

# Different effects of Pregabalin Delivery Sites in Neuropathic Pain States of Diabetic Peripheral Neuropathy and Chronic Constriction Injury

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## Disclosures



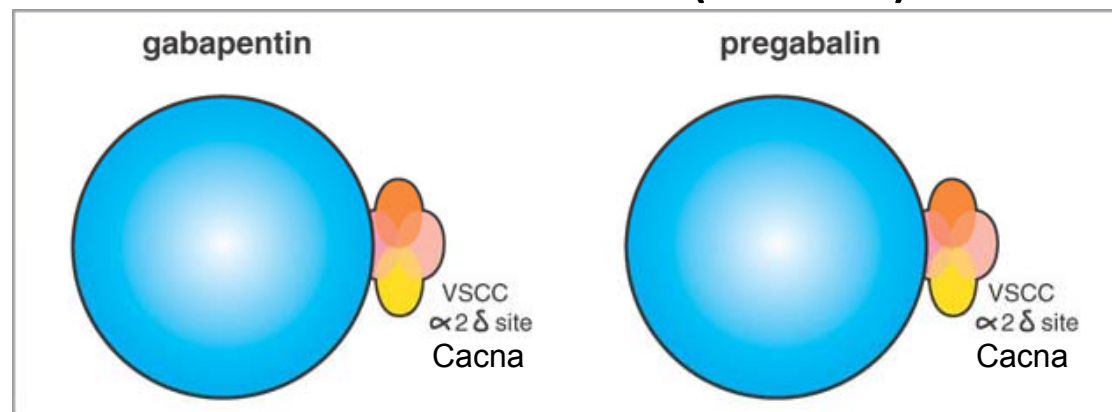
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# Background

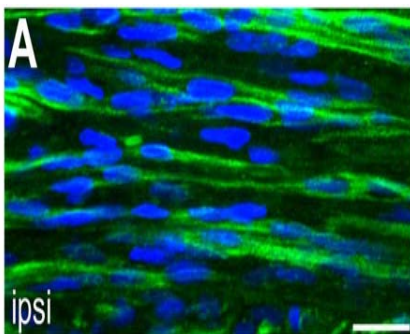
- Pregabalin is an anticonvulsant drug used for the management of neuropathic pain (NeP)
- As such, it is indicated in Canada for the management of Diabetic Peripheral Neuropathy (DPN)-related NeP
- The mechanism of pain relief with Pregabalin relates to its modulation of the  $\alpha_2\delta$  subunit of the voltage dependent calcium channel (Cacna).



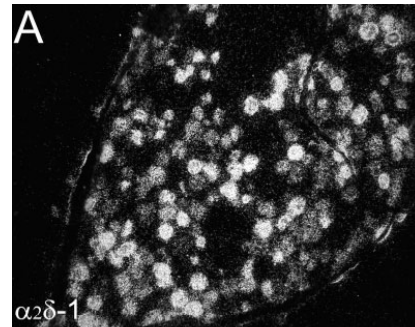
# Background

- Pregabalin is likely beneficial in other forms of NeP, such as with post-traumatic nerve injury.
- What is not clear is the main site of Pregabalin's action within the PNS or CNS.

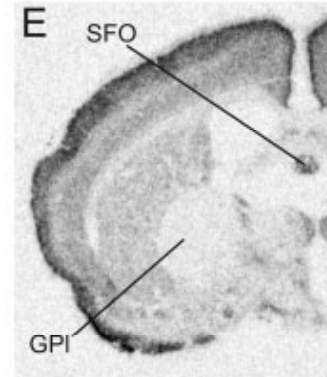
Sciatic Nerve



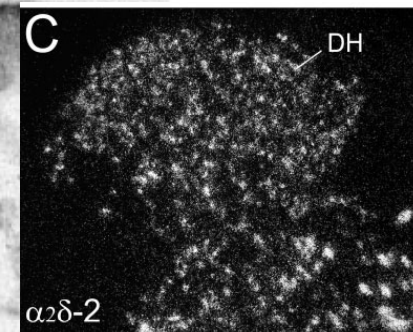
L5 DRG



Brain



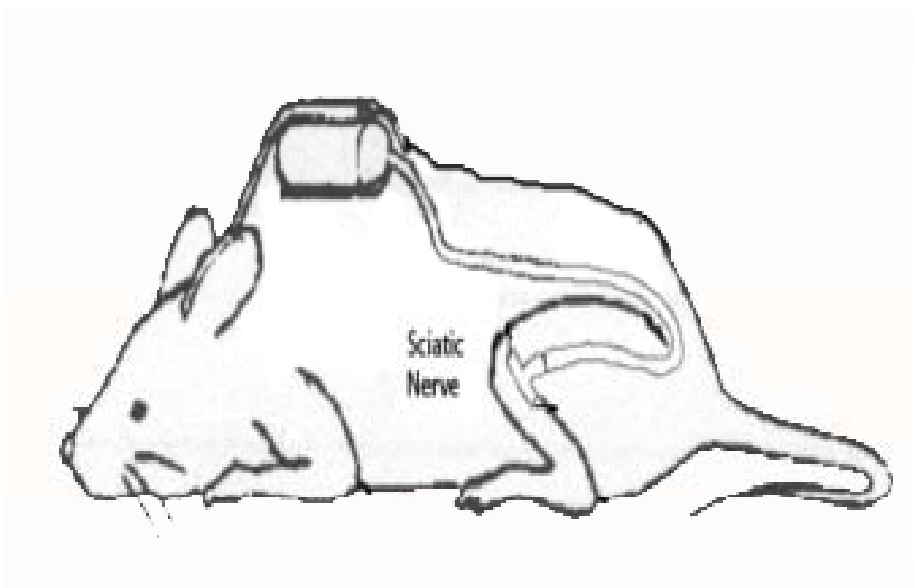
Dorsal Horn



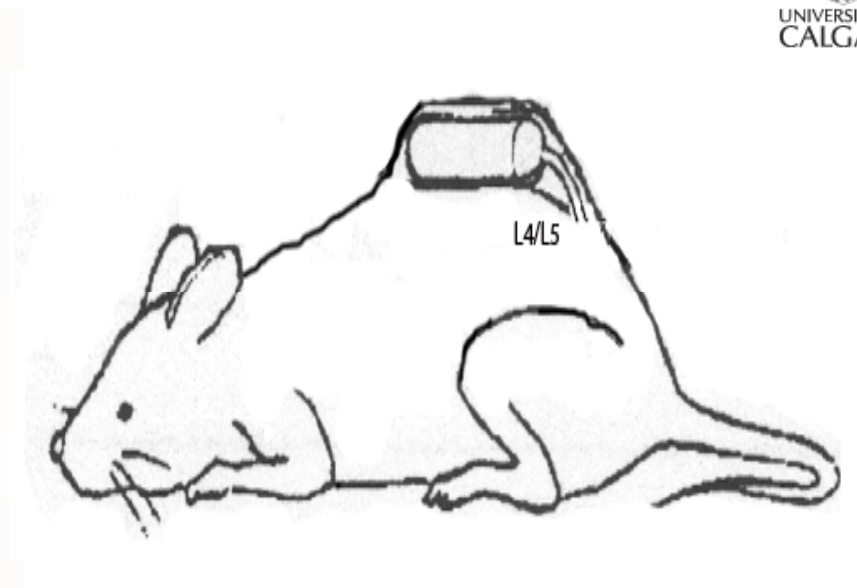
*Bauer et al, J Neurosci 2009*  
*Cole et al, J Compar Neurol 2005*

# Background

- We have developed three unique systems for drug delivery which can assist with determination of site of action and support in the prevention of systemic effects:

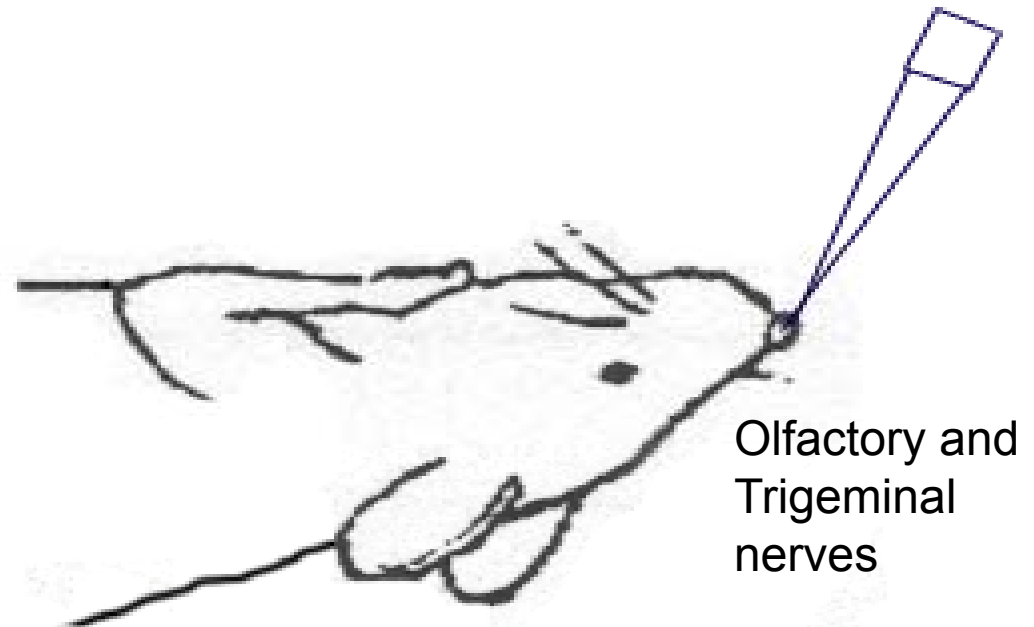


- Near Nerve (NN) delivery permitting release of a drug to a specific nerve without systemic effects



- Intrathecal (IT) delivery providing release to

- Intranasal (IN) delivery providing release to the Brain, Spinal Cord, Nerve roots and DRG.



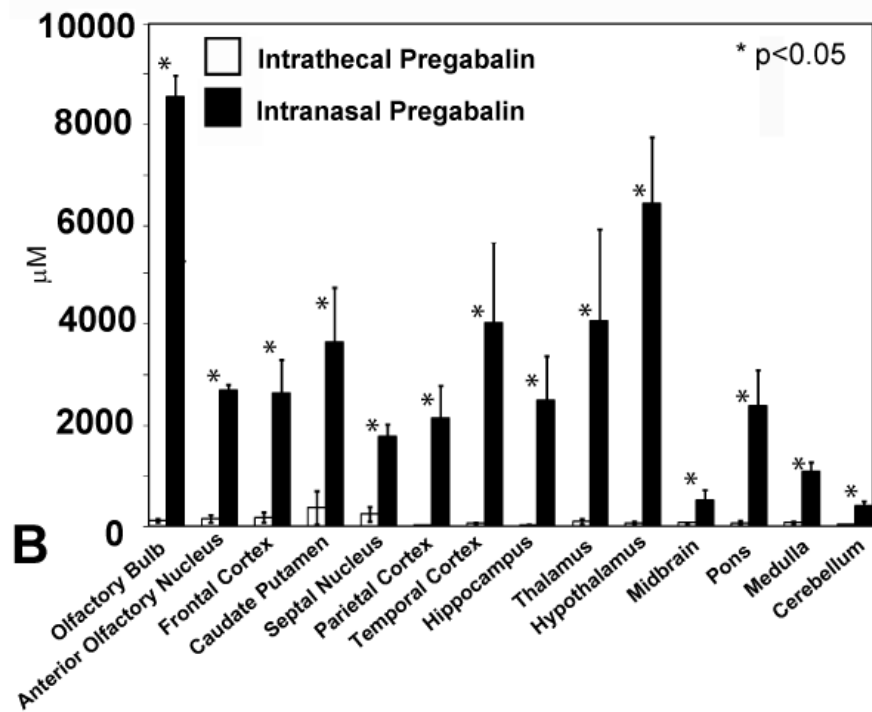
# Objective

- To assess the utility of different delivery sites of Pregabalin vs. placebo in animal models of NeP:
  - a) Chronic Constriction Injury (CCI) (post-traumatic nerve injury)
  - b) Diabetic Peripheral Neuropathy

# Methods and Results

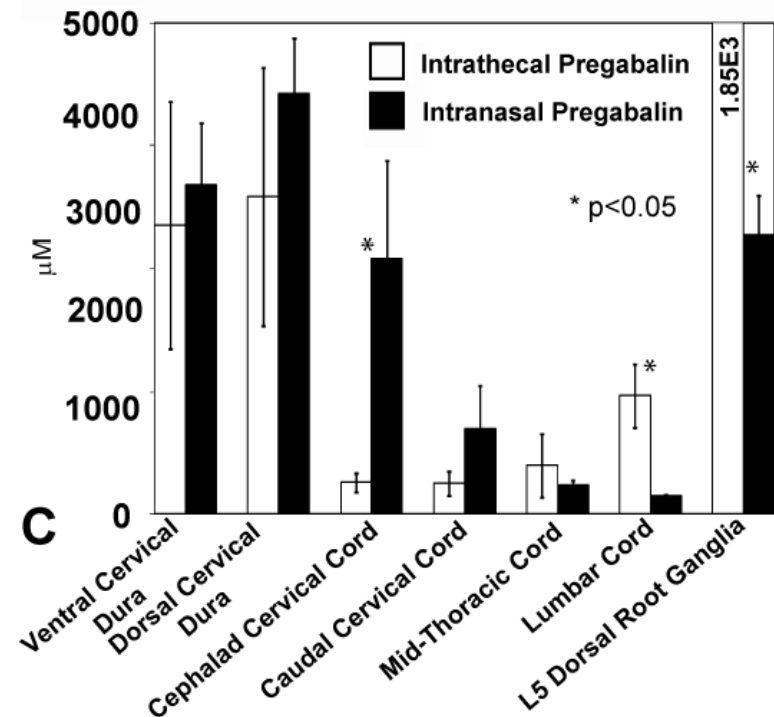
- We first gave radiolabelled Pregabalin IN and IT to quantify delivery to Brain and Spinal Cord

Intranasal and Intrathecal Pregabalin Delivery After One Hour



BRAIN

Intranasal and Intrathecal Pregabalin Delivery After One Hour



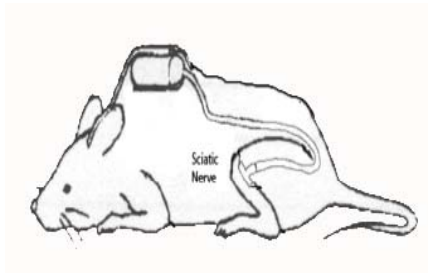
SPINAL CORD

# Methods

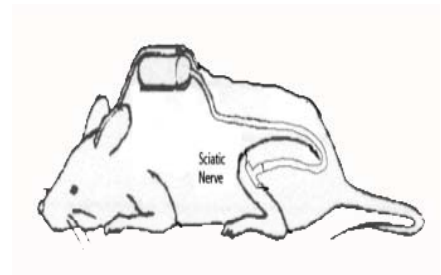
SD Male Rats  
CCI Sciatic Nerve

SD Male Rats  
Diabetic Rats

NN

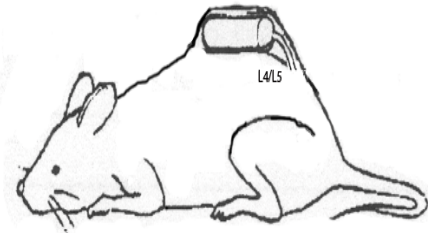


NN



Pregabalin/placebo  
administered for 14 days

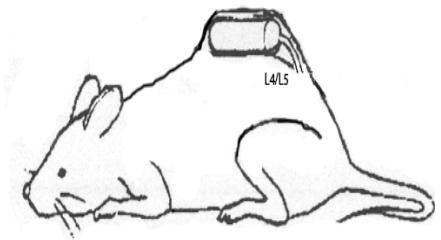
IT



Placebo

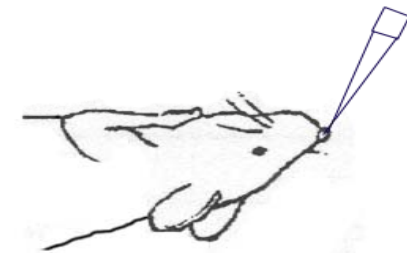
0.1  $\mu\text{mol}$ , 1.0  $\mu\text{mol}$ , 4.0  $\mu\text{mol}$ .

IT

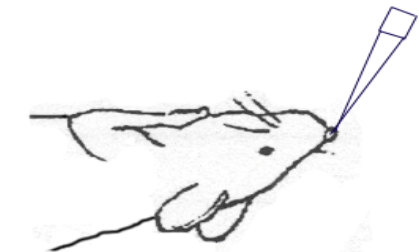


n~6 per cohort.

IN



IN



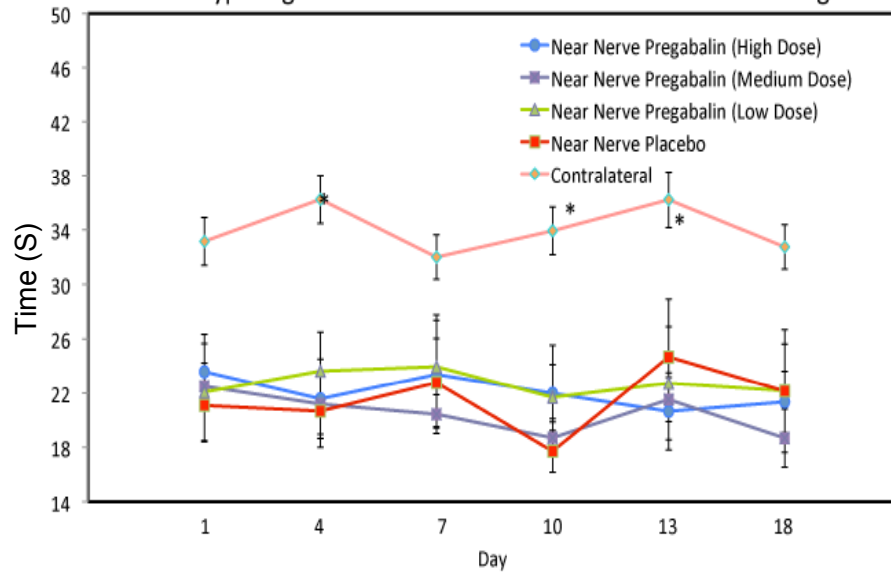
# Methods

- For both models of NeP, **sensory tests** were performed at days 0, 1, 4, 7, 10, 13, 18 of the treatment:
  - Tactile Allodynia (Quantification Aesthesiometer)
  - Thermal Hyperalgesia

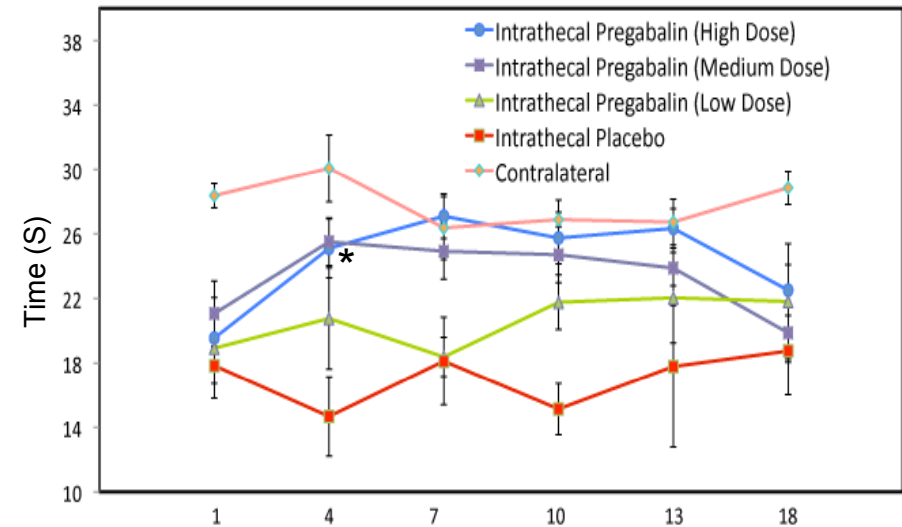


# Thermal Hyperalgesia - CCI

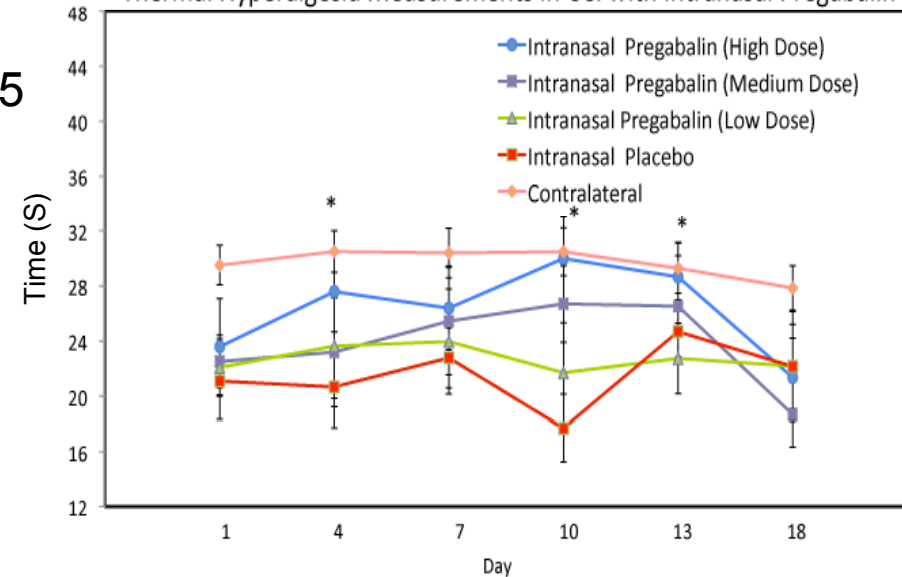
Thermal Hyperalgesia Measurements in CCI with Near Nerve Pregabalin



Thermal Hyperalgesia Measurements in CCI with Intrathecal Pregabalin



Thermal Hyperalgesia Measurements in CCI with Intranasal Pregabalin



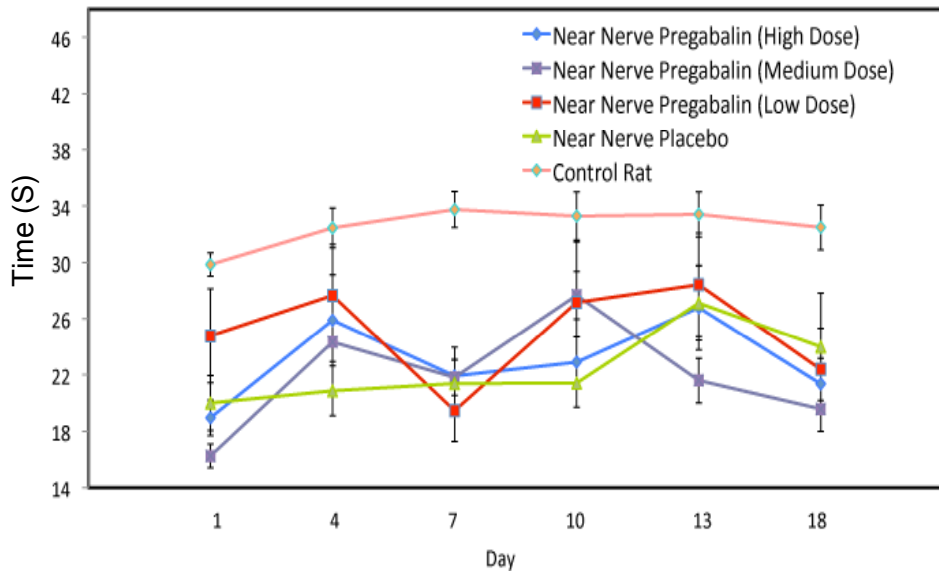
\*  $p < 0.05$

IT and IN Improvement  
Thermal Hyperalgesia  
at High doses

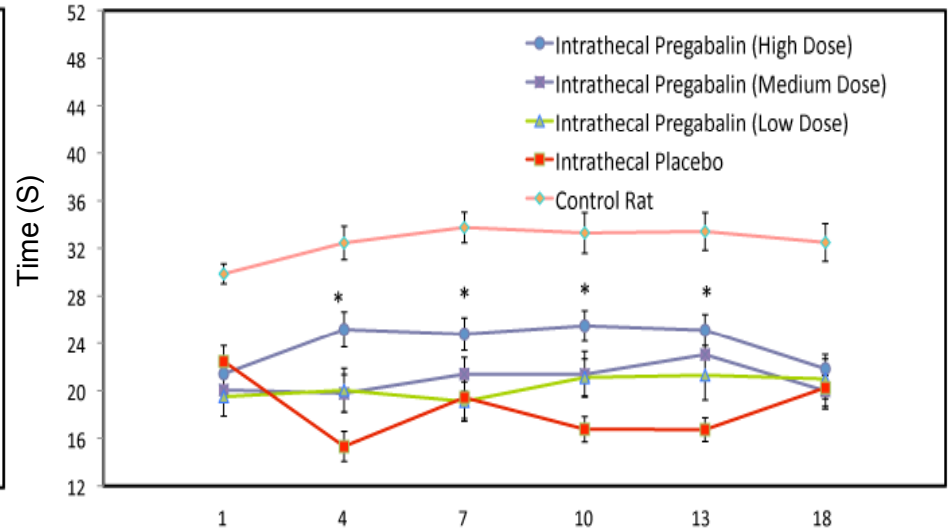
# Thermal Hyperalgesia – Diabetic Peripheral Neuropathy



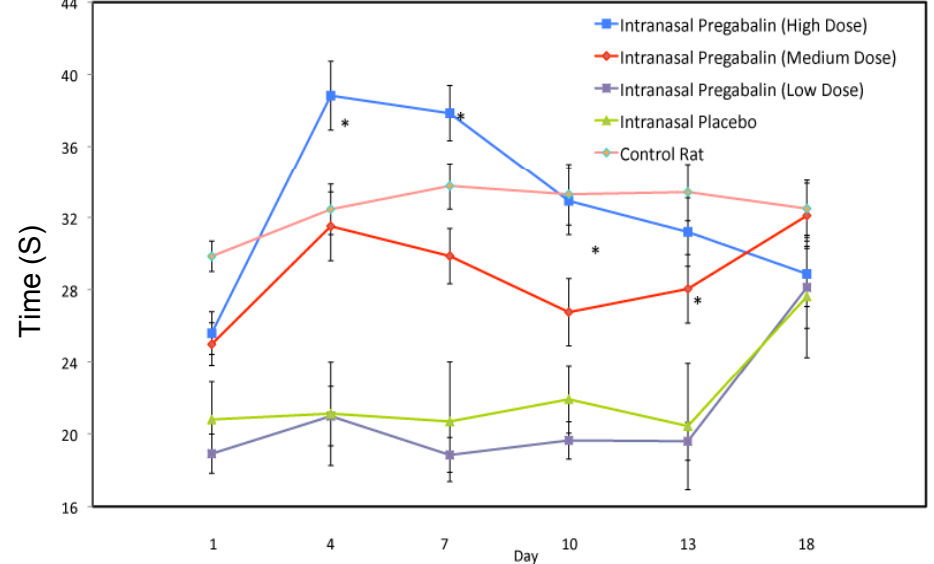
Thermal Hyperalgesia Measurements in Diabetic Peripheral Neuropathy with Near Nerve Pregabalin



Thermal Hyperalgesia Measurements in Diabetic Peripheral Neuropathy with Intrathecal Pregabalin



Thermal Hyperalgesia Measurements in Diabetic Peripheral Neuropathy with Intranasal Pregabalin

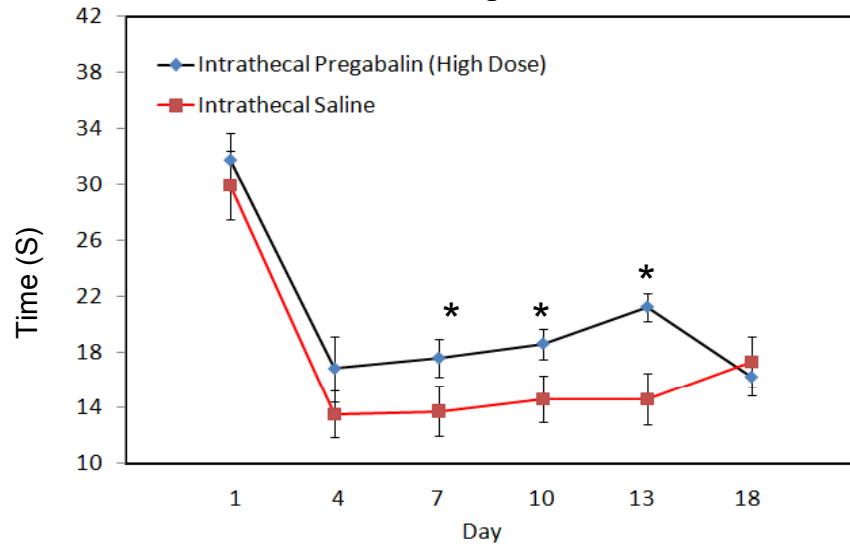


IT Improvement  
Thermal Hyperalgesia  
at High doses.  
IN High and medium  
doses  
DPN

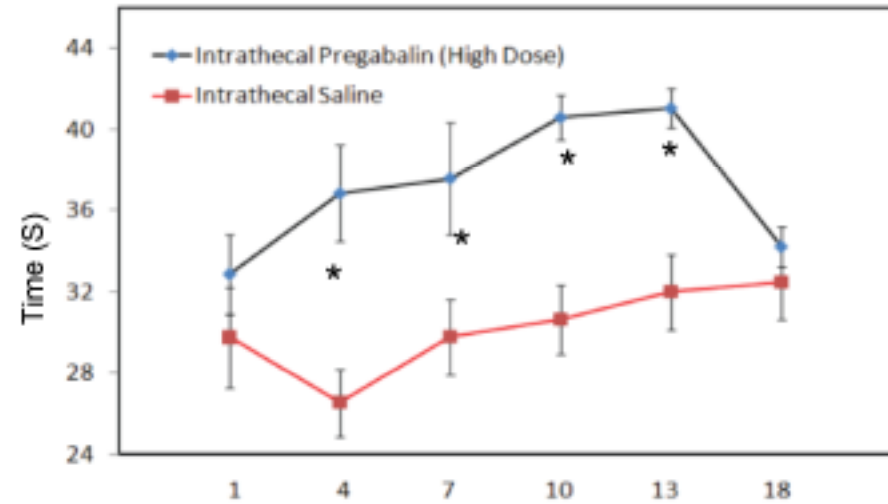
\*  $p < 0.05$

# Tactile Allodynia

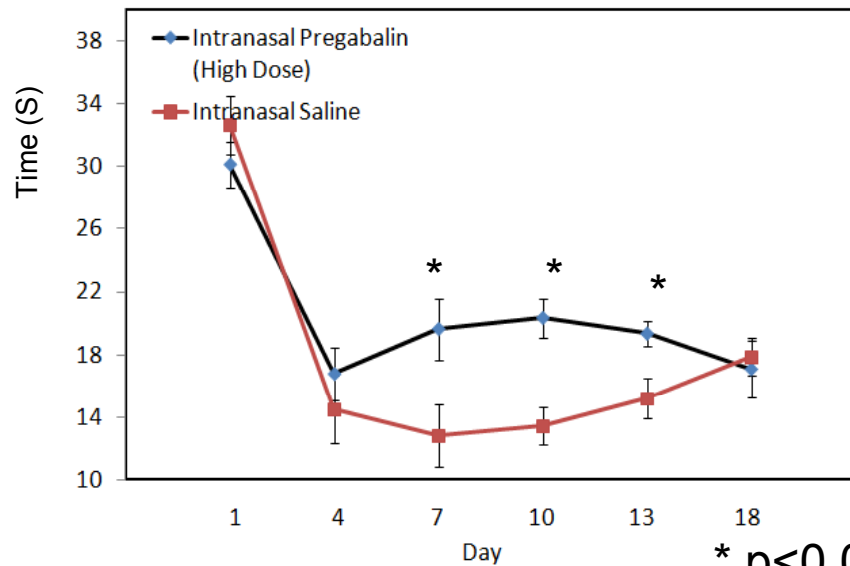
### Intrathecal Pregabalin in CCI



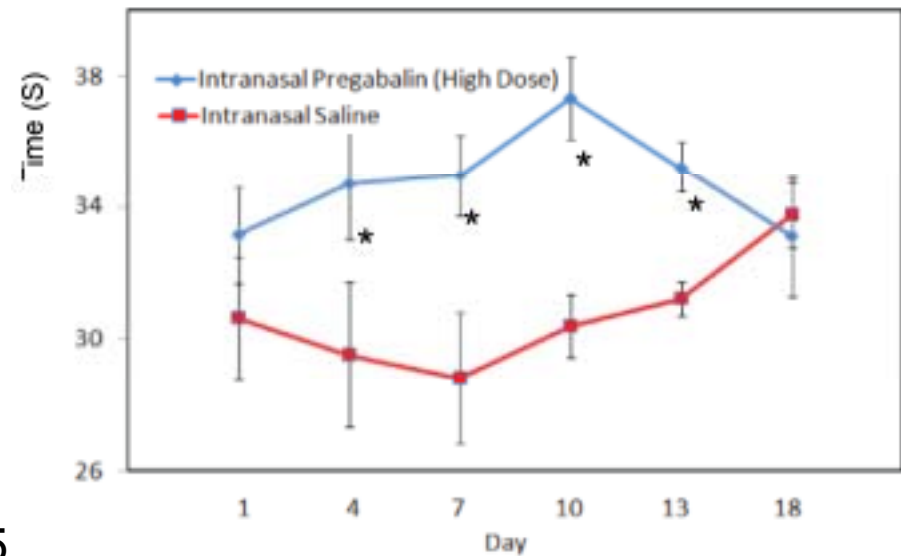
### Intrathecal Pregabalin in Diabetic Neuropathy



### Intranasal Pregabalin in CCI



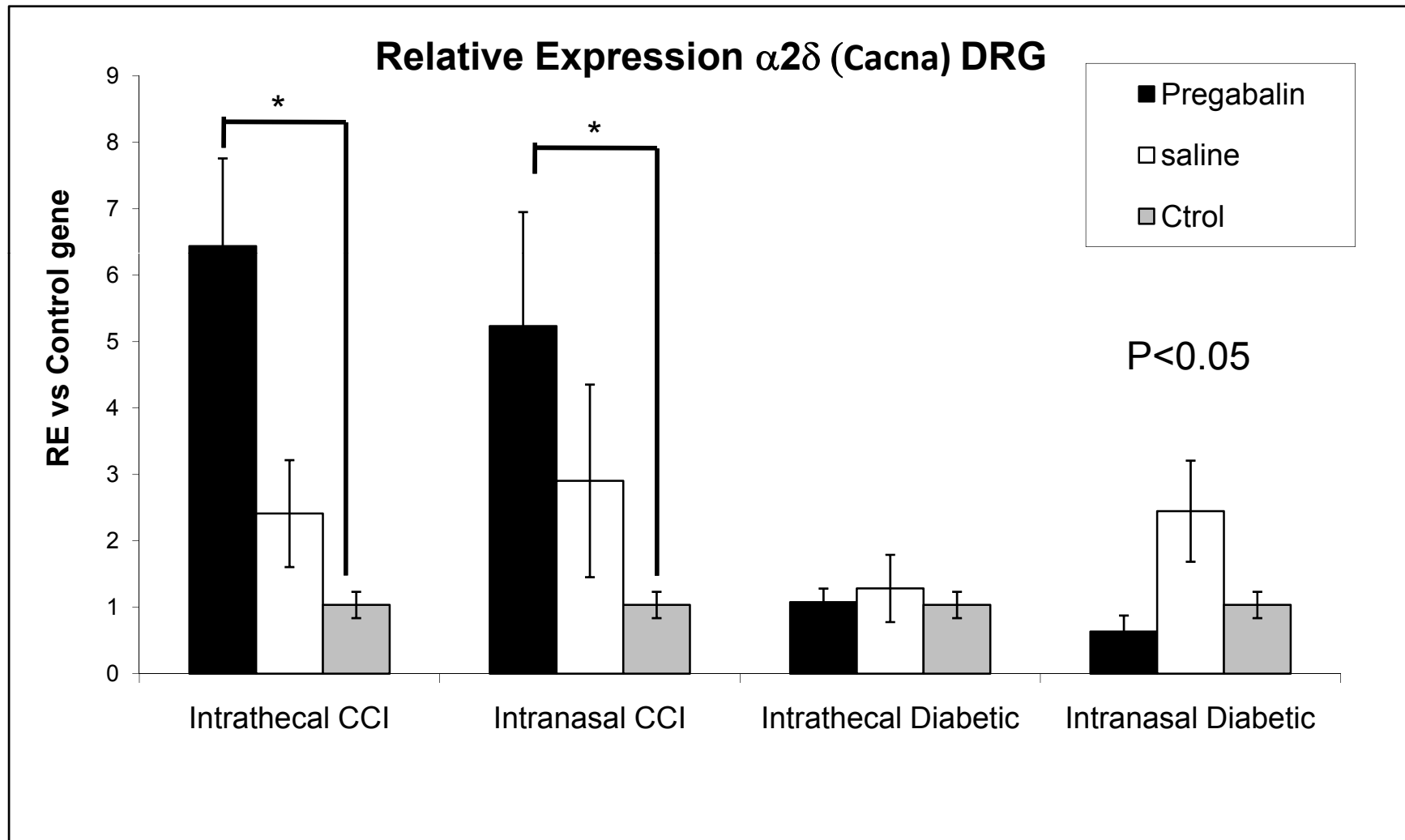
### Intranasal Pregabalin in Diabetic Neuropathy



\* p<0.05

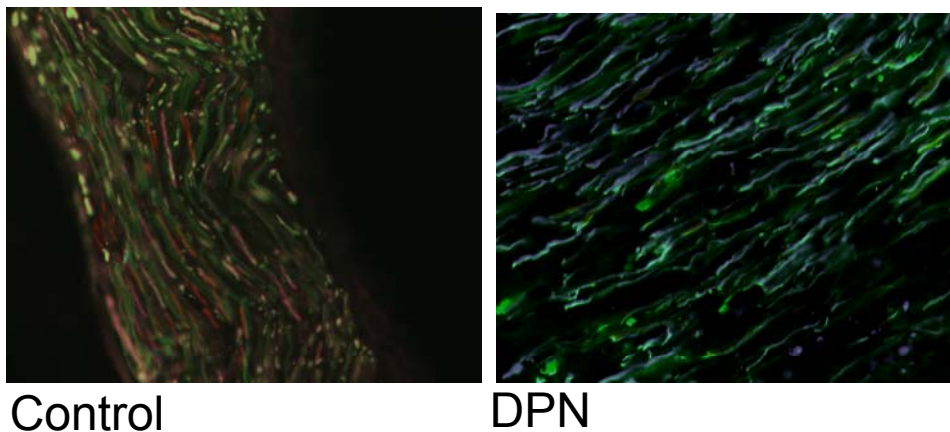
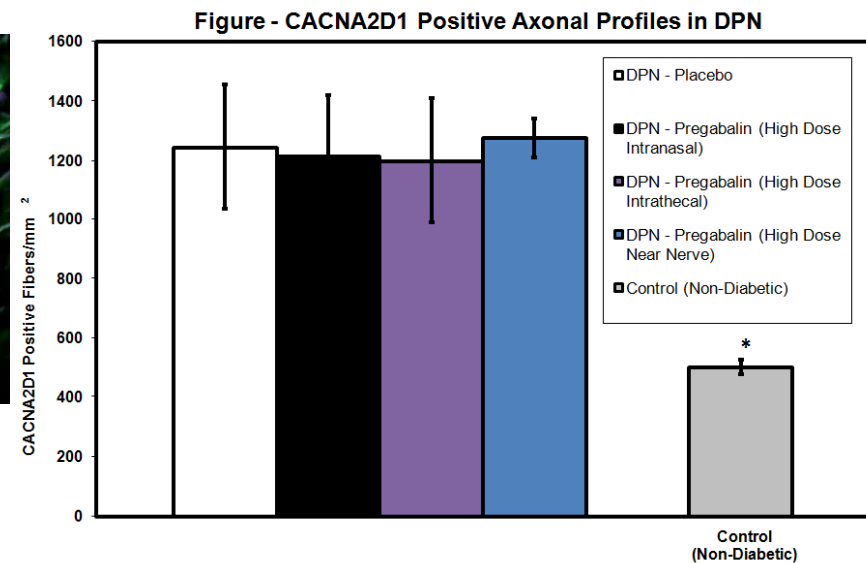
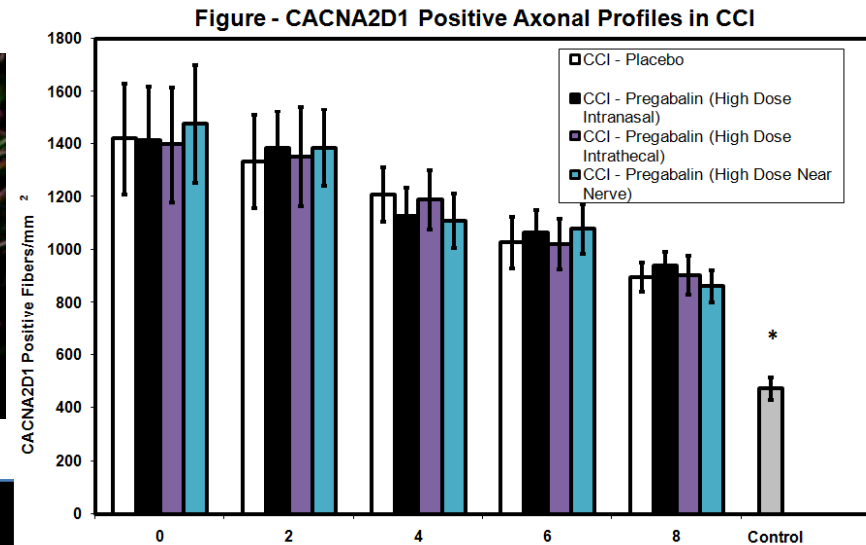
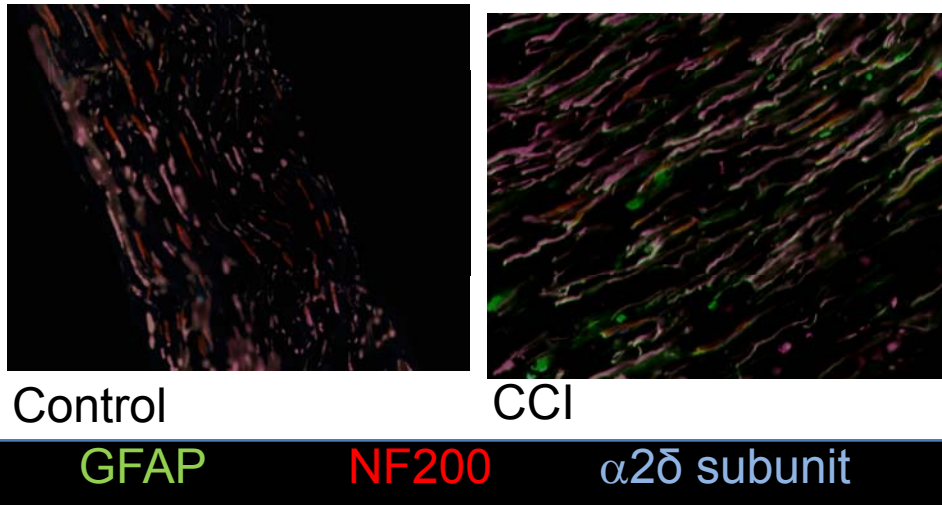
# Methods/Results

- Real-time quantitative RT-PCR was performed to quantify mRNA for the  $\alpha_2\delta$  subunit



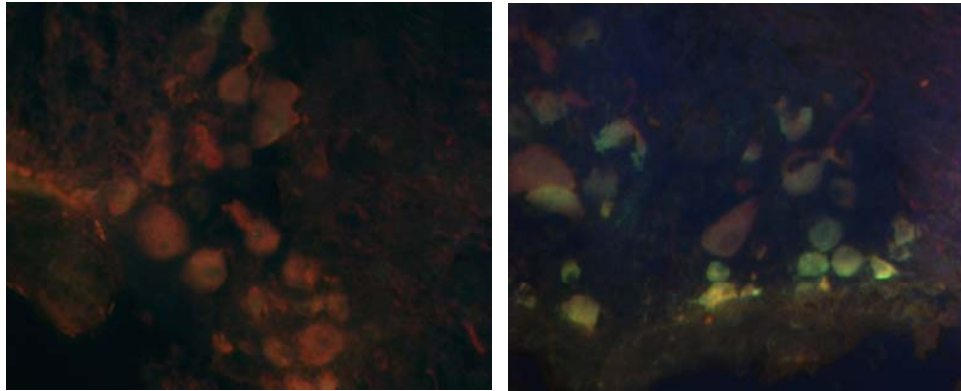
# Methods/Results

- Immunohistochemistry was performed to quantify  $\alpha_2\delta$  subunit expression in Sciatic Nerve



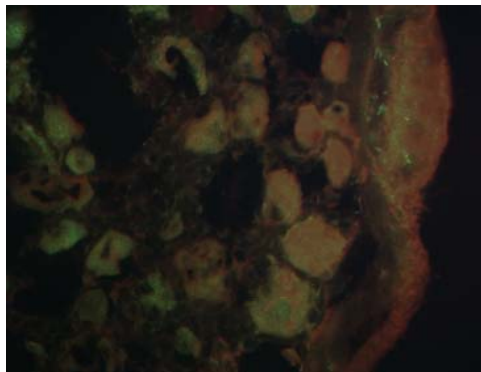
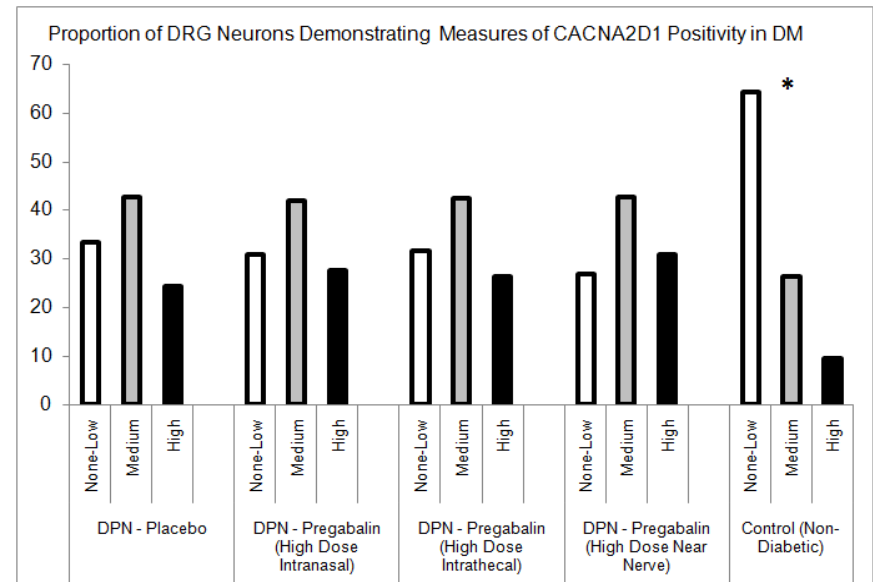
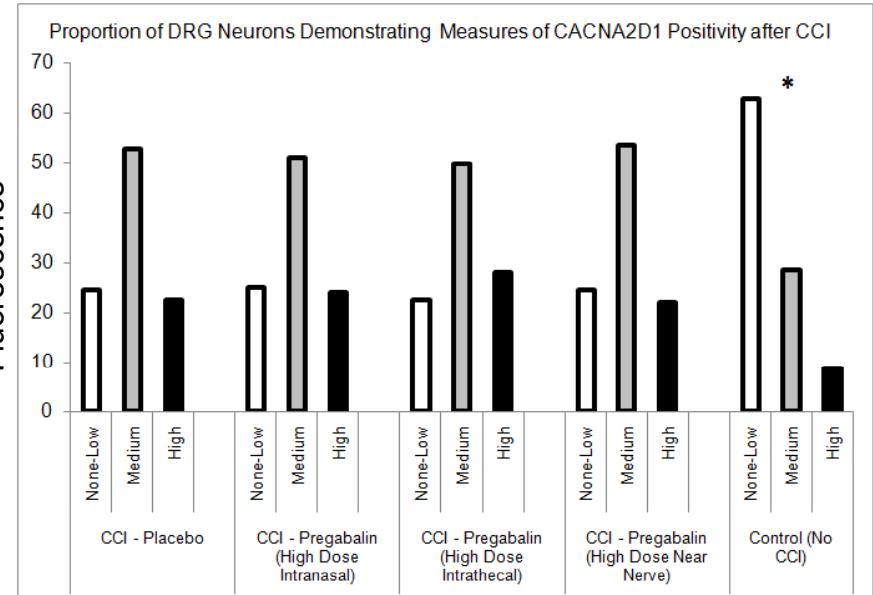
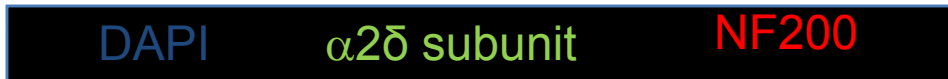
# Methods/Results

- Immunohistochemistry was performed to quantify  $\alpha_2\delta$  subunit expression in DRG

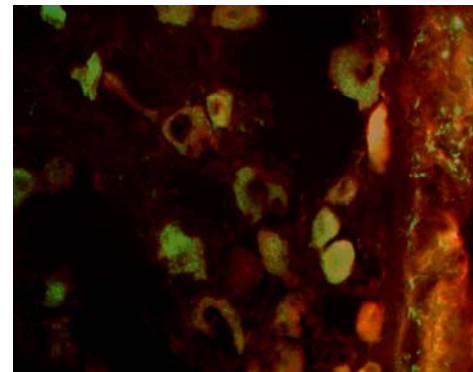


Control

CCI



Control



Diabetic

# Methods/Results

- Immunohistochemistry was performed to quantify  $\alpha_2\delta$  subunit expression in the Dorsal Horn of the Spinal Cord

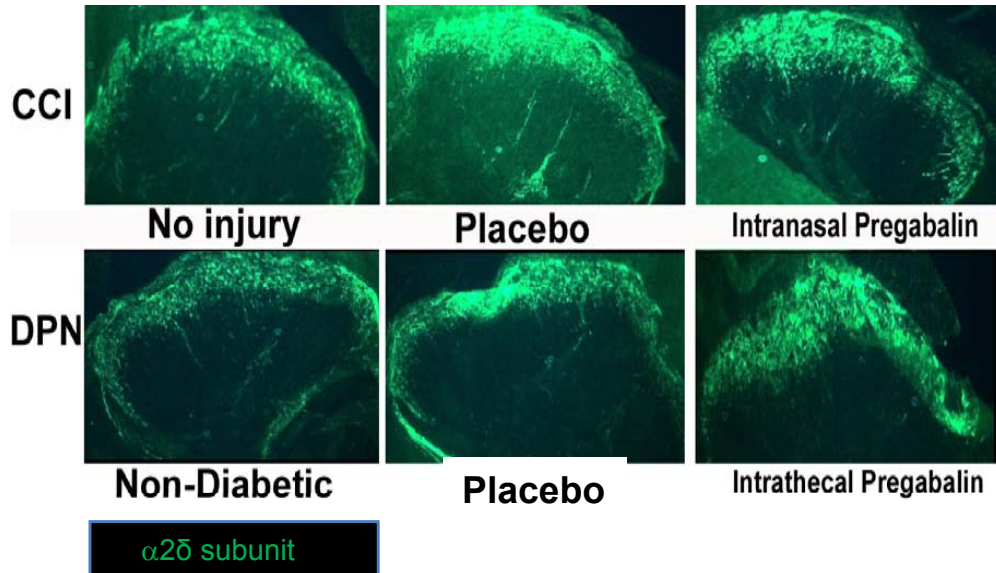


Figure  $\alpha_2\delta$  subunit Immunopositivity at the Dorsal Horn in CCI

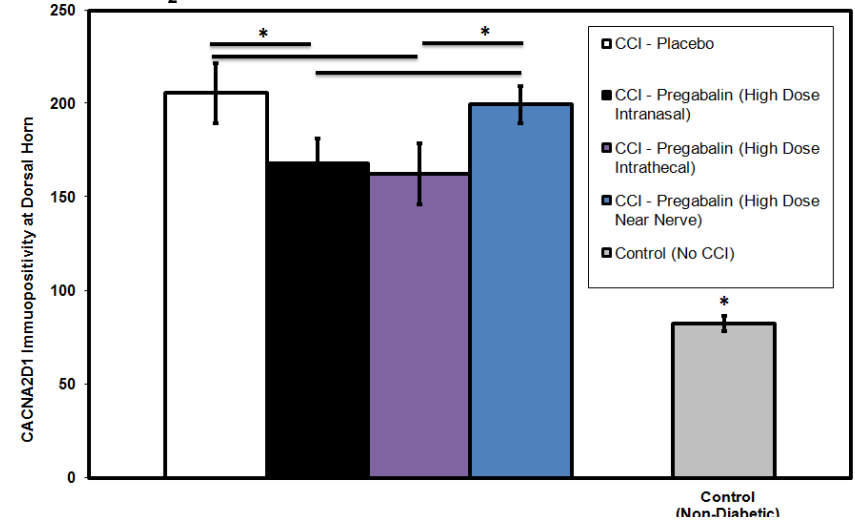
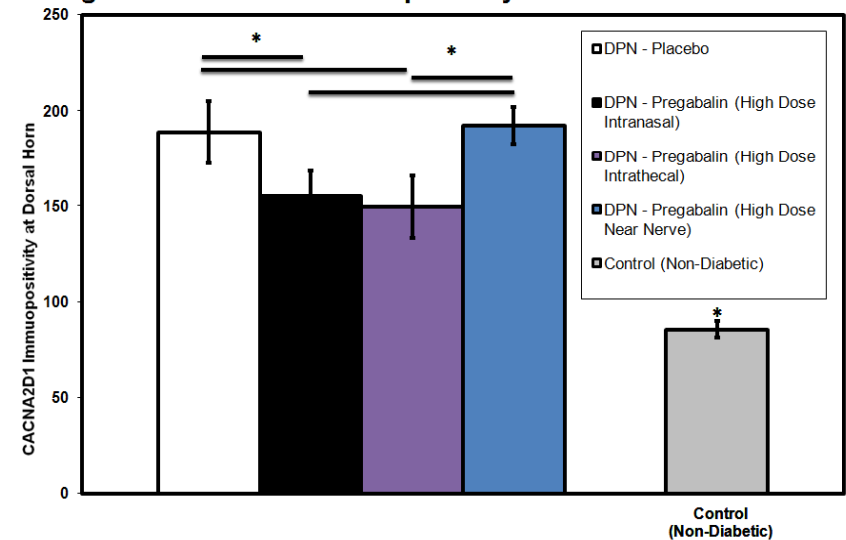


Figure  $\alpha_2\delta$  subunit Immunopositivity at the Dorsal Horn in DPN



# Conclusions

- Pregabalin is effectively delivered in CNS and PNS IN and IT.
- Pregabalin improved allodynia and hyperalgesia testing at high doses when delivered IN or IT, and not NN.
- Pregabalin did not affect  $\alpha_2\delta$  subunit mRNA within Spinal Cord or DRG.
- Pregabalin delivery did not affect  $\alpha_2\delta$  subunit protein expression in nerve or DRG.

# Conclusions

- However, Pregabalin IN or IT did decrease expression of  $\alpha_2\delta$  subunit at the Dorsal Horn in both pain models.
- Pregabalin exerts its greatest effect upon NeP within the CNS, perhaps at the Dorsal Horn by decreasing  $\alpha_2\delta$  subunit protein expression.
- Pregabalin IN may be a therapeutic avenue which could more effectively target the CNS, while avoiding systemic side effects or limited gastrointestinal absorption.

# Thanks



Dr, DW. Zochodne  
Zochodne –Toth lab members  
ARC Rocio Araujo



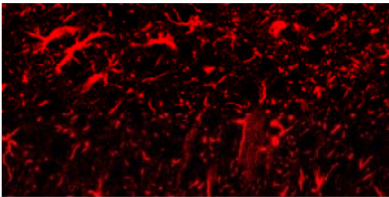
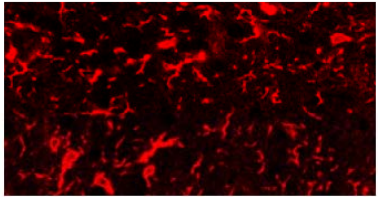
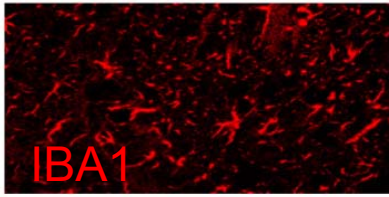
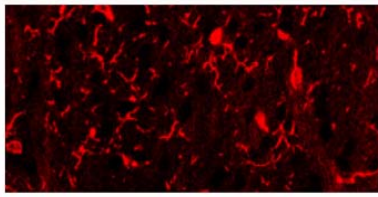
# Methods

- Male SD adult rats were subjected to CCI of Sciatic nerve
- Male SD adult rats were subjected to diabetes using STZ injections with diabetes confirmation 2 weeks later
  - Pregabalin/placebo administered for 14 days
    - » (placebo, 0.1 $\mu$ mol, 1.0 $\mu$ mol, 4.0 $\mu$ mol.) n~6 per cohort.
  - IT - using Alzet Mini Osmotic Pumps (0.5 $\mu$ l/hr.).
  - IN –80 $\mu$ l both nostrils twice a day, total 320 $\mu$ l
  - NN –using Alzet Mini Osmotic Pumps (0.5 $\mu$ l/hr.) “T-Chamber”

# Methods/Results

- Quantification of Microglia in the Dorsal Horn of the Spinal Cord revealed higher densities with NeP conditions unresponsive to intervention with pregabalin

Table - Quantitative analysis of microglia density within the dorsal horn after CCI or DPN and with pregabalin interventions

Intervention	Density (number/mm <sup>2</sup> )					
No CCI	157.0 ± 17.8	<b>CCI</b>				
CCI – Placebo	323.3 ± 14.2 (*)				<b>No injury</b>	<b>Placebo</b>
CCI – High Dose Intranasal Pregabalin	328.7 ± 17.2 (*)					
CCI – High Dose Intrathecal Pregabalin	334.0 ± 19.2(*)					
CCI – High Dose Near-Nerve Pregabalin	313.0 ± 22.3 (*)	<b>DPN</b>				
Non-Diabetic	123.0 ± 16.5				<b>Non-Diabetic</b>	<b>Placebo</b>
DPN – Placebo	191.3 ± 20.1 (*)					
DPN – High Dose Intranasal Pregabalin	210.3 ± 22.8 (*)					
DPN – High Dose Intrathecal Pregabalin	213.6 ± 17.3 (*)					
DPN – High Dose Near-Nerve Pregabalin	193.3 ± 16.8 (*)					