of side effects and increasing the tolerability of the proposed treatment in humans.

This is because LY293558 and Caramiphen both have anticonvulsant activity via **different mechanisms of action which fosters a synergistic effect**.

A rat model of nerve agent exposure applicable to the pediatric population



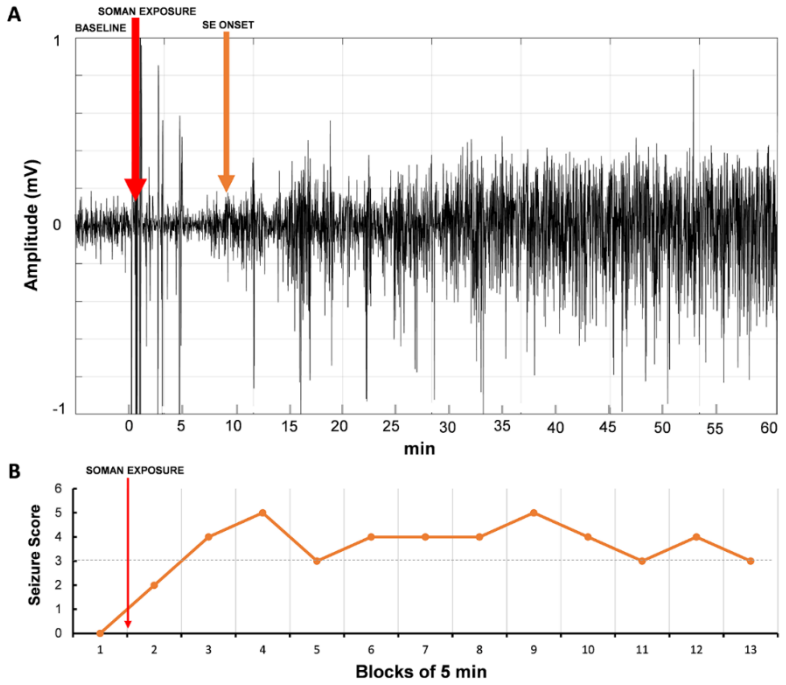
A 21 day-old rat corresponds to ~ a 4-year old child.

A 12 day-old rat corresponds to ~ an infant child.

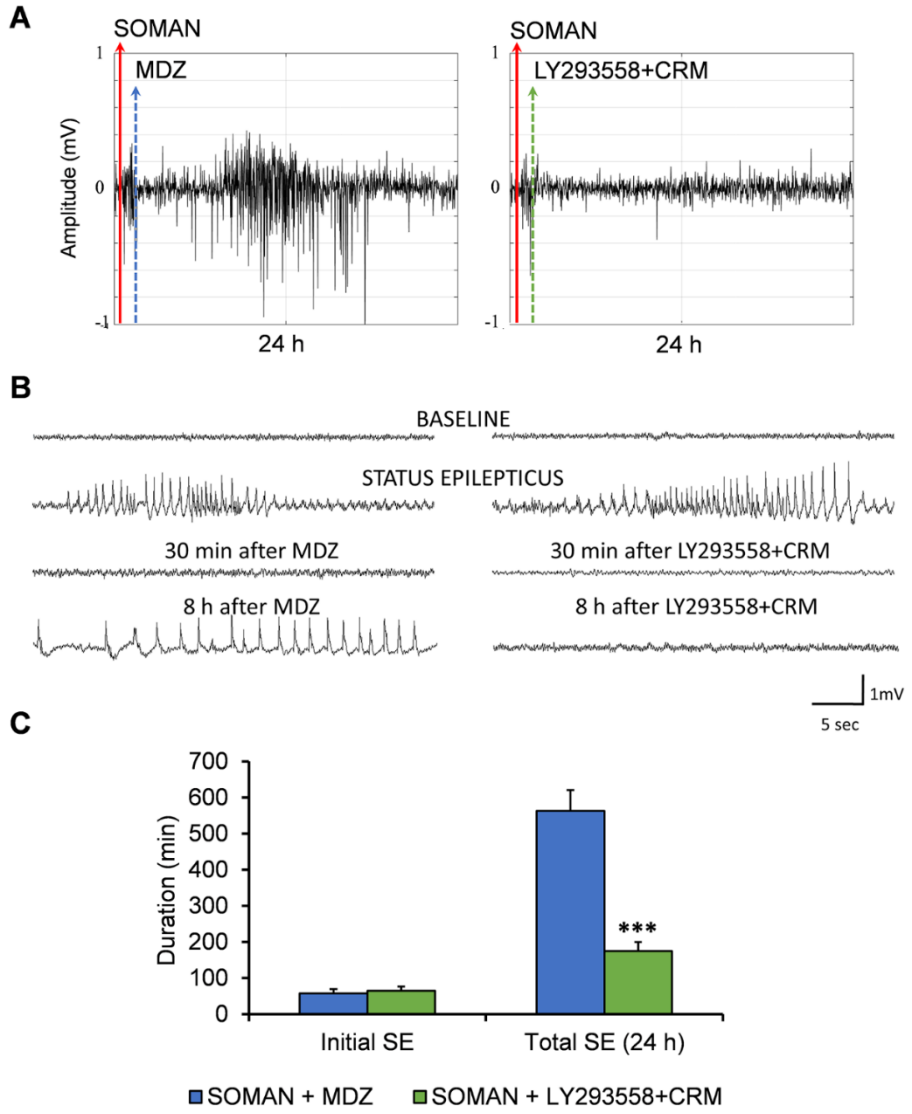
A 7 day-old rat corresponds to ~ a newborn child.

The developmental stage of the brain in the two species (Andersen, 2003), and synaptogenesis, a basic parameter of brain development, is completed within the first 3 weeks of life in the rat and about 3.5 years in the human (Pressler and Auvin, 2013).

Subcutaneous injection of P21 rats with 74.4 µg/kg soman ($1.2 \times LD_{50}$) induces robust SE



Treatment with either MDZ or LY293558+CRM stop the initial SE induced by soman exposure, however, recurrence of seizures within 24 h post-exposure is significantly less after LY293558+CRM administration

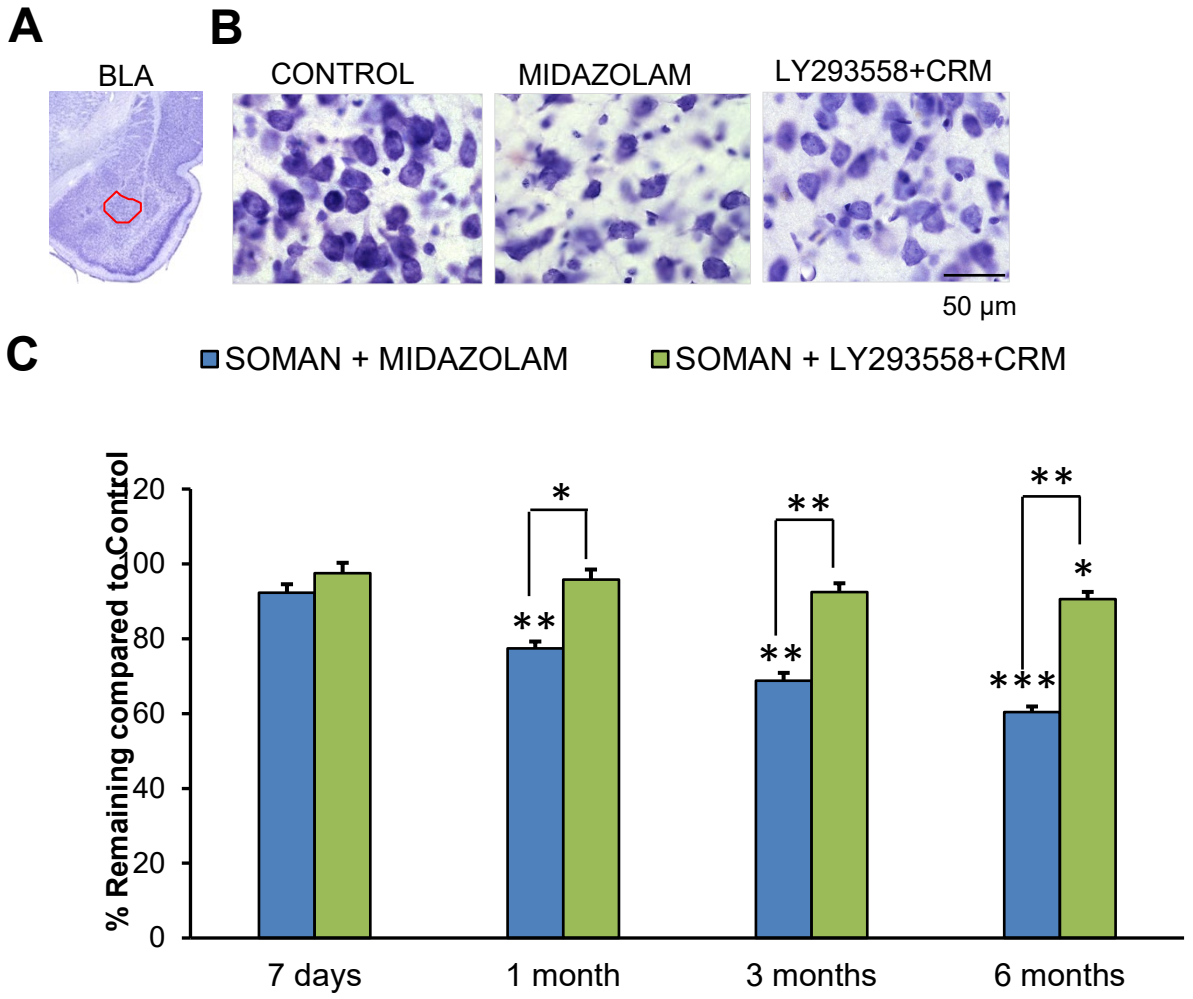


De Araujo Furtado et al. (2020) *Ann N Y Acad Sci.*

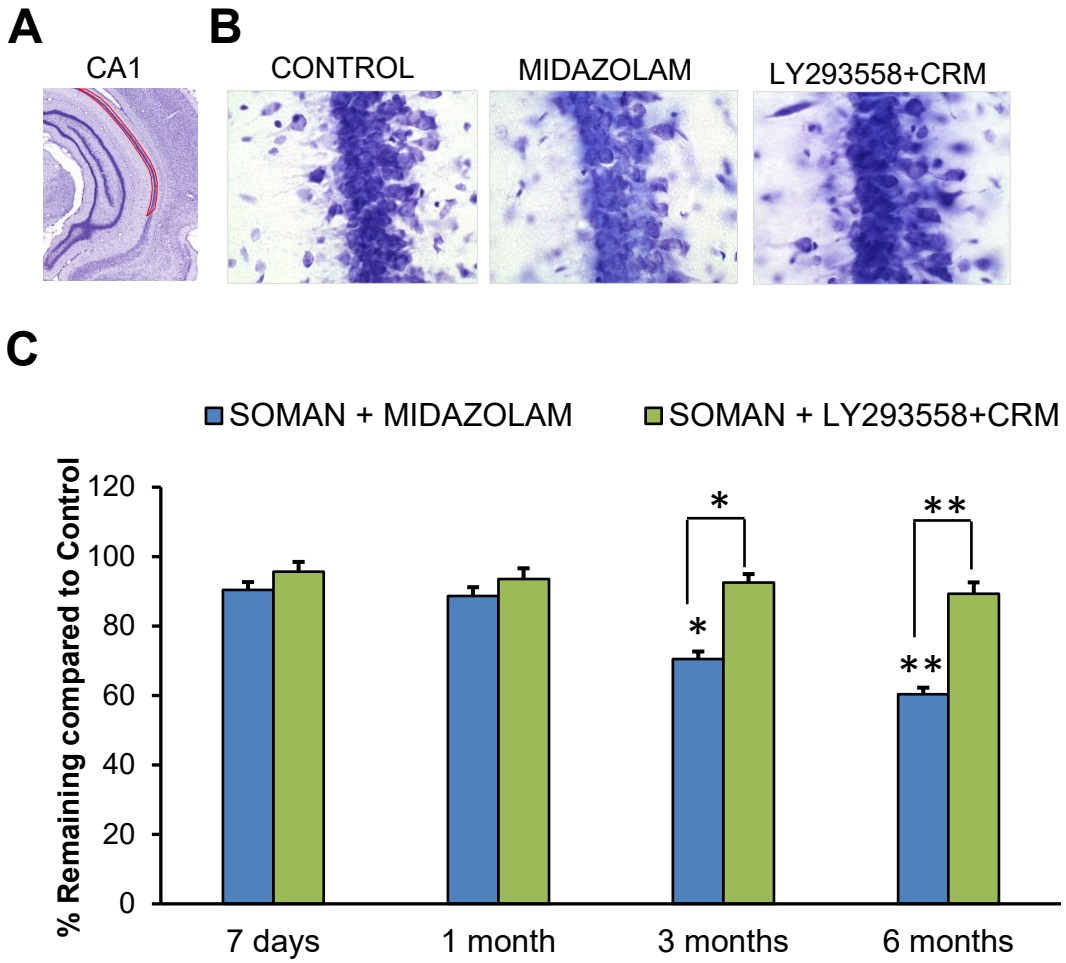
The combination of LY293558+CRM significantly increased survival rate in P21 rats exposed to 1.2X LD50 Soman compared to animals that received MDZ

	MDZ		LY293558+CRM	
Gender	Male	Female	Male	Female
Seizure Control	80% (32/40)	83% (29/35)	100% (40/40)	100% (40/40)
% survival 24h	87.5% (35/40)	88.5% (31/35)	95% (38/40)	97.5% (39/40)

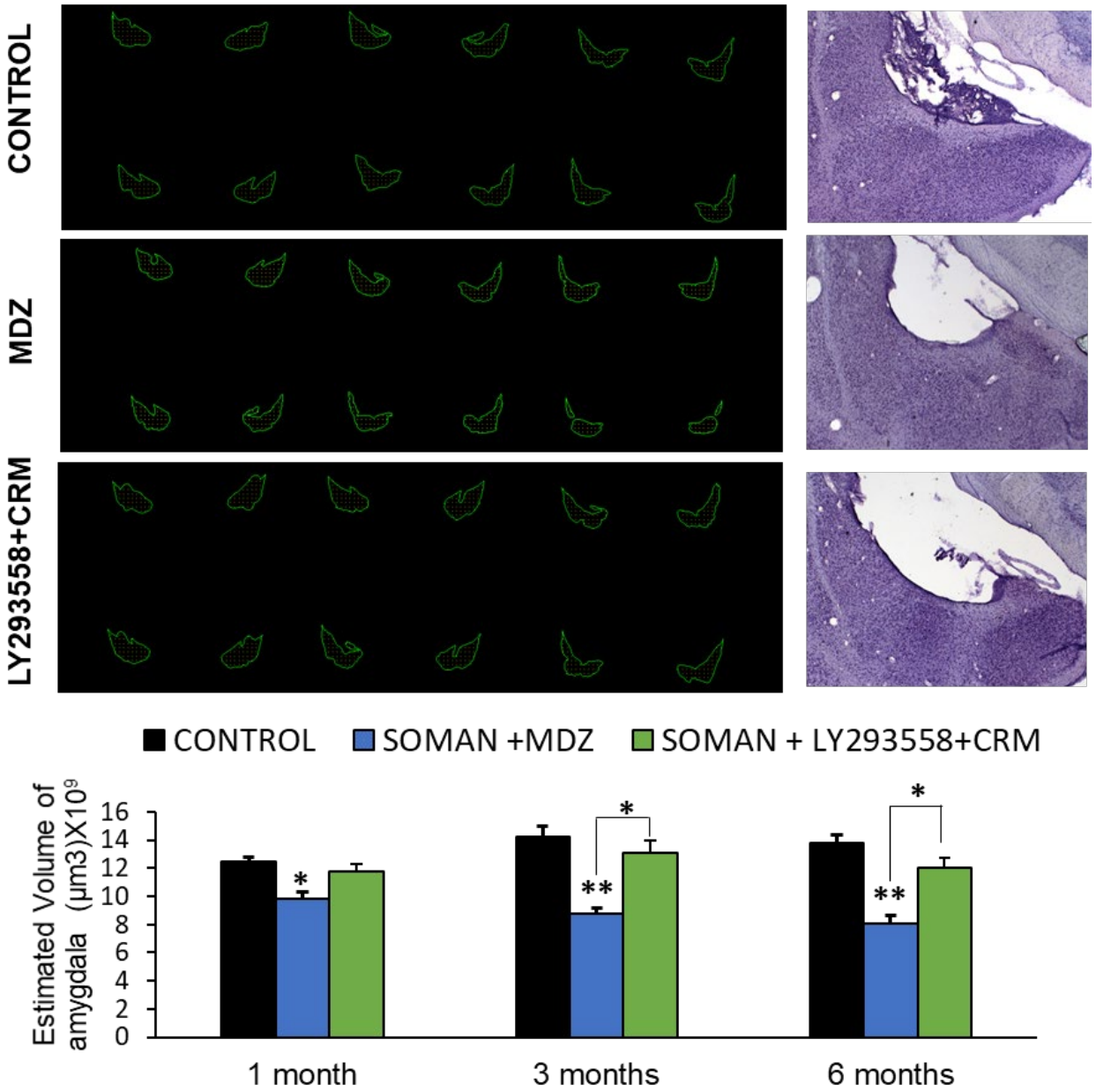
LY293558+CRM but not MDZ protects against neuronal loss in the BLA



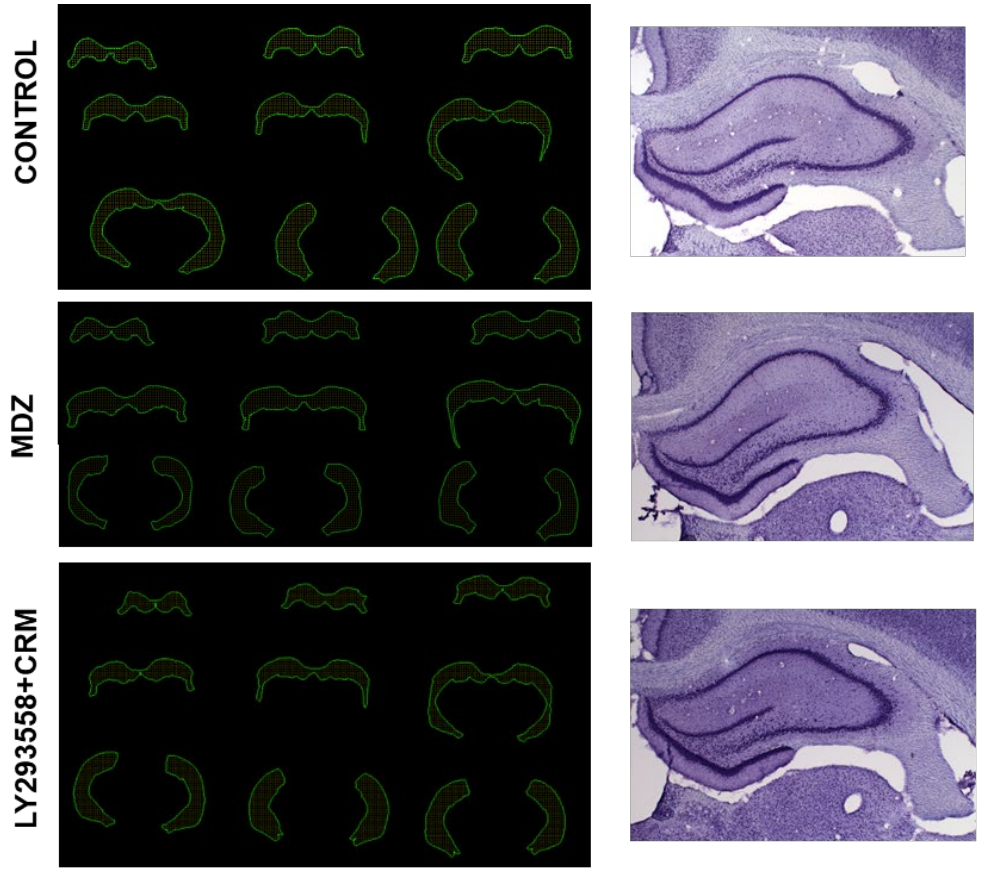
Complete protection against neuronal loss in the CA1 hippocampal area was provided by LY293558+CRM but not by MDZ treatment

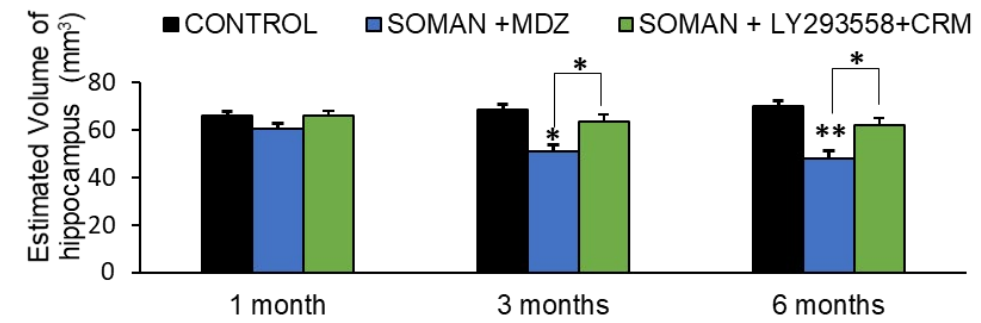


Amygdala volume is reduced 1, 3 and 6 months after soman exposure in P21 rats treated with MDZ but not in rats treated with LY293558+CRM

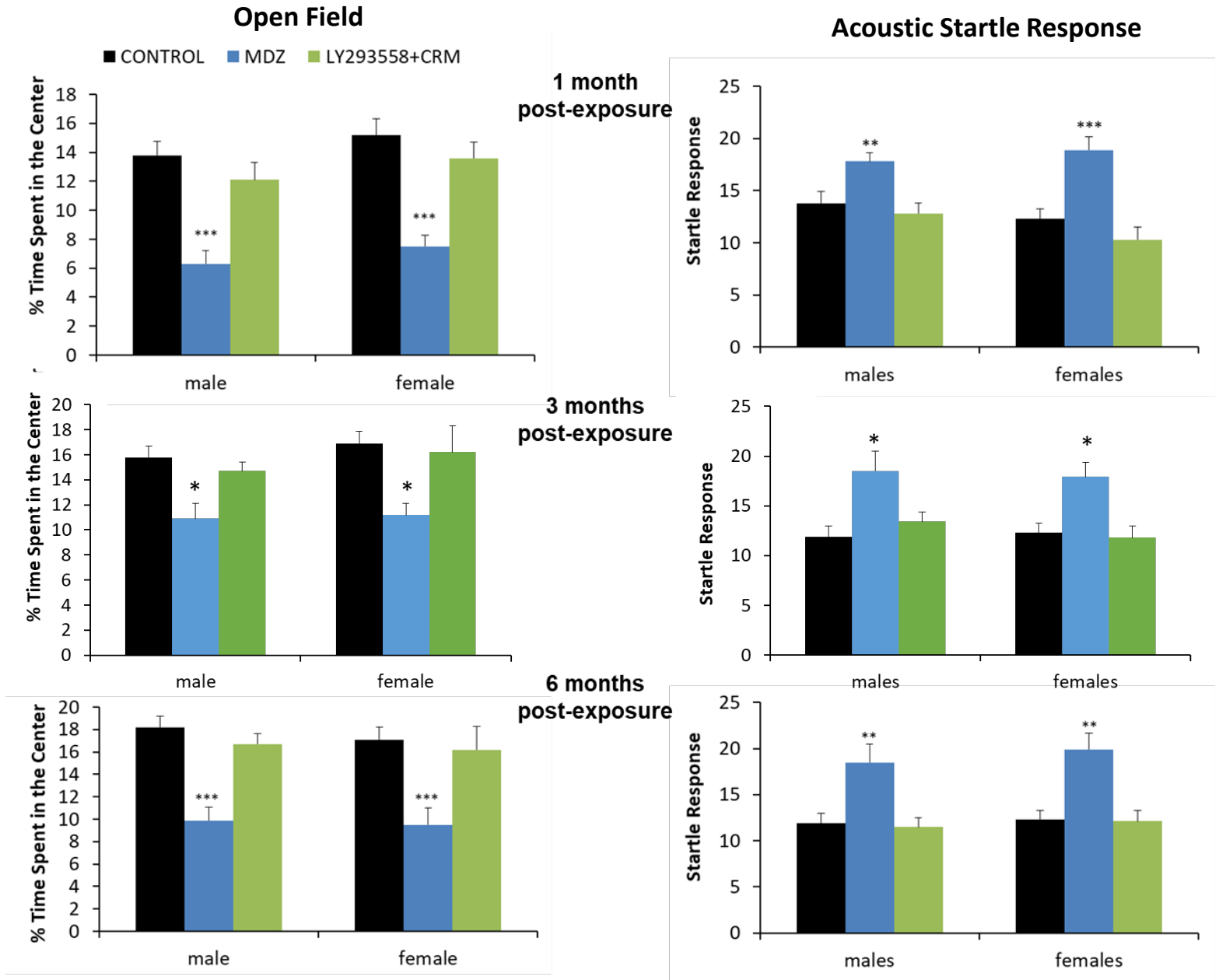


Hippocampal volume is reduced 3 and 6 months after soman exposure in P21 rats treated with MDZ but not in rats treated with LY293558+CRM





LY293558+CRM but not MDZ protected against soman-induced, long-term increases in anxiety-like behavior



Conclusions II

We found that in rat models of nerve agent exposure that are relevant to the pediatric human population, delayed administration of LY293558, an AMPA/GluK1 receptor antagonist, along with caramiphen, an antimuscarinic compound that also antagonizes NMDA receptors, provides full protection against brain damage.

In contrast, delayed midazolam administration resulted in brain damage that deteriorated over time (over a 6-month period after exposure to soman).

Future Directions

We currently investigate the efficacy of midazolam in soman-exposed young-adult and aged rats, and compare it with the efficacy of the LY293558+caramiphen combination therapy.

We expect that the completion of this research will produce a new antiglutamatergic first-line treatment for SE that can prevent brain damage even when offered with a delay, without the need of additional pharmacological (antiseizure or neuroprotective) interventions.

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