



Applications of Chemistry to Unmet Medical Needs in Neuroscience

Stephen Martin

*Department of Chemistry
The University of Texas at Austin*

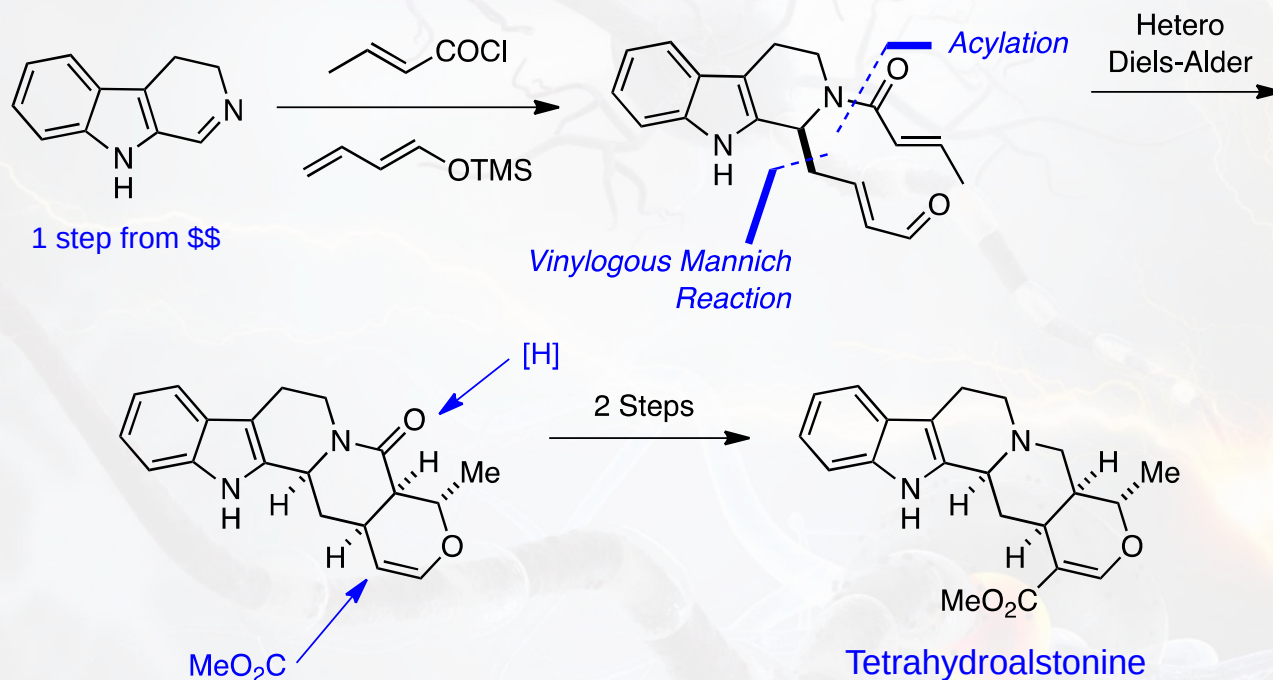
Cedars-Sinai
Los Angeles, CA
11 May 2017

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

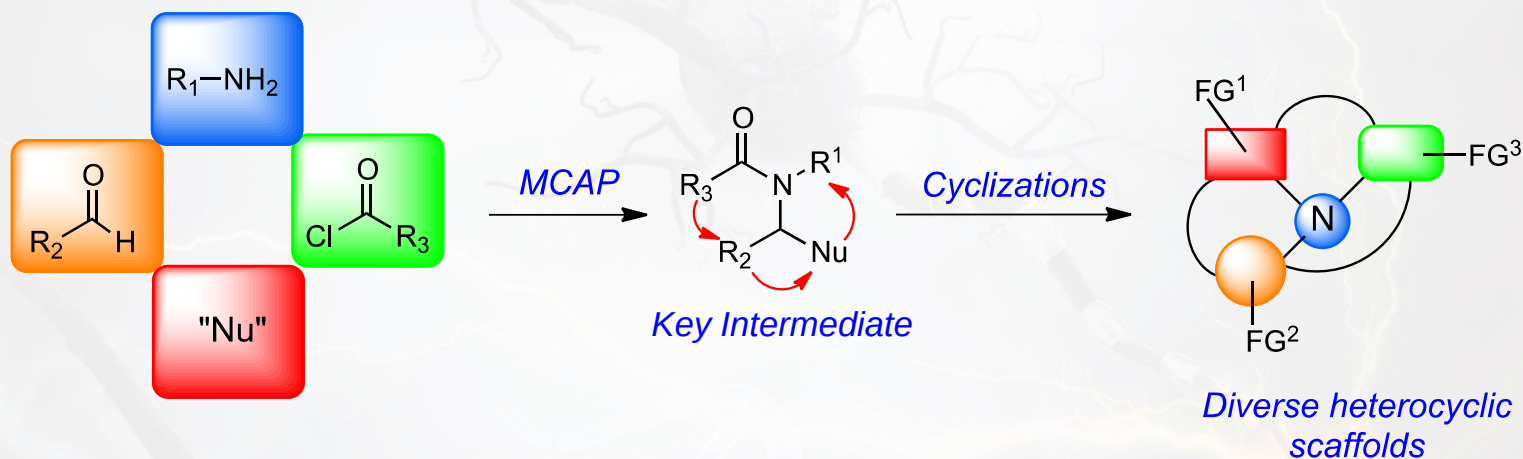
See: Benage and Hunter *J. Am. Chem. Soc.* **1988**, *110*, 5925

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



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

For lead references, see:

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

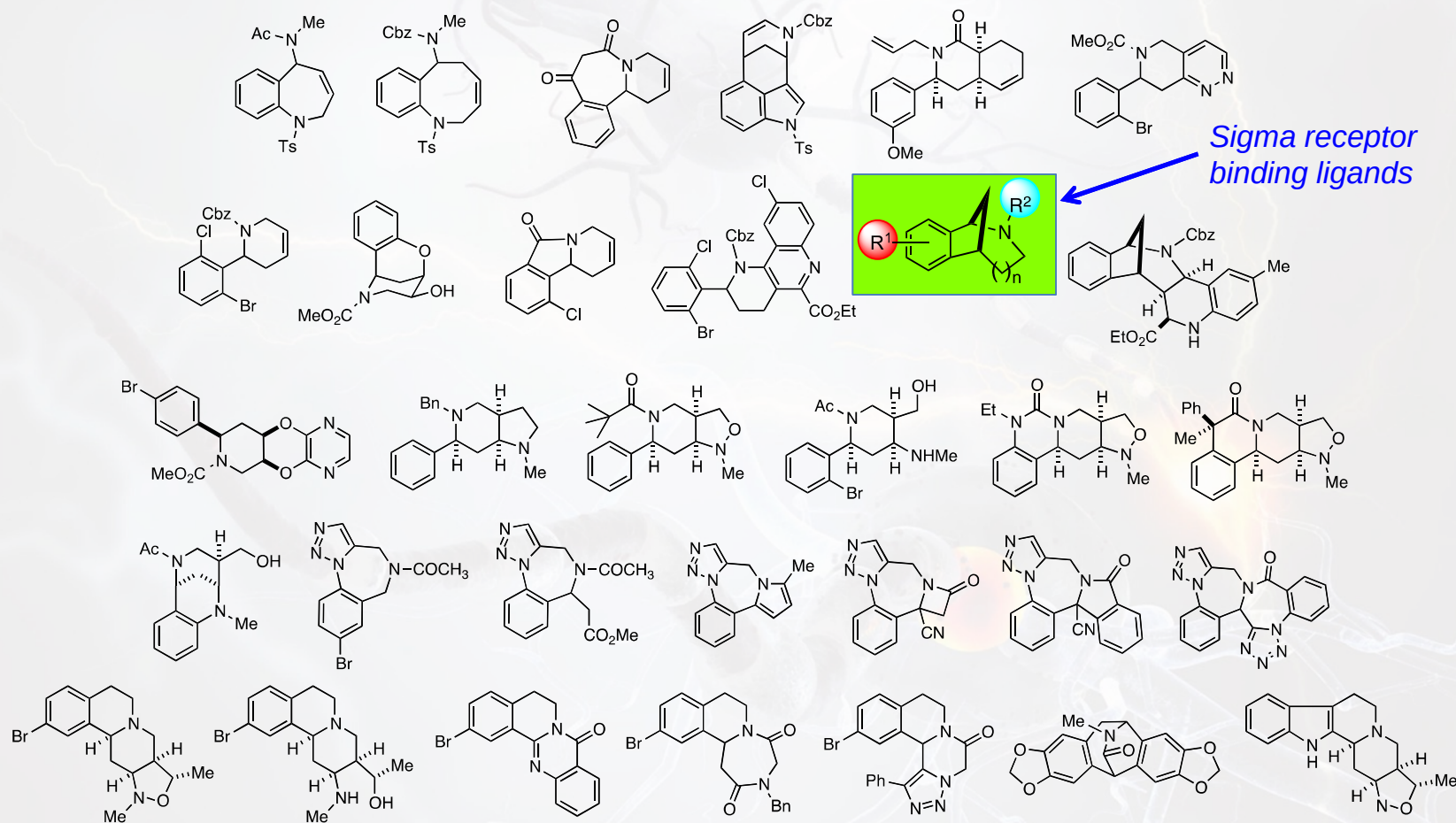
Org. Biomol. Chem. **2014**, 12, 7659

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



σ 2R/TMEM97 and Central Nervous System (CNS)

- Ø Sigma 2 receptor (σ 2R) is one of two sigma receptor subtypes. σ 2R is expressed in CNS and has been associated with neuronal function and survival, mitochondrial protection, and calcium signaling.
- Ø We (Kruse & Martin, *Proc. Natl. Acad. Sci USA*, in press) just cloned σ 2R and showed it was identical to transmembrane protein 97 (TMEM97). TMEM97 has been associated with cholesterol trafficking and homeostasis.
- Ø σ 2R/TMEM97 increasingly being implicated in disorders of the CNS (Guo, *Curr. Med. Chem.* **2015**, 22, 989).
- Ø Ill-defined relationship between σ 2R/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 σ 2R/TMEM97 Attractive as CNS Target



Ø *Some Pathological Features of Adverse CNS Conditions*

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

Ø *Known attributes of ligands that bind to σ 2R/TMEM97*

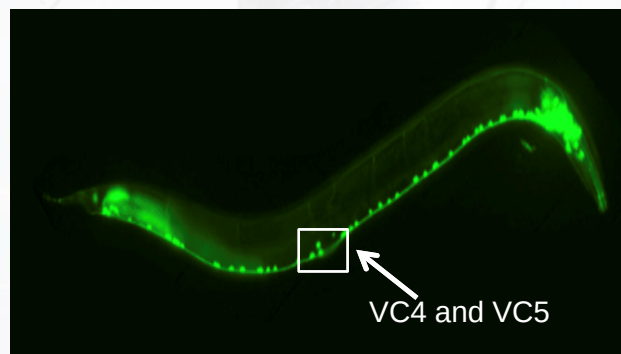
- ü Neuroprotective (axons, neurons, oligodendrocytes)
- ü Affect intracellular $[Ca^{2+}]$
- ü Affect action potential firing (*i.e.*, neuron excitability)
- ü Affect input resistance
- ü Affect LTD and LTP and synaptic plasticity
- ü Reduce proinflammatory cytokines, especially IL-1 β

Ø *What are effects of modulating σ 2R/TMEM97 in CNS?*

Discovering Neuroprotective Compounds



C. elegans as a Model Organism for Compound Screening



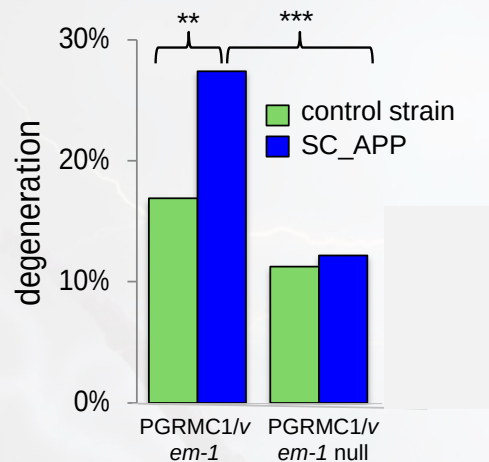
Transgenic (SC_APP) strain

- Ø *C. elegans* has orthologs of about two-thirds of human genes
 - Ø Single copy of human amyloid precursor protein (APP)
 - Ø Exhibits age-related, AD-like neurodegeneration, especially of VC4 and VC5 cholinergic neurons
 - Ø Cholinergic neurons labeled with green fluorescent protein for visualization
- Ø Model to screen $\sigma 2R$ /TMEM97 binding ligands for neuroprotective activity*

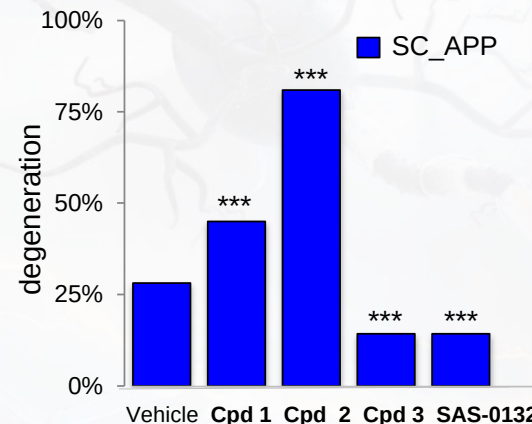
with Jon Pierce-Shimomura (University of Texas, Austin)



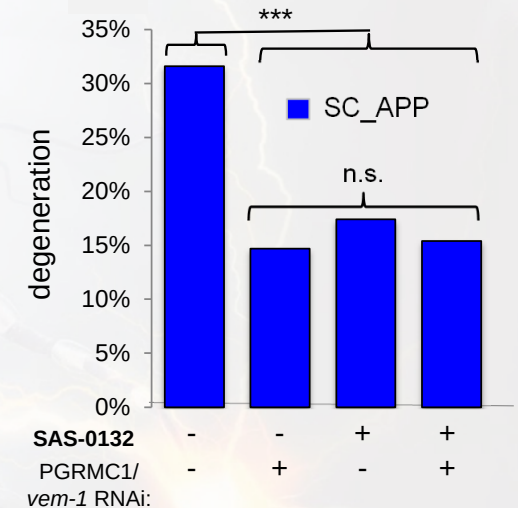
A. Transgenic worm and *vem-1* (PGRMC1) knockout



B. Transgenic worm treated with σ 2R ligands



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



- Ø *C. elegans* SC_APP transgenic worm contains single copy of full length hAPP69 and exhibits greater neurodegeneration than control (WT)
- Ø *C. elegans vem-1* (PGRMC1 ortholog) knockout (Null) exhibits reduced neurodegeneration vs WT
- Ø σ 2R binding ligands may *increase* or *decrease* neurodegeneration
- Ø RNAi studies show neuroprotective σ 2R binding ligands also inhibit PGRMC1-mediated pathway

σ 2R/TMEM97 modulation can be neuroprotective!

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

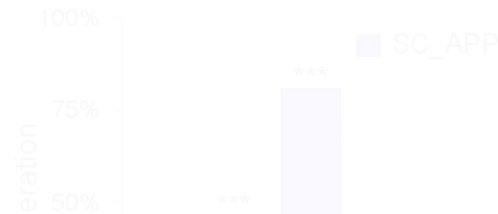
Assay for Neuroprotection



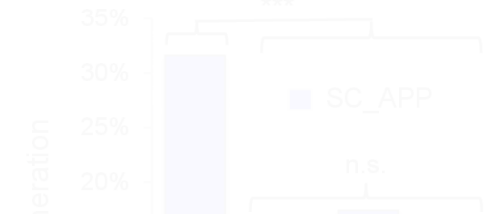
A. Transgenic worm and *vem-1* (PGRMC1) knockout



B. Transgenic worm treated with σ 2R ligands



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



What are effects of modulating σ 2R/TMEM97 upon neurodegenerative disease?

- *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
- σ 2R binding ligands may *increase or decrease* neurodegeneration
- RNAi studies show neuroprotective σ 2R binding ligands also inhibit PGRMC1-mediated pathway

σ 2R/TMEM97 modulation can be neuroprotective!

J. Neurochem. 2017, 140, 561

Alzheimer's Disease: An Unmet Need



- Ø Alzheimer's disease (AD) is a devastating terminal disease
 - ² 5 million Americans now living with AD
 - ² 6th leading cause of death in US
- Ø Standard-of-care drugs not well-tolerated and provide only temporary, mild cognitive enhancement for some patients.



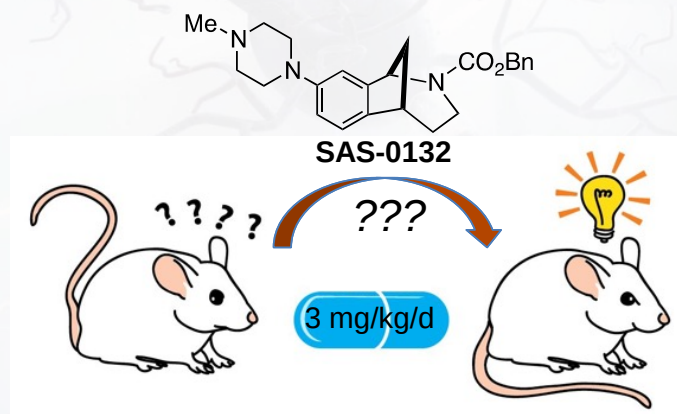
- Ø About 100 companies had >220 drugs in development in 2013, but no new drug since 2003 and few in Phase III trials; *estimated market opportunity >\$20 billion/year.*

***No clear winners and new approaches needed,
so what is effect of modulating $\sigma R/TMEM97$ 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

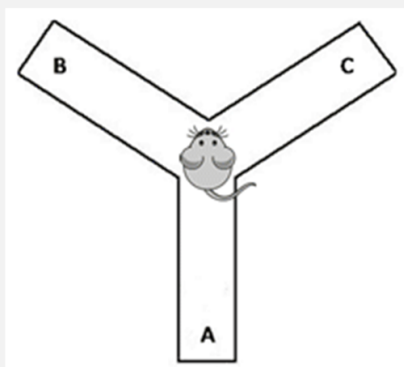
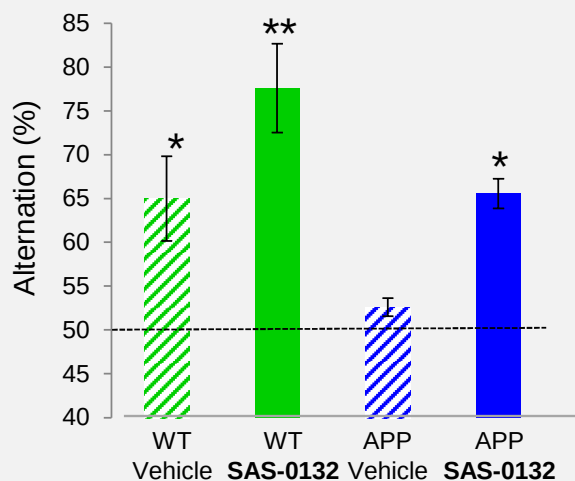
- Ø APP mice and their wild-type littermates aged to 6.7 mos
- Ø Dosed (SC) daily with **SAS-0132** and vehicle
- Ø Learning and memory tests begun after 30 days of dosing

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

SAS-0132: Cognition and Inflammation

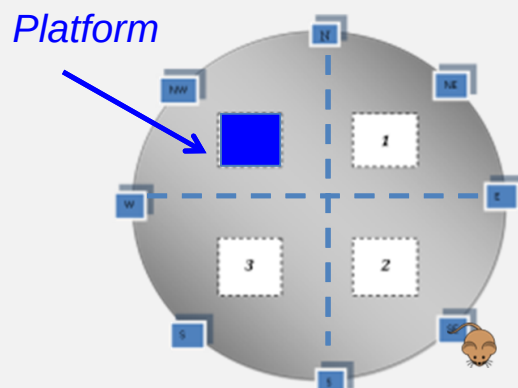
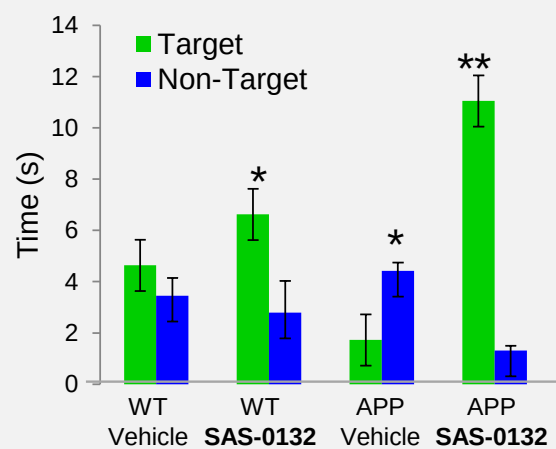


Y-Maze (Spatial Memory)



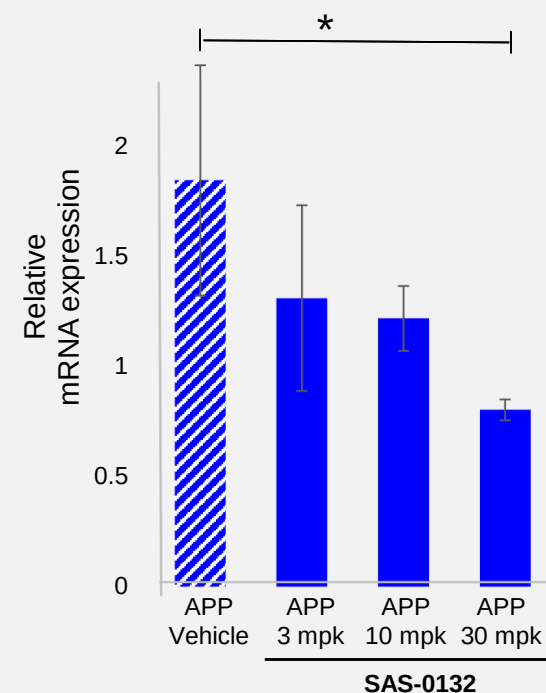
SAS-0132 improves recall of visitations in WT and APP mice

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-1 β levels

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

Traumatic Brain Injury ~ Unmet Need



Ø *What is traumatic brain injury (TBI)*

- 2 Complex brain injury due to external mechanical force
- 2 Adverse effects to cognition, motor function, sensation, and emotion
- 2 Major risk factor for AD

Ø *Diverse patient population and causes*

- 2 All ages affected with 1.7 M new cases annually in the US; 10 M worldwide
- 2 Explosive blasts, falls, vehicle accidents, sports injuries, assault
- 2 Predicted to be third leading cause of disability and death by 2020



Ø *No approved drug bearing label claim for the treatment of TBI*

- 2 Estimated market in US is \$2.7 B annually

Ø *Mitigating secondary brain injury critical to improving outcomes*

- 2 "Holy Grail" is neuroprotective drug that promotes neurogenesis

TBI: First Proof-of-Principle

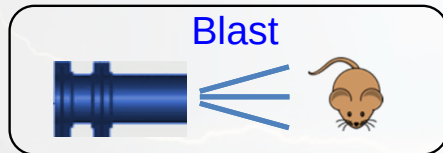


Blast Injury (Mild, diffuse)



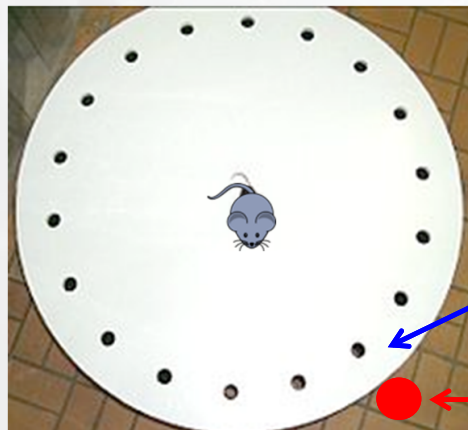
Sham

VS



Blast

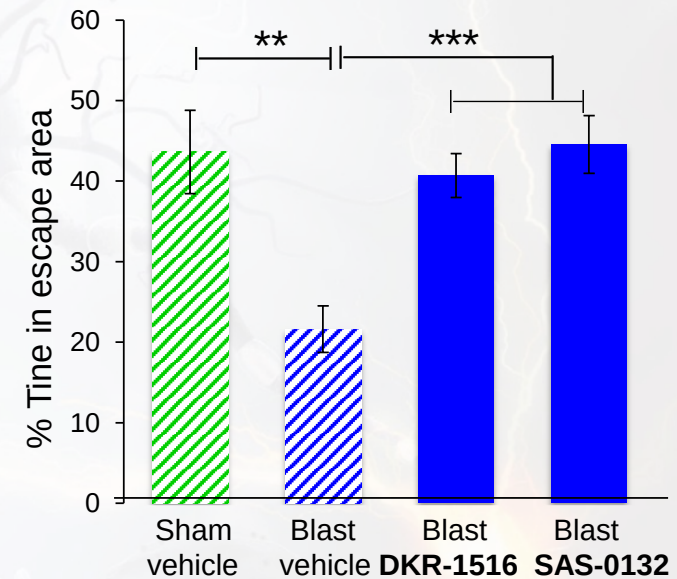
Barnes Maze



Escape
hole

Visual
cue

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).

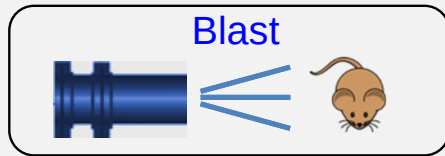
First evidence that modulating $\sigma 2R$ /TMEM97 has beneficial effect in TBI

with Andrew Pieper, University of Iowa

Traumatic Brain Injury: 1st Animal Model

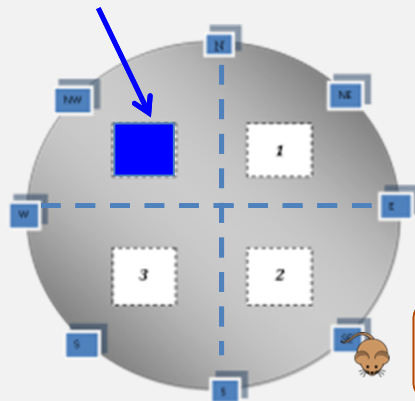


VS

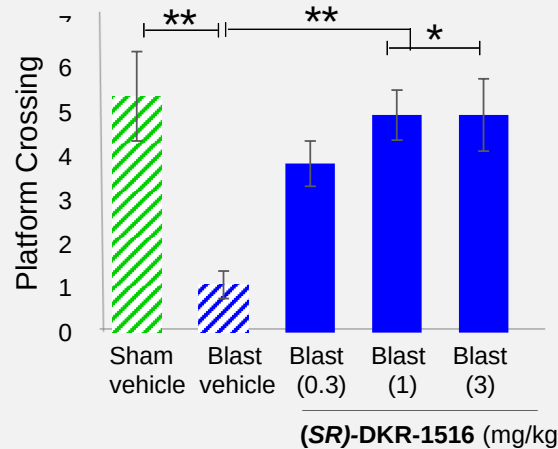


Morris Water Maze (Spatial and Long Term Memory)

Platform

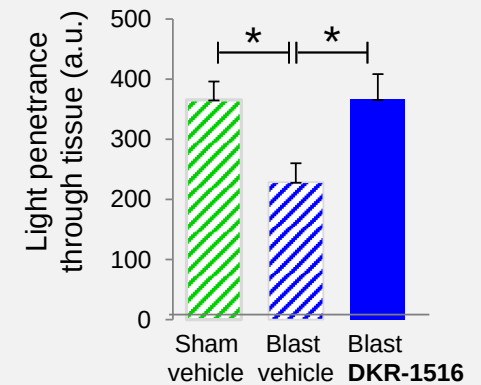


Morris Water Maze



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

Silver staining



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

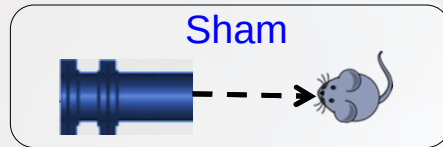
DKR-1516 promising lead to treat TBI by new mechanism

with Andrew Pieper, University of Iowa

TBI: 2nd Animal Model



Injury (Moderate–Severe)



VS

Controlled cortical impact



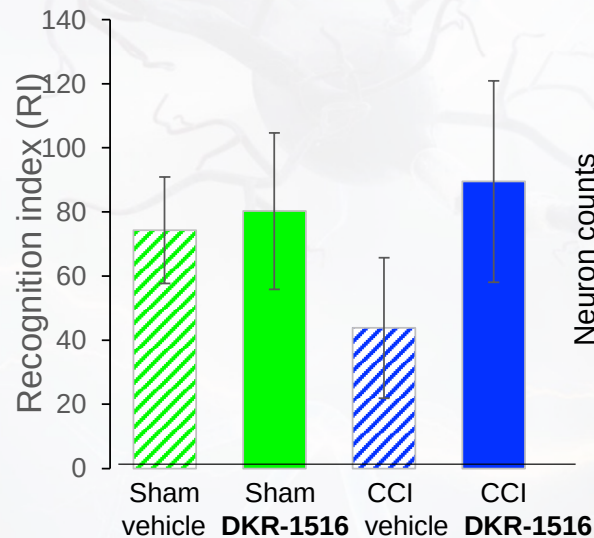
Novel Object Recognition



then

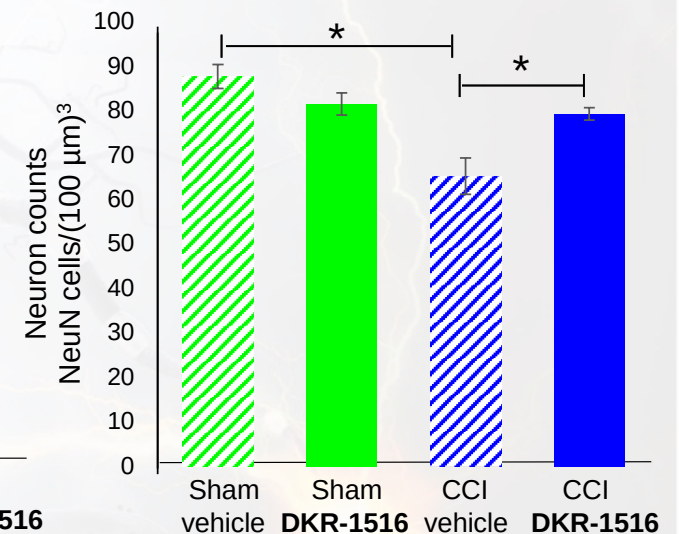


NOR Performance



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

Neuron (Cortex) Survival



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

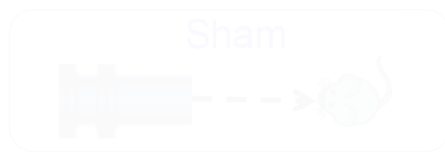
Modulating σ 2R/TMEM97 improves cognition, neuron and oligodendrocyte survival after TBI in CCI animal model (moderate-severe injury)

with Dan Liebl, University of Miami

TBI: 2nd Animal Model



Injury (Moderate–Severe)



Sham

vs



Controlled cortical impact

NOR Performance



Neuron (Cortex) Survival



Are σ 2R/TMEM97 modulators brain penetrant and how do they work?



then



Recognition

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

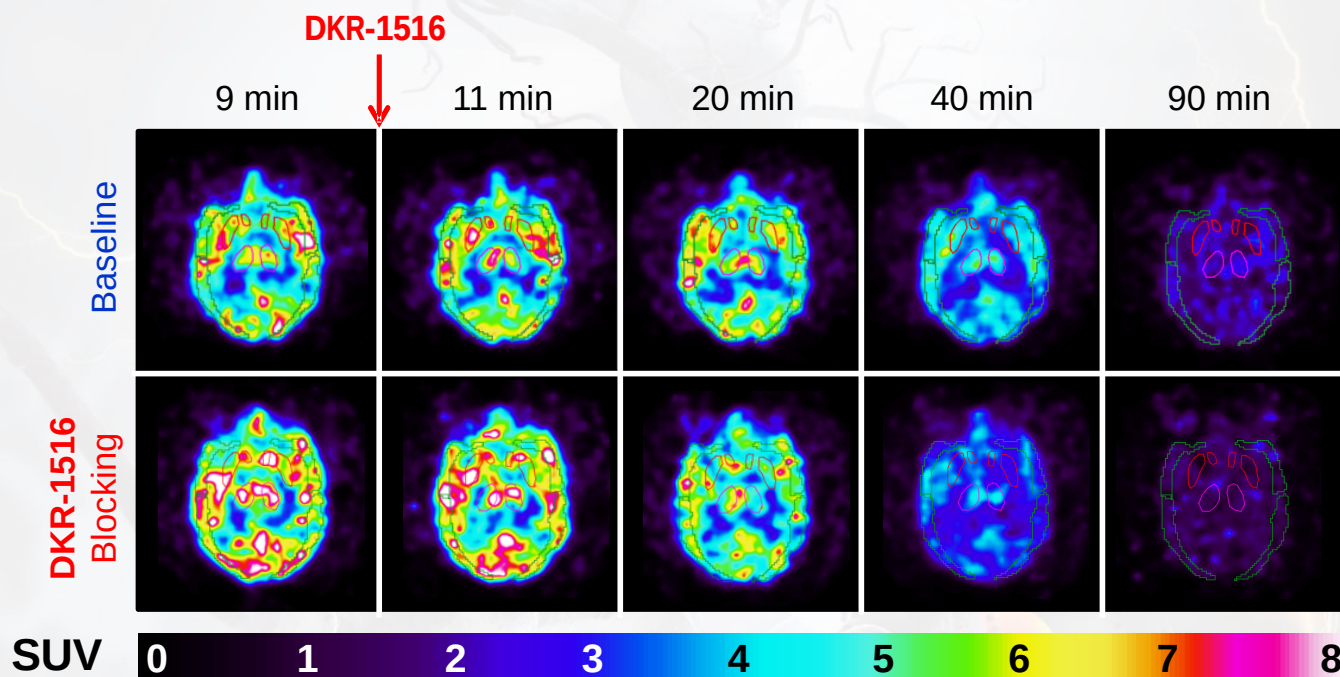
Modulating σ 2R/TMEM97 improves cognition, neuron and oligodendrocyte survival after TBI in CCI animal model (moderate-severe injury)

with Dan Liebl, University of Miami

Target Engagement Confirmed



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



Ø *Standard uptake values (SUV) show*

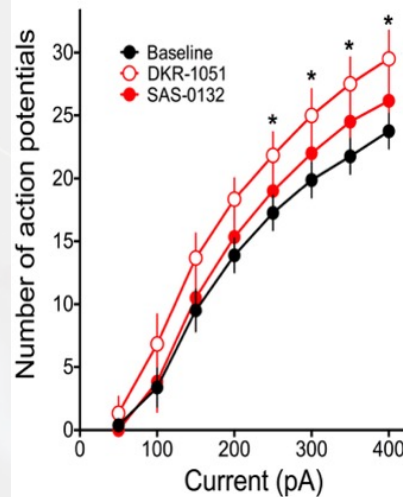
- 2 **DKR-1516** rapidly crosses blood-brain barrier and binds $\sigma_2\text{R}$ in brain of rhesus monkey
- 2 **DKR-1516** more selective than CTX, phase Ib clinical lead for AD (Cognition Therapeutics)

with Bob Mach, The University of Pennsylvania

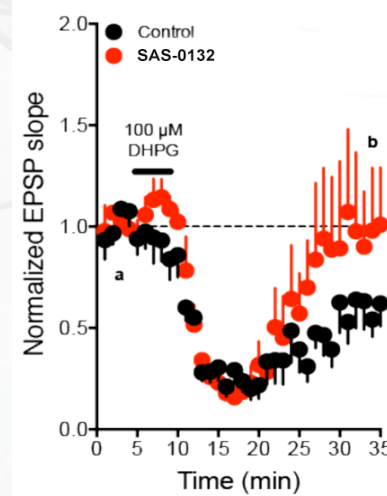
σ 2R/TMEM97 and Electrophysiology



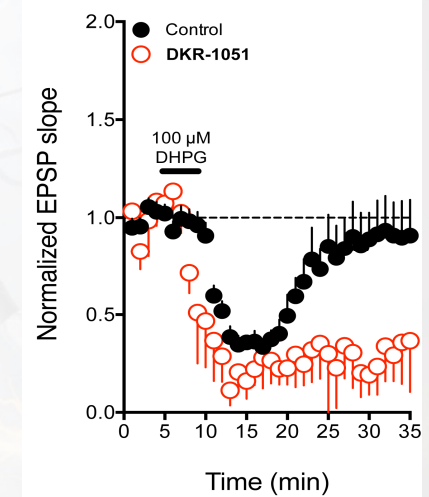
σ 2R/TMEM97 Ligands Modulate Excitability, Plasticity, $[Ca^{2+}]$



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



SAS-0132 inhibits long-term depression (LTD)



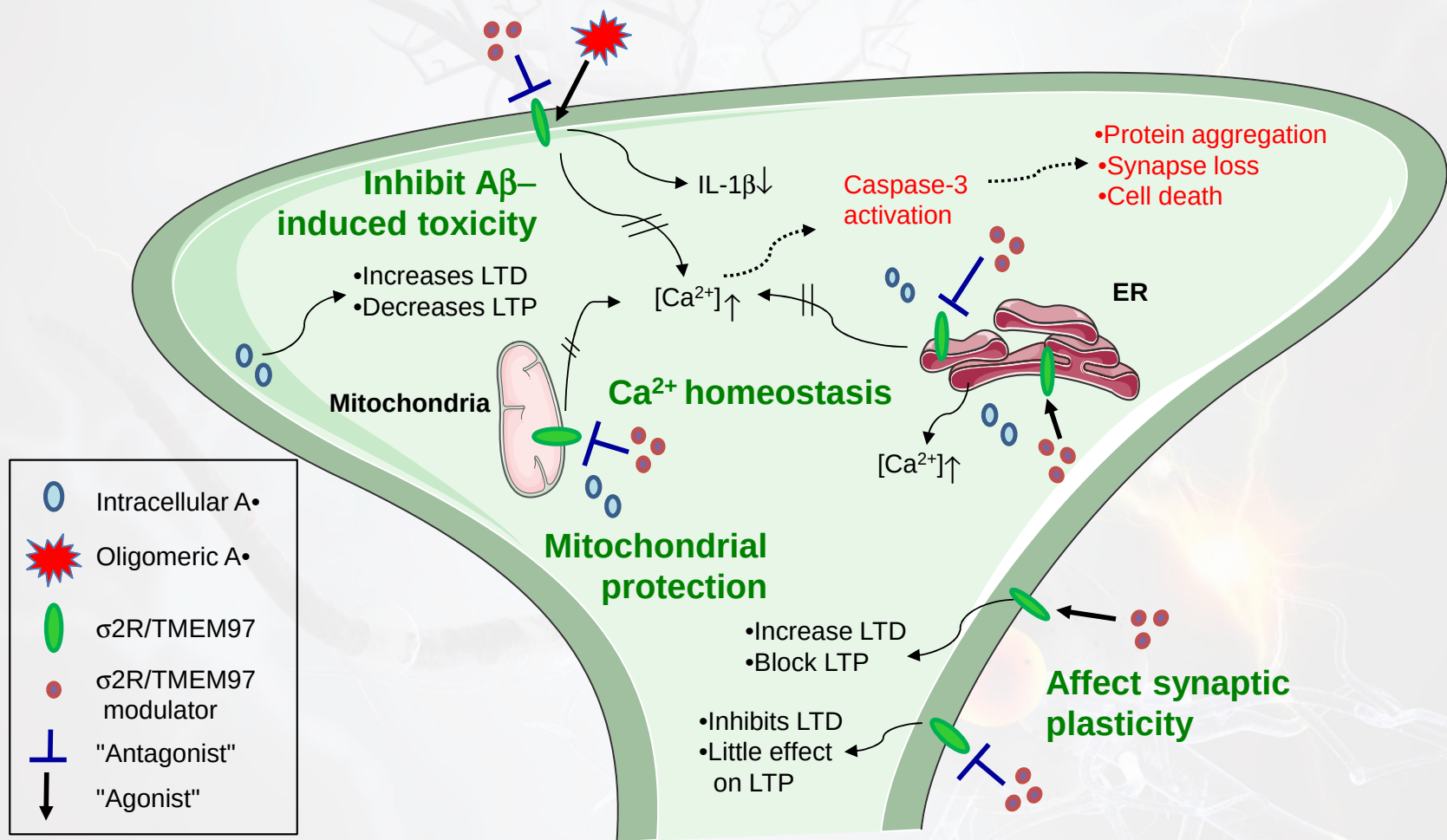
DKR-1051 increases mGluR-dependent LTD

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

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

with Dan Johnston (The University of Texas, Austin)

σ 2R/TMEM97 Modulators~Mode of Action



*Inhibiting σ 2R/TMEM97 blocks A β -mediated synaptotoxicity and reduces IL-1 β .
Modulating σ 2R/TMEM97 regulates plasticity (LTD and LTP) and [Ca²⁺].*

Alcohol Dependence: An Unmet Need



Ø Alcohol Use Disorder (AUD) is a Major Global Health Problem

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

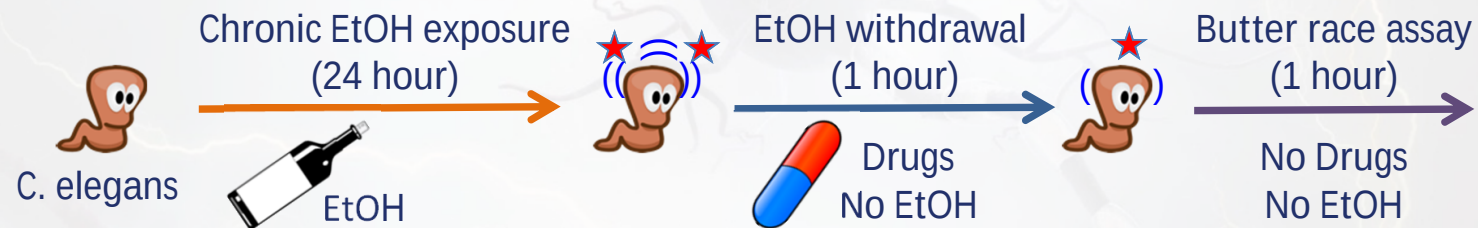
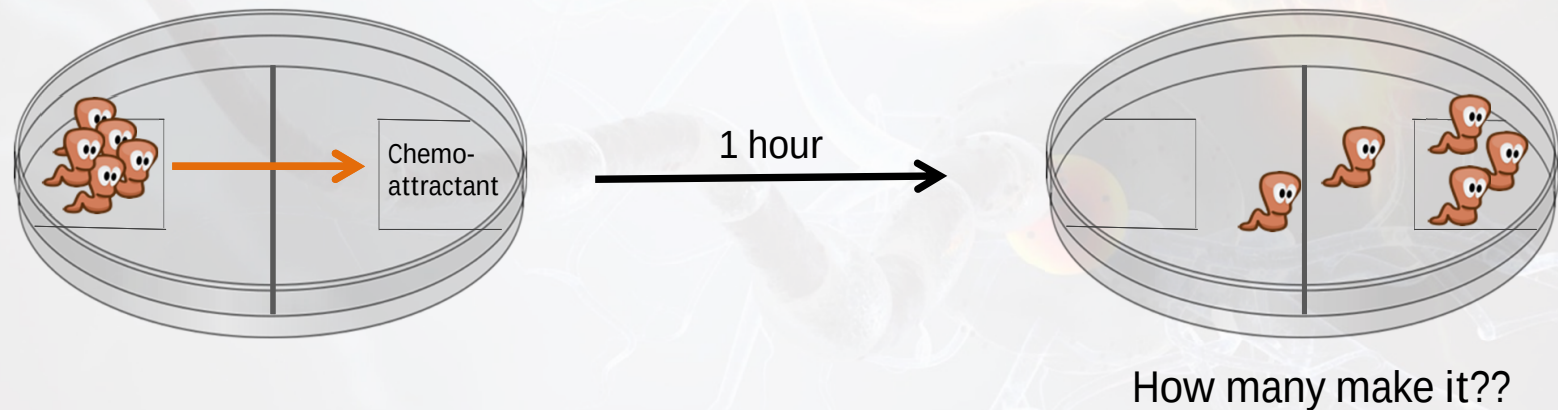


Ø *No effective treatments and new therapeutic approaches needed*

σ 2R/TMEM97 and Ethanol Withdrawal



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

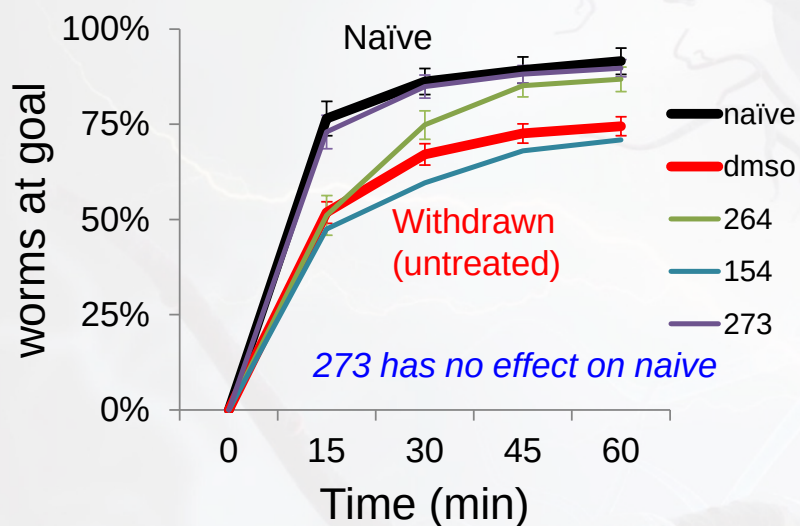


with Scott and Pierce-Shimomura – University of Texas, Austin

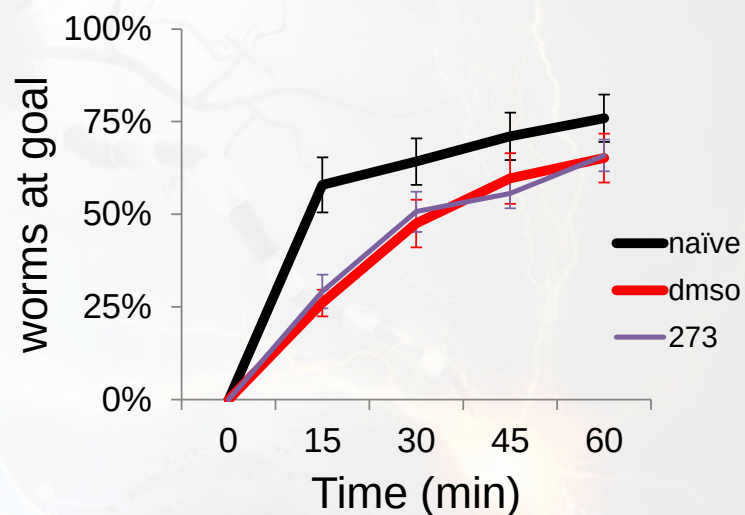
Mode of Action Mediated by PGRMC1



C. elegans - Wild-type



C. elegans – *vem-1* (PGRMC1) null



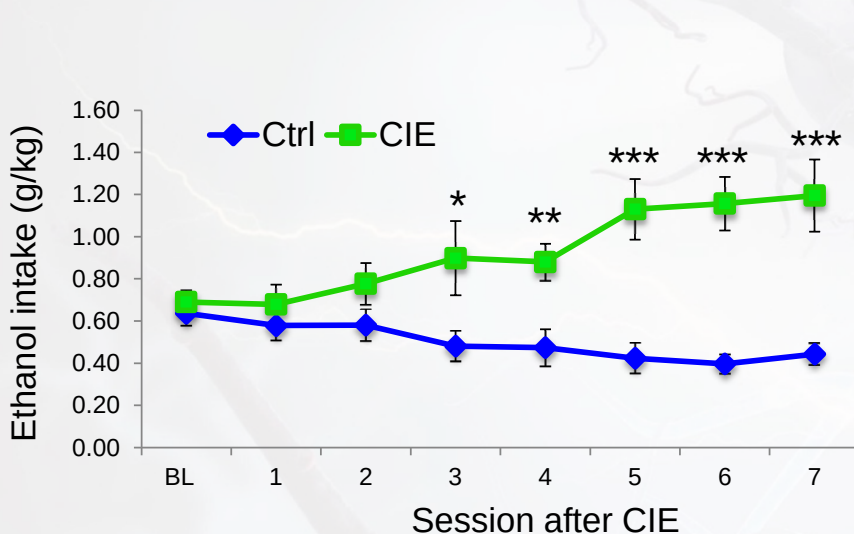
Because *vem-1* (PGRMC1) knockout is not affected by treatment, $\sigma 2R$ ligands also appear to act via PGRMC1-mediated pathway

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

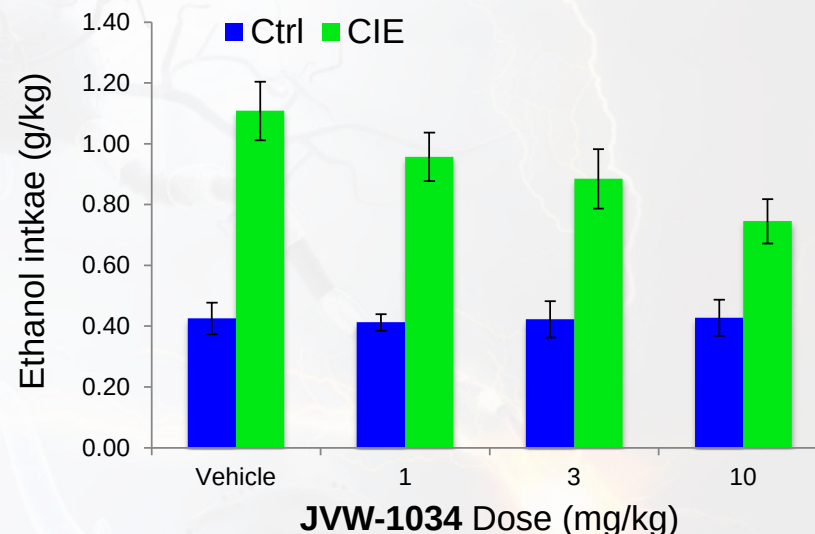
Alcohol Dependence: Proof-of-Principle



JVW-1034 Reduces EtOH Consumption in EtOH-Dependent Rats



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



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

First evidence that modulating $\sigma 2R$ /TMEM97 has beneficial effect in animal model for alcohol dependence

with Valentina Sabino (Boston University)

Neuropathic Pain: An Unmet Need



Ø Effective Drugs to Treat Chronic and Neuropathic Pain Lacking

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

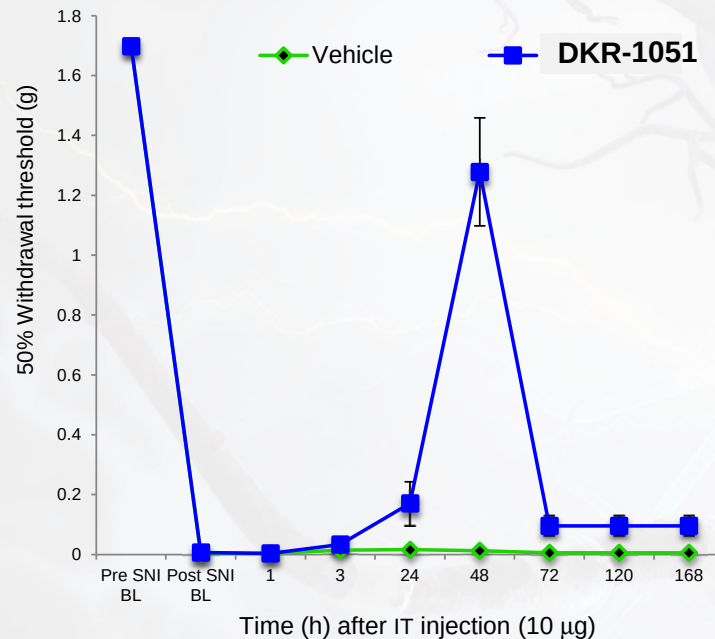


Ø ***No effective treatments and new targets and approaches needed***

σ 2R/TMEM97 Ligands Attenuate Pain



DKR-1051 Reduces Neuropathic Pain



- 2 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)
- 2 Notably, effect of **DKR-1051** is completely blocked by **SAS-0132** (data not shown)
- 2 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 σ 2R/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 $\sigma 2R$ /TMEM97 IS attractive target in neuroscience

- Ø Compounds safe and selective (low off target binding to other CNS proteins)
- Ø Confirmed rapid target engagement in rodent and nonhuman primate brain
- Ø Modulators
 - 2 reduce axon degeneration and improve neuron and oligodendrocyte survival
 - 2 regulate neuron excitability and synaptic plasticity (LTD and LTP)
 - 2 reduce proinflammatory cytokines such as IL-1 β
 - 2 improve cognitive performance in healthy mice
 - 2 improve cognitive performance animal model of Alzheimer's disease
 - 2 improve cognitive performance in two animal models of TBI (blast and CCI)
 - 2 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

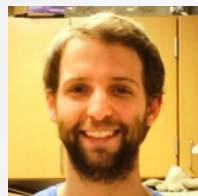


Synthetic/Medicinal Chemistry

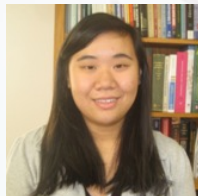
The University of Texas, Austin



Jim Sahn



Tim Hodges



Jessica Chan

C. elegans Models

The University of Texas, Austin



Jon Pierce-Shimomura



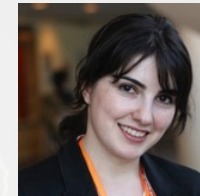
Luisa Scott

Alzheimer's Disease

Stanford University



Mehrdad Shamloo



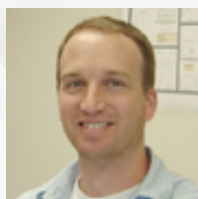
Pooneh Ardestani

Neurophysiology

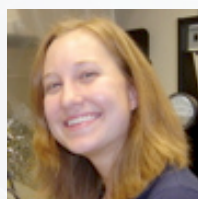
The University of Texas, Austin



Dan Johnston



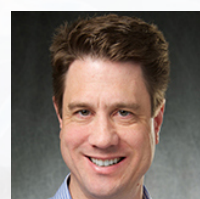
Darrin Brager



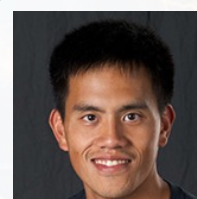
Brandy Routh

Traumatic Brain Injury

University of Iowa



Andrew Pieper



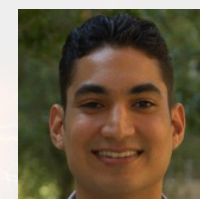
Terry Yin

Pain

University of Texas, Dallas



Ted Price



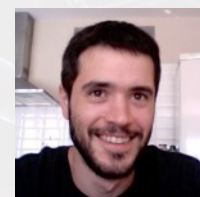
Galo Mejia

Alcoholism

Boston University



Valentina Sabino

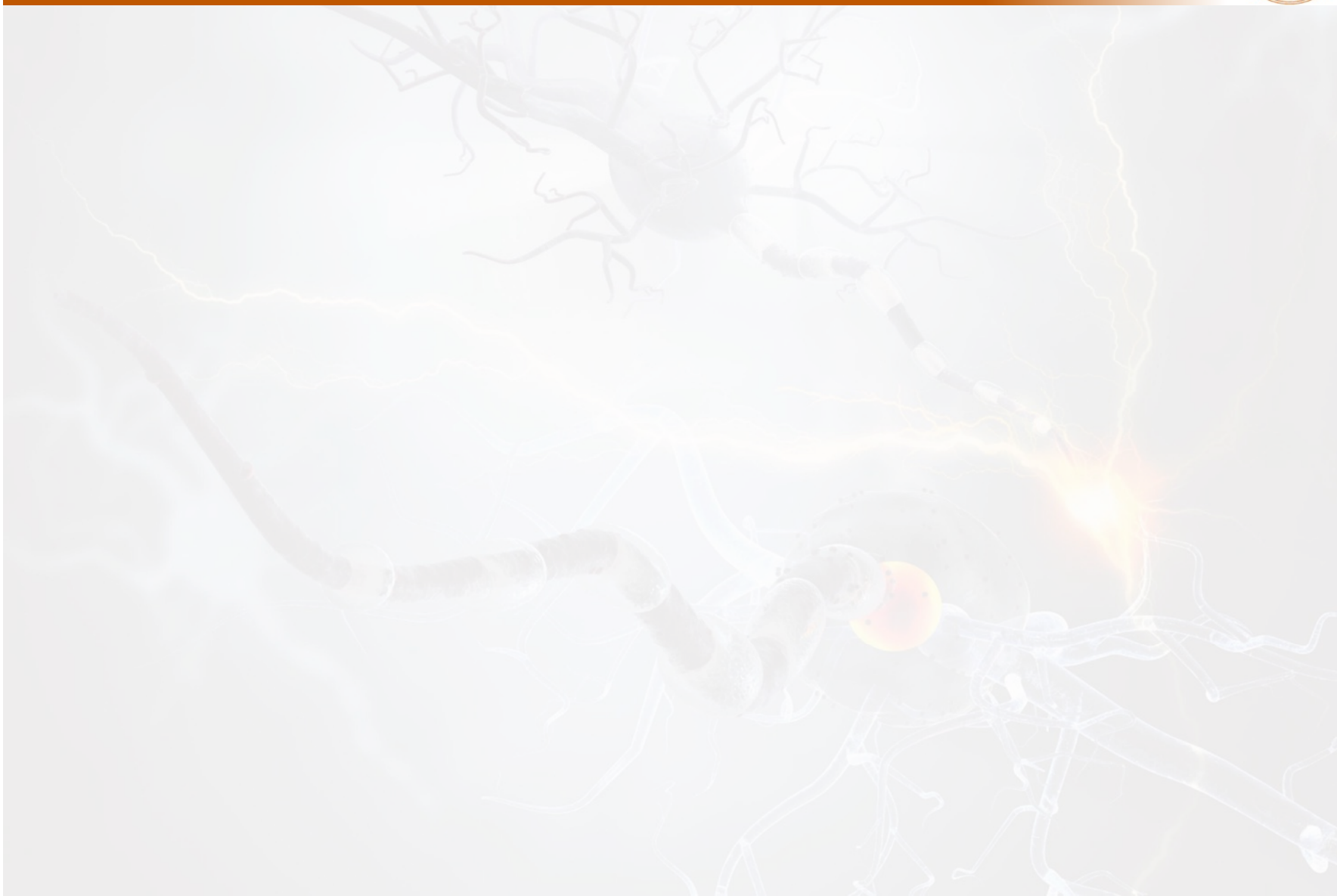


Antonio Ferragud





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
Jinbin Xu ~ Washington University, St. Louis
Ted Price ~ University of Texas, Dallas
Daniel Johnston ~ University of Texas, Austin
Boris Zemelman ~ University of Texas, Austin
Robert Mach ~ University of Pennsylvania
Wayne Bowen ~ Brown University
Andrew Kruse ~ Harvard University





And Thank You

Thanks Again To All
For Listening

For Listening



Ø *What we have learned about σ 2R/TMEM97 modulators*

- ü No toxic effects observed (MTD- and extended dosing)
- ü Improve cognitive performance in mice in two TBI models
- ü Reduce axonal degeneration
- ü Protect neurons and oligodendrocytes

Ø *How do they work?*

Unmet Medical Needs in Neuroscience

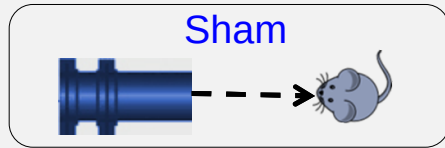


Ø *The Sad Truth*

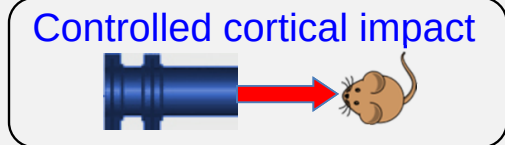
- 2 Huge unmet medical need for effective drugs in neuroscience
 - § Alzheimer's disease
 - § Traumatic brain injury
 - § Huntington's disease
 - § Substance abuse and addiction
 - § Pain and neuropathic pain
 - § Numerous others
- 2 Many approaches to develop drugs to treat CNS diseases and disorders have failed over the past 25 years.
- 2 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



VS



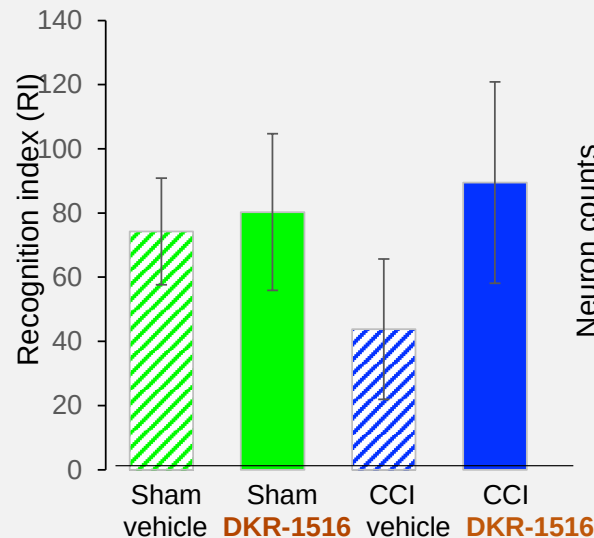
Novel Object Recognition



then

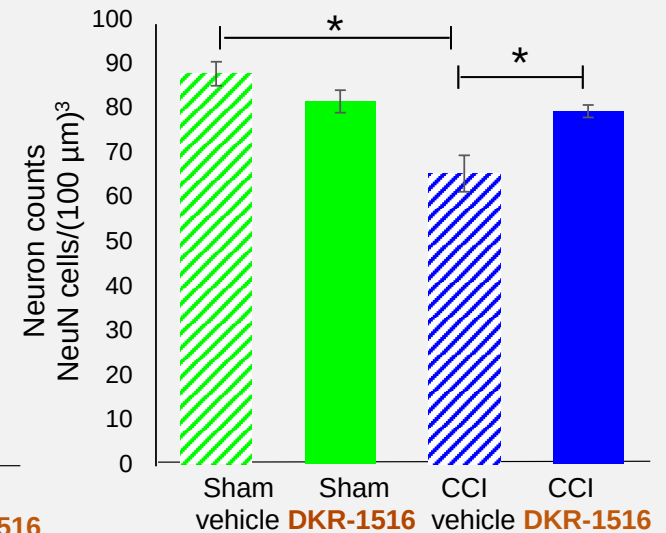


Novel Object Recognition



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 oligodendrocyte (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)

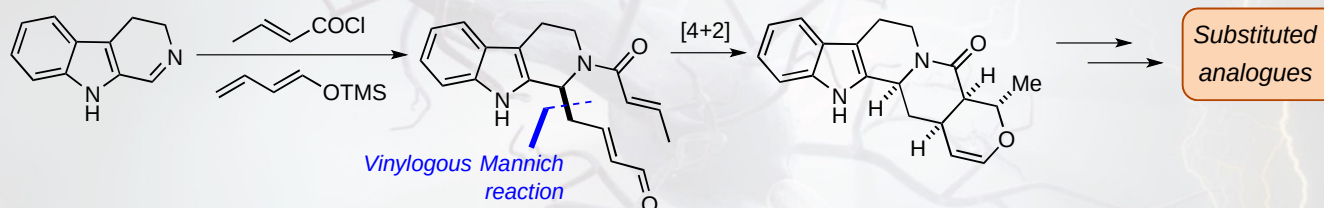
- Ø Sigma 2 receptor (σ 2R) is one of two sigma receptor subtypes. It is expressed in CNS and has been associated with neuronal function and survival, mitochondrial protection, and calcium signaling.
- Ø We (Kruse & Martin, unpublished) just cloned this receptor and showed it was identical to a transmembrane protein associated with cholesterol trafficking and homeostasis.
- Ø σ 2R 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 σ 2R
upon other neurodegenerative conditions?*

It All Begins With Chemistry

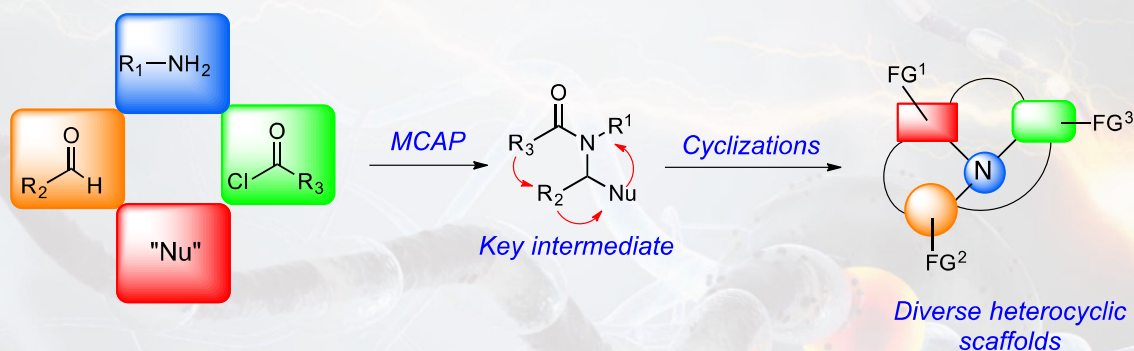


Rapid Scaffold Assembly: Are There Other Applications?



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



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

For reviews, see:

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

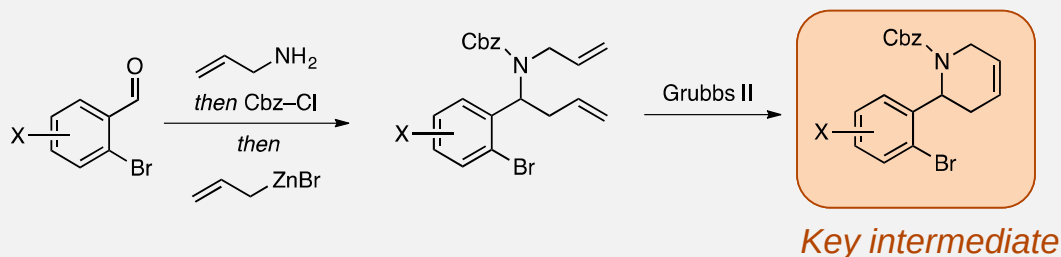
Sahn, Granger, Martin *Org. Biomol. Chem.* **2014**, 12, 7659

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

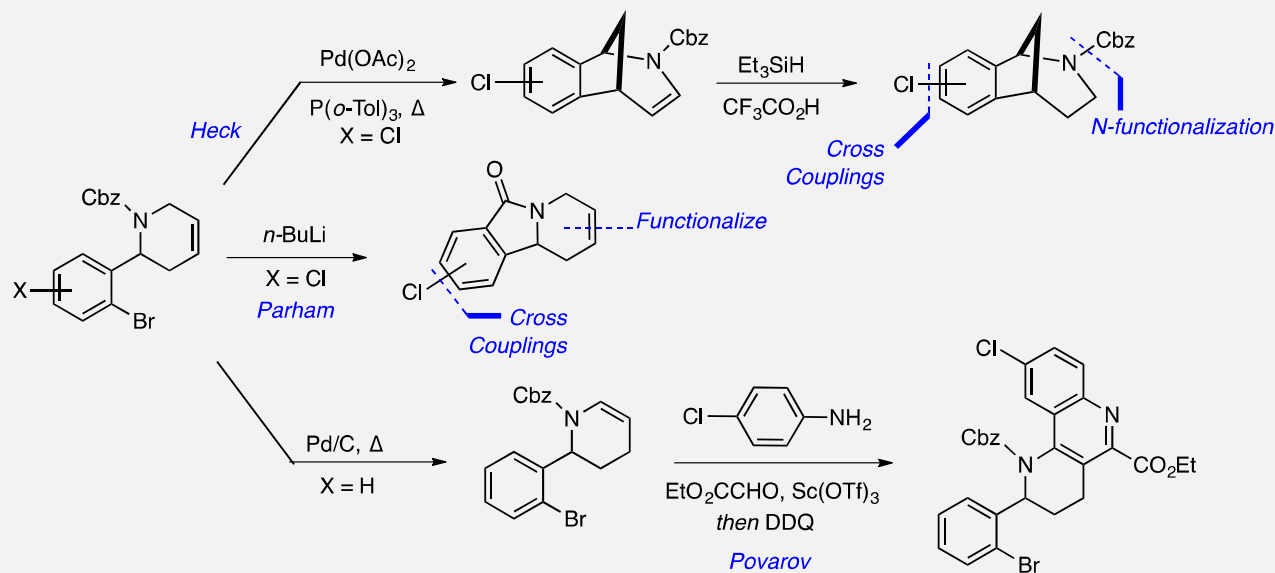
New Tactic for DOS Exemplified



Tandem MCAP/RCM to Generate Pivotal Scaffold



Skeletal Diversification



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