



NEURALSTEM INC.

**“The neurogenic antidepressant compound, NSI-189,  
shows potential as a broad neurotrophic agent”**

Karl Johe, Ph.D.  
Chief Scientific Officer

NEURALSTEM, INC.

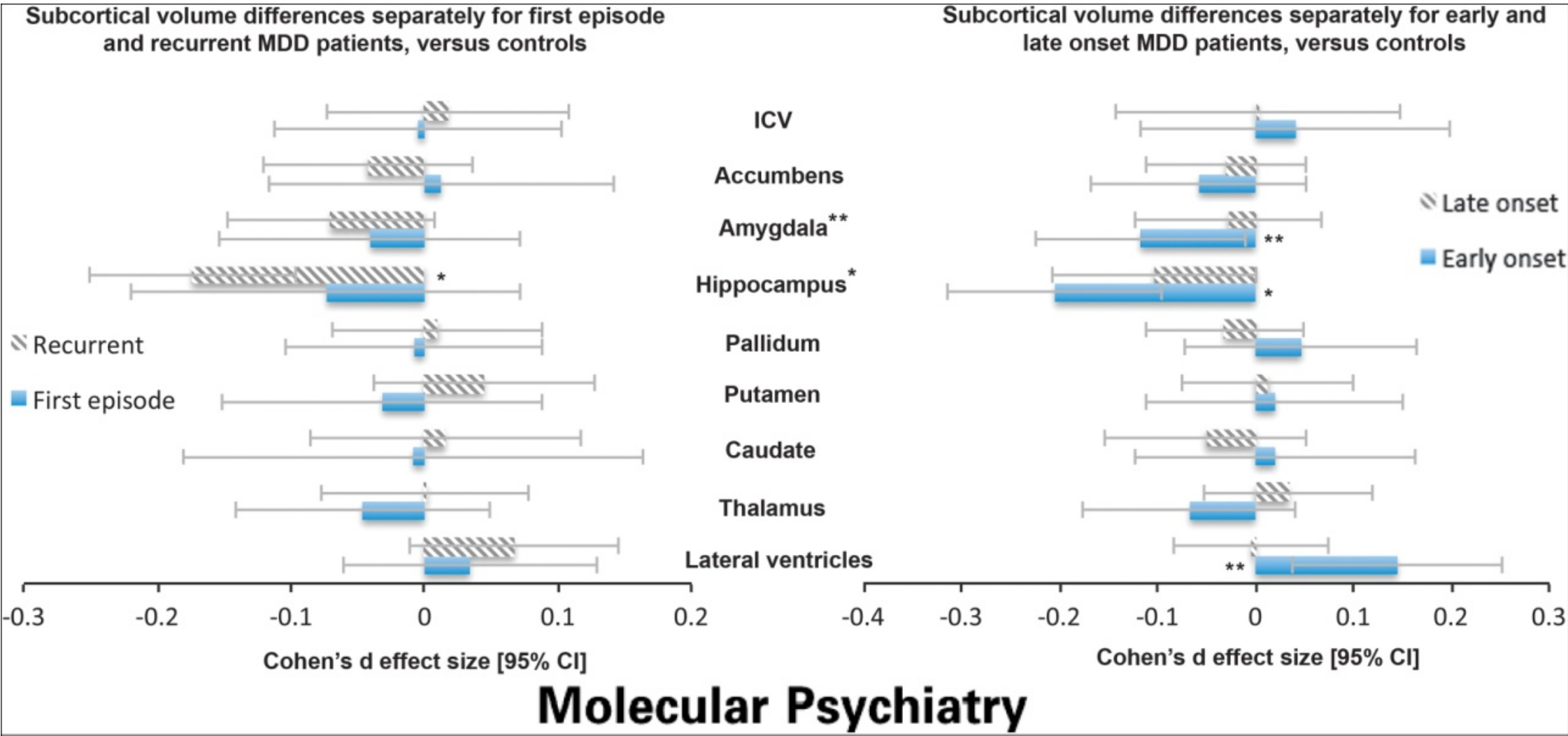
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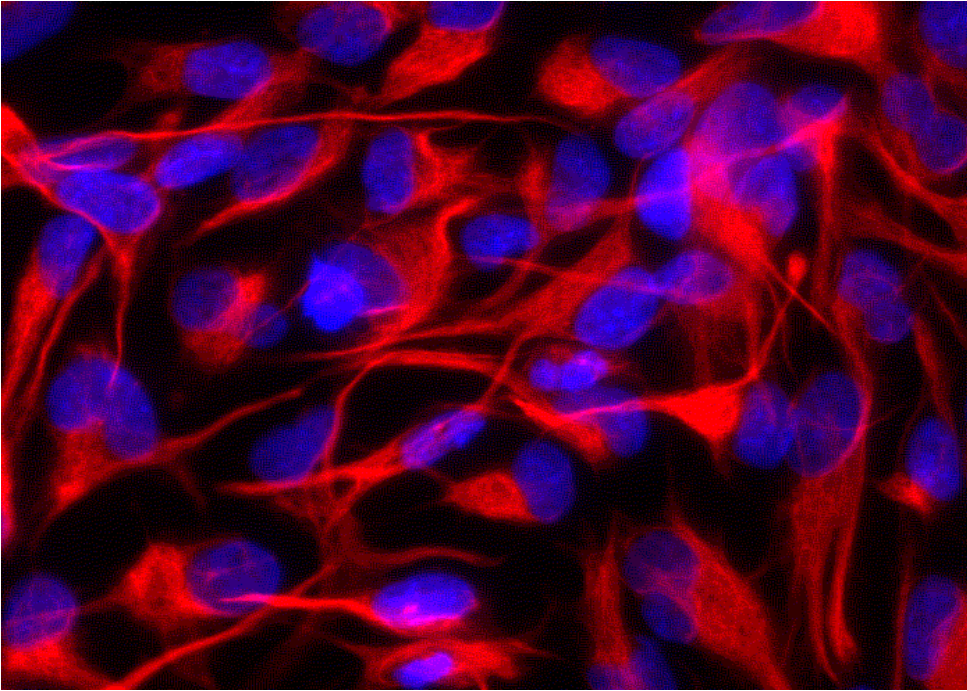
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Dr. Karl Johe is an employee of Neuralstem, Inc. and holds patents and financial interests in Neuralstem, Inc.

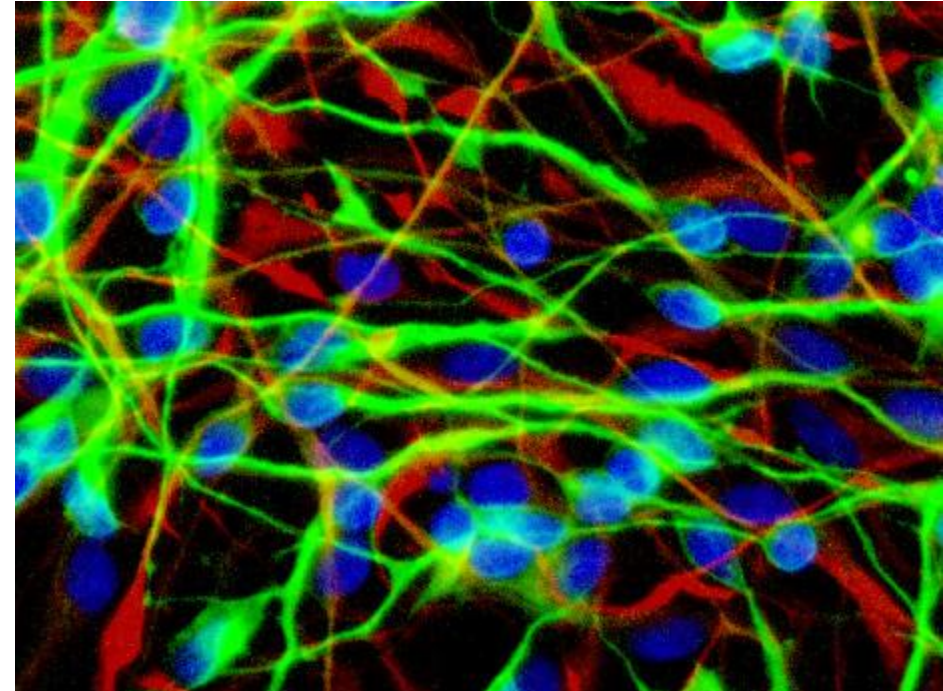


# Human Hippocampal (HI) Neurogenesis *in vitro* Platform Enables Neurogenic Drug Discovery



**+ Mitogen**

Neural Stem Cells (Red)/  
Nuclei (Blue)



**- Mitogen for 7 Days**

Neurons (Green)/  
Glia (Red)/  
Nuclei (Blue)

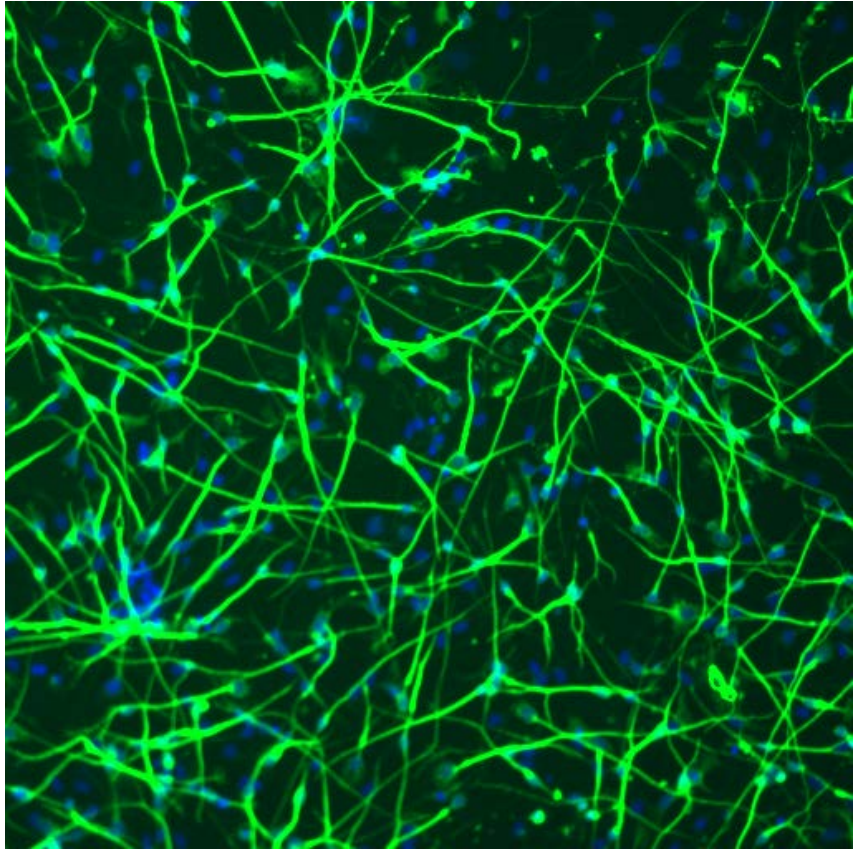
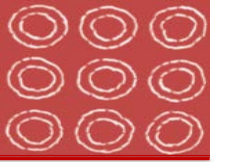


- Libraries selected to target neurogenesis
  - Kinases & phosphatases
  - Nuclear receptors
  - Peptide mimetics
  - GPCRs
- Five structural libraries chosen for diversity (scaffolds)
- Selected ~2000 compounds per library
  - Predict in advance for CNS-Availability
  - Cover max chemical space within each library

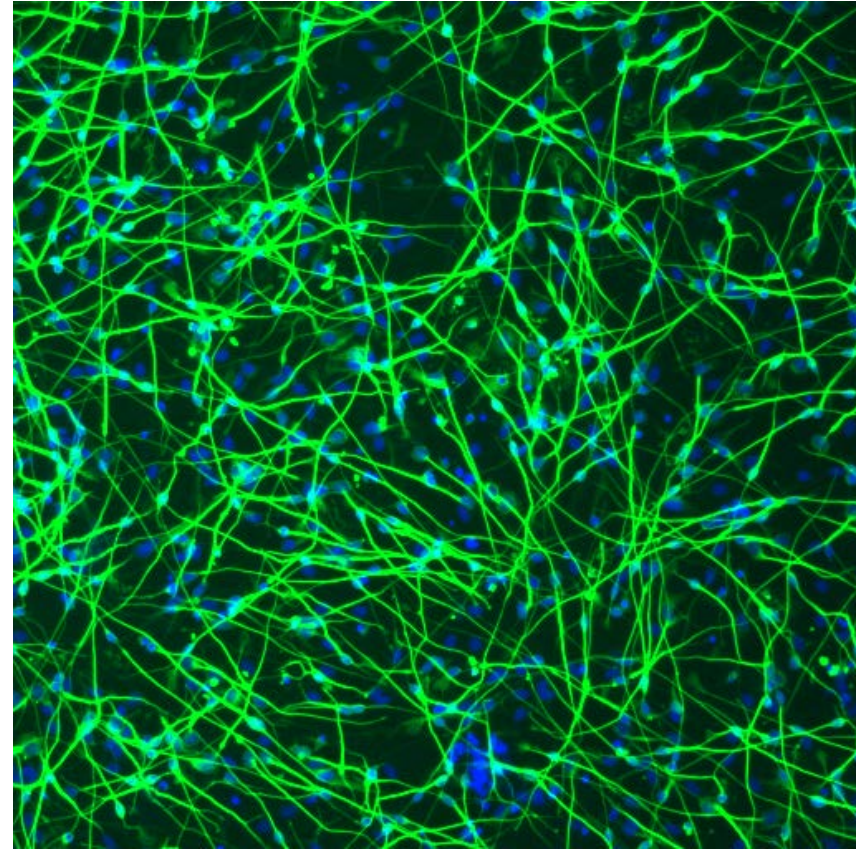


# Compound-Induced Neurogenesis

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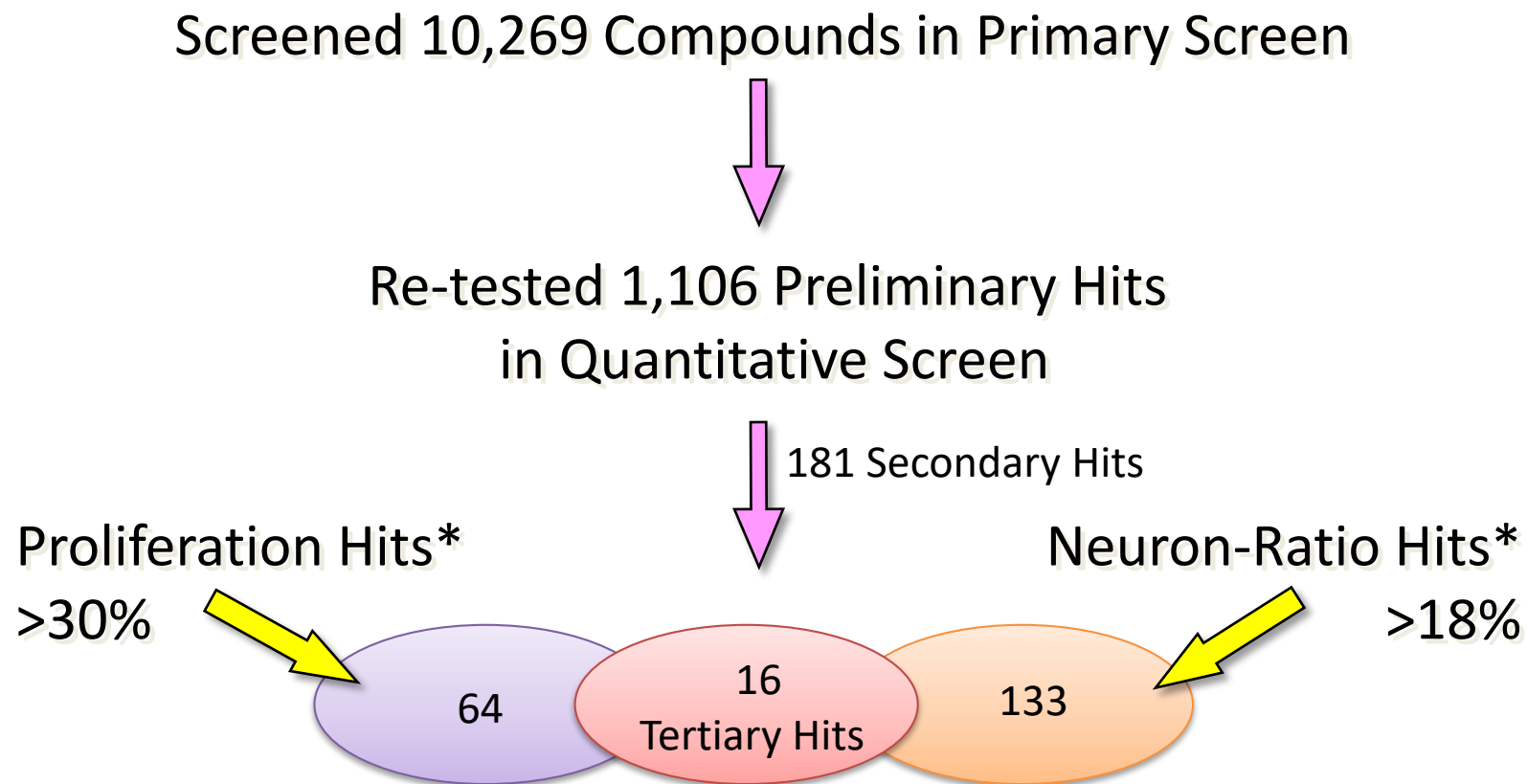


Vehicle Control

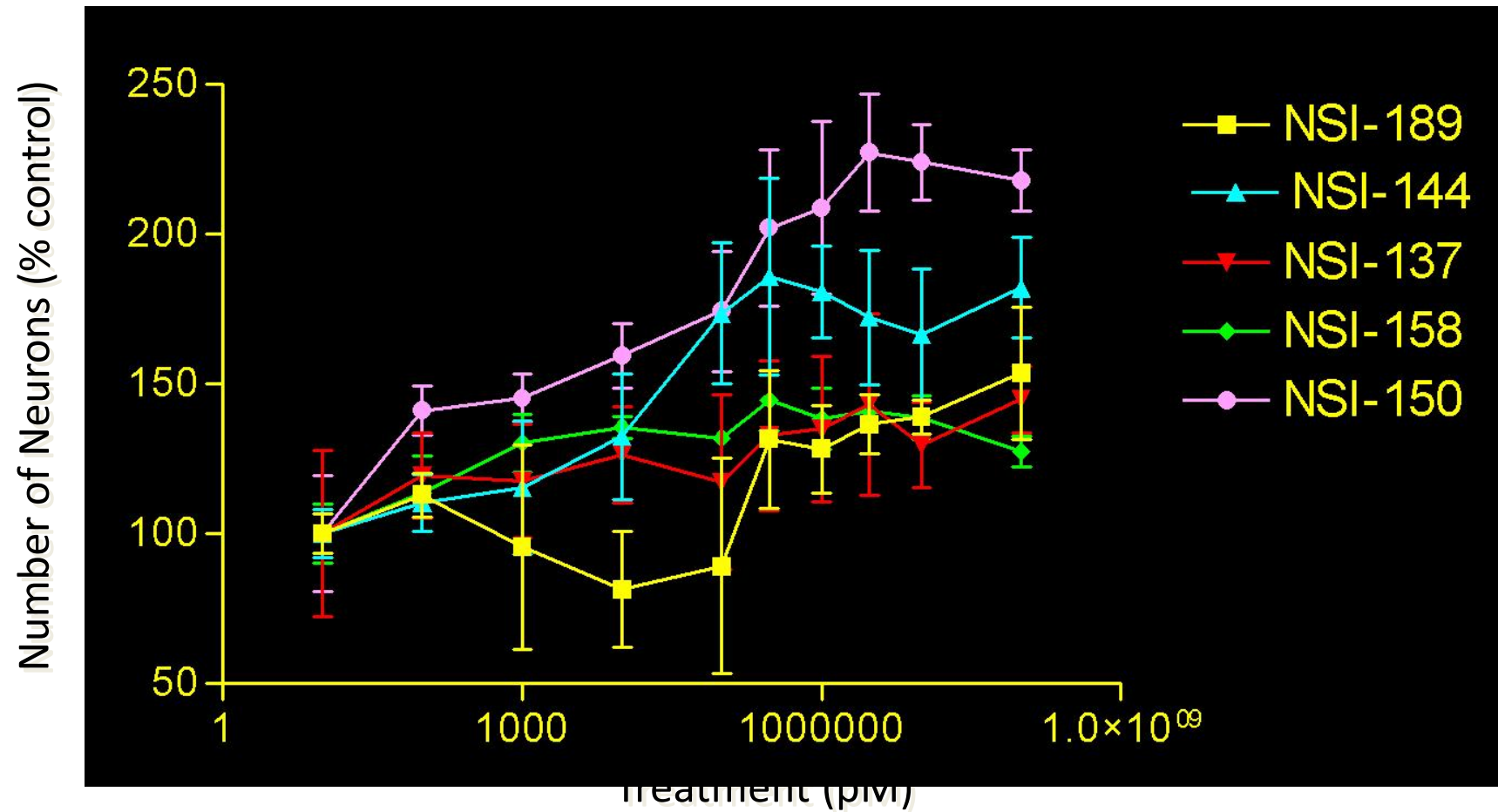


NSI-106

# Primary Screen and Additional Filters

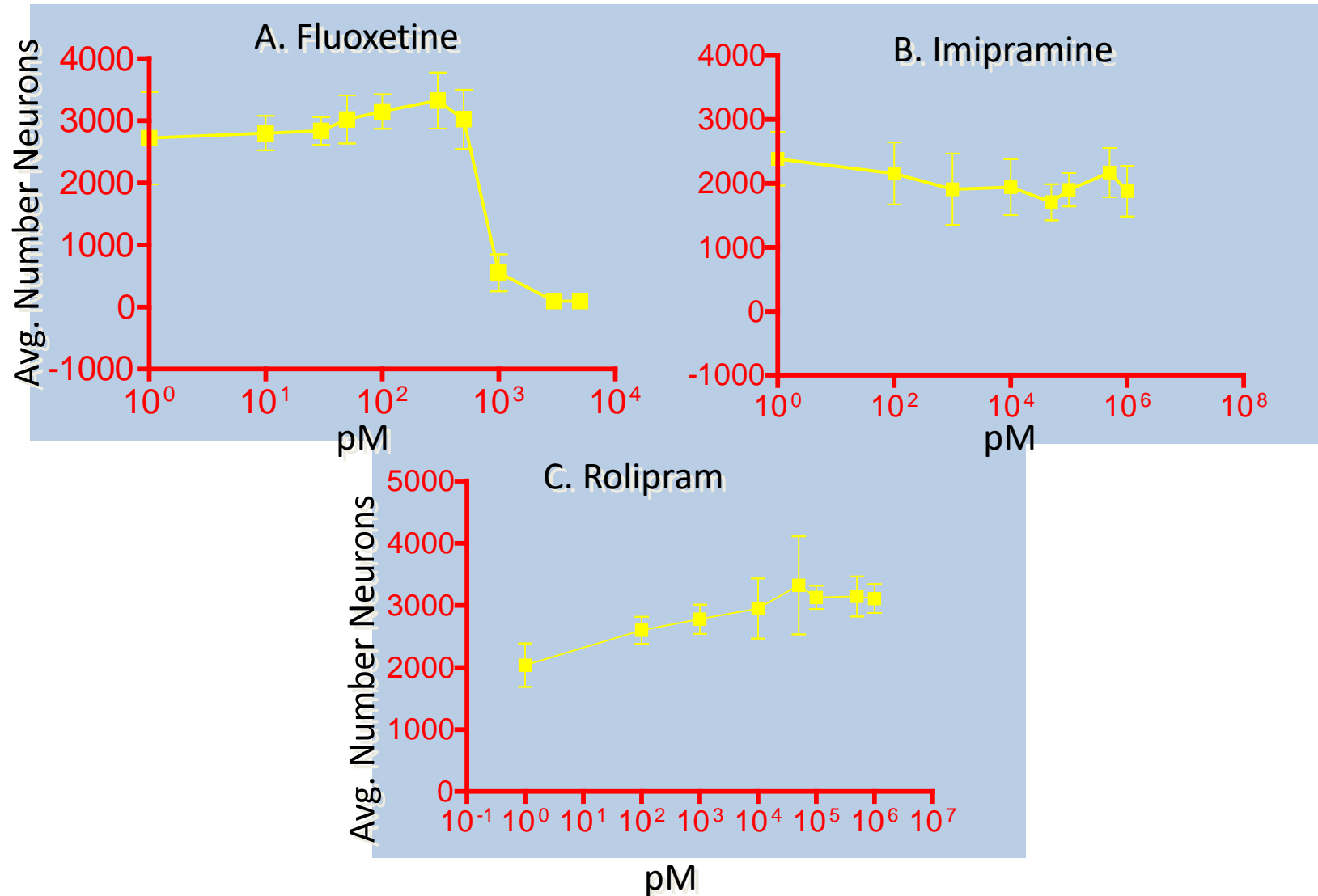
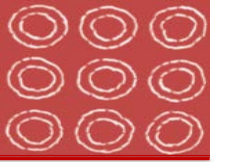


# Leads Increase Neuron Number in a 7-day Neurogenesis Assay

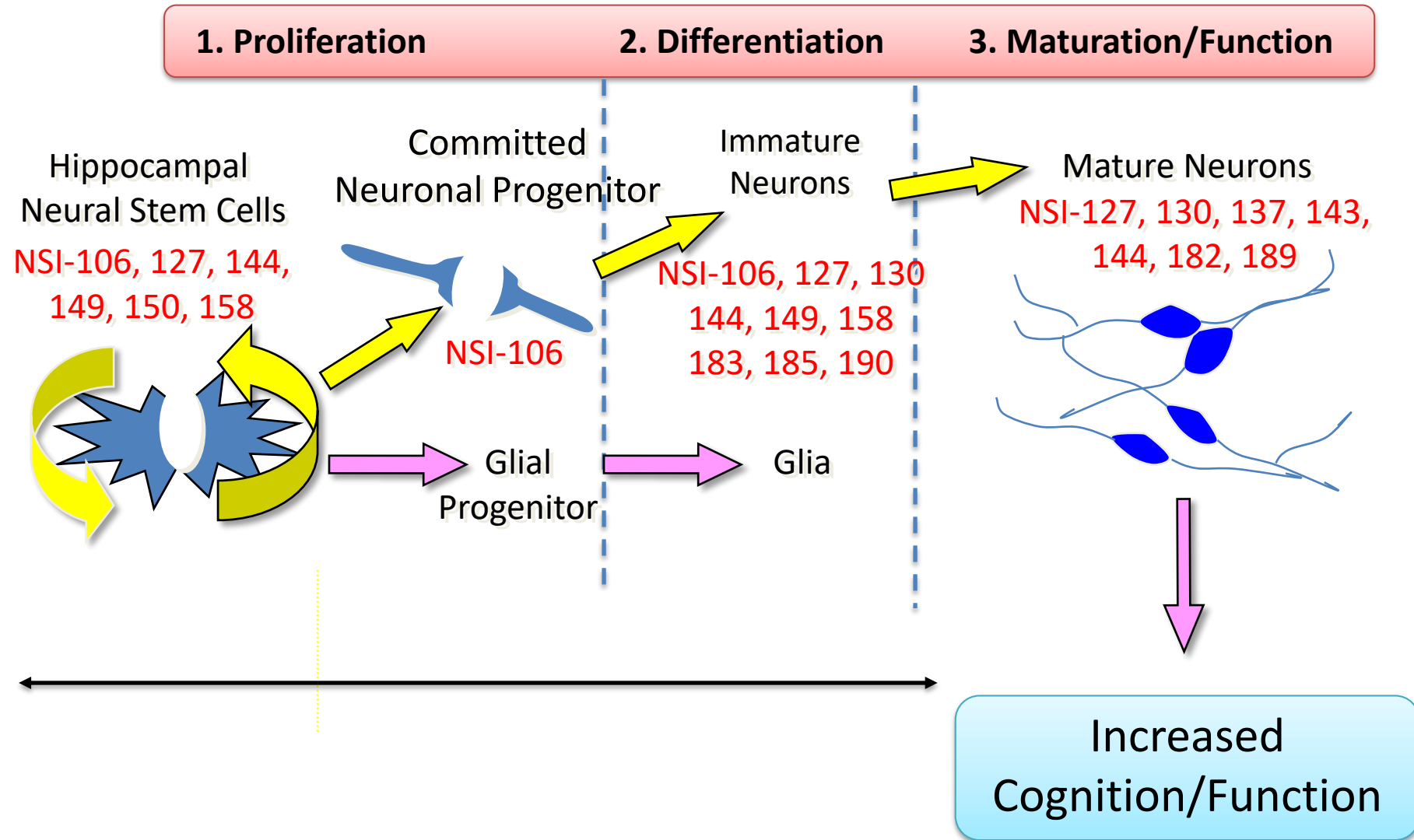




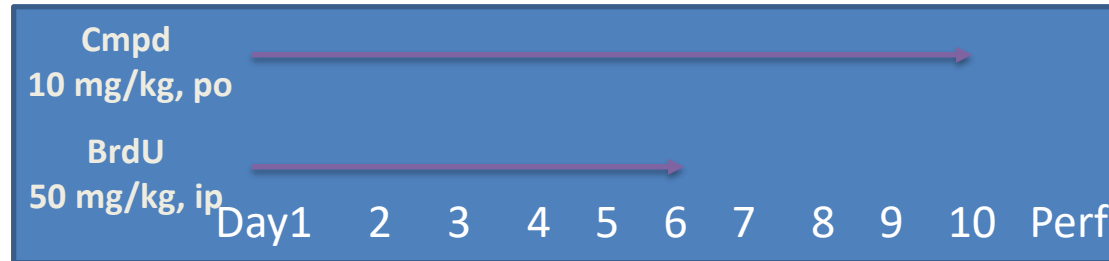
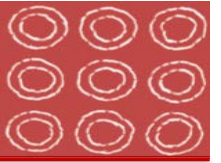
# Effect of Antidepressants on Primary Stem Cell Neurogenesis Screen



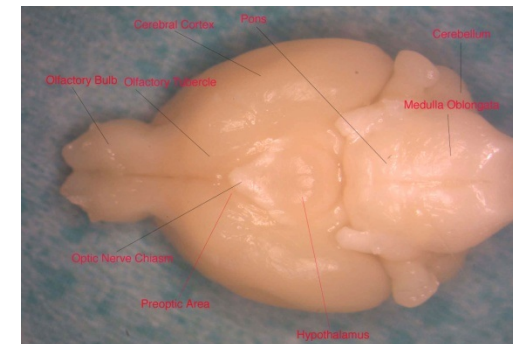
# Leads Affect Multiple Potential Sites of Action During Stages of Neurogenesis



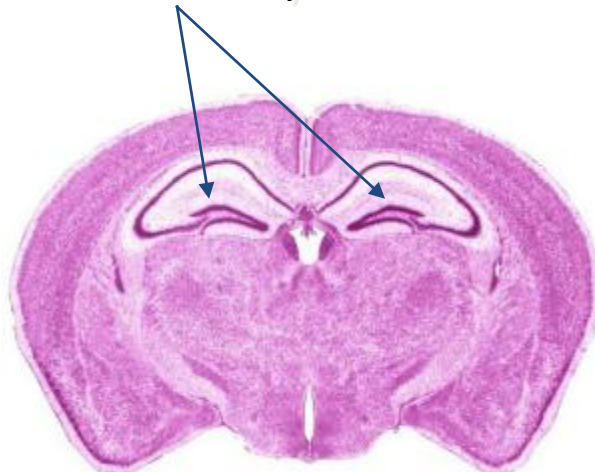
# *in vivo* Neurogenesis Assay: Protocol



1) Perfuse & Remove Brain on Day 11



Dentate Gyrus



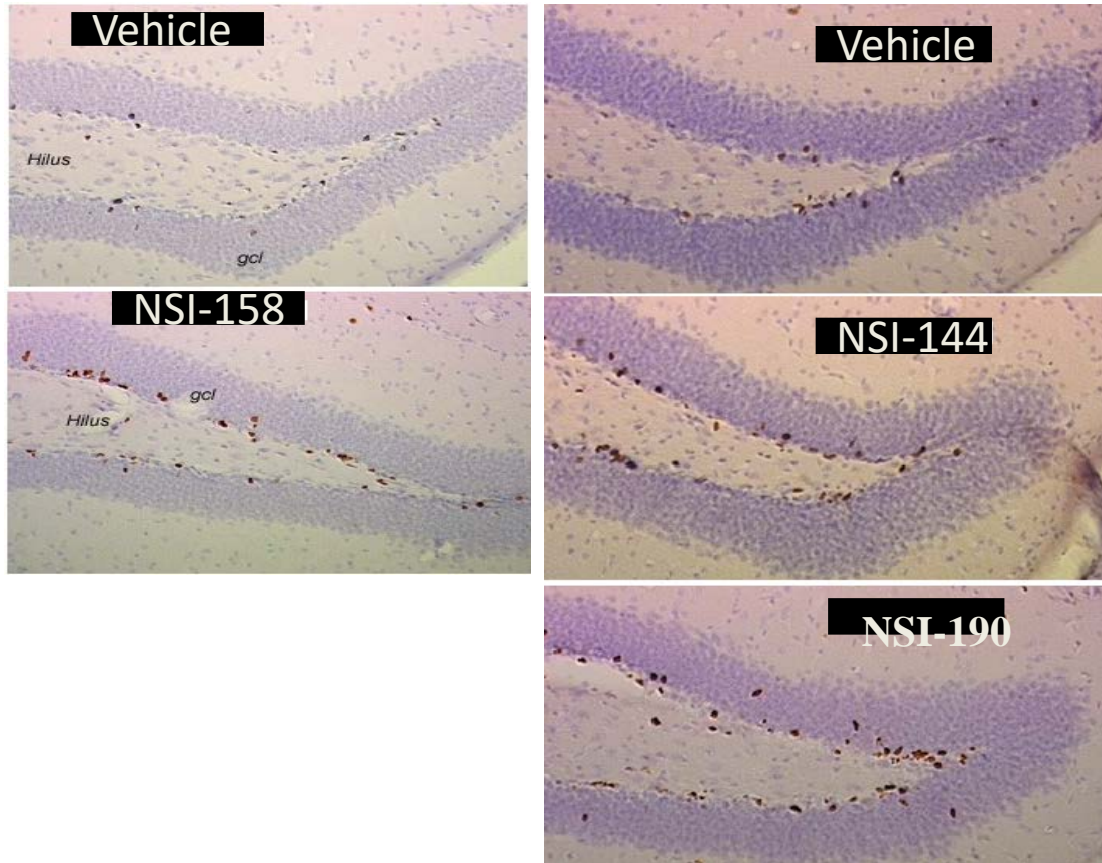
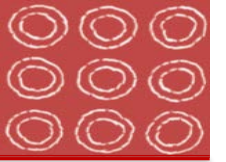
2) Do coronal sections (30um)

3) Immuno-stain BrdU-labeled cells

4) Count BrdU+ cells in 10 sections/dentate gyrus/mouse

5) Stain for neuronal phenotype

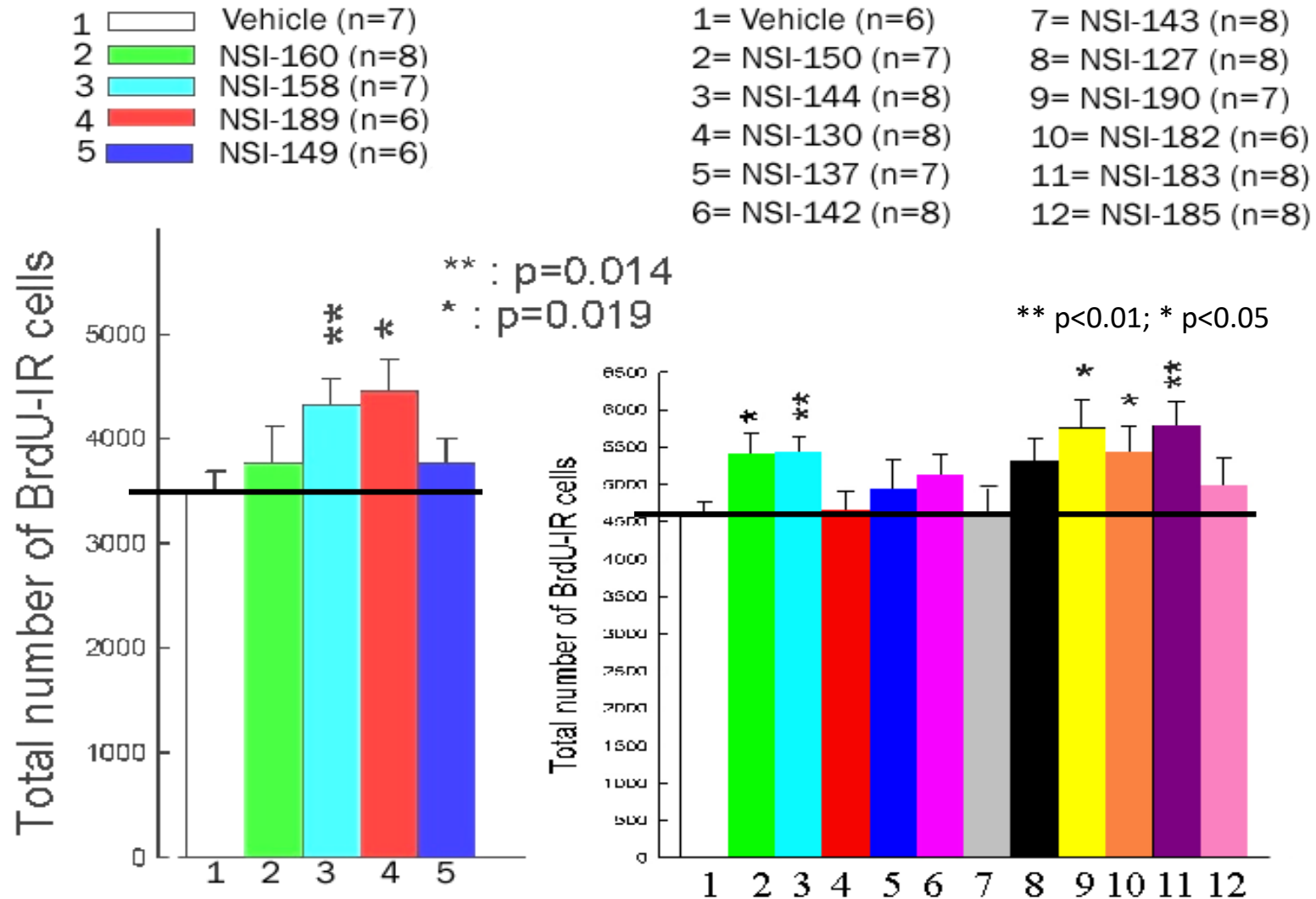
# Leads (10 mg/kg) Stimulate Neurogenesis in Healthy Adult Mouse



\*Conducted by Cerep & NeuroDetective Inc.

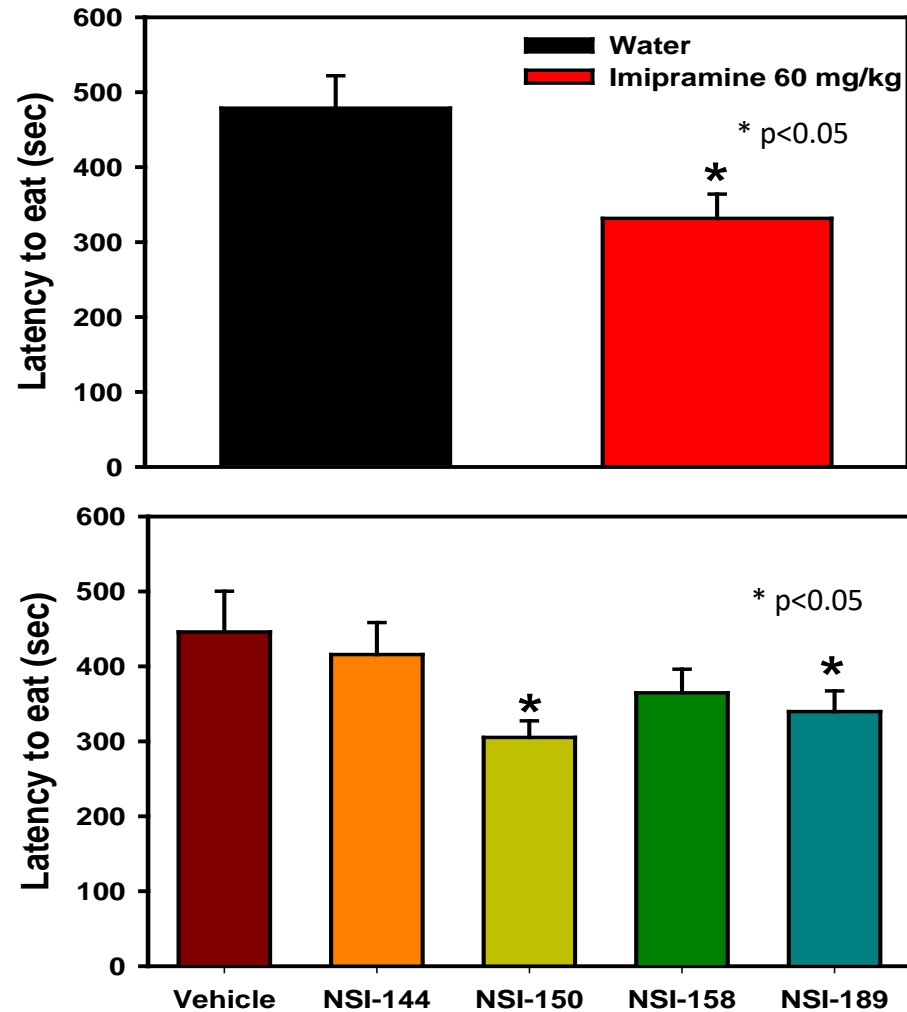
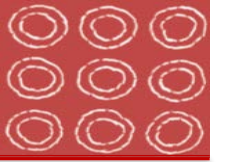


# Leads (10 mg/kg) show significant neurogenic effects in healthy adult mouse dentate gyrus



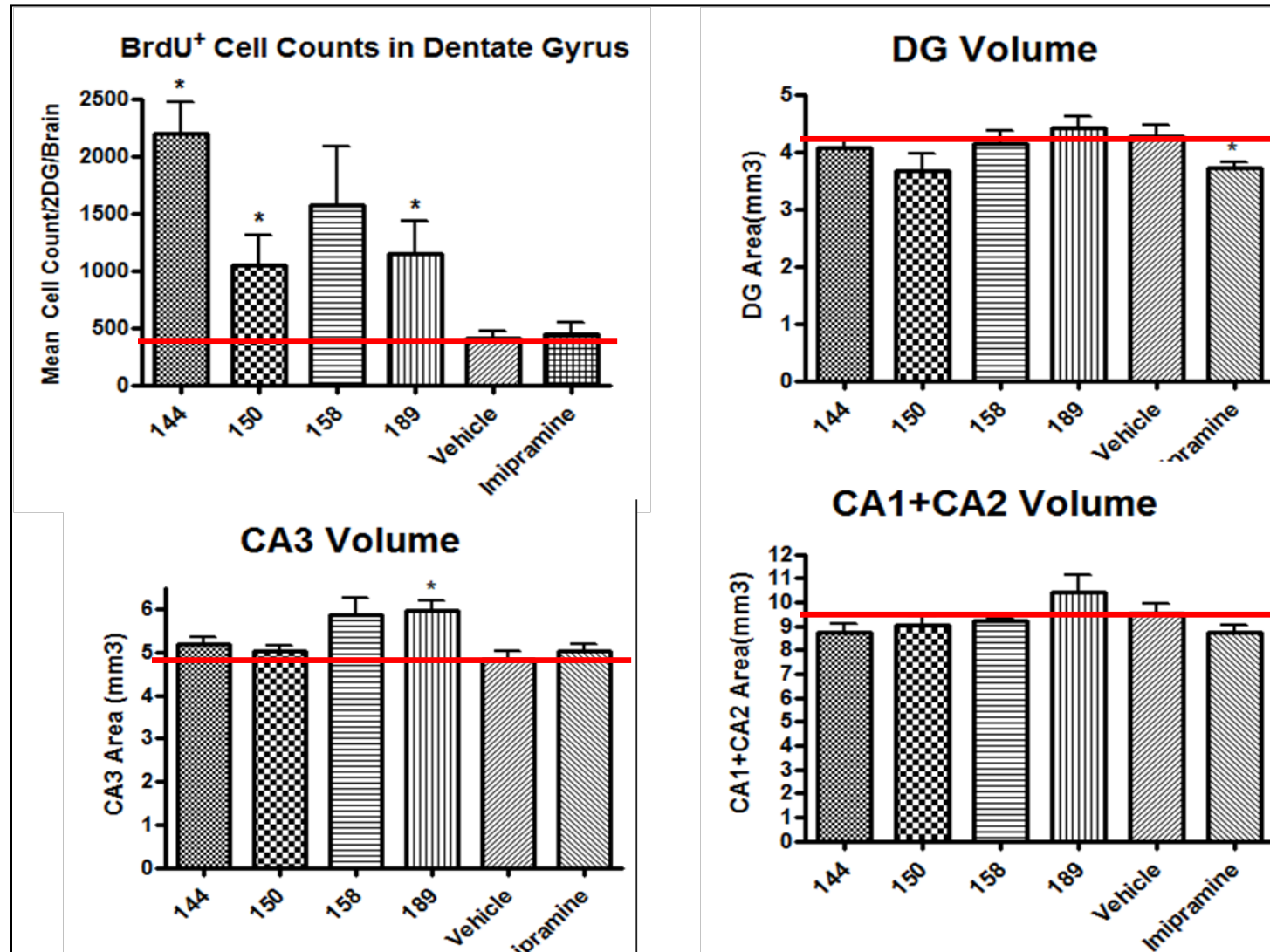
\*Conducted by Cerep & NeuroDetective Inc.

# Leads show anti-depression activity in models of depression: chronic novelty suppressed feeding



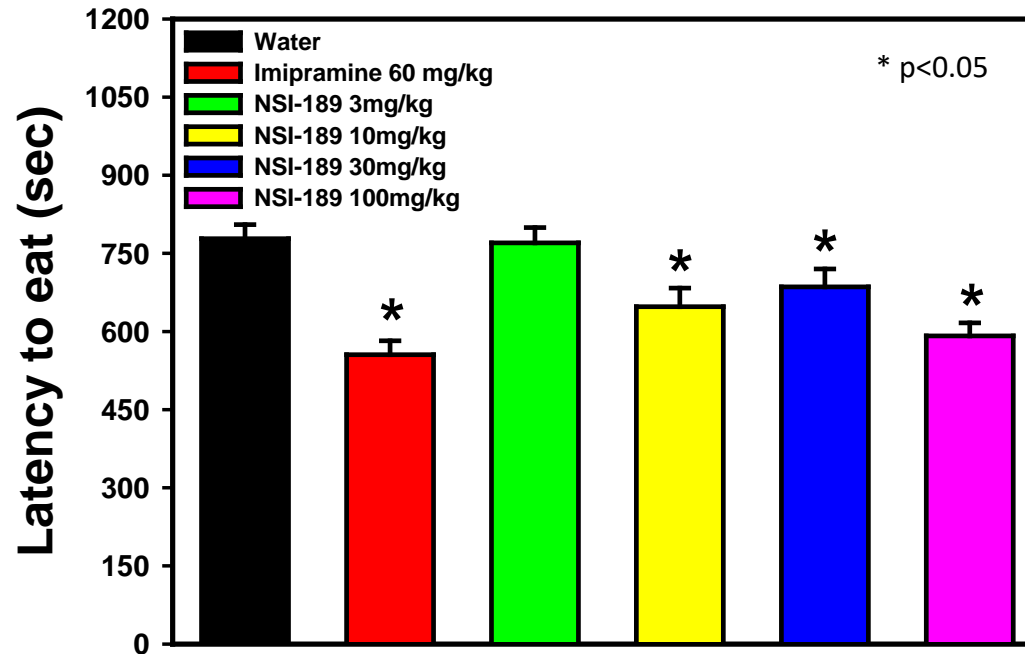
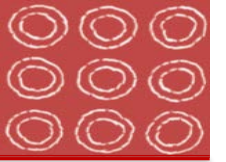
N=15-20/group; Conducted by PsychoGenics, Inc.

# Leads stimulate neurogenesis and increase HI volume



\*30 mg/kg and imipramine at 60 mg/kg for 28d p.o.; Histology analysis conducted by NeuroDigiTech

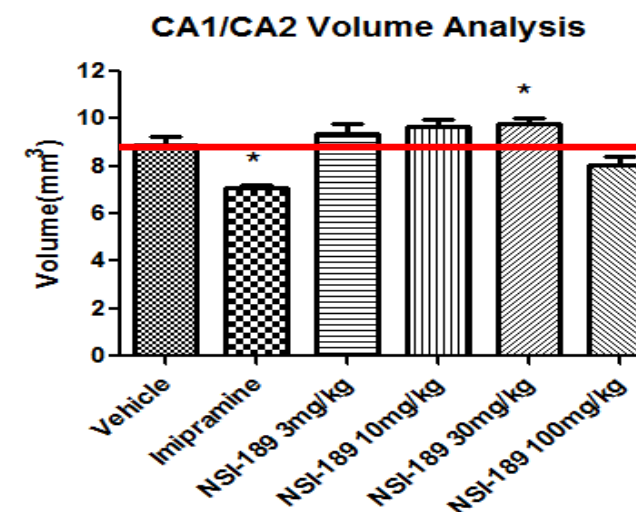
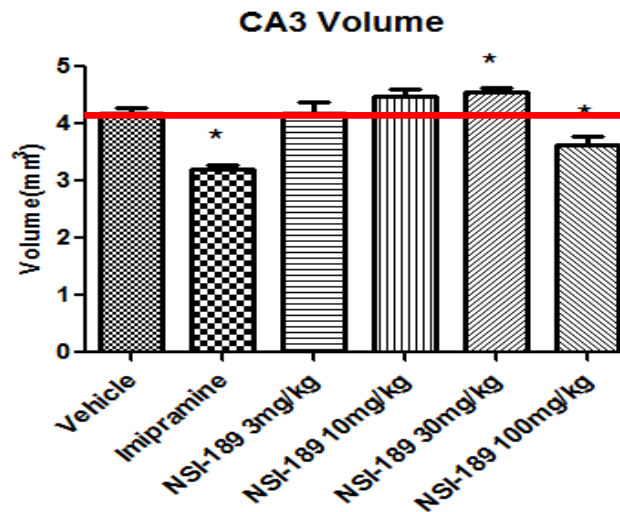
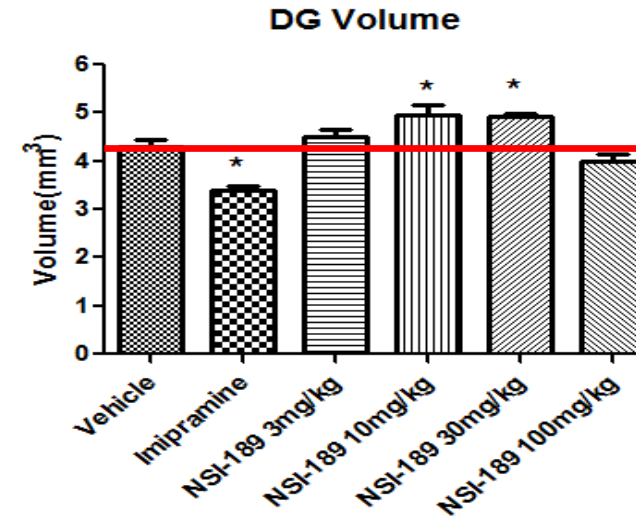
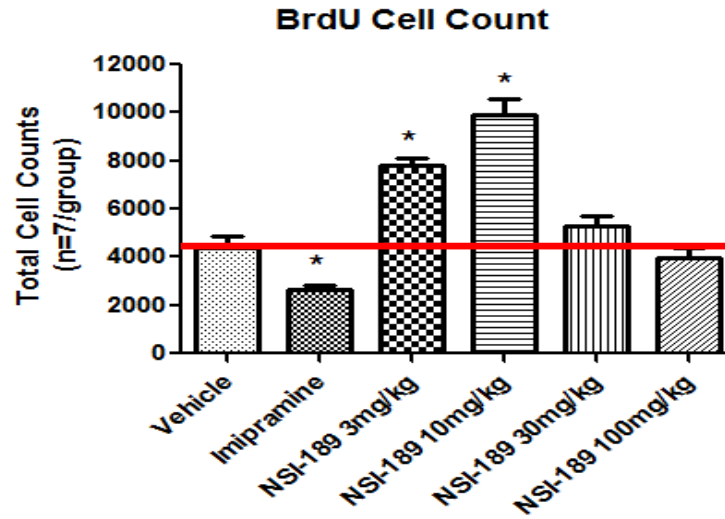
# Effective in chronic novelty suppressed feeding model



- NSI-189 (10-100 mg/kg) and imipramine significantly decreased the latency to eat compared to vehicle (water) after 28 days of oral dosing.
- No significant treatment effects on either body weight or neurological observation were seen.



# Increases neurogenesis and HI volume



# Screening Path to NSI-189



10,269 Small Molecule Compounds through Primary Screen



16 Neurogenic Compounds *in vitro*



16 Tested for Acute Tox in Mice



15 Tested for Neurogenesis  
in Healthy, Adult Mice



7 Orally Active Neurogenic Leads  
(3 Structural Classes)



**1 Development Candidate Selected  
NSI-189**

- Goal was to develop a drug that promotes proliferation of endogenous neural stem cells and increases their differentiation into new neurons
  - Replenishes neural circuitry and enhances cognition in CNS diseases



# **A Phase 1B, Randomized, Double-Blind, Placebo-Controlled, Multiple-Dose Escalation Study Evaluating the Effects of NSI-189 Phosphate, a Neurogenic Compound, in Patients with Major Depressive Disorder (MDD)**

Maurizio Fava, M.D., Karl Johe, Ph.D., Lev G. Gertsik, MD, Larry Ereshefsky, PharmD, Bettina Hoeppepner, Ph.D., Martina Flynn, David Mischoulon, M.D., Ph.D., Gustavo Kinrys, M.D., and Marlene Freeman, M.D.



# Phase 1b Study Design

- Double-blind, randomized, placebo-controlled, multiple-dose study with three ascending cohorts (N=24)

<b>Cohort 1</b>	N=8 (6 drug, 2 placebo)	40 mg QD
<b>Cohort 2</b>	N=8 (6 drug, 2 placebo)	40 mg BID
<b>Cohort 3</b>	N=8 (6 drug, 2 placebo)	40 mg TID
<b>Drug Treatment: 28 days</b>		<b>No-Drug Follow up: 35, 42, 49, 70, 84</b>

## Participants

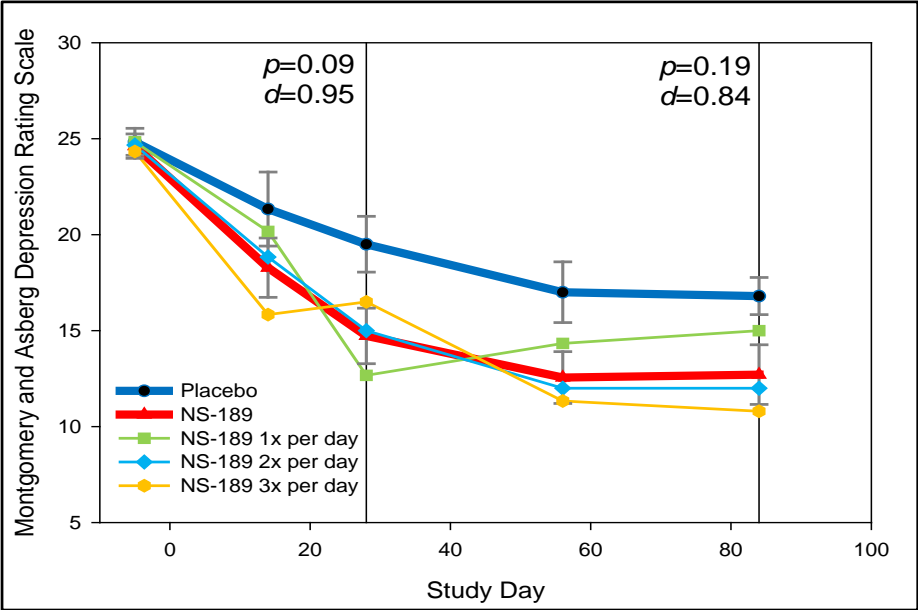
- Male or female, 18 to 60 years of age, diagnosed with MDD, recurrent, as per DSM-IV-TR confirmed by SCID-CT
- Patients at screening could be taking an antidepressant medication(s), or have a history of taking antidepressant medication(s) in the past for their depressive disorder
- At least two prior depressive episodes (including current episode)



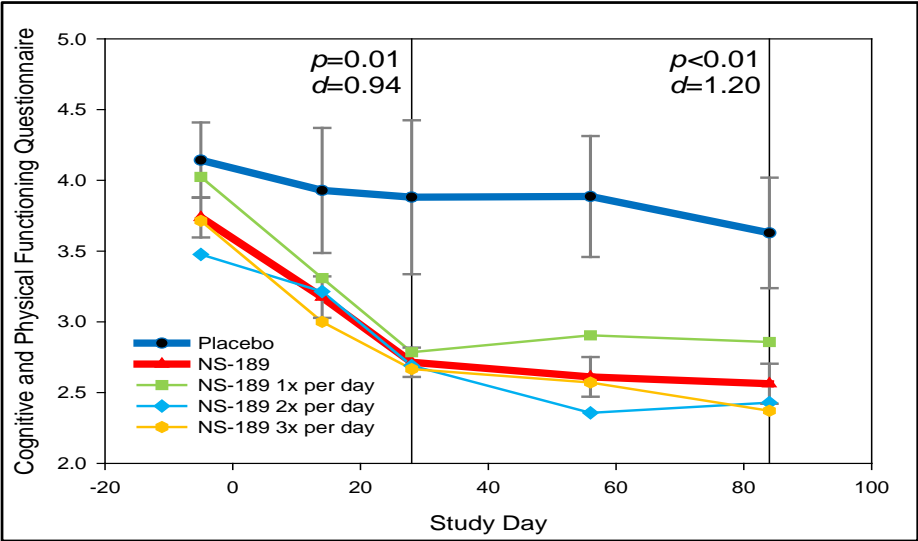
# Positive Phase 1b clinical outcomes



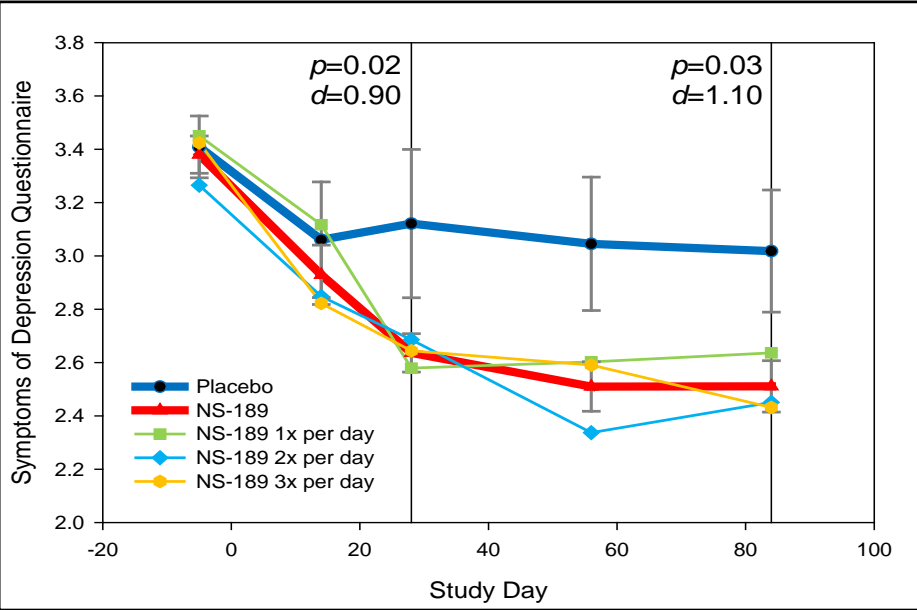
MADRS



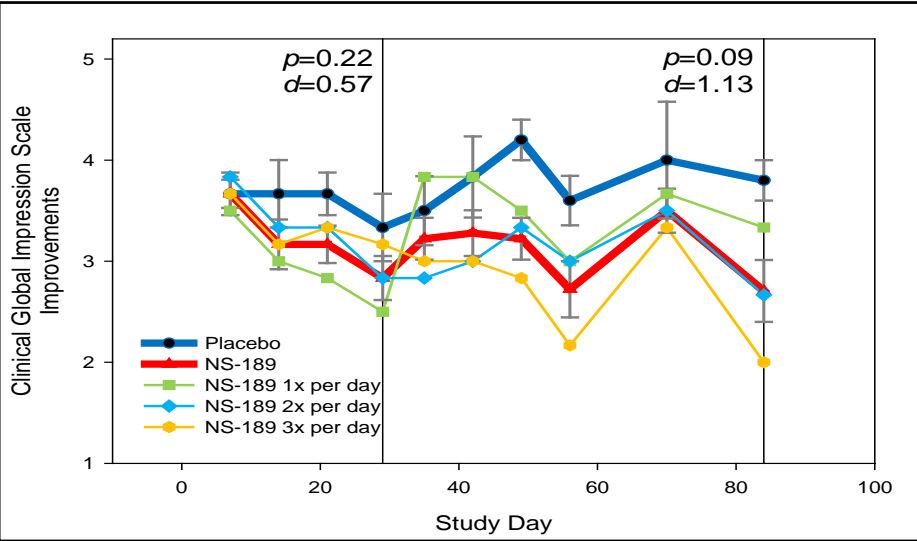
CPFQ



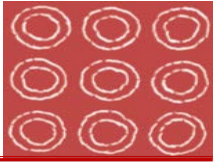
SDQ



CGI-I

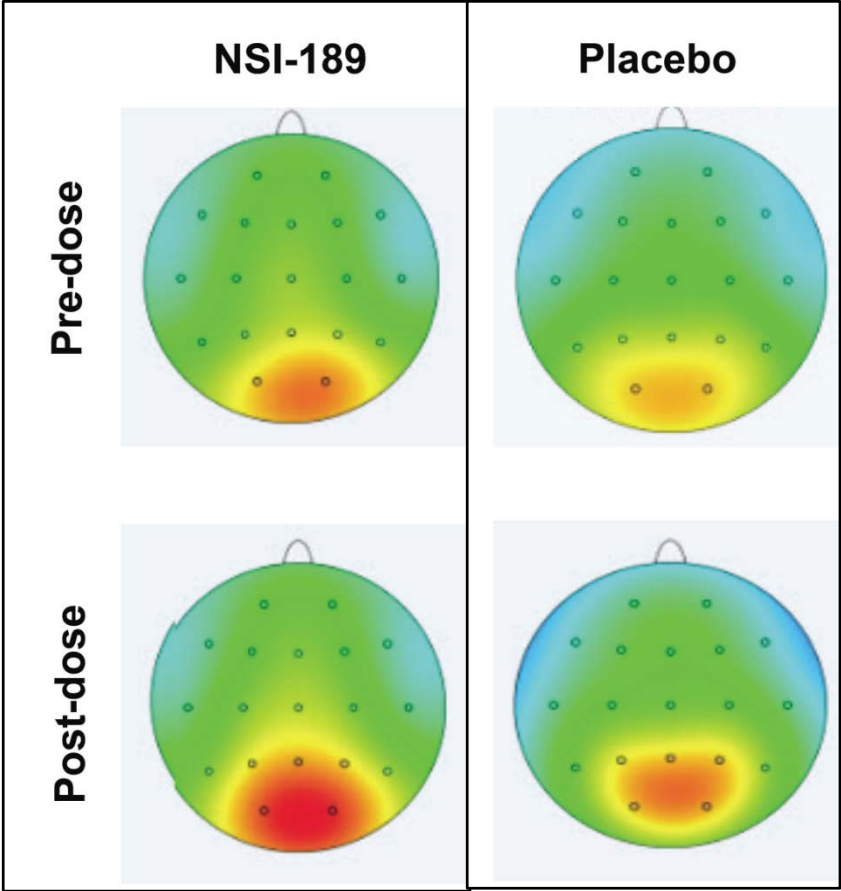


# Positive biomarker results



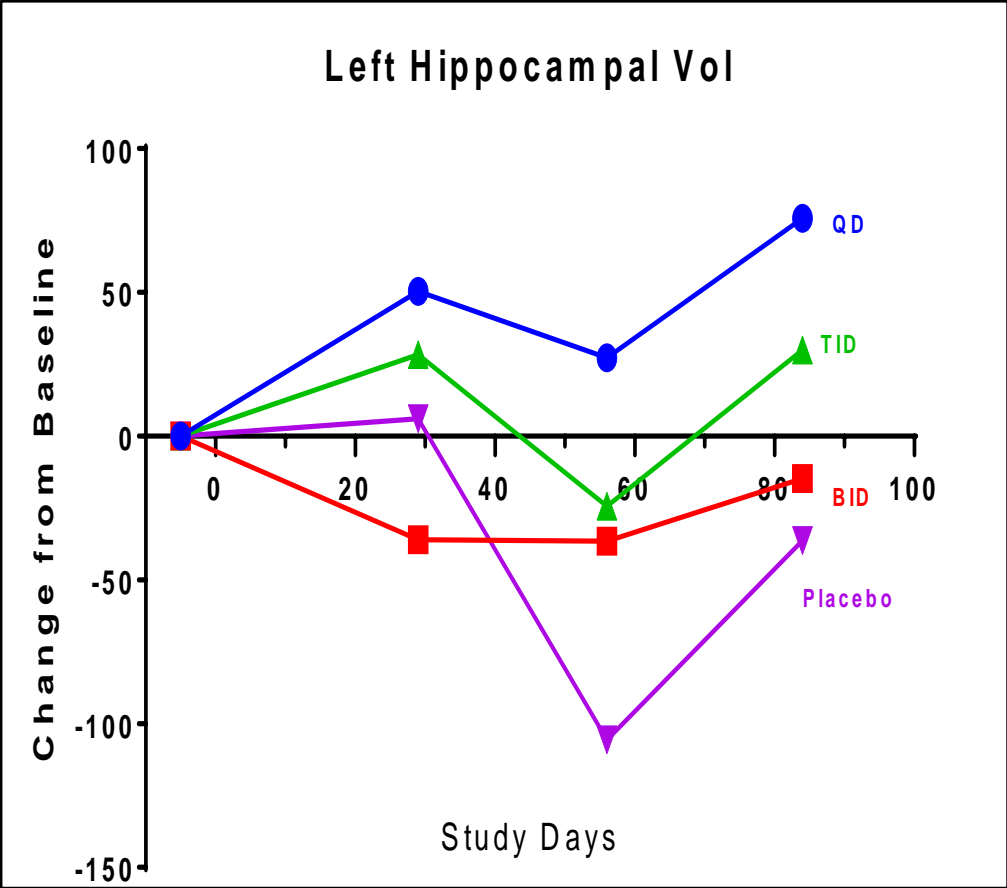
## qEEG

Topographs of High Frequency alpha (10-12 Hz): Day 28 from Baseline



Left posterior temporal (T5) ( $t=2.45$ ,  $p=0.02$ )  
Left parietal regions (P3) ( $t=3.31$ ,  $p=0.004$ )

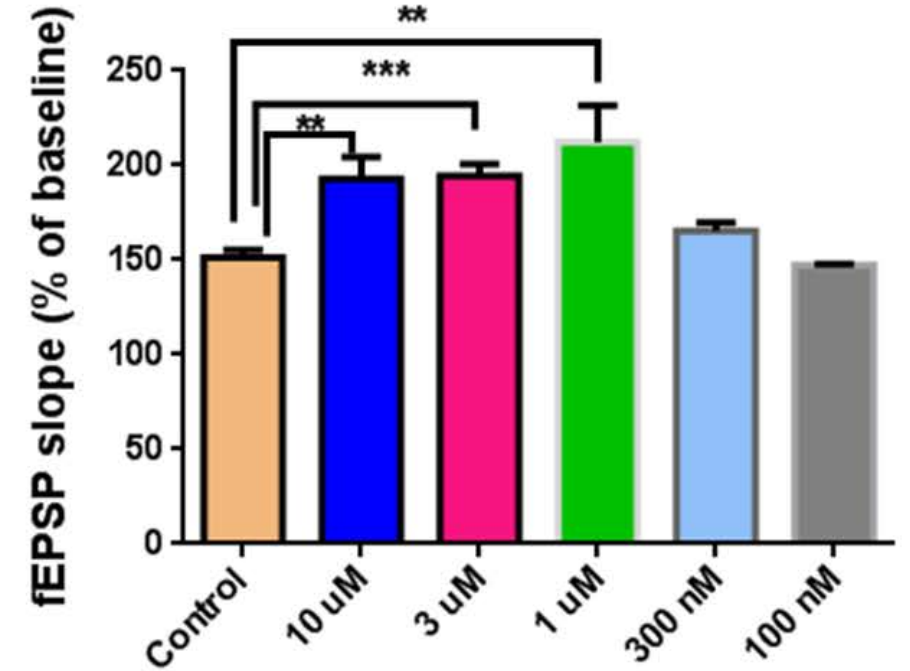
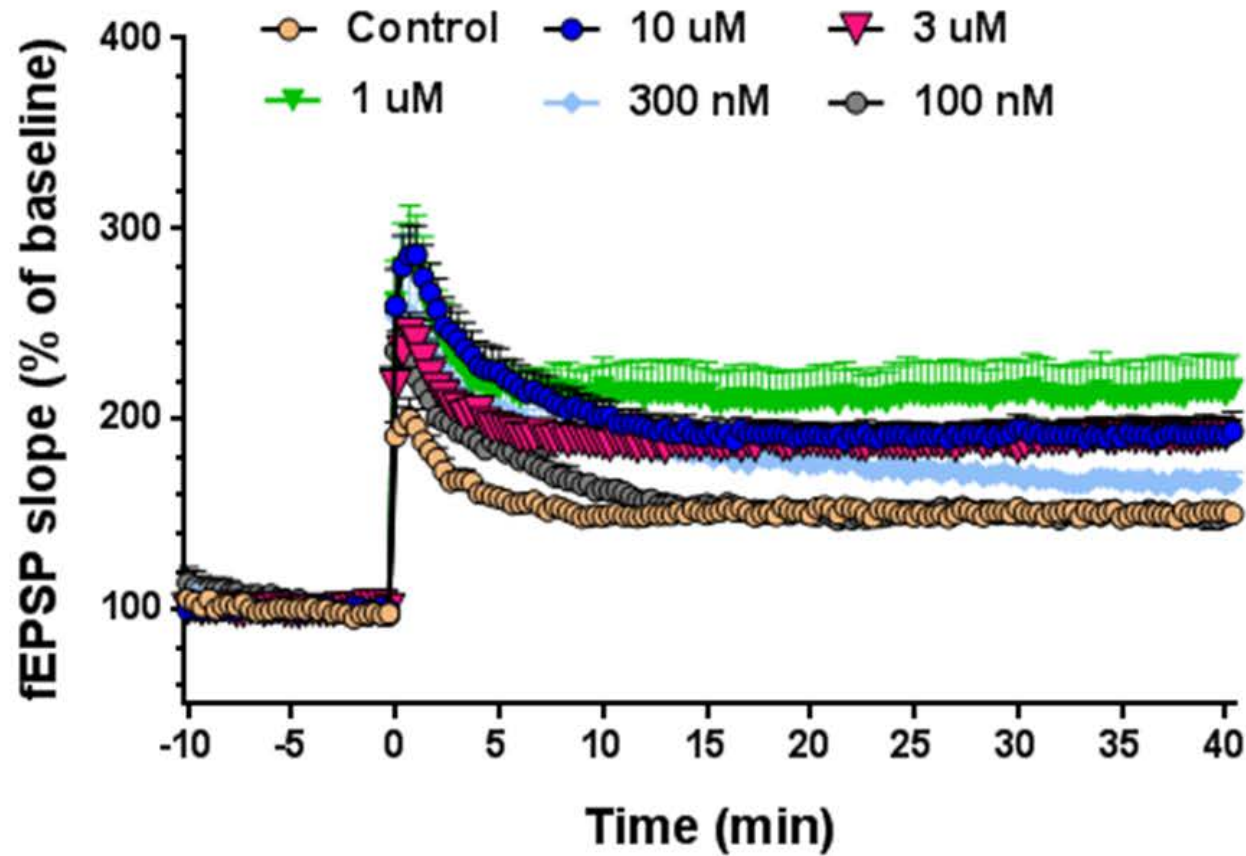
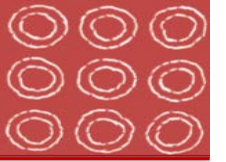
## MRI



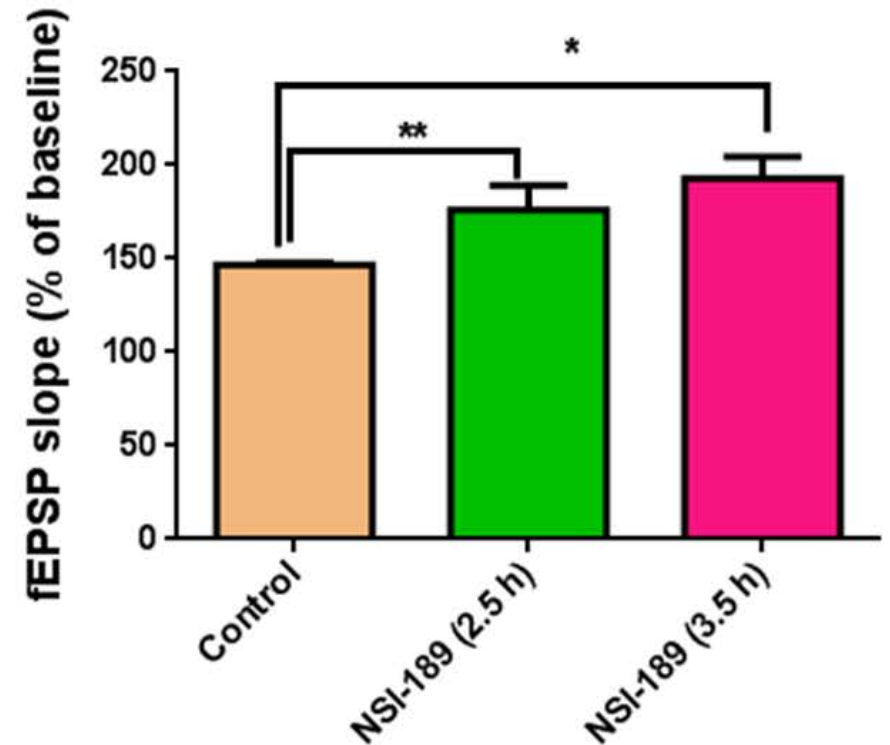
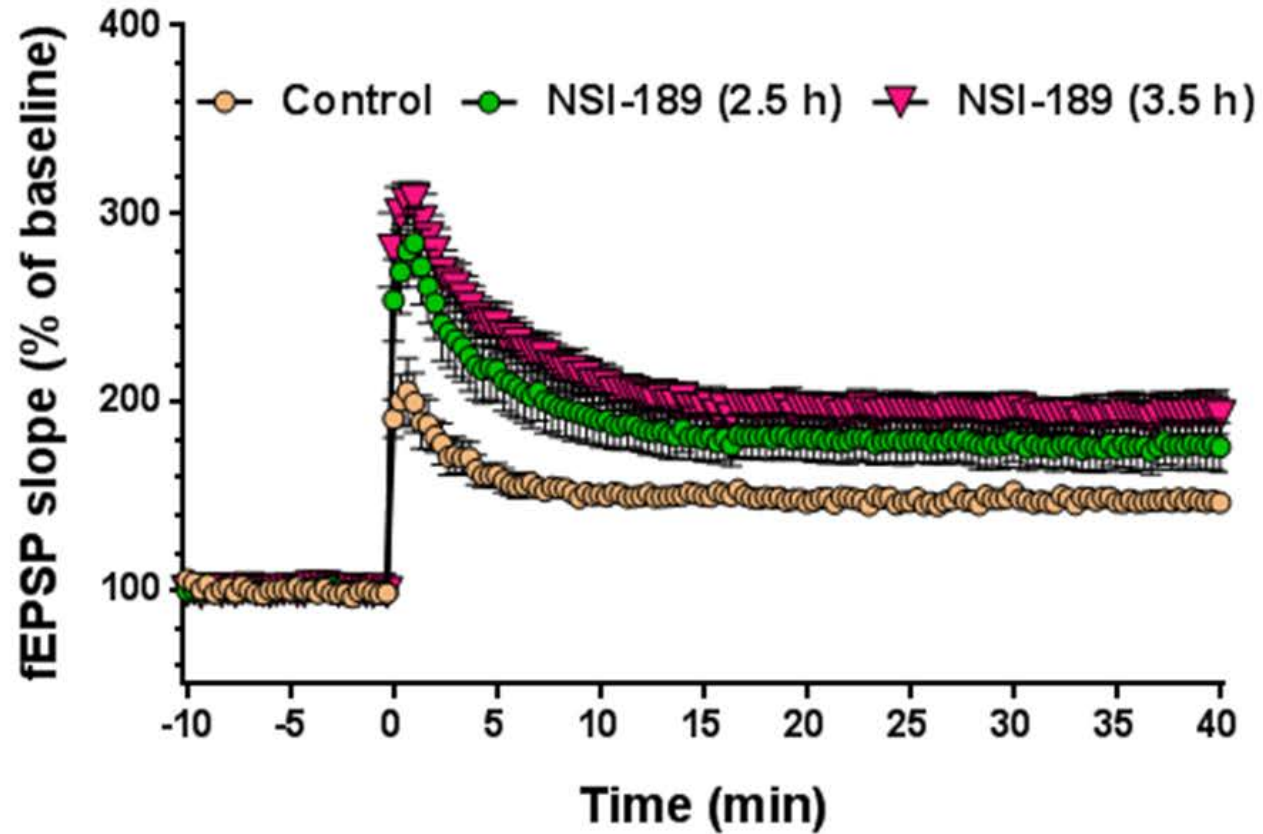
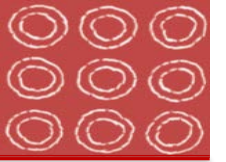
## Blood

Pt. ID	Designa tion	Probabilty
501	PR	1.000
502	R	1.000
504	R	1.000
505	R	1.000
506	R	1.000
508	NR	0.032
602	R	1.000
603	R	0.961
604	PR	1.000
605	PR	1.000
606	PR	0.998
608*	PR*	0.000*
627	R	0.980
701	R	1.000
702	R	0.987
703	R	0.000
705	NR	0.031
706	R	1.000
707	R	0.995

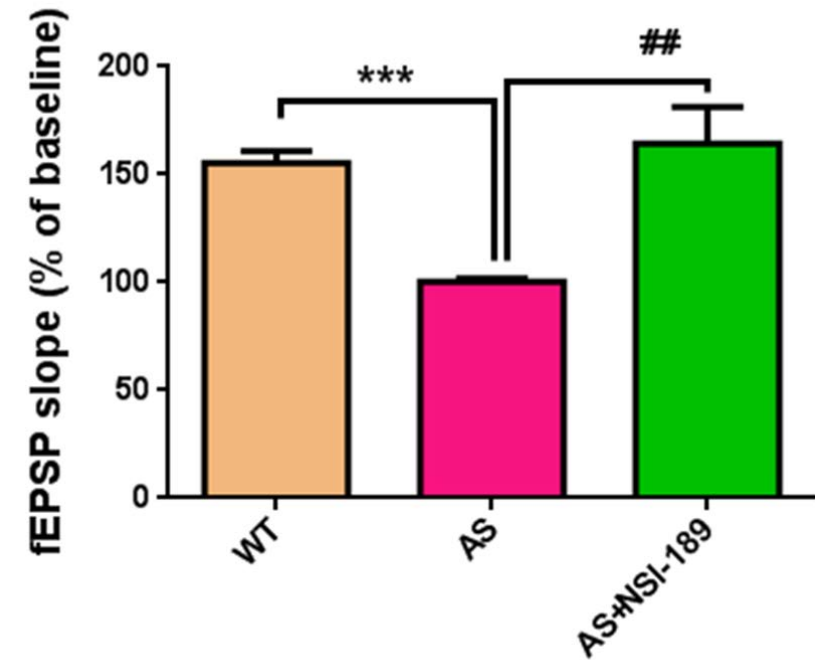
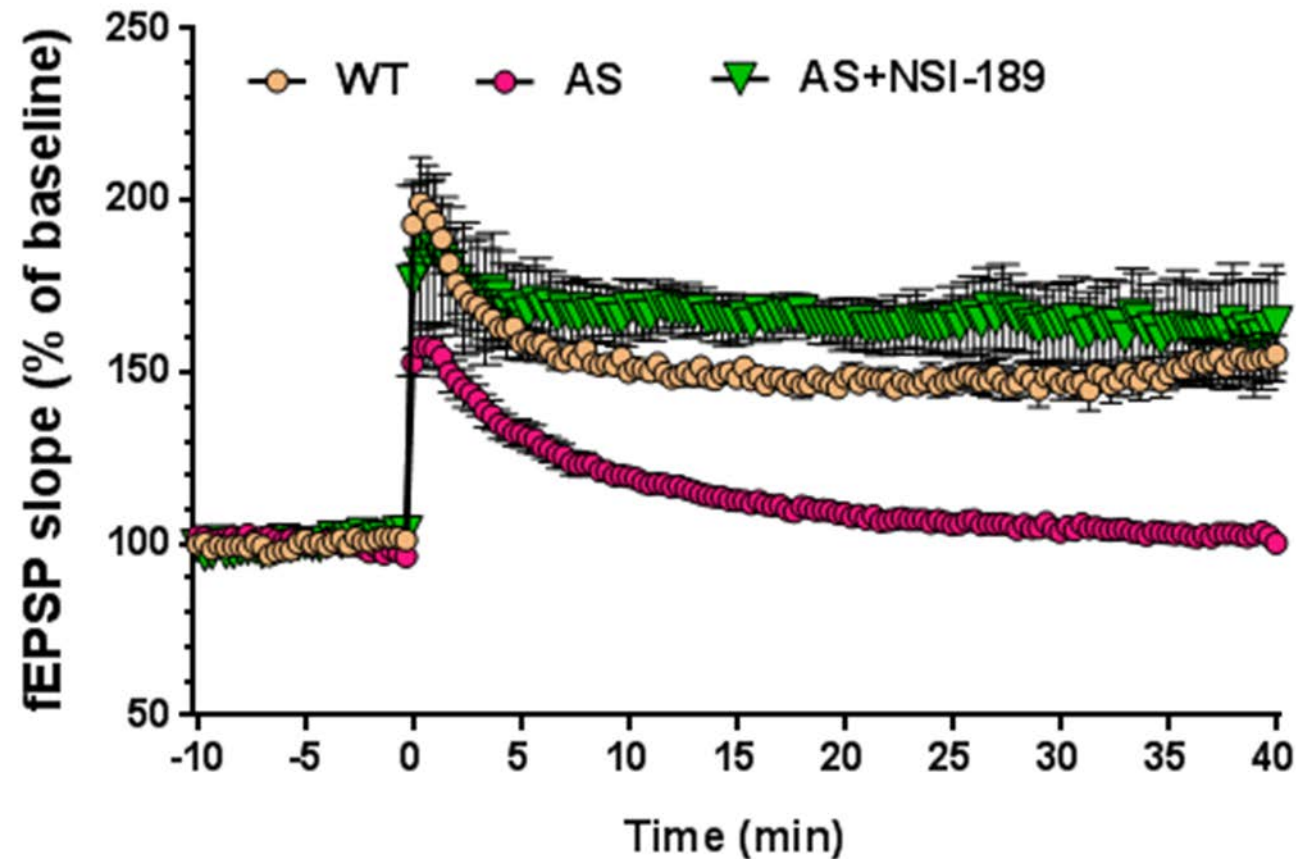
# NSI-189 produces a **concentration-dependent** enhancement of LTP magnitude



# NSI-189 produces a **time-dependent** enhancement of LTP magnitude



# NSI-189 **restores LTP** in hippocampal slices from Angelman Syndrome model

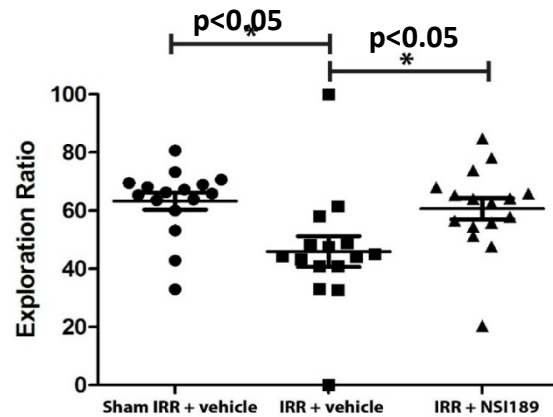


Yan Liu<sup>1</sup>, Michael P. Hefferan<sup>2</sup>, Karl Johe<sup>2</sup>, Xiaoning Bi<sup>1</sup>, Michel Baudry<sup>1</sup>

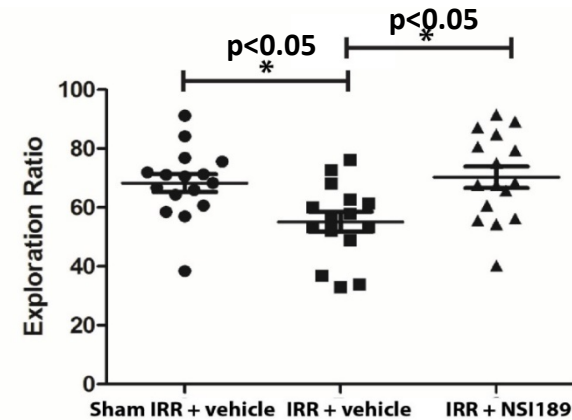
# NSI-189 ameliorates **cognitive deficit** in radiation-induced brain injury model



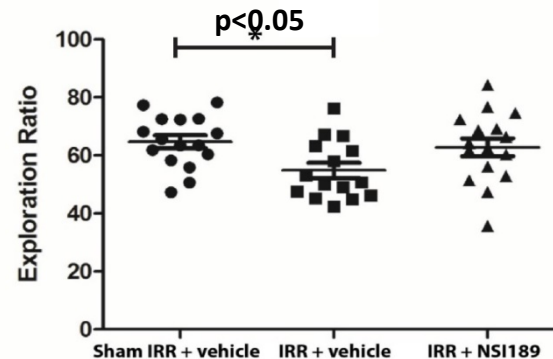
Spatial Cognitive Impairment  
Novel Place Recognition Task



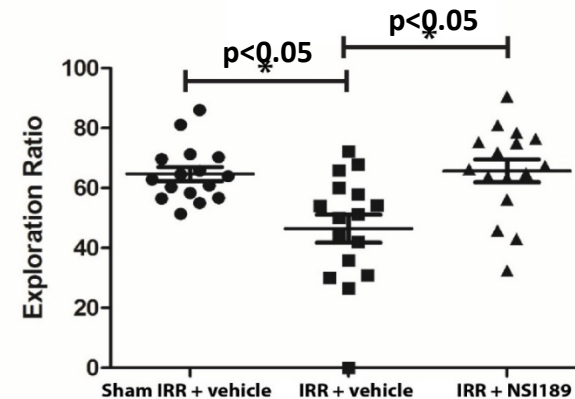
Episodic Memory Retention  
Novel Object Recognition Task



Spatial Memory Retention  
Object in Place Task



Spatiotemporal Episodic Memory Retention  
Temporal Order Task

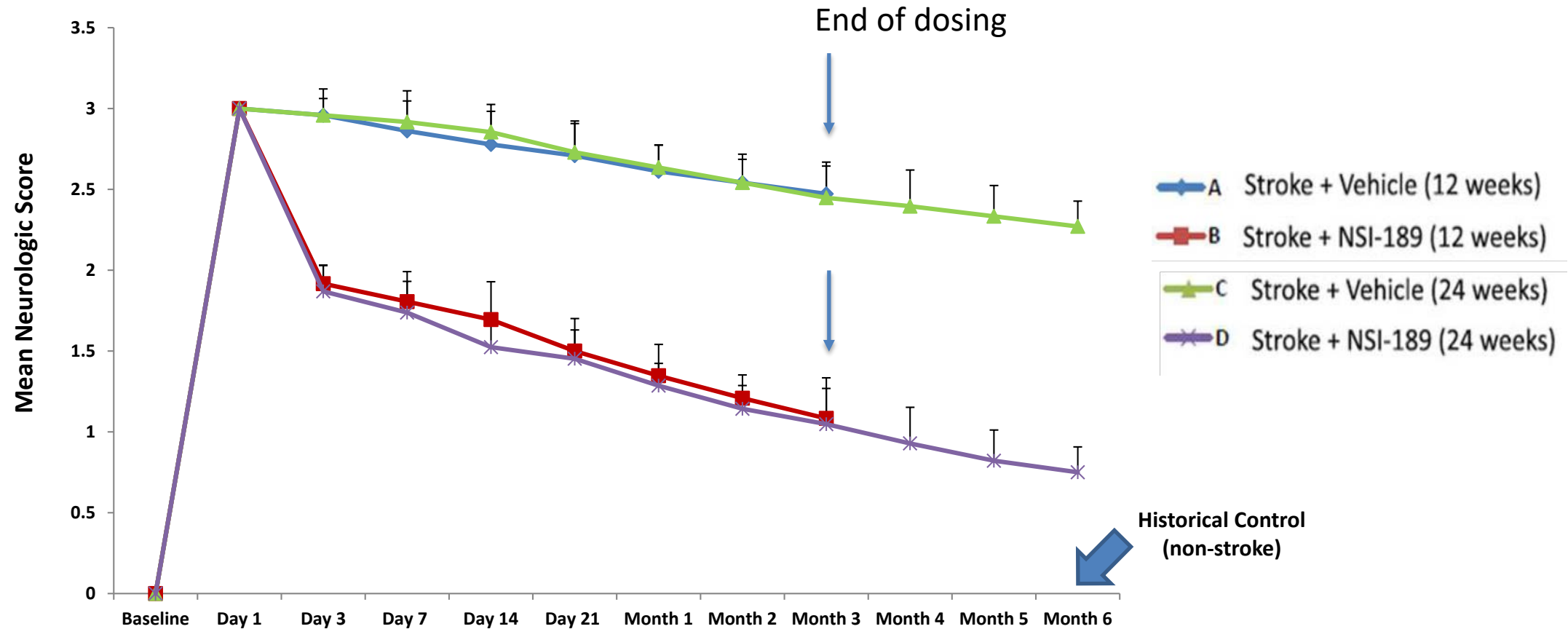




# NSI-189 ameliorates **motor deficit** in ischemic stroke model



## Bederson Neurologic Test

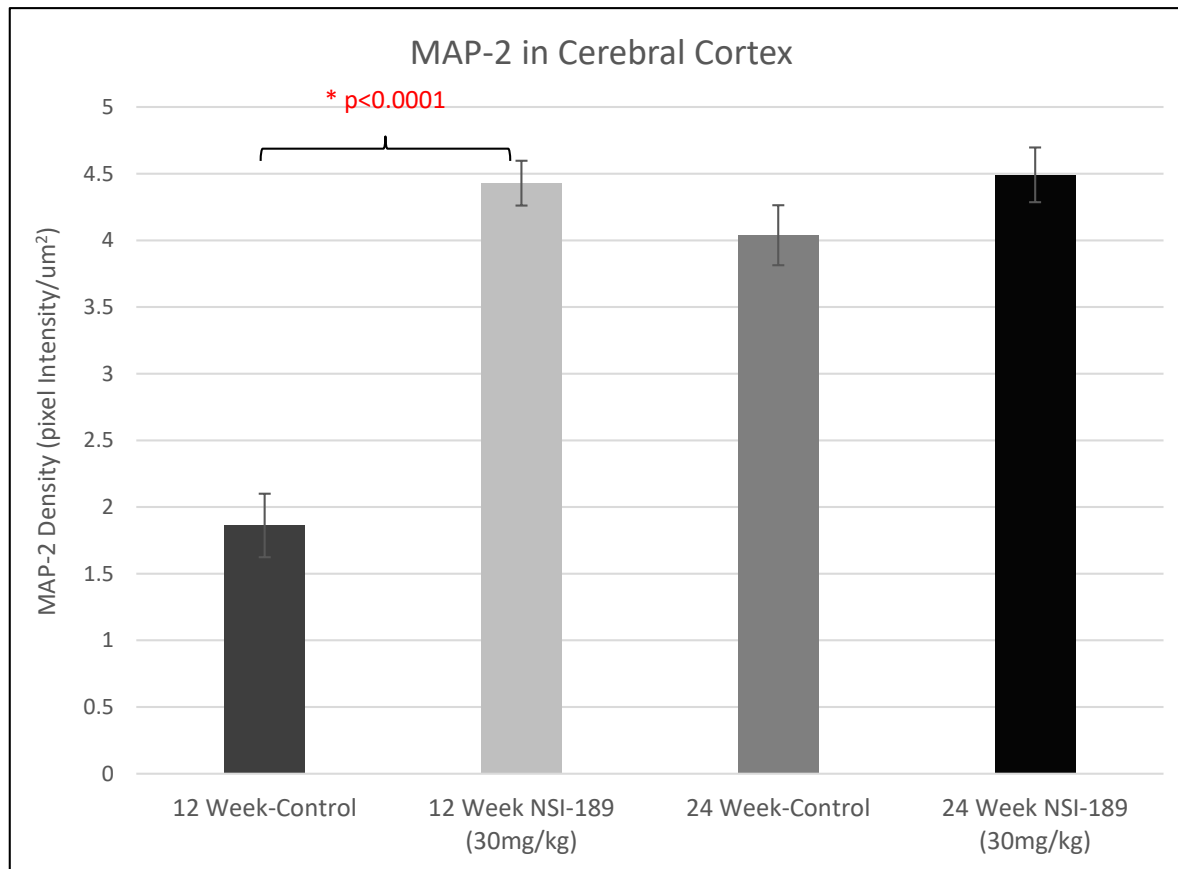


\*p's < 0.05: B, D vs. A, C

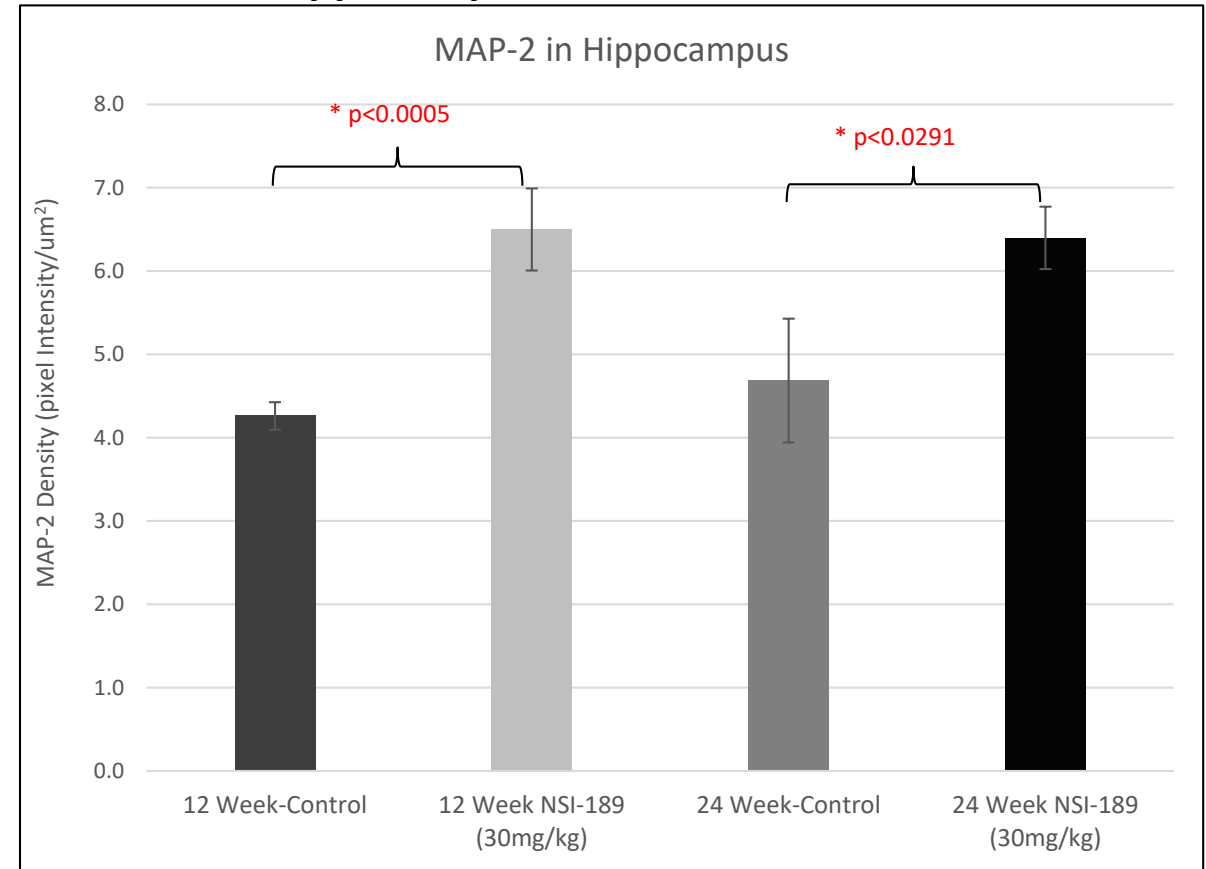
# NSI-189 induces **synaptic remodeling** in ischemic stroke brain



## A. MAP-2 in Cerebral cortex



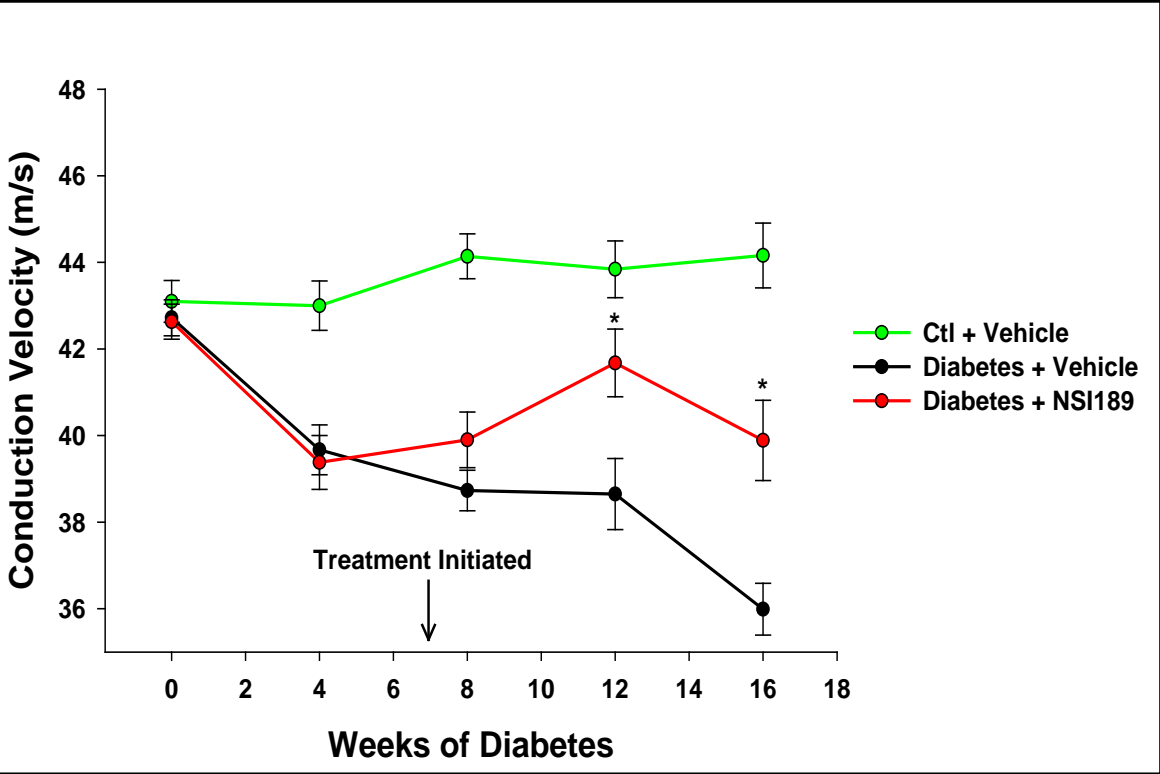
## B. MAP-2 in Hippocampus



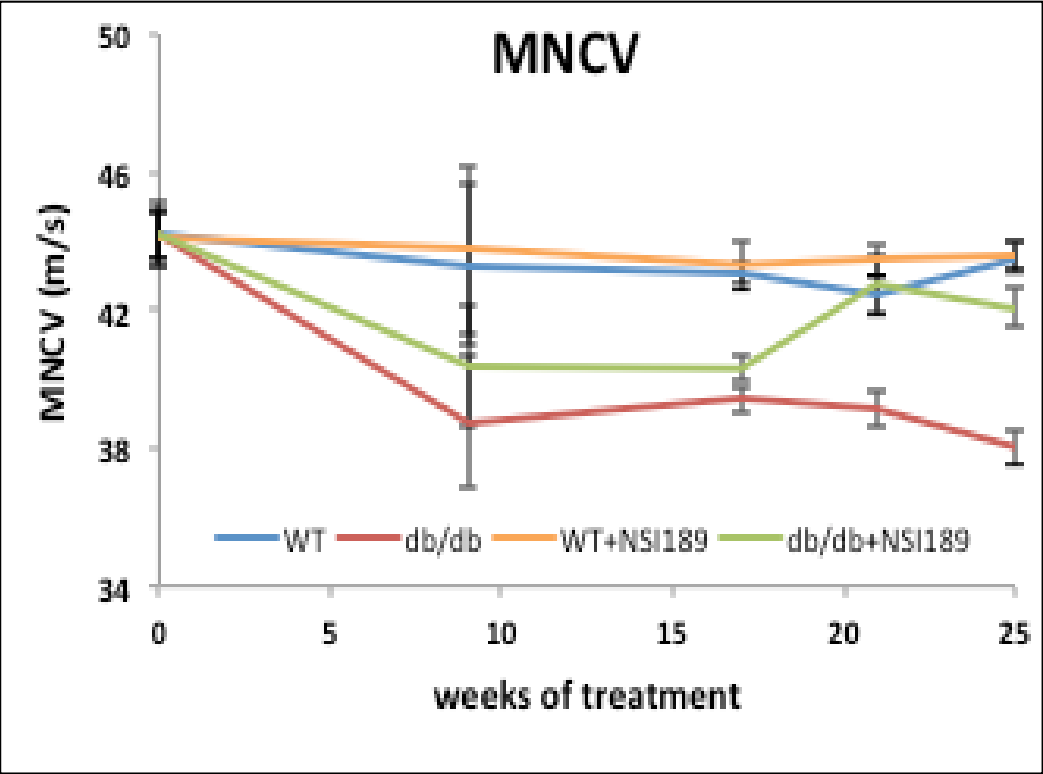
# NSI-189 increases motor **nerve conduction velocity** in type 1 and 2 diabetic models



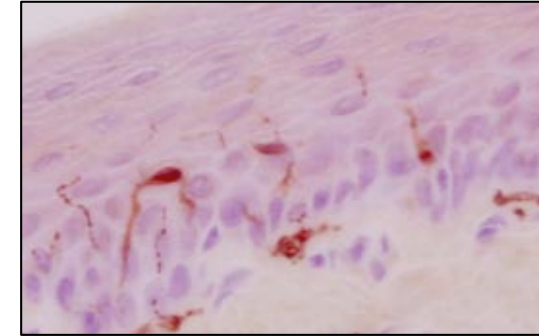
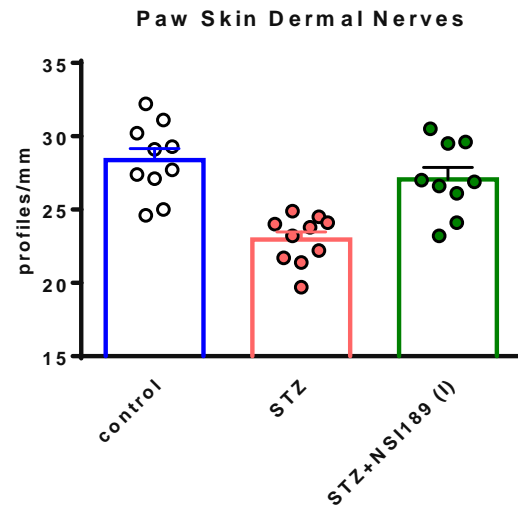
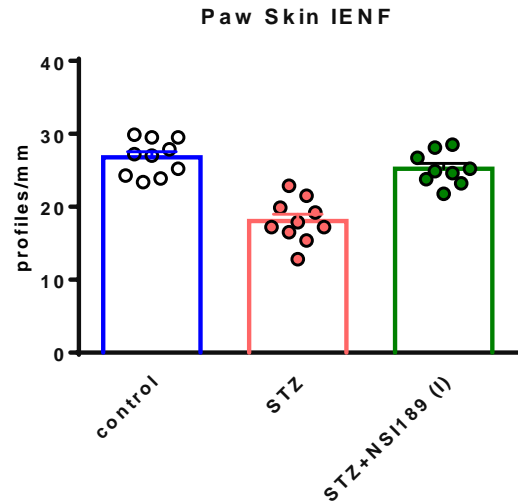
Type 1 Diabetes Model—Intervention Study



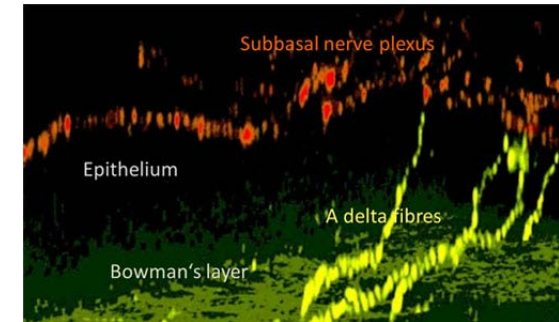
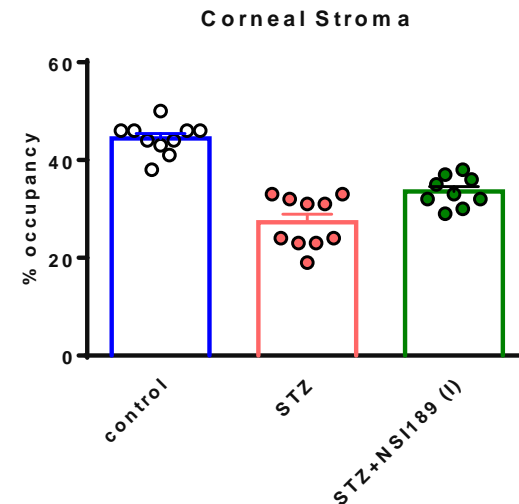
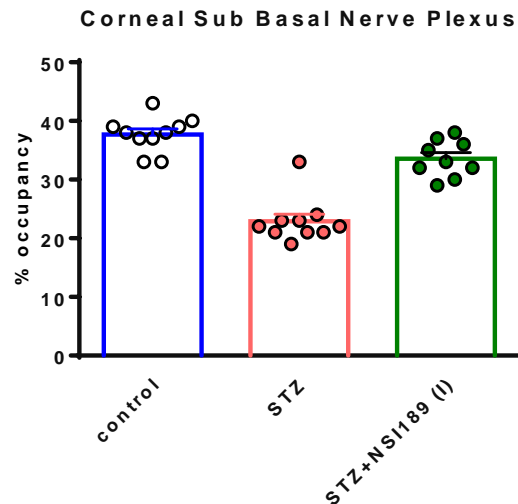
Type 2 Diabetes Model—Prevention Study



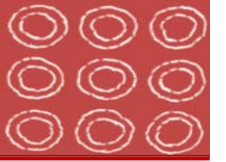
# NSI-189 increases small peripheral **nerve fiber density** in type 1 diabetic model



Number of PGP 9.5-stained profiles in the epidermis (intra-epidermal nerve fibers: IENF)



3D reconstruction of nerve structure in the mouse cornea: A delta fibers (yellow) penetrating the Bowman's layer and spreading into the sub-basal nerve plexus (red)



- Orally active neurogenic, synaptogenic, neurotrophic compound for treatment of depression, cognitive impairment, and neurodegeneration
  - Increases hippocampal neurogenesis, hippocampal volume, synaptogenesis, and synaptic plasticity in normal and/or lesioned brain
  - Novel, unknown, pharmacology, not mediated by 5-HT or norepinephrine re-uptake inhibition, or by BDNF release
  - Requires protein synthesis
- 28-day Phase 1b clinical study in MDD showed very large effect size across multiple clinical scales and with correlating biomarker changes
- MDD Phase 2 efficacy study with 220 patients, results in 3Q 2017