National Institute of Neurological Disorders and Stroke

NIH CounterACT Program

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

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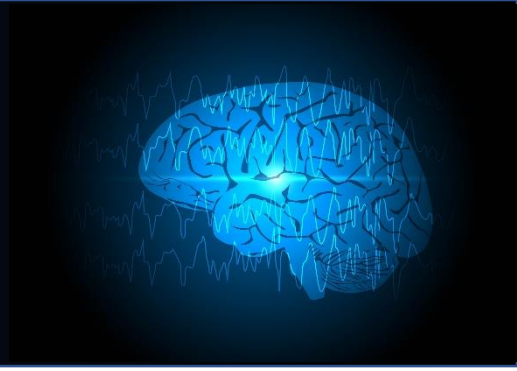
Preclinical Research Gaps in Chemical-Induced Seizures

Pamela Lein, PhD, Professor and Chair, University of California, Davis

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 or the University of California.

Disclosure

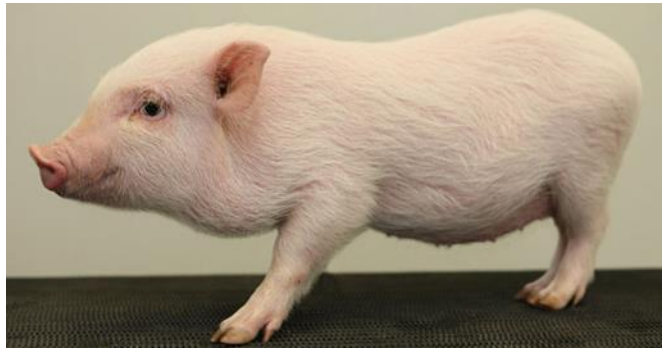
This certifies that I, Pamela Lein, have a financial relationship that is relevant to the subject matter of the presentation.
I am one of four authors on United States Patent 10,426,786 B2, Rogawski et al., *Mitigation of Epileptic Seizures by Combination Therapy Using Benzodiazepines and Neurosteroids*, filed October 1, 2019.

The identification of more effective medical countermeasures for chemical-induced seizures will necessarily require preclinical research

- **Key preclinical research gaps**
  - **Preclinical models**
  - **Mechanistic gaps**
  - **Relative contribution of chemical toxicity *vs*. *status epilepticus* (SE) to chronic neurotoxic effects**
- ***Implications for development of broad-spectrum vs. targeted therapeutics***

Preclinical research gap: Models

- **Animal models used to study chemical-induced seizures and OP neurotoxicity**
  - Chicken (*Gallus domesticus*)
  - Mouse (*Mus musculus*)
  - Guinea pig (*Cavia porcellus*)
  - Rabbit (*Oryctolagus cuniculus*)
  - Rat (*Rattus norv*egicus)
  - Swine (*Sus scrofa*), including the mini-pig
  - Dog (*Canis lupus familiaris*)
  - Non-human primates (*Rhesus macaque*)

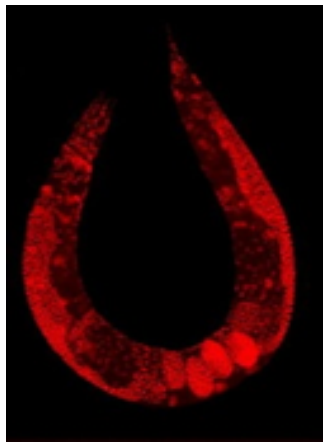


Neurotoxic outcomes observed in animal models of chemical-induced seizures

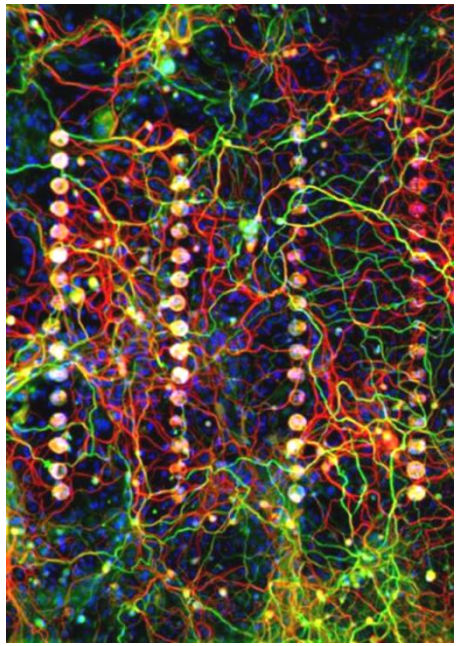
- **Acute seizures**
  - High proportion of animals develop seizures following acute intoxication
  - Seizures look and behave like nerve agent-induced seizures in humans
  - Anti-seizure and neuroprotective efficacy of anti-seizure medications decrease with increased delay between seizure onset and drug administration
  - Standard of care (atropine, oxime and benzodiazepine) administered after delayed times expected in civilian mass casualty do not prevent chronic neurotoxic outcomes
- **Chronic neurotoxicity**
  - Persistent neuropathology
  - Spontaneous recurrent seizures and abnormal EEG
  - Behavioral effects
    - Impaired cognitive behavior
    - Altered anxiety-like behavior
    - Depression-like behavior

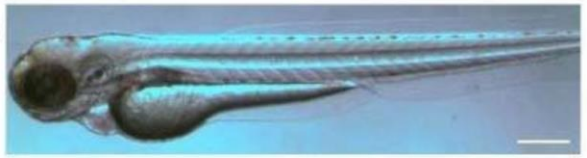
Alternative models

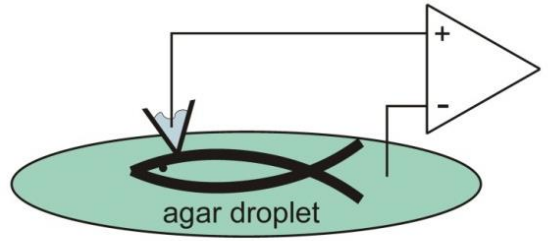
Primarily used for drug discovery purposes

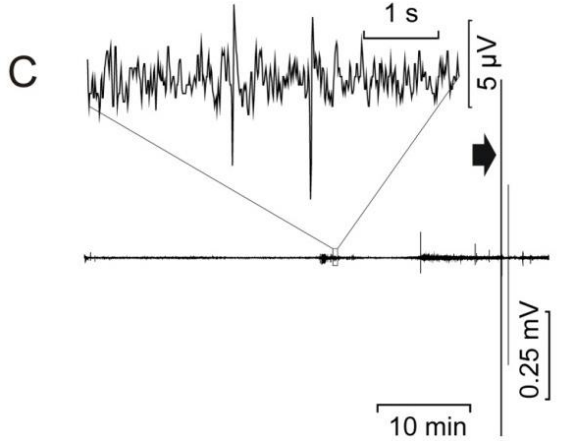


- Flatworms (*Planaria*)
- Nematode (*Caenorhabditis elegans*)
- Fruit fly (*Drosophila melanogaster*)
- Zebrafish (*Danio rerio*)
- Human iPSC-derived neurons
- *In silico* approaches









Neurotoxic effects modeled using alternative models

- **Acetylcholinesterase activity – inhibition and regeneration**
  - ***In silico*, cell culture, simple animal models**
- **Acute chemical-induced hyperexcitability**
  - **Cell culture, simple animal models**
- **To date, it has been very challenging to model chronic neurotoxic effects of chemical-induced seizures in alternative models**

Limitations of the preclinical animal models

- **Relevance of exposure paradigms to civilian mass casualty scenario**
  - **Pretreatment and/or immediate post-treatment often needed to keep most animals alive following intoxication with doses that cause seizures**
  - **Very steep dose-response curve often observed**
- **Species (and strain) differences**
  - **Acetylcholinesterase (AChE)**
  - **Cytochrome P450s**
  - **Scavenging enzymes**
    - ***ES1*$^{-/-}$ mouse lacks plasma carboxylesterase**
  - **Immune and neuroinflammatory responses vary significantly from human responses, particularly true of mice**
- **Most animal models do not faithfully recapitulate all aspects of human behavior, especially affective disorders**

Technical challenges of preclinical models

- **Variable potency between lots of chemical threat agents**
- **Variability of exposure paradigms between research groups**
- **Lack of consensus regarding quantitative outcome measures for assessing:**
  - **Seizure activity**
  - **Neuropathological endpoints**
  - **Behavioral outcomes**
  - **Therapeutic responsiveness**

Do the preclinical models of chemical-induced seizures meet FDA requirements for the animal rule?

- Are animal models of chemical-induced seizures predictive of human responses?
  - Predictive of the acute and chronic neurotoxic effects of chemical threat agents?
  - Predictive of the efficacy of therapeutic candidates?
  - What criteria do we use to address predictability?
- Are there key features of the human experience that are lacking in animal models?
  - Responses in adult female animals
  - Responses in neonatal animals
  - Psychological stress (PTSD)?
- How do we improve the validity of animal models
  - External (generalizability to human disease)
  - Internal (elimination of bias, increased scientific rigor)
- Do we need a “single” standardized animal model? Is it even possible to develop a single animal model?

Preclinical research gap: Mechanisms

- **Molecular mechanisms by which chemically and mechanistically diverse chemical threat agents trigger acute seizures**
  - **AChE inhibition *vs*. GABA receptor antagonism *vs*. hyperstimulation of glutamate signaling**
    - **Relative contribution of these basic mechanisms vary between threat agent chemotypes?**
    - **What receptor subtypes and/or other molecular targets (e.g., glutamate transporters) mediate chemical effects on excitability?**

- **Poor correlation between *in vitro* anticholinesterase potency and published LD_{50} values**
- ***In vivo* neurotoxicity studies reported that different OPs gave rise to different toxicological profiles even at doses that caused comparable AChE inhibition**
- **AChE knockout mice are *more* vulnerable to OP neurotoxicity than wild type mice**

Sensitivity of AChE deficient mice to OP toxicity

AChE genotype	DFP	CPO	VX
+/+	>2.5	3.5	0.024
+/–	2.5	2.5	0.017
–/–	<2.5	0.5	0.011

Duysen et al., 2001, JPET 299:528-535.

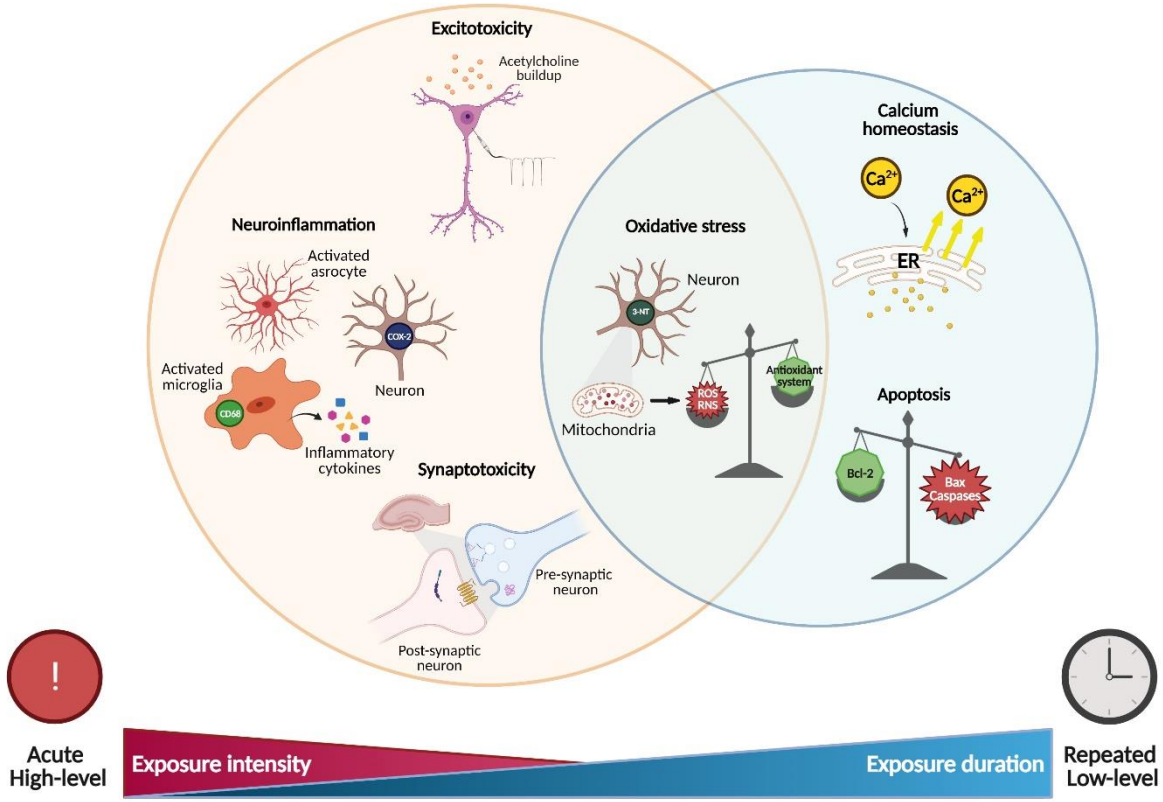
Preclinical research gap: Mechanisms

- **Mechanisms underlying transition to *status epilepticus* (SE)**
  - **Preclinical evidence suggests involvement of diverse receptor subtypes**
    - **M1 muscarinic cholinergic receptor (mAChR) implicated in paraoxon-induced hyperexcitability in basolateral amygdala principal neurons** [Miller et al., 2017, JPET 360:23-32]
    - **M1/M3 mAChR shown to activate presynaptic endocannabinoid type 1 receptors (CB1Rs) to inhibit excitatory neurotransmission in hippocampal slice cultures exposed to OP nerve agents** [Hoffman et al., 2019, Neuropharm 155:113-120]
    - **Extrasynaptic $GABA_AR$ influence duration of OP-induced SE** [Dhir et al., 2020, Ann NY Acad Sci 1480:183-206; Lumley et al., 2019, Epilepsia Open 4(3):382-396]
    - **AMPA, kainate and glutamate receptor activity causally linked to sustaining OP-induced seizures** [Apland et al., 2018, Neurotox Res 34:511-524; Rojas et al, 2020, Neurobiol Dis 140:104863; Niquet et al., 2019, Epilepsy Behav 101(Pt B):106367; Spampanato et al., 2020, JPET 375:59-68; McCarren et al., 2018, Epilepsy Res 141:1-12]
  - **Outstanding questions include:**
    - **Whether and how the functions of these receptor subtypes vary in a region- and/or time-dependent manner during the evolution of OP-induced seizures**
    - **The role of glia in in the initiation and propagation of OP-induced seizures**

Preclinical research gap: Mechanisms

- **Pathogenic mechanisms linking acute SE to long-term adverse neurological outcomes**
  - **Oxidative stress**
  - **Neuroinflammation**
  - **Calcium dysregulation**
  - **Blood-brain barrier (BBB) impairment**
  - **Altered patterns of synaptic connectivity/dendritic arborization**
  - **Genetic/epigenetic changes**
- **Clinical and experimental evidence implicate mechanisms in the pathogenesis of epilepsy, cognitive deficits and affective disorders resulting from causes not related to chemical threat agents**
- **There is significant preclinical evidence demonstrating that acute OP intoxication causes all of the above responses in the brain. However,**
  - **These responses have not been extensively studied in models of chemical-induced seizures caused by acute intoxication with chemical threat agents other than OPs**
  - **Limited evidence demonstrating these are causally linked to persistent neuropathology, but this often varies between brain regions**
  - **Even less evidence establishing a causal link between these responses and adverse neurological outcomes, including SRS, EEG abnormalities, cognitive deficits**

Outstanding questions re: mechanisms linking acute SE to delayed, persistent adverse neurological outcomes



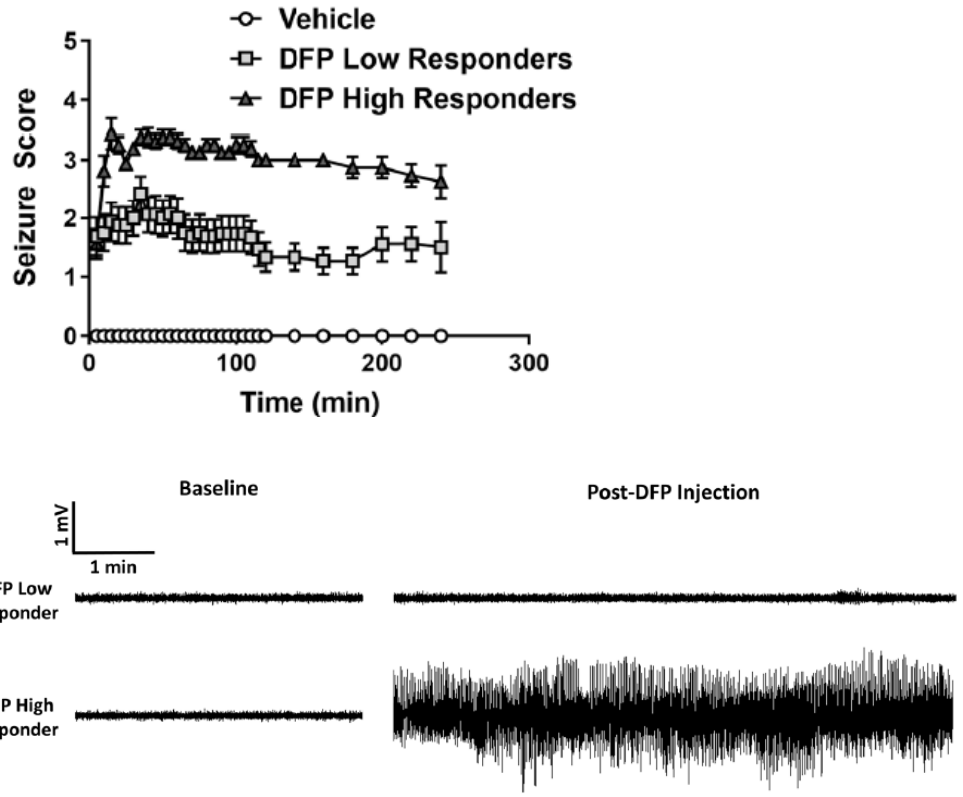
- These mechanisms are inter-connected
  - Which mechanism(s) are driving the neurotoxic outcome?
  - How do they vary across different exposure paradigms?
  - How do they vary regionally?
  - How do they vary with time post-exposure?
- All these mechanisms can be neurotoxic or neuroprotective
  - How to distinguish between these?
  - How to preserve beneficial effects while mitigating disease-promoting effects?

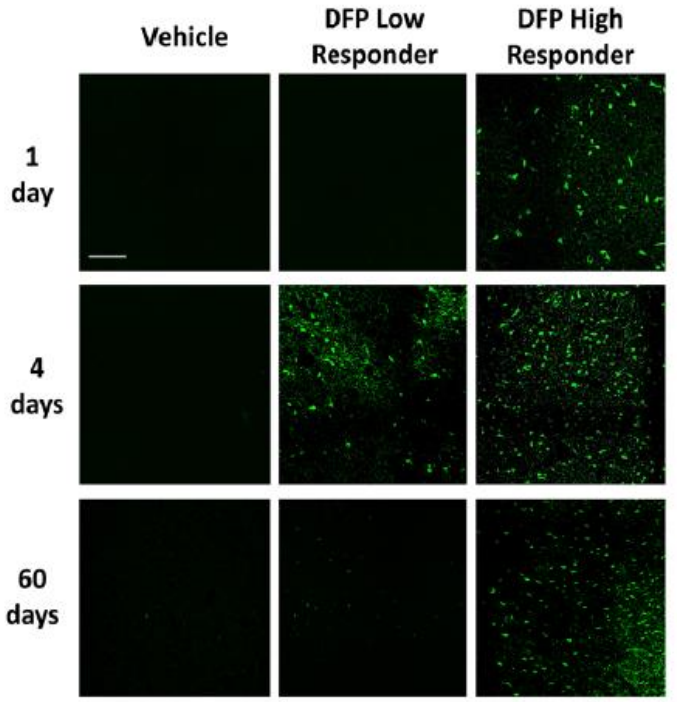
Tsai and Lein, 2021, Current Opinions in Toxicology, 26:49-60.

Preclinical research gap: Understanding the relative contribution of chemical toxicity *vs*. SE to chronic neurotoxic effects

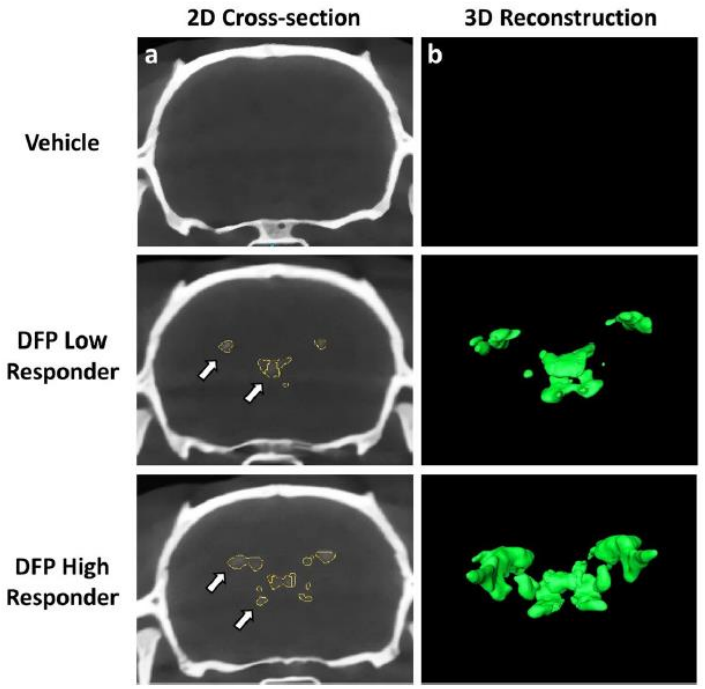
- **Generally believed that brain damage observed after chemical-induced SE is primarily caused by prolonged seizure activity**
  - **Many research groups have demonstrated a strong correlation between seizure severity/duration with the extent of brain damage**
- **However,**
  - **Preclinical studies demonstrated that anti-seizure activity did not necessarily correlate with protection against neuronal cell death 24 h after acute intoxication of rats with DFP**
    - **Memantine exacerbated seizure severity, but significantly reduced neuronal cell death**
    - **Conversely, dexmedetomidine enhanced seizure suppression but conferred no significant neuroprotection** **[Spampanato et al., 2020, JEPET 375:59-68]**

- **In another study of DFP-intoxicated rats** [Gonzalez et al., 2020, Arch Toxicol 94:2149-2162]
  - A subpopulation of animals exhibited minimal to no seizures despite brain AChE inhibition comparable to that of animals with DFP-induced SE
  - The brains of non-seizing animals exhibited significant neuropathology





FluoroJade C labeling (green) of degenerating neurons

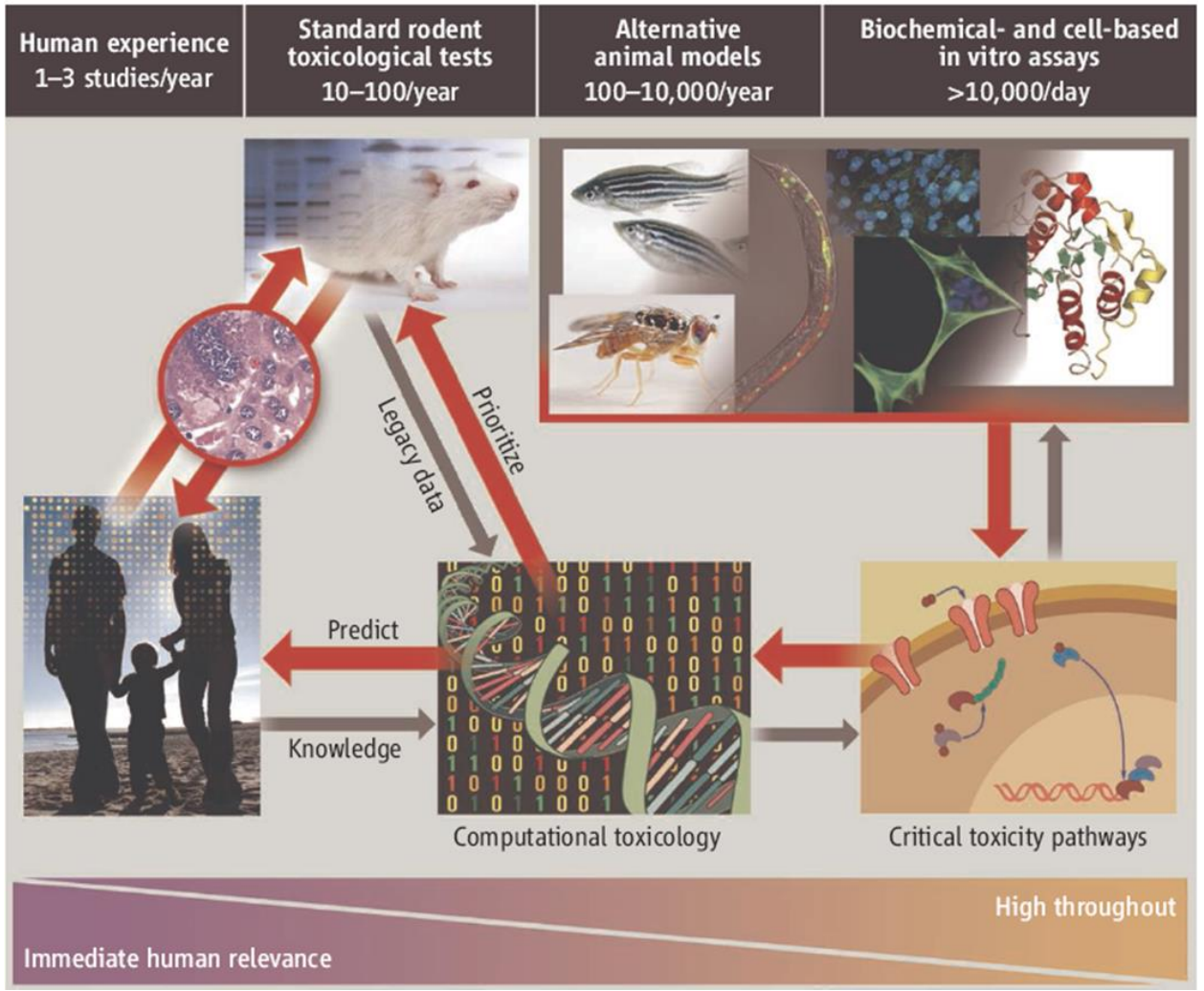


Micro-CT analyses of mineralization in the thalamus

Preclinical research gaps: Summary

- **Further research is needed to:**
  - Establish the relevance of preclinical models to the human condition
    - Strengths, weaknesses, limitations
    - How can we best leverage the preclinical data to inform clinical studies?
  - Understand impact of variable exposure paradigms on neurotoxic and therapeutic outcomes
  - Better characterize mechanisms underlying acute seizure activity and transition to SE following acute intoxication with chemical threat agents
    - Are there conserved mechanisms across chemically and mechanistically distinct chemical threat agents?
    - Important for developing broad-spectrum antidotes
  - Identify mechanisms causally linked to persistent adverse neurological outcomes
  - Appreciate the relative contribution of chemical toxicity vs. SE to acute and chronic neurotoxicity
    - Important for understanding clinical risks to exposed humans who do not develop seizures

Questions?


Human experience
1–3 studies/year
Standard rodent toxicological tests
10–100/year
Alternative animal models
100–10,000/year
Biochemical- and cell-based in vitro assays
>10,000/day
Legacy data
Prioritize
Predict
Knowledge
Computational toxicology
Critical toxicity pathways
High throughout
Immediate human relevance