Applications of Chemistry to Unmet Medical Needs in Neuroscience

Stephen Martin

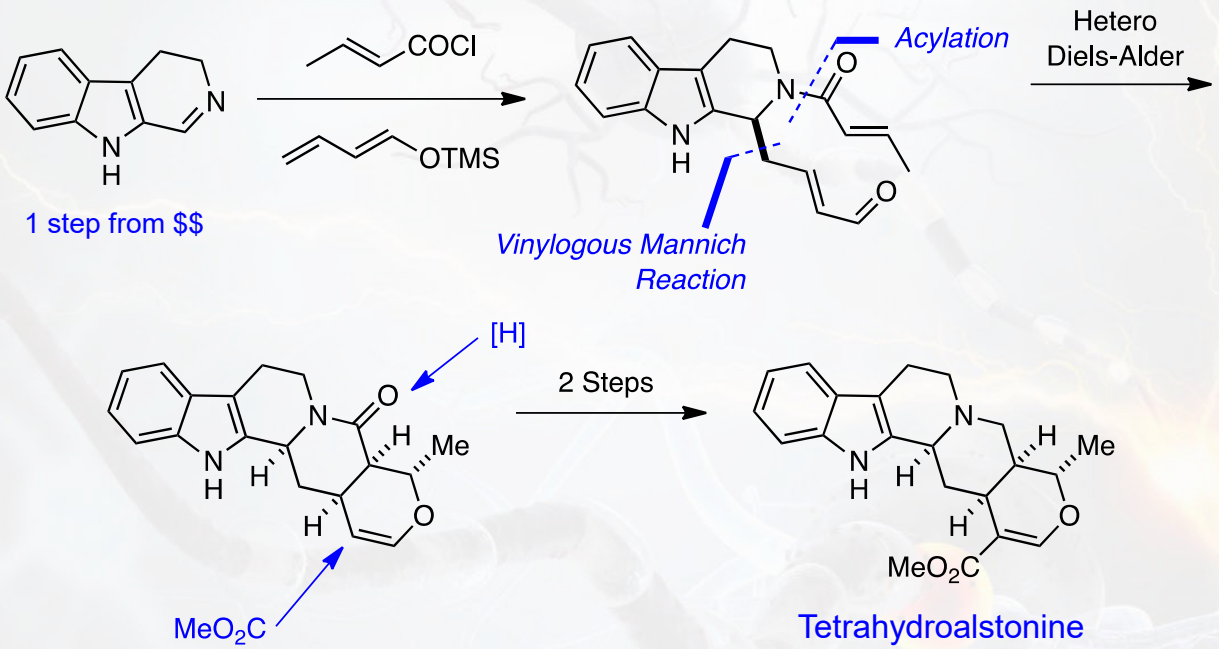
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Los Angeles, CA
11 May 2017

Disclosure: Cofounder of NuvoNuro

It All Begins With Chemistry

Synthetic challenge gives birth to " vinylogous Mannich reaction

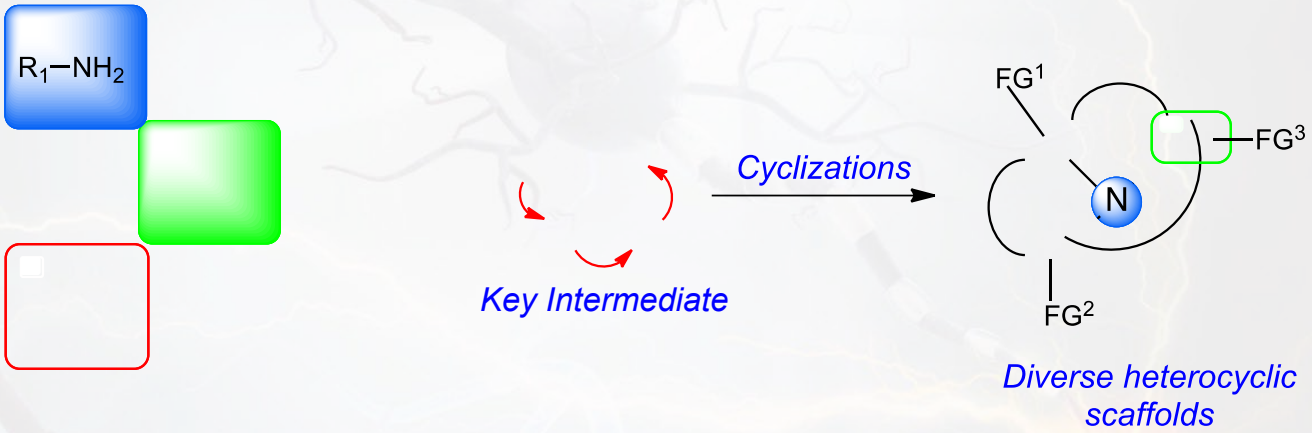


Tandem vinylogous Mannich–Diels Alder reaction: rapid entry to pentacyclic indole derivatives

See: Benage and Hunter *J. Am. Chem. Soc.* **1988**, *110*, 5925
For review of applications of vinylogous Mannich reaction, see: Martin, *Acc. Chem. Res.* **2006**, *39*, 433.

Inspired Novel Approach to "Library" Synthesis

DOS via Tandem Mannich-Based MCAP/Cyclizations



- Four different, readily available inputs may be used in *multicomponent assembly process (MCAP)*
- Enantioselectivity in C=N additions (For review, see Friestad, *Tetrahedron* **2007**, *63*, 2541)
- Numerous cyclization manifolds available, including Diels-Alder and dipolar cycloadditions, transition metal-catalyzed reactions (Heck, RCM, Pauson-Khand, etc), Dieckmann, aldol, etc
- Functional groups (FG) serve as handles for further elaboration and diversification

For lead references, see:

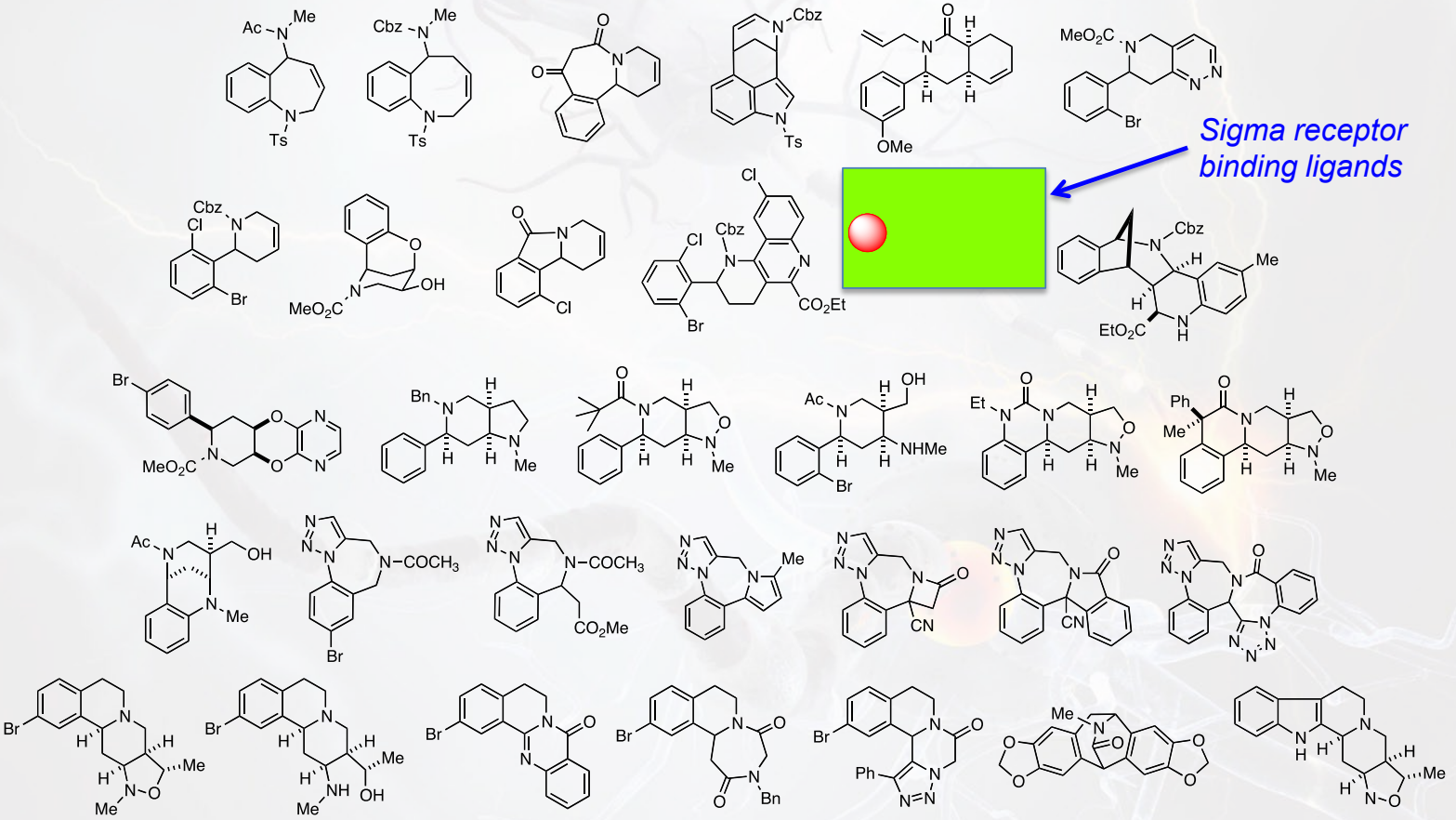
Adv. Heterocyclic Chem. **2013**, *110*, 74

Org. Biomol. Chem. **2014**, *12*, 7659

See also: Nielsen, Schreiber *Angew. Chem. Int. Ed.* **2007**, *47*, 48

Heterocyclic Scaffolds: Therapeutic Leads

Aryl Methylamino and Piperidine Compounds ~ Privileged Scaffolds



About 8 of the top 200 selling pharmaceuticals are α-aryl amine derivatives and more than 70 FDA approved drugs contain piperidine substructures

New Target for Unmet Needs in Neuroscience

σ2R/TMEM97 and Central Nervous System (CNS)

- Sigma 2 receptor (σ2R) is one of two sigma receptor subtypes. σ2R is expressed in CNS and has been associated with neuronal function and survival, mitochondrial protection, and calcium signaling.

- We (Kruse & Martin, *Proc. Natl. Acad. Sci USA,* in press) just cloned σ2R and showed it was identical to transmembrane protein 97 (TMEM97). TMEM97 has been associated with cholesterol trafficking and homeostasis.

- σ2R/TMEM97 increasingly being implicated in disorders of the CNS (Guo, *Curr. Med. Chem.* **2015**, *22*, 989).

- Ill-defined relationship between σ2R/TMEM97 and progesterone receptor membrane component 1 (PGRMC1), which is also expressed in CNS and involved in neuroprotection and axonal migration and cholesterol biosynthesis. Upregulated after brain and spinal cord injury.

Why is σ2R/TMEM97 Attractive as CNS Target

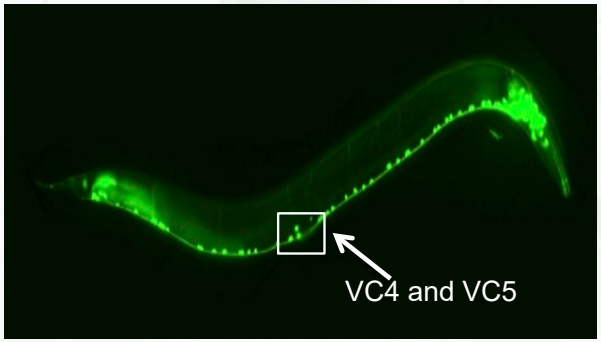
- *Some Pathological Features of Adverse CNS Conditions*
  - Neurodegeneration and cell death
  - Loss of [Ca^{2+}] regulation or homeostasis
  - Neuron hyperexcitability
  - Loss of synaptic plasticity from imbalance between synaptic long term depression (LTD) and long term potentiation (LTP)
  - Release of proinflammatory cytokines and chronic inflammation

- *Known attributes of ligands that bind to σ2R/TMEM97*
  - ✓ Neuroprotective (axons, neurons, oligodendrocytes)
  - ✓ Affect intracellular [Ca^{2+}]
  - ✓ Affect action potential firing (*i.e.,* neuron excitability)
  - ✓ Affect input resistance
  - ✓ Affect LTD and LTP and synaptic plasticity
  - ✓ Reduce proinflammatory cytokines, especially IL-1β

- *What are effects of modulating σ2R/TMEM97 in CNS?*

Discovering Neuroprotective Compounds

C. elegans as a Model Organism for Compound Screening



Transgenic (SC_APP) strain

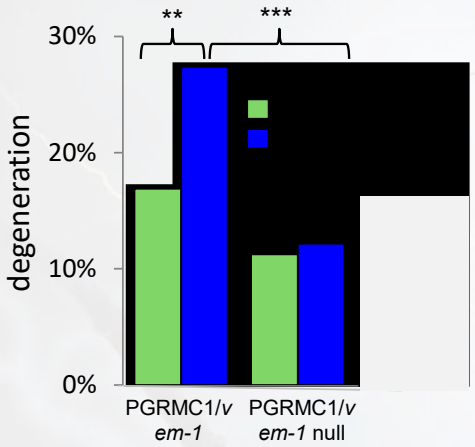
- *C. elegans* has orthologs of about two-thirds of human genes
- Single copy of human amyloid precursor protein (APP)
- Exhibits age-related, AD-like neurodegeneration, especially of VC4 and VC5 cholinergic neurons
- Cholinergic neurons labeled with green fluorescent protein for visualization

➢ *Model to screen σ2R/TMEM97 binding ligands for neuroprotective activity*

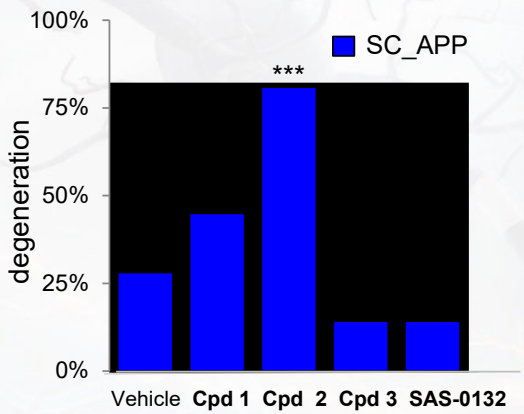
with Jon Pierce-Shimomura (University of Texas, Austin)

Assay for Neuroprotection

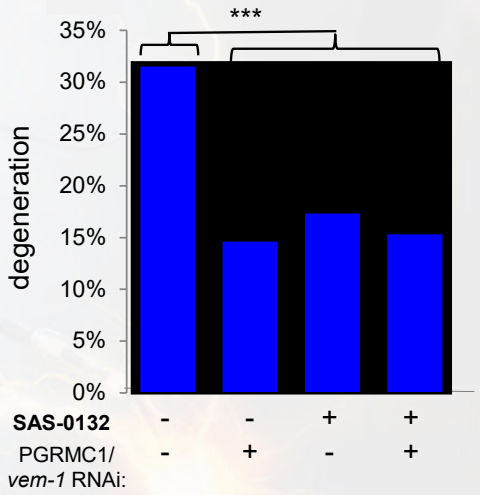
A. Transgenic worm and *vem-1* (PGRMC1) knockout



B. Transgenic worm treated with σ2R ligands



C. Transgenic worm and *vem-1* (PGRMC1) RNAi



- *C. elegans* SC_APP transgenic worm contains single copy of full length hAPP69 and exhibits greater neurodegeneration than control (WT)
- *C. elegans vem-1* (PGRMC1 ortholog) knockout (Null) exhibits reduced neurodegeneration vs WT
- σ2R binding ligands may *increase* or *decrease* neurodegeneration
- RNAi studies show neuroprotective σ2R binding ligands also inhibit PGRMC1-mediated pathway

σ2R/TMEM97 modulation can be neuroprotective!

Yi, Sahn, Ardestani, Evans, Scott, Chan, Iyer, Crisp, Zuniga, Pierce, Shamloo *J. Neurochem.* **2017**, *140*, 561

What are effects of modulating σ2R/TMEM97 upon neurodegenerative disease?

Are

Alzheimer's Disease: An Unmet Need

- Alzheimer's disease (AD) is a devastating terminal disease
  - 5 million Americans now living with AD
  - 6th leading cause of death in US
- Standard-of-care drugs not well-tolerated and provide only temporary, mild cognitive enhancement for some patients.

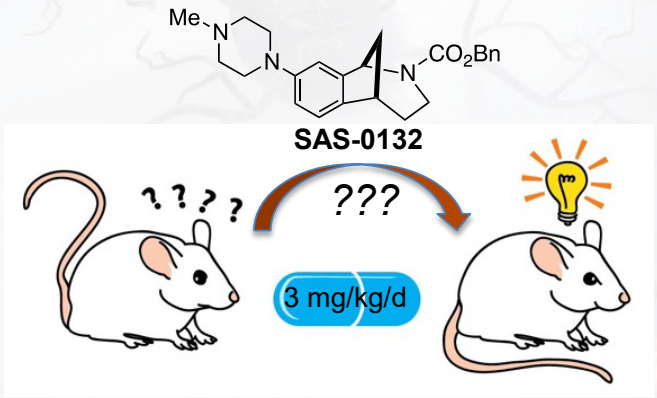


- About 100 companies had >220 drugs in development in 2013, but no new drug since 2003 and few in Phase III trials; *estimated market opportunity >$20 billion/year*.

No clear winners and new approaches needed, so what is effect of modulating σR/TMEM97 in AD models?

What Is Effect of SAS-0132 in AD

*Testing **SAS-0132** in Transgenic APP Mouse Model*



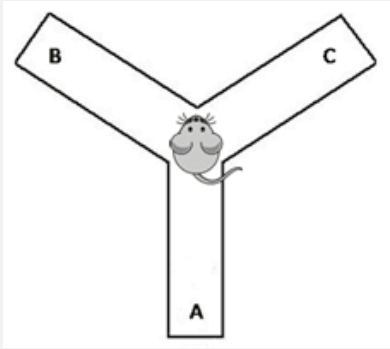
Transgenic APP mouse model
- Thy1-hAPP Lond/Swe
- Industry accepted model for AD

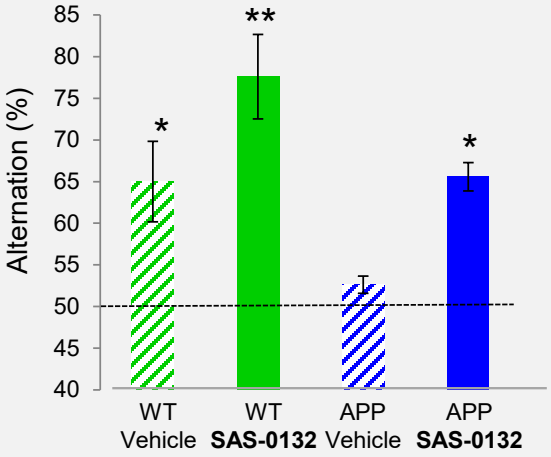
- APP mice and their wild-type littermates aged to 6.7 mos
- Dosed (SC) daily with **SAS-0132** and vehicle
- Learning and memory tests begun after 30 days of dosing

Yi, Sahn, Ardestani, Evans, Scott, Chan, Iyer, Crisp, Zuniga, Pierce, Shamloo *J. Neurochem.* **2017**, *140*, 561

SAS-0132: Cognition and Inflammation

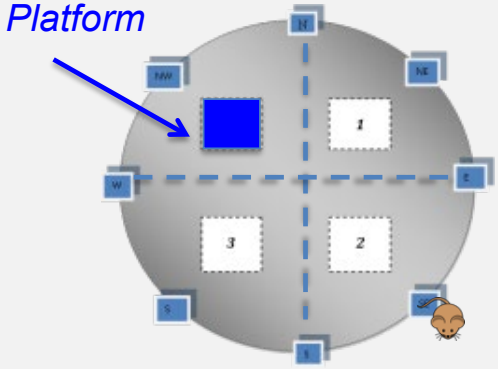
Y-Maze (Spatial Memory)

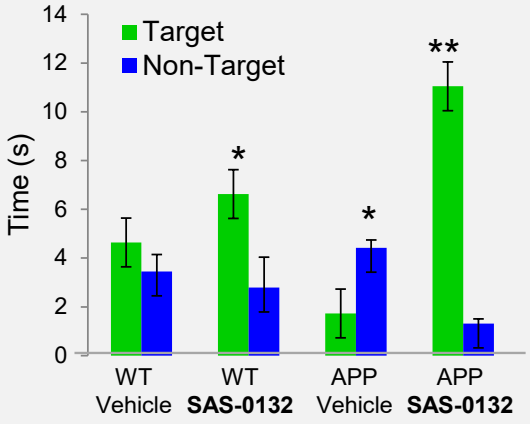




SAS-0132 improves recall of visitations in WT and APP mice

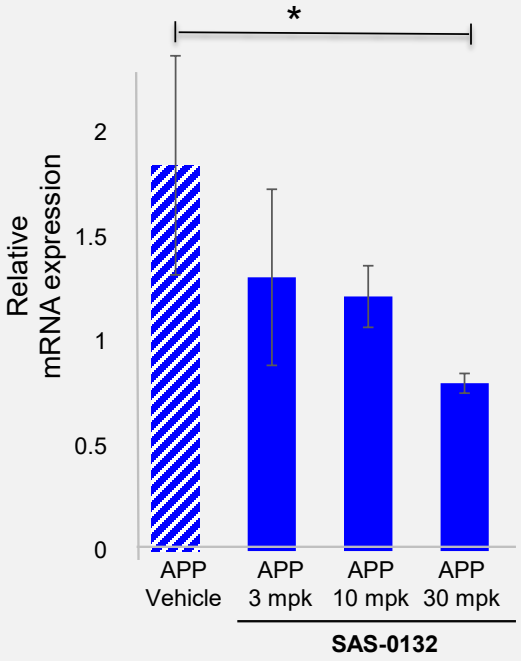
Morris Water Maze (Spatial and Long Term Memory)





SAS-0132 enhances memory of target location in WT and APP mice

Proinflammatory Cytokines Reduced



SAS-0132 significantly reduces IL-1β levels

Yi, Sahn, Ardestani, Evans, Scott, Chan, Iyer, Crisp, Zuniga, Pierce, Shamloo *J. Neurochem.* **2017**, *140*, 561

Traumatic Brain Injury ~ Unmet Need

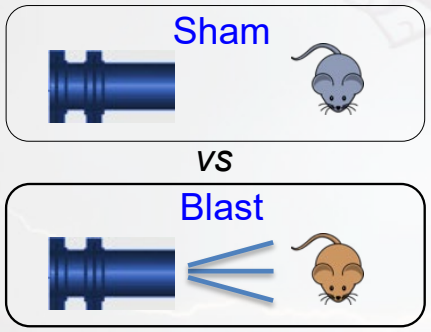
- *What is traumatic brain injury (TBI)*
  - Complex brain injury due to external mechanical force
  - Adverse effects to cognition, motor function, sensation, and emotion
  - Major risk factor for AD
- *Diverse patient population and causes*
  - All ages affected with 1.7 M new cases annually in the US; 10 M worldwide
  - Explosive blasts, falls, vehicle accidents, sports injuries, assault
  - Predicted to be third leading cause of disability and death by 2020



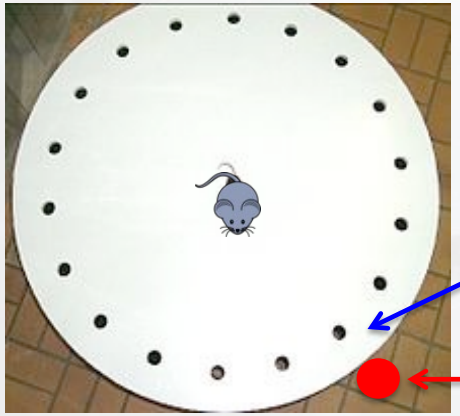
- *No approved drug bearing label claim for the treatment of TBI*
  - Estimated market in US is $2.7 B annually
- *Mitigating secondary brain injury critical to improving outcomes*
  - "Holy Grail" is neuroprotective drug that promotes neurogenesis

TBI: First Proof-of-Principle

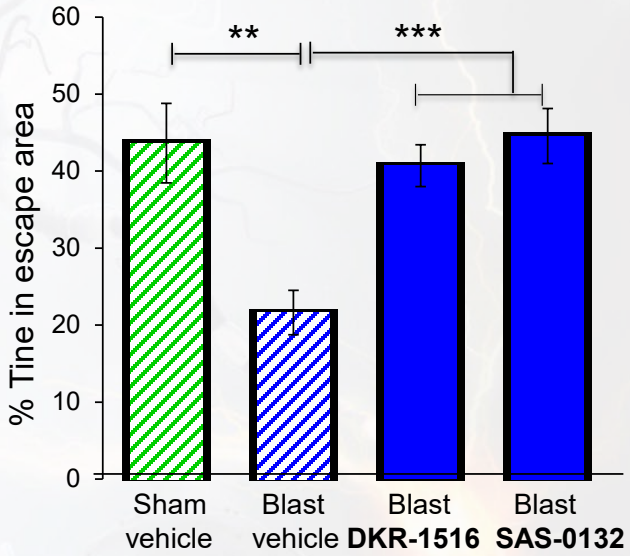
Blast Injury (Mild, diffuse)



Barnes Maze



Barnes Maze Performance

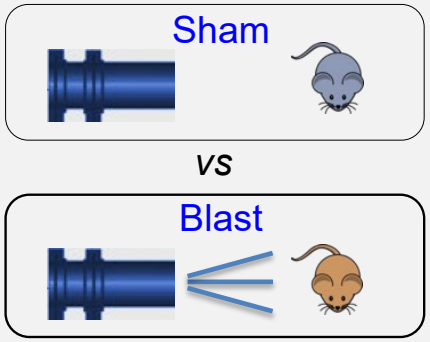


DKR-1516 and **SAS-0132** (10 mg/kg/d for 6 d beginning 24 hpi) improve performance in male mice (C57BL/6J) after air blast relative to control (Sham).

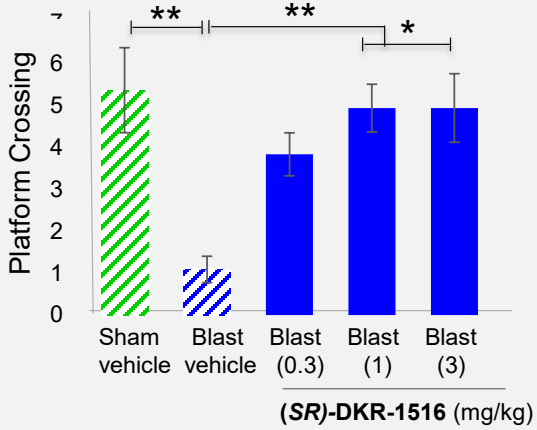
First evidence that modulating σ2R/TMEM97 has beneficial effect in TBI

with Andrew Pieper, University of Iowa

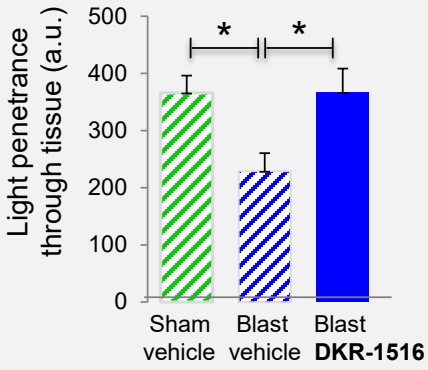
Traumatic Brain Injury: 1st Animal Model



Morris Water Maze

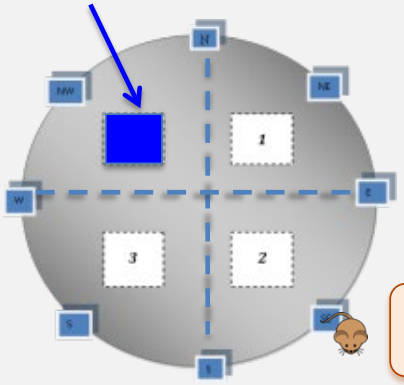


Silver staining



Morris Water Maze (Spatial and Long Term Memory)

Platform



DKR-1516 (0.3–3 mg/kg/d for 6 d beginning 24 hpi) increases spatial and long term memory to sham levels

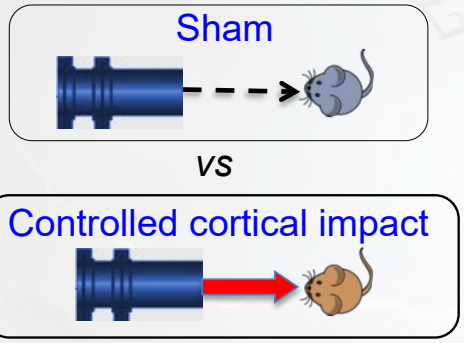
DKR-1516 (3 mg/kg) decreases axonal degeneration to sham level

***DKR-1516** promising lead to treat TBI by new mechanism*

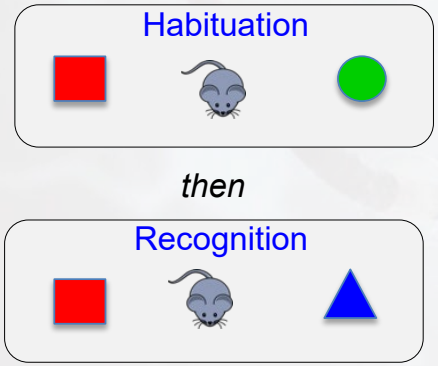
with Andrew Pieper, University of Iowa

TBI: 2nd Animal Model

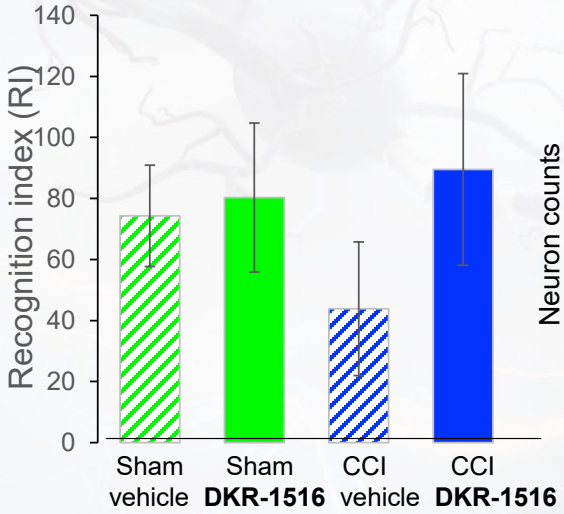
Injury (Moderate–Severe)



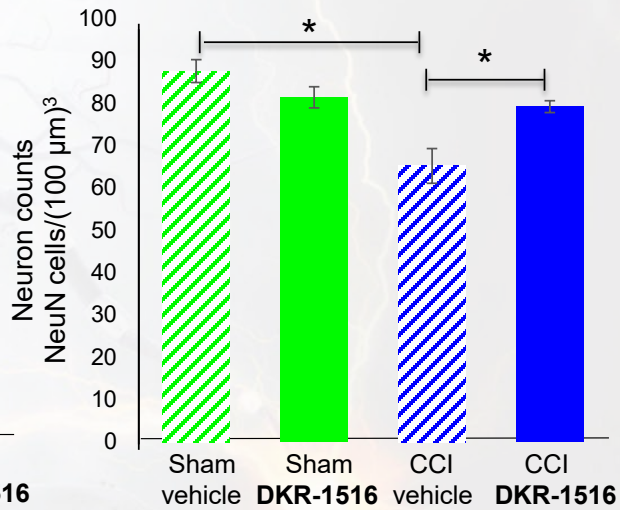
Novel Object Recognition



NOR Performance



Neuron (Cortex) Survival



DKR-1516 (3 mg/kg/d for 6 d beginning 1 hpi) improves performance in male mice (C57BL/6J) after CCI injury

DKR-1516 (3 mg/kg/d) decreases neuron and oligodendrocyte (not shown) death to Sham level

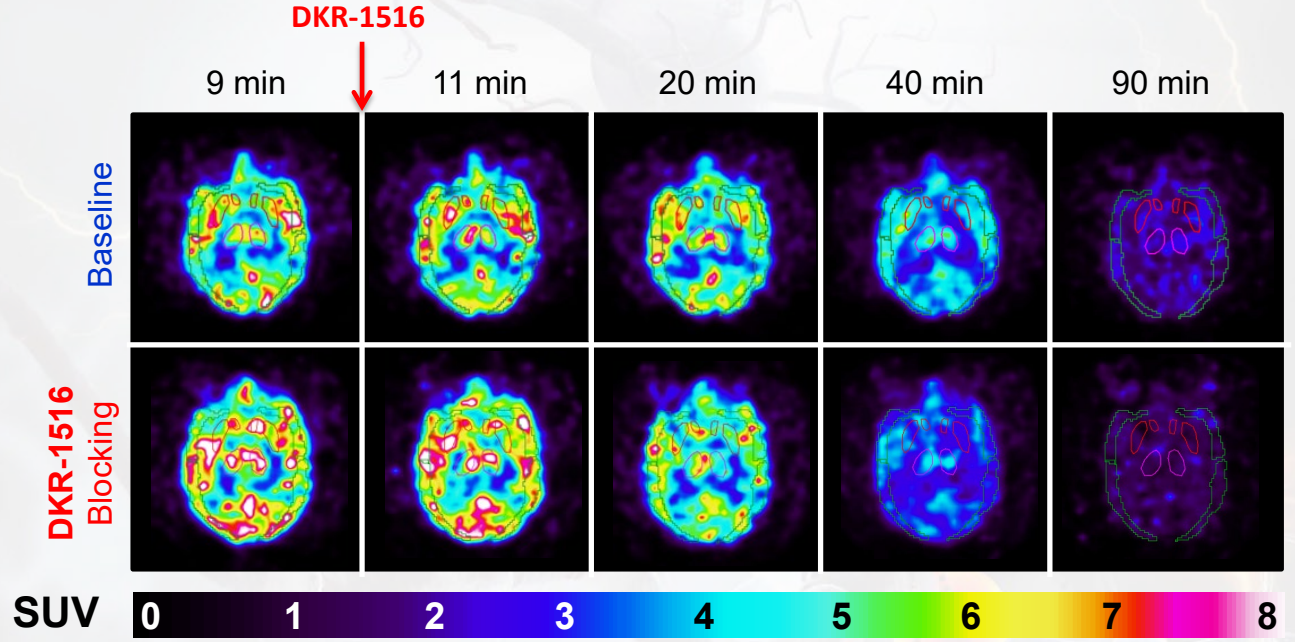
Modulating σ2R/TMEM97 improves cognition, neuron and oligodendrocyte survival after TBI in CCI animal model (moderate-severe injury)

with Dan Liebl, University of Miami

Are σ2R/TMEM97 modulators brain penetrant and how do they work?

Target Engagement Confirmed

Positron Emission Tomography Using [^{11}C]CTX in Monkey

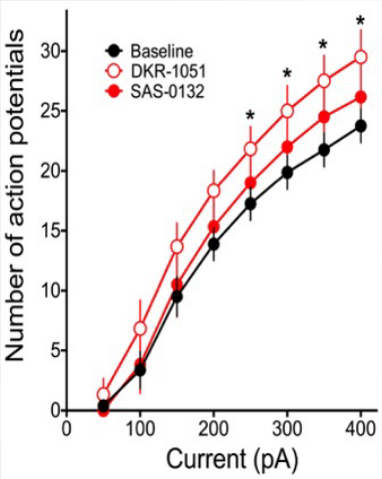


- *Standard uptake values (SUV) show*
  - **DKR-1516** rapidly crosses blood-brain barrier and binds σ2R in brain of rhesus monkey
  - **DKR-1516** more selective than CTX, phase Ib clinical lead for AD (Cognition Therapeutics)

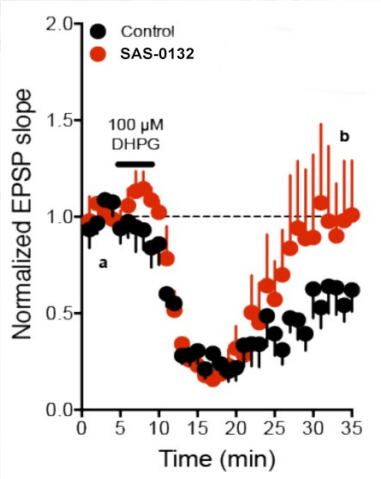
with Bob Mach, The University of Pennsylvania

σ2R/TMEM97 and Electrophysiology

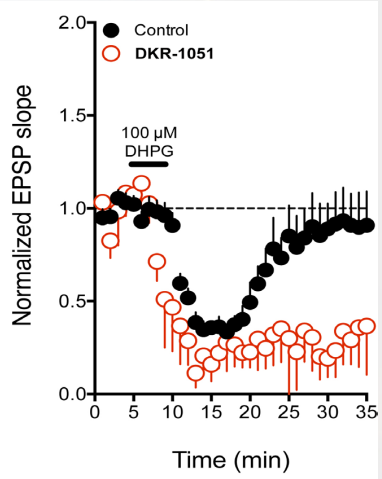
σ2R/TMEM97 Ligands Modulate Excitability, Plasticity, [Ca^{2+}]



SAS-0132 has little effect on action potential firing (APF), but **DKR-1051** *increases* APF



SAS-0132 *inhibits* long-term depression (LTD)



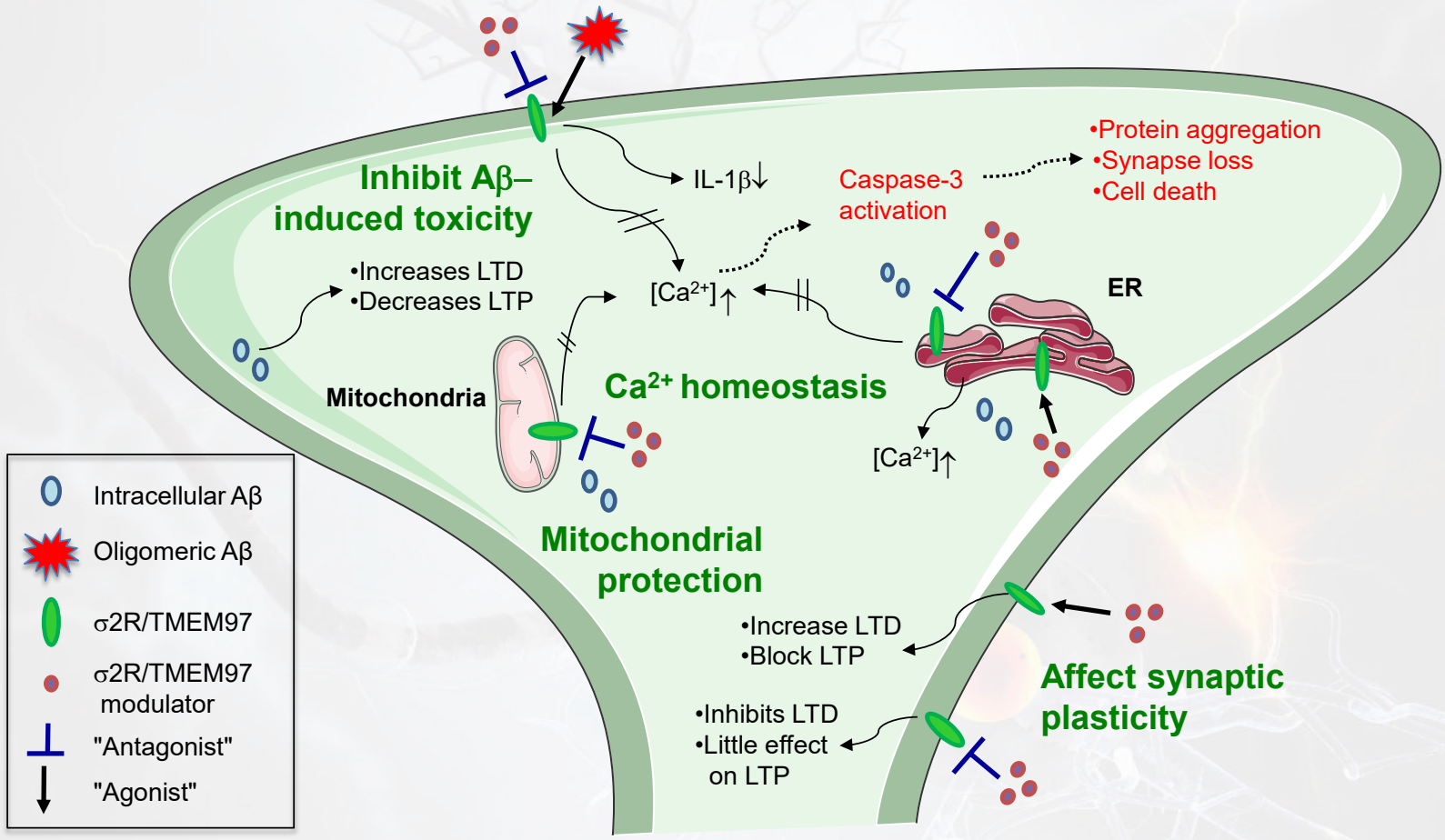
DKR-1051 *increases* mGluR-dependent LTD

In hippocampal CA1 neurons (6-mo C57BL/6J male mice),

- ***SAS-0132*** *blocks mGluR-dependent LTD but has little effect on LTP*
- ***DKR-1051*** *blocks LTP, and this is partly reversed by* ***SAS-0132***
- ***DKR-1051*** *increases APF, input resistance, and LTD*
- ***DKR-1051*** *stimulates release of [Ca^{2+}] from ER that is suppressed by* ***SAS-0132***

with Dan Johnston (The University of Texas, Austin)

σ2R/TMEM97 Modulators~Mode of Action



Inhibiting σ2R/TMEM97 blocks Aβ-mediated synaptotoxicity and reduces IL-1β. Modulating σ2R/TMEM97 regulates plasticity (LTD and LTP) and [Ca²⁺].

Alcohol Dependence: An Unmet Need

- Alcohol Use Disorder (AUD) is a Major Global Health Problem
  - Over 16.3 million adult Americans had an AUD in 2014
  - Nearly 88,000 people in the US die from alcohol related causes annually; 4th leading preventable cause of death
  - In 2012 3.3 million deaths (5.9%) globally attributable to alcohol consumption
  - Alcohol misuse problems cost the US $249 billion in 2010

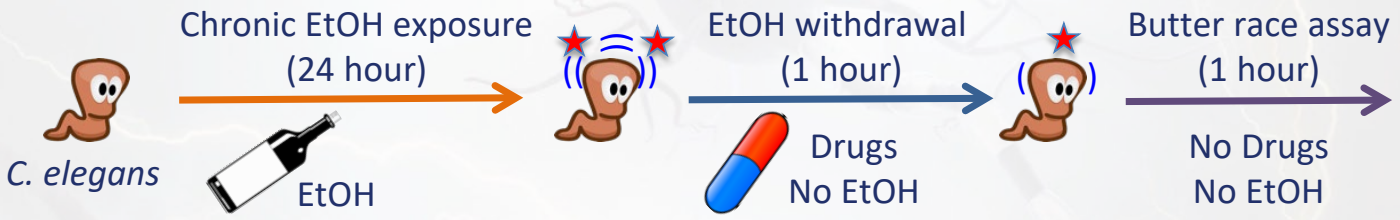


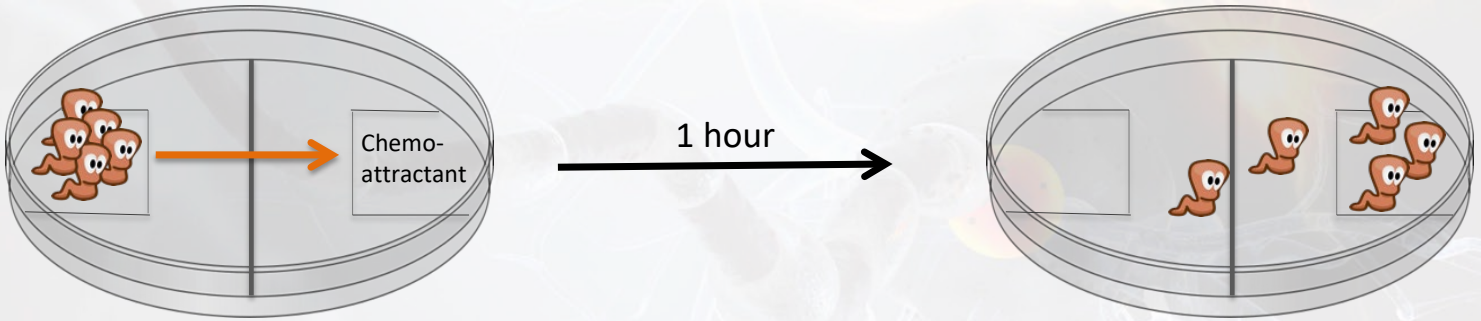


- ***No effective treatments and new therapeutic approaches needed***

σ2R/TMEM97 and Ethanol Withdrawal

Screening in C. elegans: *Experimental Method and Timeline*



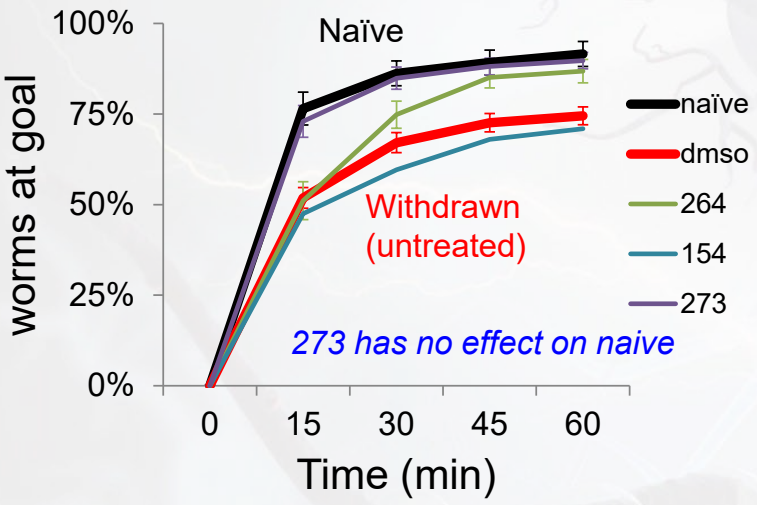


How many make it??

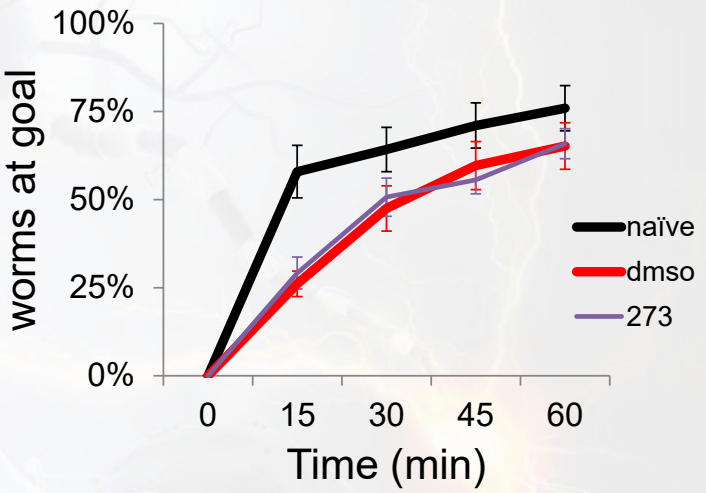
with Scott and Pierce-Shimomura – University of Texas, Austin

Mode of Action Mediated by PGRMC1

C. elegans - Wild-type



C. elegans – vem-1 (PGRMC1) null

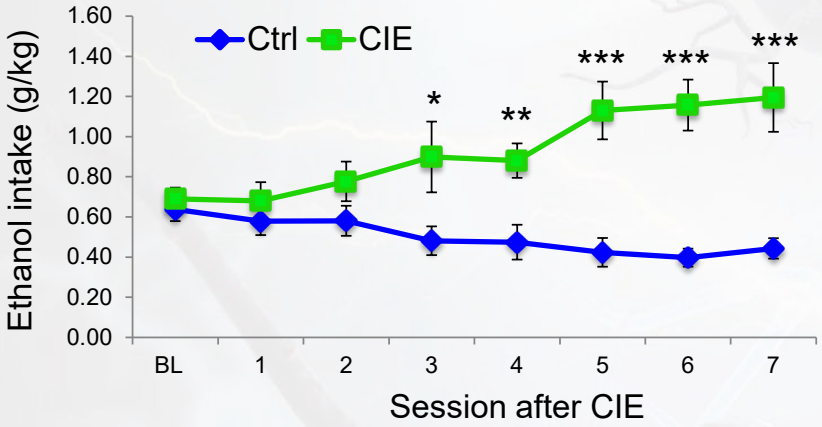


Because vem-1 *(PGRMC1) knockout is not affected by treatment, σ2R ligands also appear to act via PGRMC1-mediated pathway*

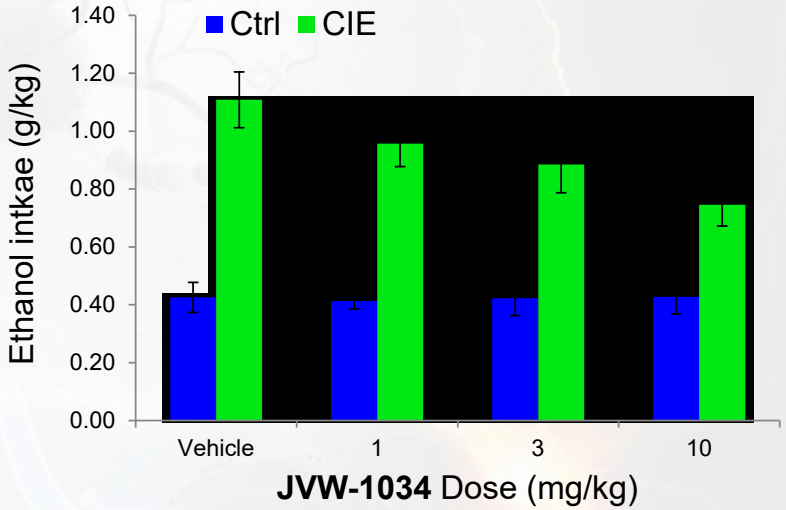
with Scott and Pierce-Shimomura – University of Texas, Austin

Alcohol Dependence: Proof-of-Principle

JVW-1034 Reduces EtOH Consumption in EtOH-Dependent Rats



Rats made EtOH dependent by exposure to chronic intermittant ethanol vapors (CIE)



JVW-1034 decreases EtOH consumption in rats in dose dependent fashion (did not alter water intake)

First evidence that modulating σ2R/TMEM97 has beneficial effect in animal model for alcohol dependence

with Valentina Sabino (Boston University)

Neuropathic Pain: An Unmet Need

- Effective Drugs to Treat Chronic and Neuropathic Pain Lacking
  - Chronic and neuropathic pain affected more than 100 million Americans in 2011
  - Neuropathic pain markedly reduces quality of life and is associated with many diseases, including diabetes, cancer, HIV and
  - Opioids not always effective and use contributes to abuse, addiction, etc
  - Average cost per patient with neuropathic pain in US in 2012 was $28,000/y
  - Annual cost of chronic pain in US in 2011 estimated to be $635 billion

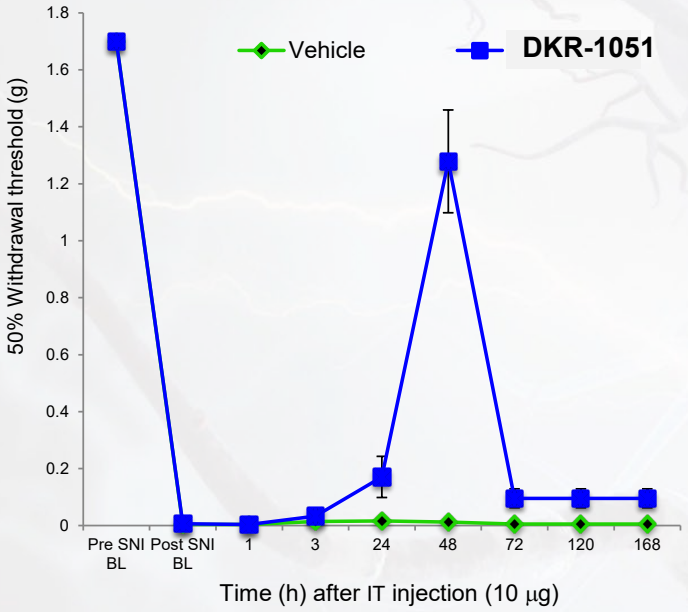




- ***No effective treatments and new targets and approaches needed***

σ2R/TMEM97 Ligands Attenuate Pain

DKR-1051 *Reduces Neuropathic Pain*



- In mice, **DKR-1051** dramatically decreases neuropathic pain associated with spared nerve injury (SNI); slow onset after intrathecal injection, but long duration (48 h). Similar for IV (data not shown)
- Notably, effect of **DKR-1051** is completely blocked by **SAS-0132** (data not shown)
- Gold-standard drug for neuropathic pain, Neurontin (gabapentin), is meaningfully effective for only 2-3 h at significantly higher dose than used for **DKR-1051** (not shown).

First evidence that modulating σ2R/TMEM97 has beneficial effect in animal model for neuropathic pain

with Ted Price (University of Texas at Dallas)

And It All Ends With Biology

Why σ2R/TMEM97 IS attractive target in neuroscience

- Compounds safe and selective (low off target binding to other CNS proteins)
- Confirmed rapid target engagement in rodent and nonhuman primate brain
- Modulators
  - reduce axon degeneration and improve neuron and oligodendrocyte survival
  - regulate neuron excitability and synaptic plasticity (LTD and LTP)
  - reduce proinflammatory cytokines such as IL-1β
  - improve cognitive performance in healthy mice
  - improve cognitive performance animal model of Alzheimer's disease
  - improve cognitive performance in two animal models of TBI (blast and CCI)
  - show promise in alcohol dependence and neuropathic pain

How is this approach different from others

Novel biological target

Multipotential preclinical leads

IT HAS NOT FAILED!!

Thanks to Those Who Made It Happen

Synthetic/Medicinal Chemistry
The University of Texas, Austin


Jim Sahn


Tim Hodges


Jessica Chan

C. elegans Models
The University of Texas, Austin


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Ted Price


Galo Mejia

Alcoholism
Boston University


Valentina Sabino


Antonio Ferragud

Texas Health Catalyst
Accelerating the translation of research to health products

THE Welch FOUNDATION

BrightFocus Foundation

PDSP
NIMH Psychoactive Drug Screening Program

DARRELL K ROYAL RESEARCH FUND for ALZHEIMER'S DISEASE

materia
CATALYSTS FOR CHANGE

NATIONAL INSTITUTES OF HEALTH

SANOFI

And Thank You

For Listening

And Thanks to Collaborators and Funders

Collaborators

Michael Doyle ~ Trinity University
Horst Kessler ~ Technische Universität München
Gabriel Waxsman ~ Washington University, St. Louis
Jonathan Pierce ~ University of Texas, Austin
Mehrdad Shamloo ~ Stanford University
Andrew Pieper ~ University of Iowa
Daniel Liebl ~ University of Miami
Valentina Sabino ~ Boston University

Brian Roth ~ University of North Carolina
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Daniel Johnston ~ University of Texas, Austin
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Robert Mach ~ University of Pennsylvania
Wayne Bowen ~ Brown University
Andrew Kruse ~ Harvard University



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P&G
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materia Catalysts for Change
BrightFocus Foundation
Texas Health Catalyst
Accelerating the translation of research to health products
SANOFI
PDSP
NIMH Psychoactive Drug Screening Program

Givaudan

And Thank You

For Listening

Thanks Again To All
Partner Universities



σ2R/TMEM97 and TBI

- *What we have learned about σ2R/TMEM97 modulators*
  - ✓ No toxic effects observed (MTD- and extended dosing)
  - ✓ Improve cognitive performance in mice in two TBI models
  - ✓ Reduce axonal degeneration
  - ✓ Protect neurons and oligodendrocytes

- *How do they work?*

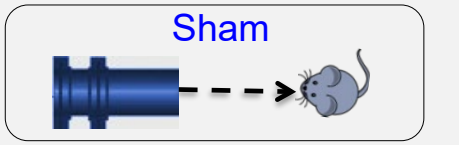
Unmet Medical Needs in Neuroscience

➢ *The Sad Truth*

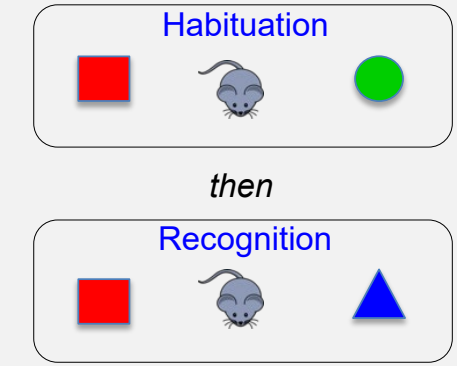
- ✧ Huge unmet medical need for effective drugs in neuroscience
  - Alzheimer's disease
  - Traumatic brain injury
  - Huntington's disease
  - Substance abuse and addiction
  - Pain and neuropathic pain
  - Numerous others
- ✧ Many approaches to develop drugs to treat CNS diseases and disorders have failed over the past 25 years.
- ✧ Collectively, annual cost to society is 100s of billions USD

What are the effects of modulating Sig2R/TMEM97 upon neurodegenerative disease?

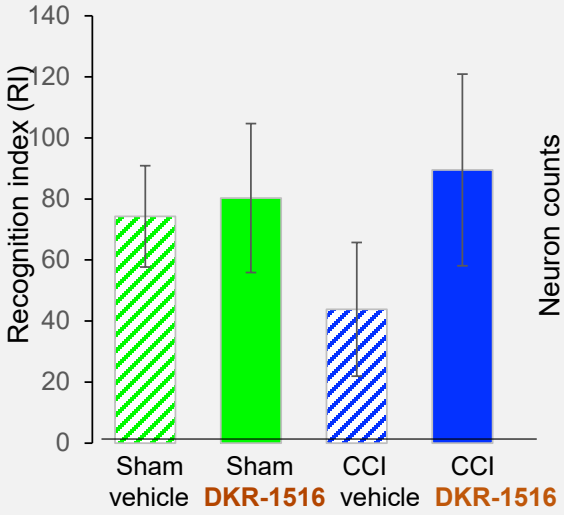
Traumatic Brain Injury: 2nd Animal Model



Novel Object Recognition

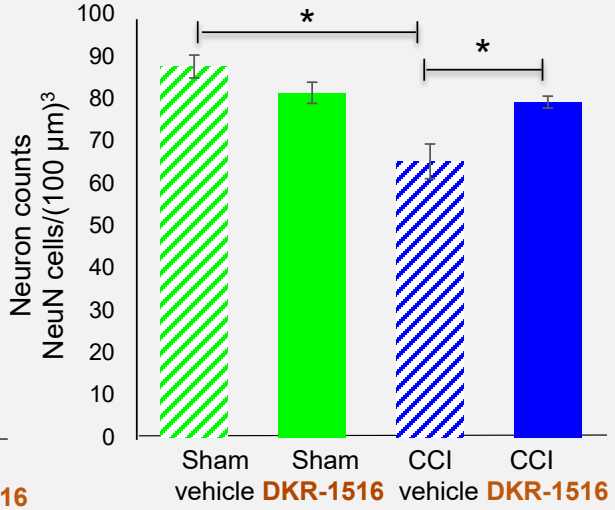


Novel Object Recognition



DKR-1516 (3 mg/kg/d) improves performance in male mice (C57Bl/6j) after CCI injury

Neuron (Cortex) Survival



DKR-1516 (3 mg/kg/d) decreases neuron and oliogodendrocyte (not shown) cell death

Modulating Sig2R/TMEM97 has beneficial effect in two animal models for TBI

with Dan Liebl, University of Miami

New Target for Unmet Needs in Neuroscience

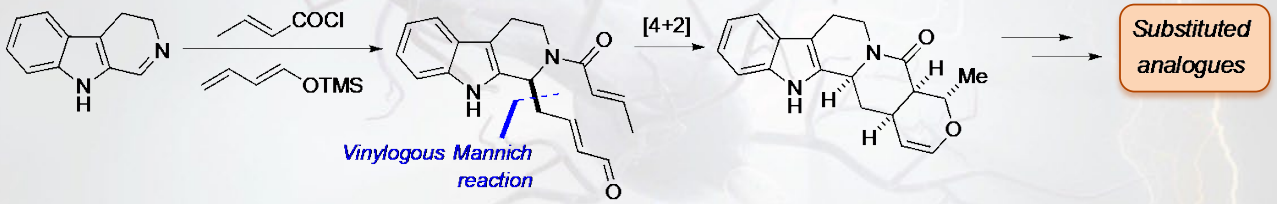
Sigma 2 Receptor and Central Nervous System (CNS)

- Sigma 2 receptor (σ2R) is one of two sigma receptor subtypes. It is expressed in CNS and has been associated with neuronal function and survival, mitochondrial protection, and calcium signaling.
- We (Kruse & Martin, unpublished) just cloned this receptor and showed it was identical to a transmembrane protein associated with cholesterol trafficking and homeostasis.
- σ2R increasingly implicated in CNS disorders (Guo, *Curr. Med. Chem.* **2015**, *22*, 989), including Alzheimer's disease (Martin, Sahn, Shamloo, *J. Neurochem*. **2017**, *140*, 561)

What are effects of modulating σ2R upon other neurodegenerative conditions?

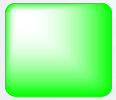
It All Begins With Chemistry

Rapid Scaffold Assembly: Are There Other Applications?



Benage, Hunter *J. Am. Chem. Soc.* **1988**, *110*, 5925. Hunter, Benage, Geraci, Mortimore *J. Am. Chem. Soc.* **1991**, *113*, 6161.

Multicomponent Assembly Process: Novel Approach to Molecular Libraries



Key intermediate

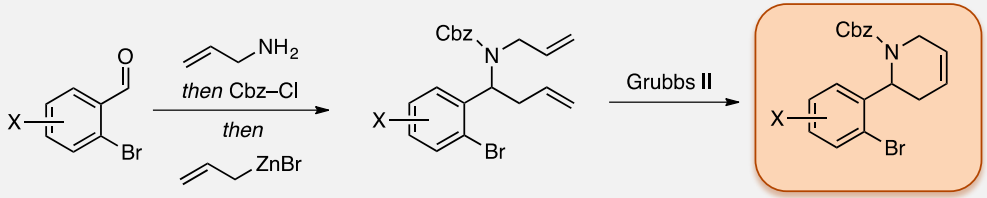
affolds

- Four different, readily available inputs may be used in *multicomponent assembly process (MCAP)*
- Numerous cyclization manifolds available, including Diels-Alder and dipolar cycloadditions, transition metal-catalyzed reactions (Heck, RCM, Pauson-Khand, etc), Dieckmann, aldol, etc
- Functional groups (FG) serve as handles for further elaboration and diversification

For reviews, see:
Martin *Adv. Heterocyclic Chem.* **2013**, *110*, 74
Sahn, Granger, Martin *Org. Biomol. Chem.* **2014**, *12*, 7659
See also: Nielsen, Schreiber *Angew. Chem. Int. Ed.* **2007**, *47,* 48

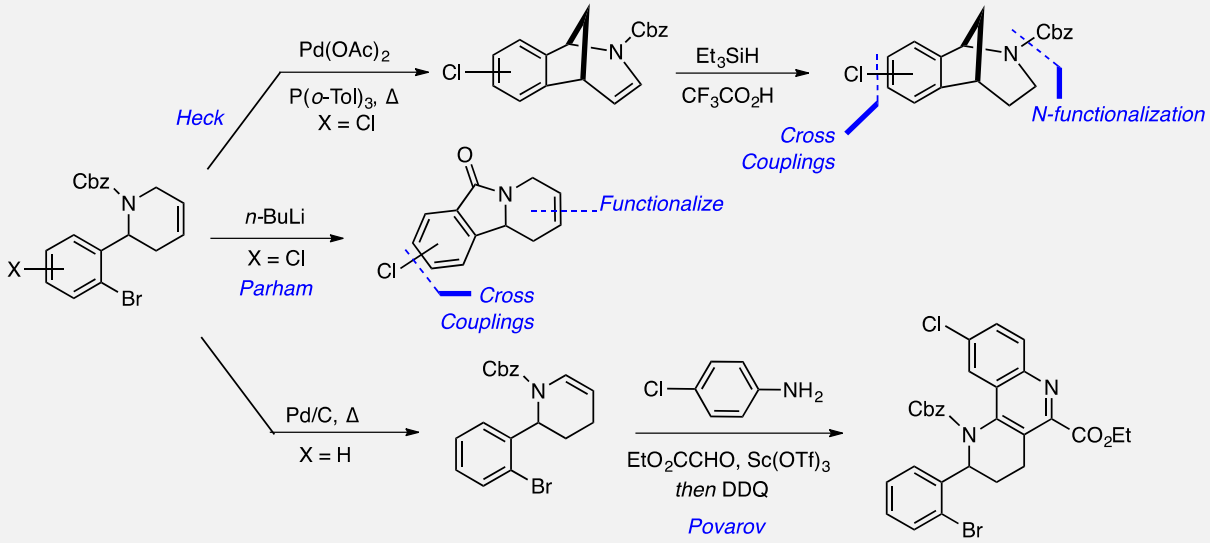
New Tactic for DOS Exemplified

Tandem MCAP/RCM to Generate Pivotal Scaffold



Key intermediate

Skeletal Diversification



Sahn, Su *Org. Lett.* **2011**, *13*, 2590. Sahn *ACS Combi. Sci.* **2012**, *14*, 496.