Cannabinoid-mediated modulation of neuropathic pain and microglial accumulation in a model of murine type I diabetic peripheral neuropathic pain

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Disclosures

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Background

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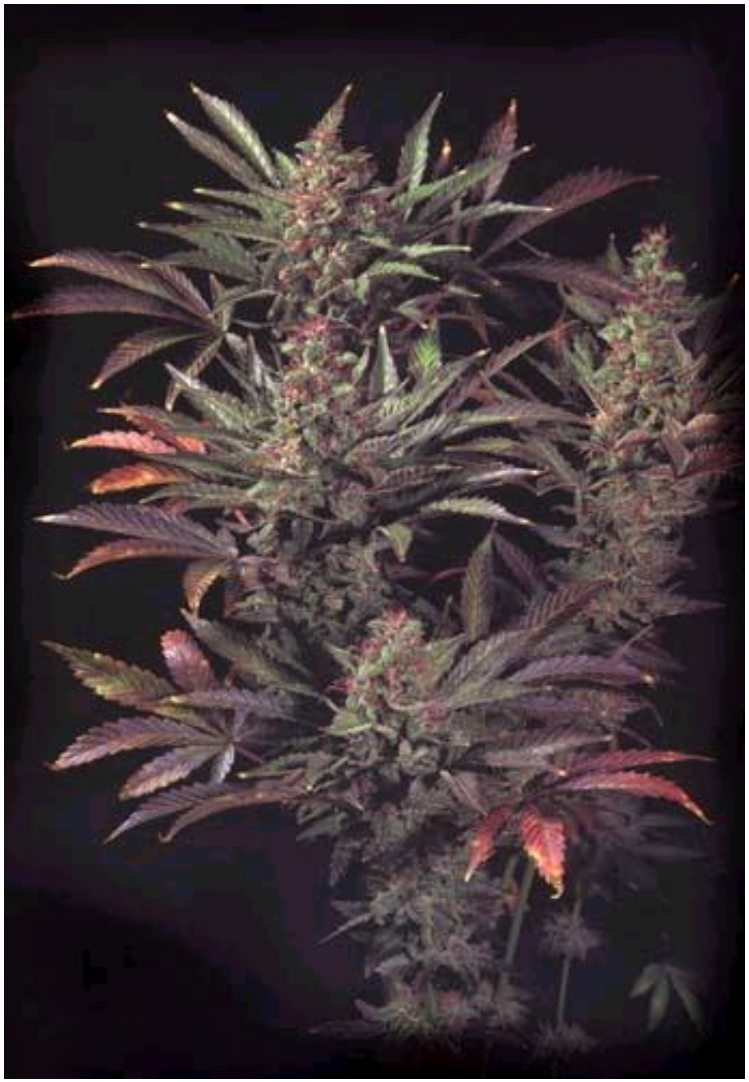
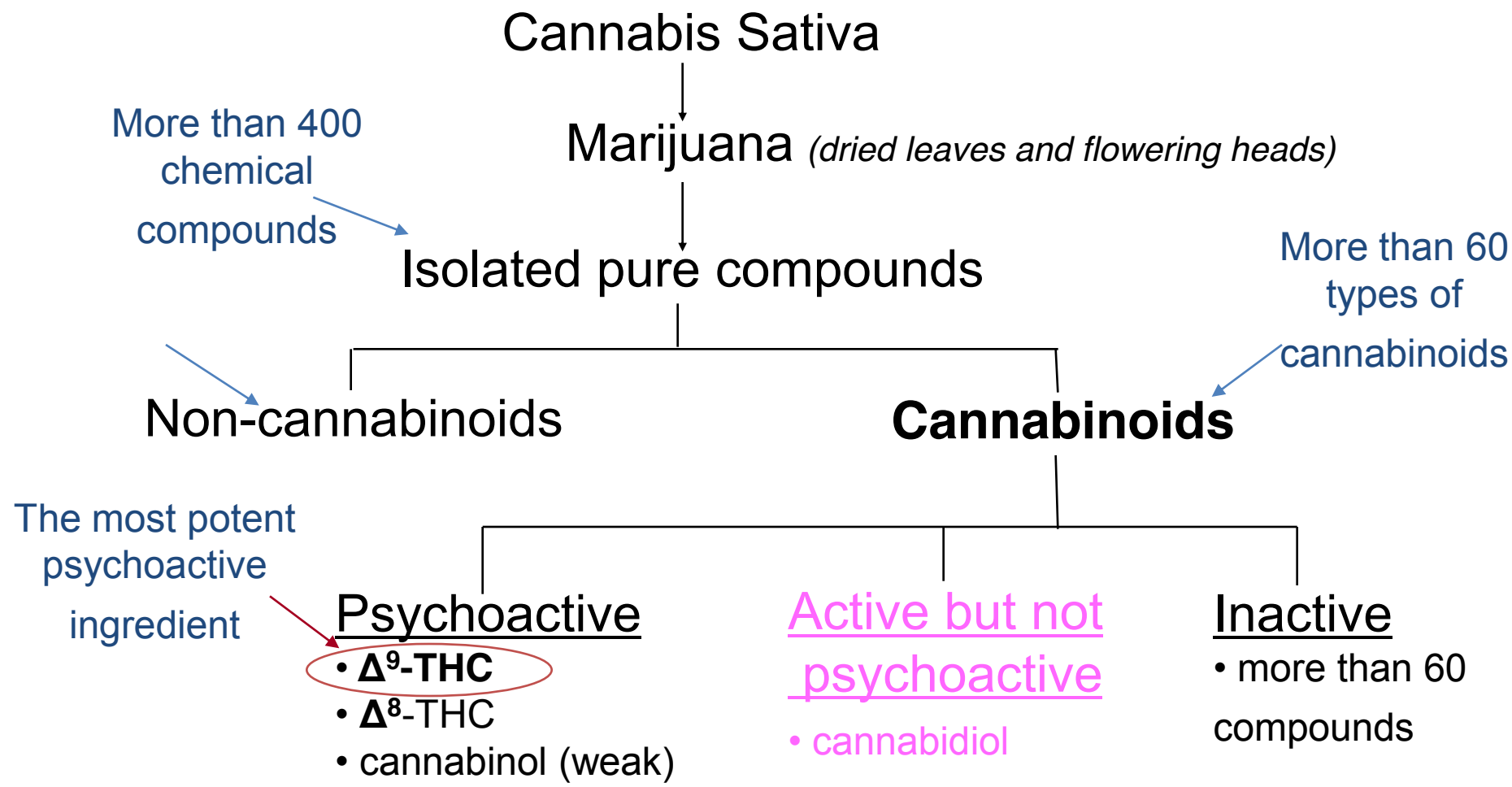
- Recent evidence has demonstrated a prominent role of microglial cells in neuropathic pain states.
- One potential therapeutic option gaining clinical acceptance is the cannabinoids, for which cannabinoid receptors (CB) are expressed on neurons and microglia
- The mechanism of pain relief with cannabinoids is unclear, but may relate to microglial suppression
- A common cause of neuropathic pain is diabetic peripheral neuropathy (DPN)

Cannabinoids in Neuropathic Pain



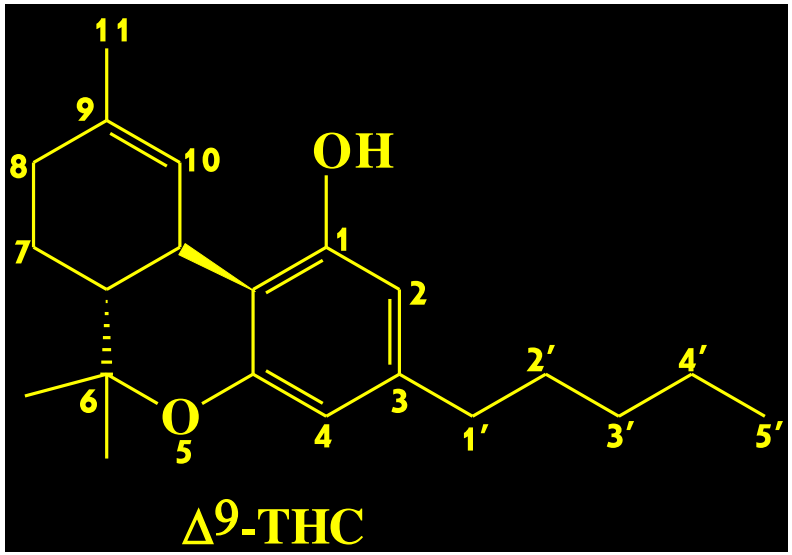
Compounds with pharmacological profile similar to ∆-9-THC (main psychoactive component in marijuana)

Cannabinoids in Neuropathic Pain

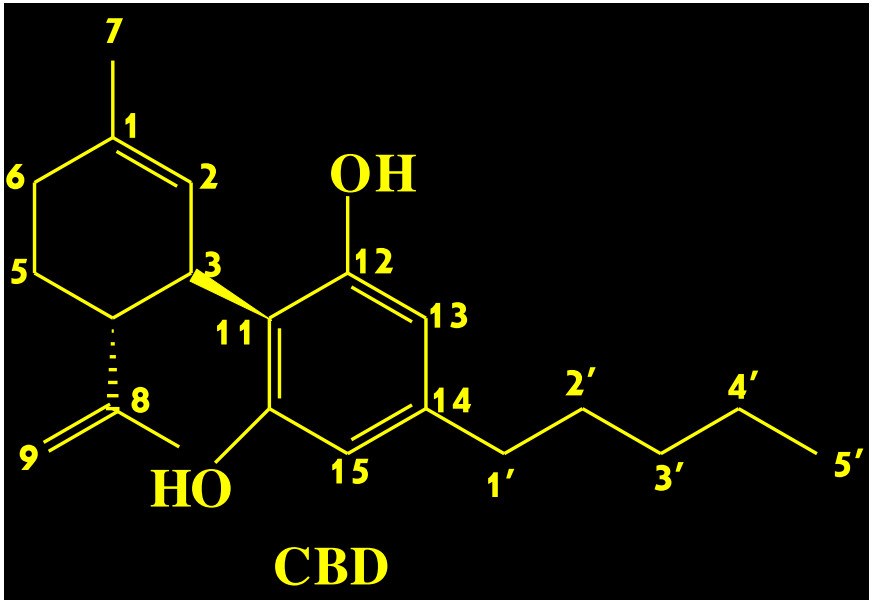


Cannabinoids in Neuropathic Pain

Principal Cannabinoids



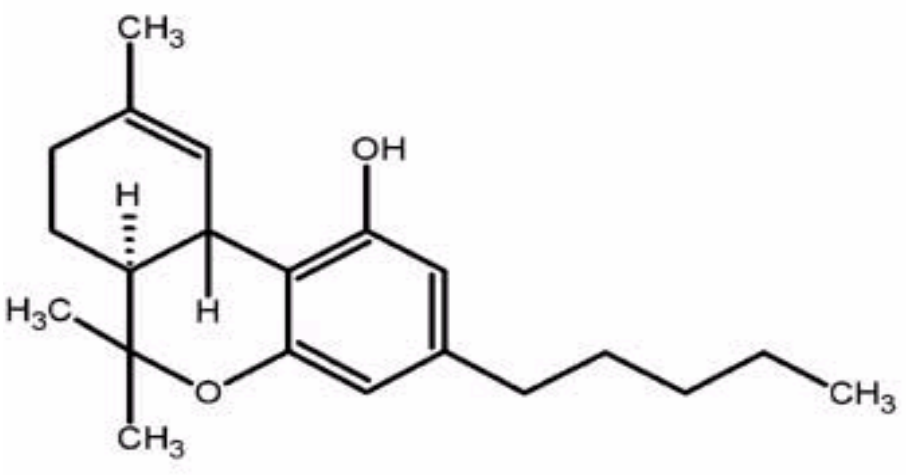
Δ^9-THC



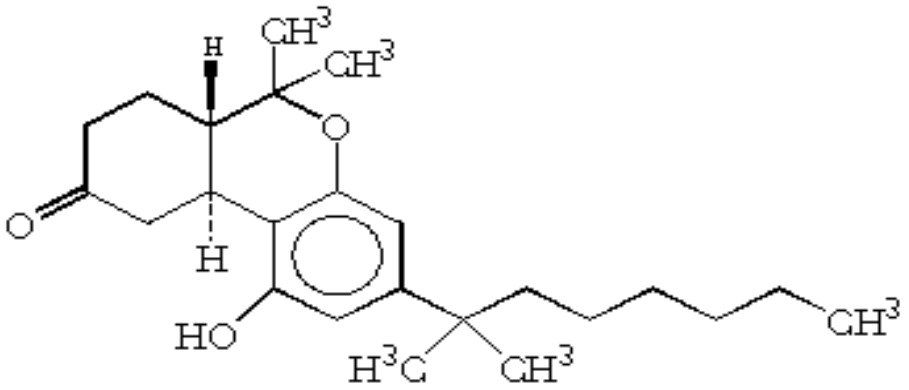
CBD

Resorcinol-type ring with a terpene moiety derivative (around 70 identified)

Cannabinoids in Neuropathic Pain



Tetrahydrocannabinol (THC) $C_{21}H_{30}O_2$
Image by Erowid, © 2002 Erowid.org



Nabilone (THC analogue) $C_{24}H_{36}O_3$
Product monograph. ICN Canada Ltd. 2002

Cannabinoids in Neuropathic Pain

Cannabinoids: 3 classes

1) Endogenous to animals:

Anandamide; nolandin; 2-AG; NADA, virodhamine

2) Endogenous to cannabis plants:

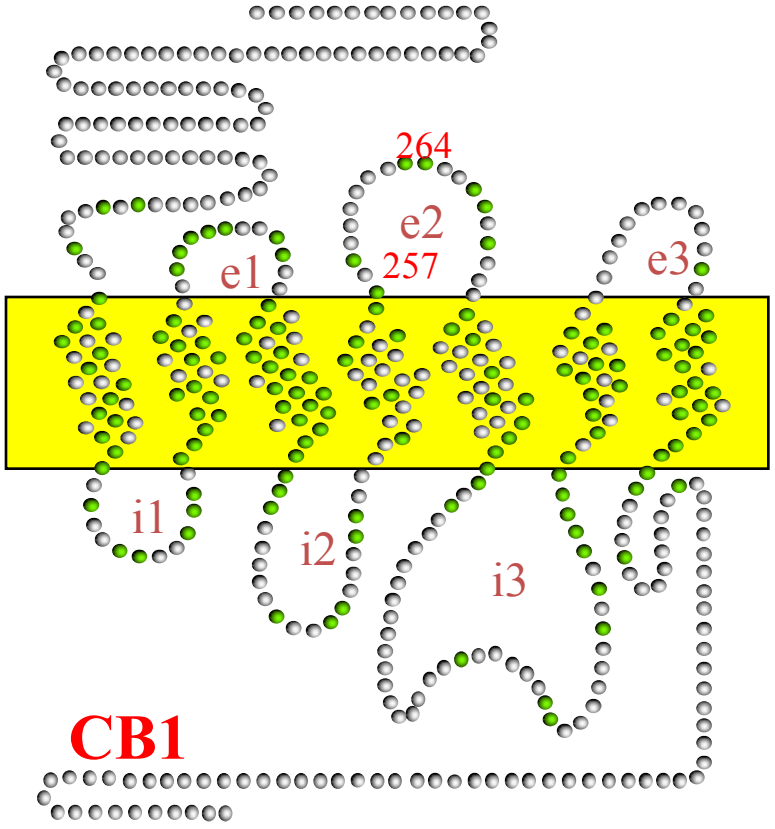
Δ9-THC; cannabidiol; cannabinol; etc

3) Synthetic:

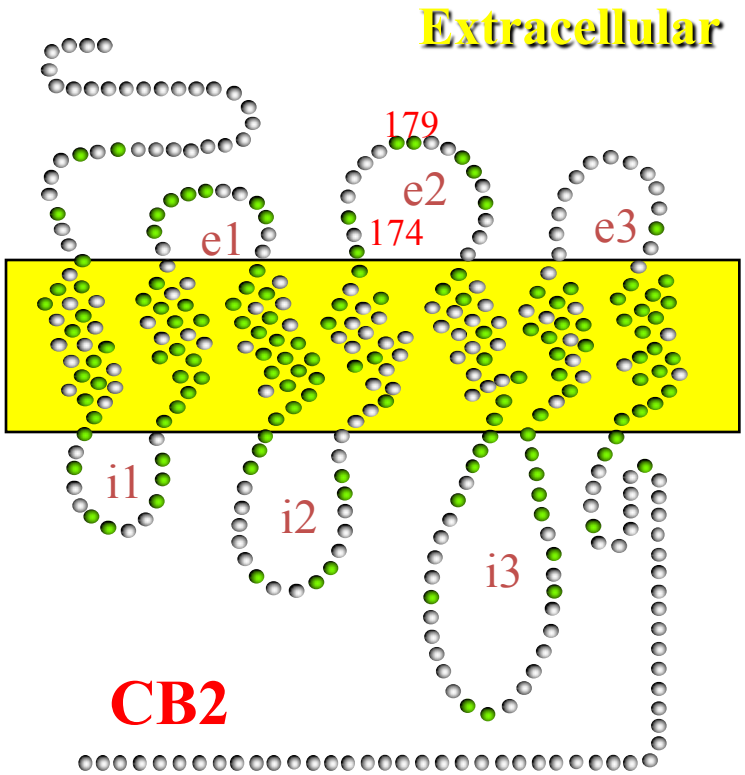
WIN55212,2; HU210; CPP55940; nabilone; etc

Cannabinoids in Neuropathic Pain

Two Cannabinoid Receptors: CB1 & CB2

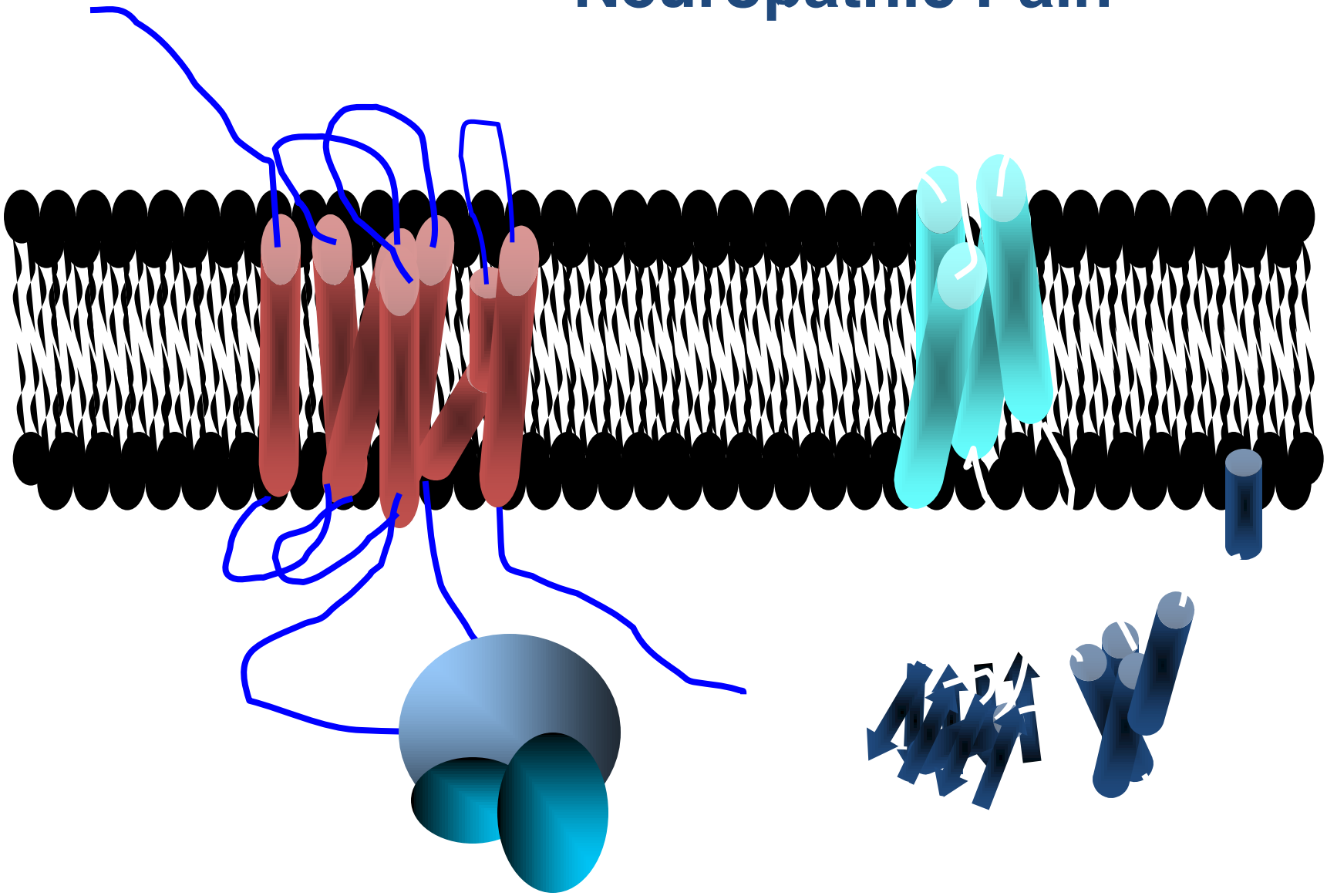


472 amino acids



360 amino acids

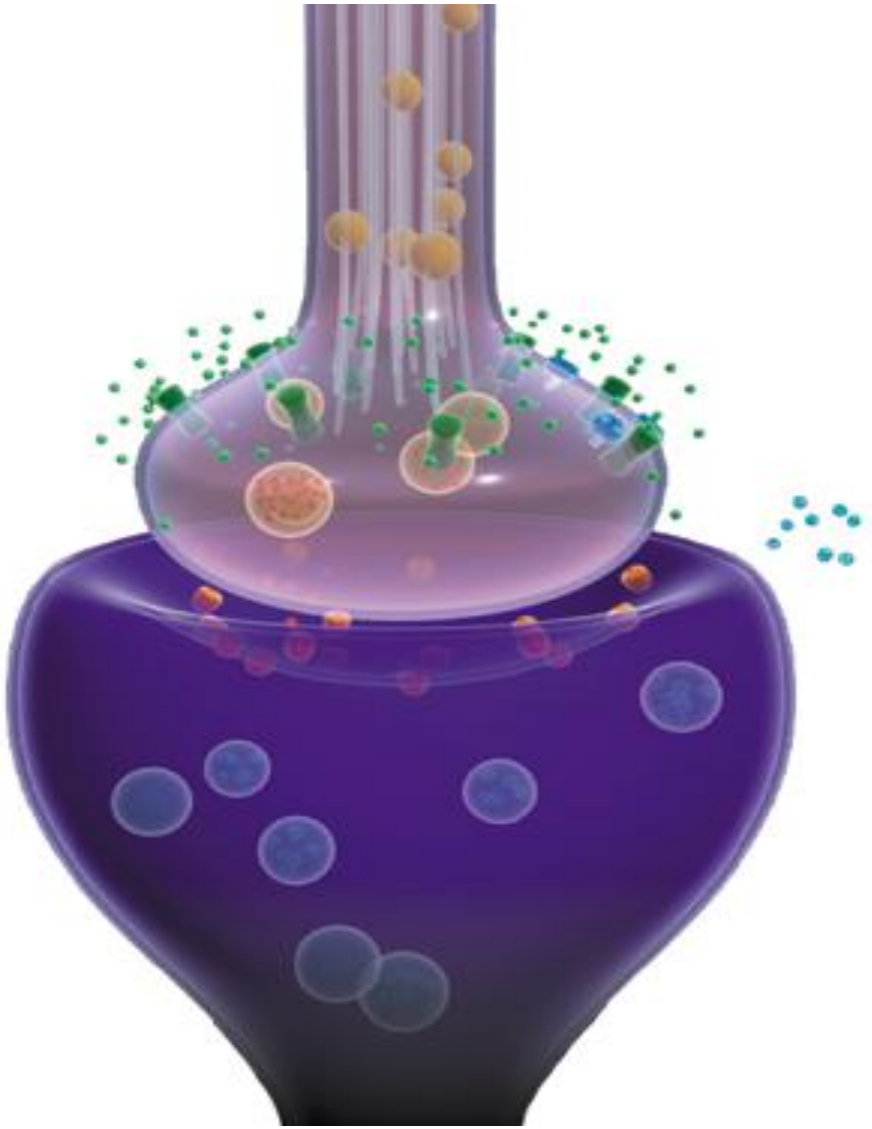
Cannabinoids in Neuropathic Pain



Cannabinoids in Neuropathic Pain

Exogenous cannabinoid such as nabilone act on presynaptic CB1 receptors, similar to endocannabinoids

Endogenous CB1 ligands act in reverse from classical neurotransmitters by serving **as retrograde synaptic messengers**

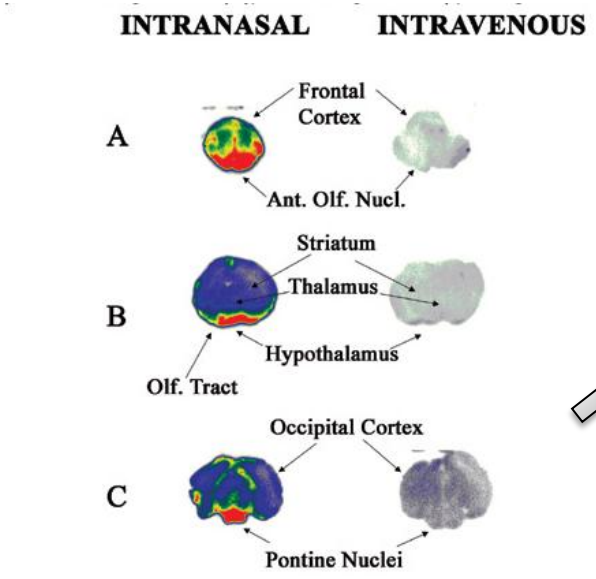


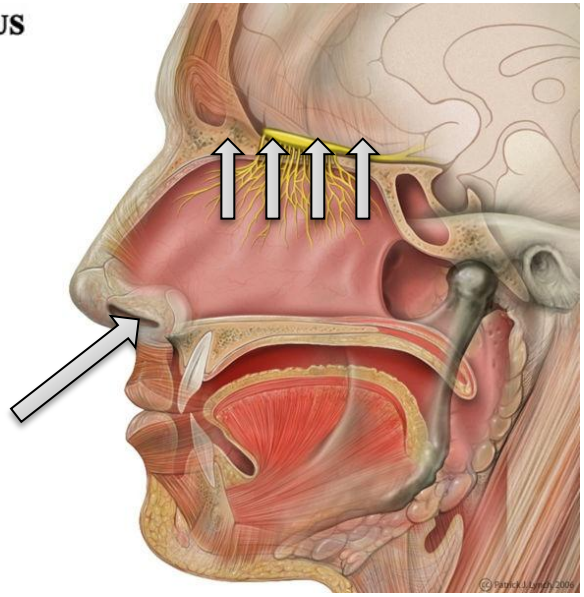
Background

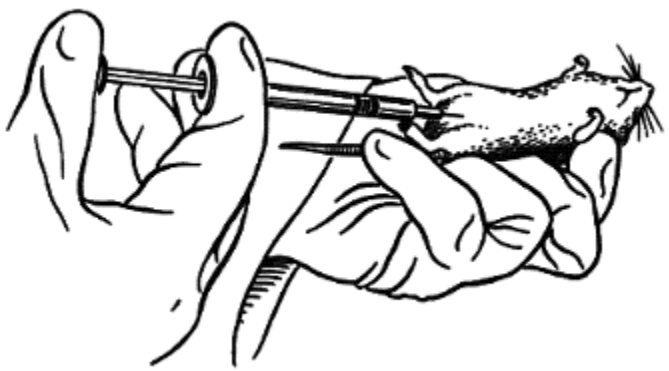
We used two separate methods of delivery of the cannabinoid agents:

1) Intranasal – to specifically provide delivery to the nervous system and avoid systemic delivery

2) Intraperitoneal – to specifically provide systemic delivery which includes the nervous system







Background

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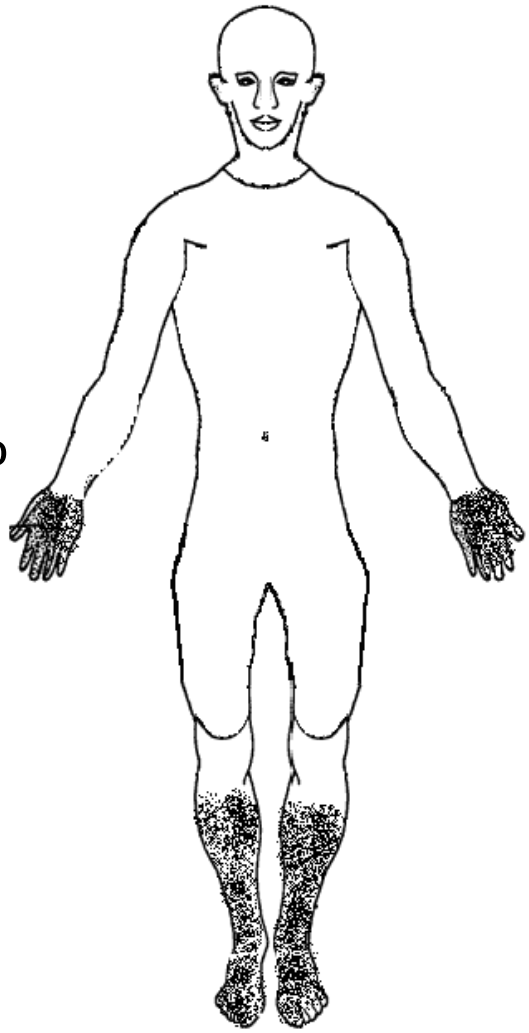
- Diabetes Mellitus is the most common cause of peripheral neuropathy in the world due to increaseing prevalence of diabetes over the past 3 decades

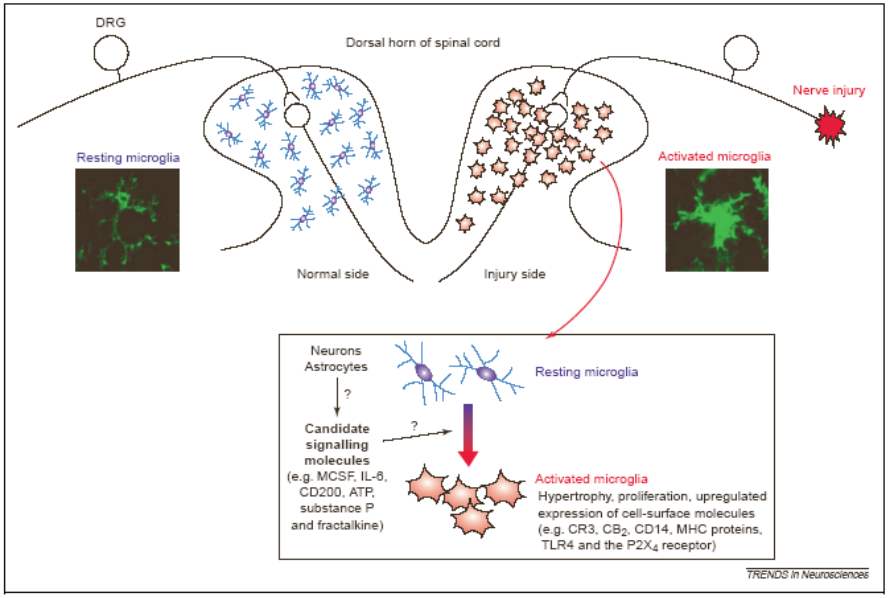
- DPN causes neuropathic pain in ~50% of patients with diabetes presenting to tertiary care (Toth and Au, Pain, 2008)

- Although there are indicated medications for management of neuropathic pain in DPN, more options for relief are needed to better meet patients' expectations

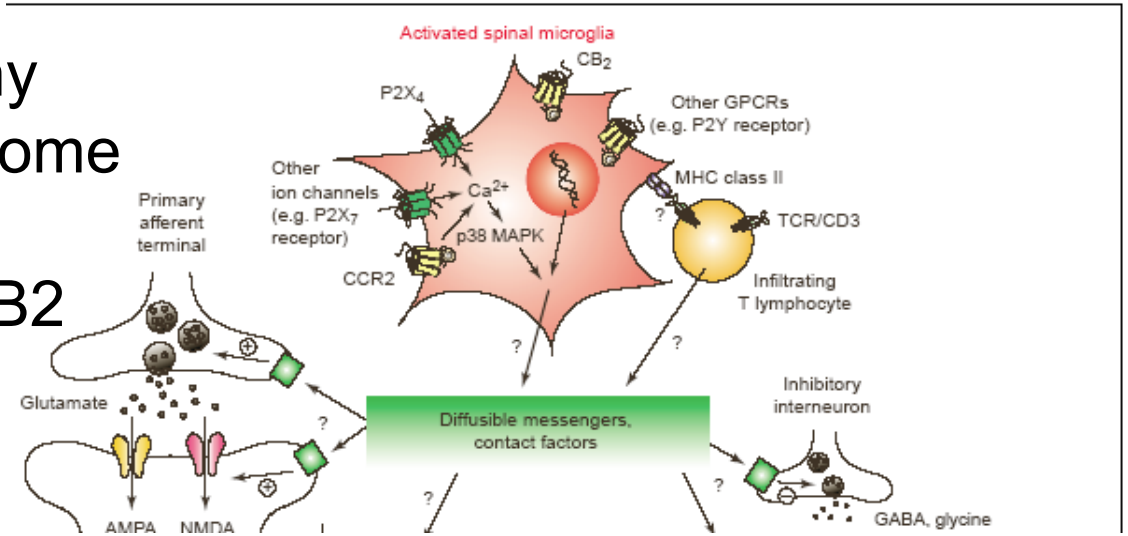


Background

- Microglia activate within the dorsal spinal cord after nerve injury, but they also activate after development of diabetes mellitus as well



- Microglia express many receptors after they become activated, including the cannabinoid receptor CB2



Objectives

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- To assess the potential efficacy of cannabinoids in the management of neuropathic pain related to DPN
- To determine the impact of cannabinoid agents upon microglia in the spinal cord of mice with DPN and neuropathic pain

Methods

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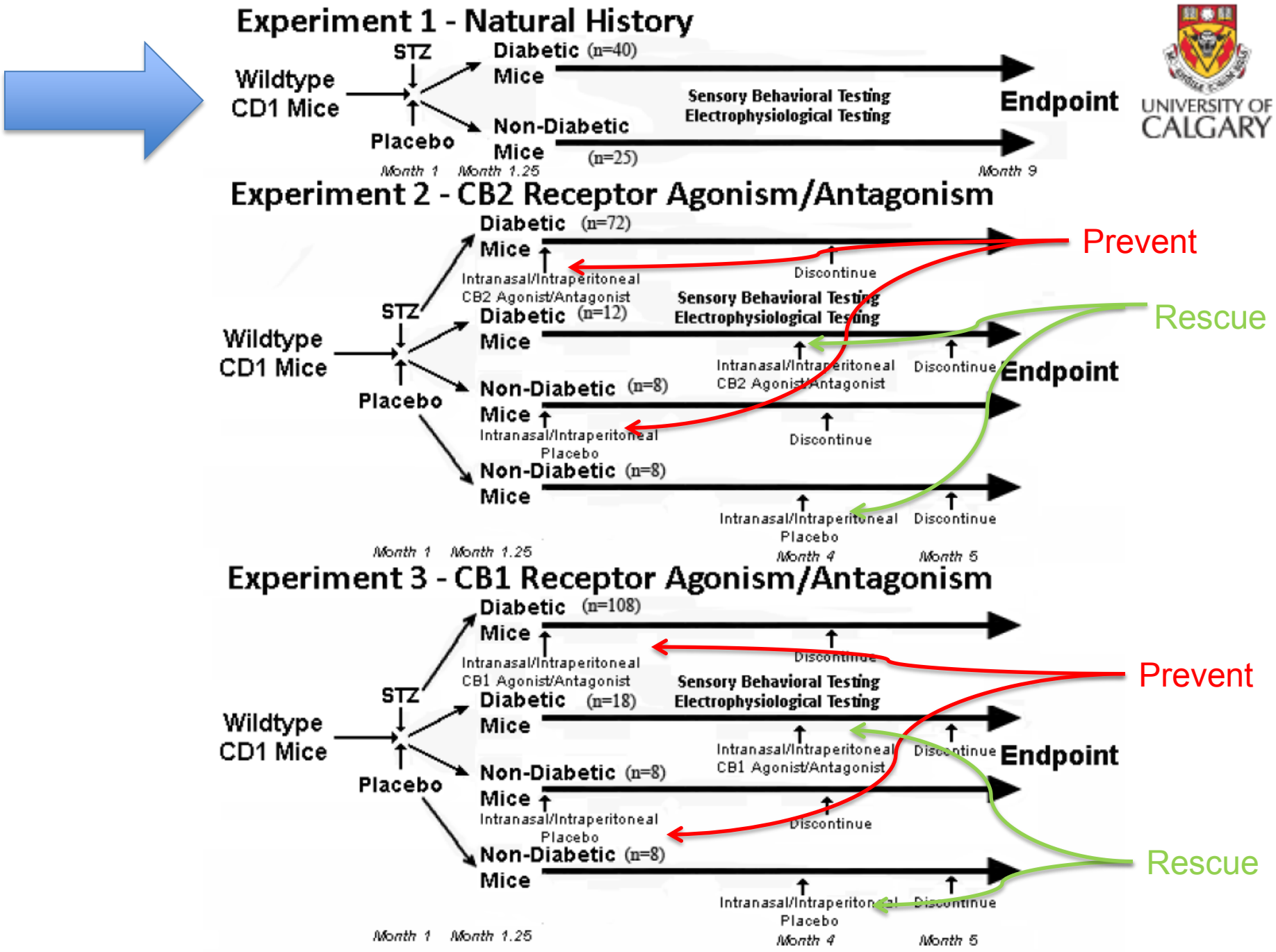
• We studied CD1 male wildtype mice receiving I.P. streptozotocin to induce an animal model of type 1 diabetes and DPN. Mice not developing hyperglycemia were excluded

• Intranasal dosing was generally 1/10 that of intraperitoneal dosing

• We used FITC fluorescence conjugation to fluorescently tagged molecules of cannabidiol and nabilone delivered through intranasal and intraperitoneal delivery for localization

We studied sensory behavioral testing to identify presence of tactile allodynia (Dynamic Plantar Aesthesiometer (Ugo-Basile, Milan)) and thermal hyperalgesia (mobile radiant heat source (Hargreaves apparatus) at multiple time points

• We then identified microglial densities in the spinal cord using immunohistochemistry to Iba-1 (microglial marker) and complemented this with CB1, CB2 and the downstream marker p-p38MAPK immunohistochemistry. Complementary Western blotting was performed for the same proteins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Wildtype CD1 Mice
STZ
Placebo
Diabetic (n=40) Mice
Non-Diabetic Mice (n=25)
Sensory Behavioral Testing
Electrophysiological Testing
Endpoint
Month 1
Month 1.25
Month 9
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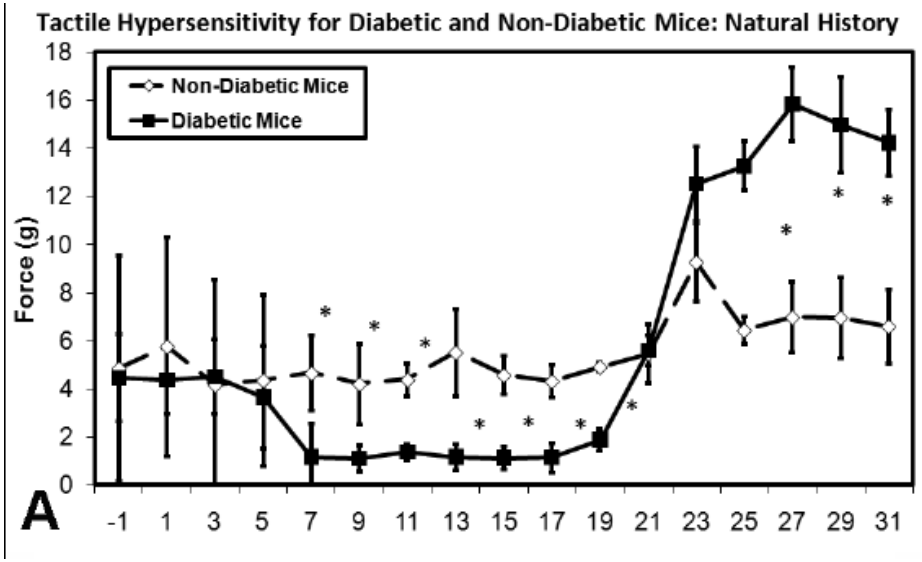
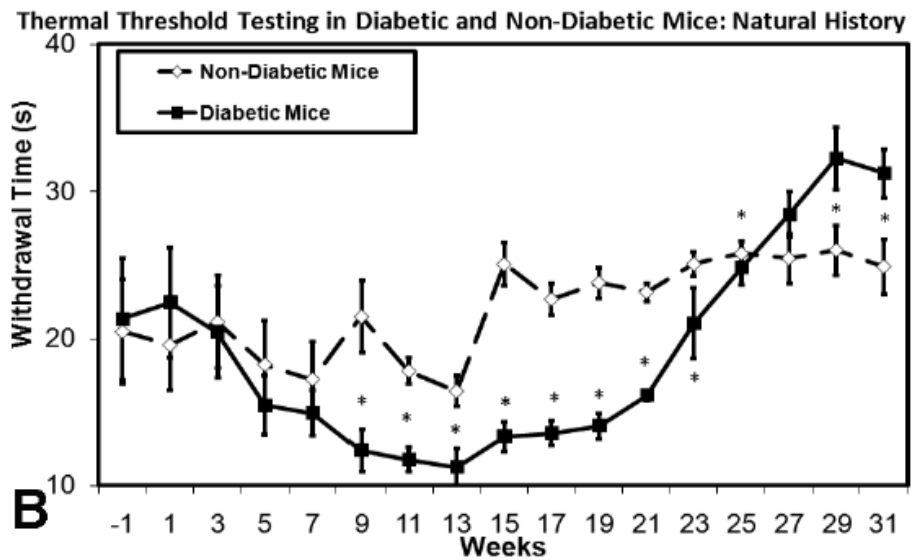
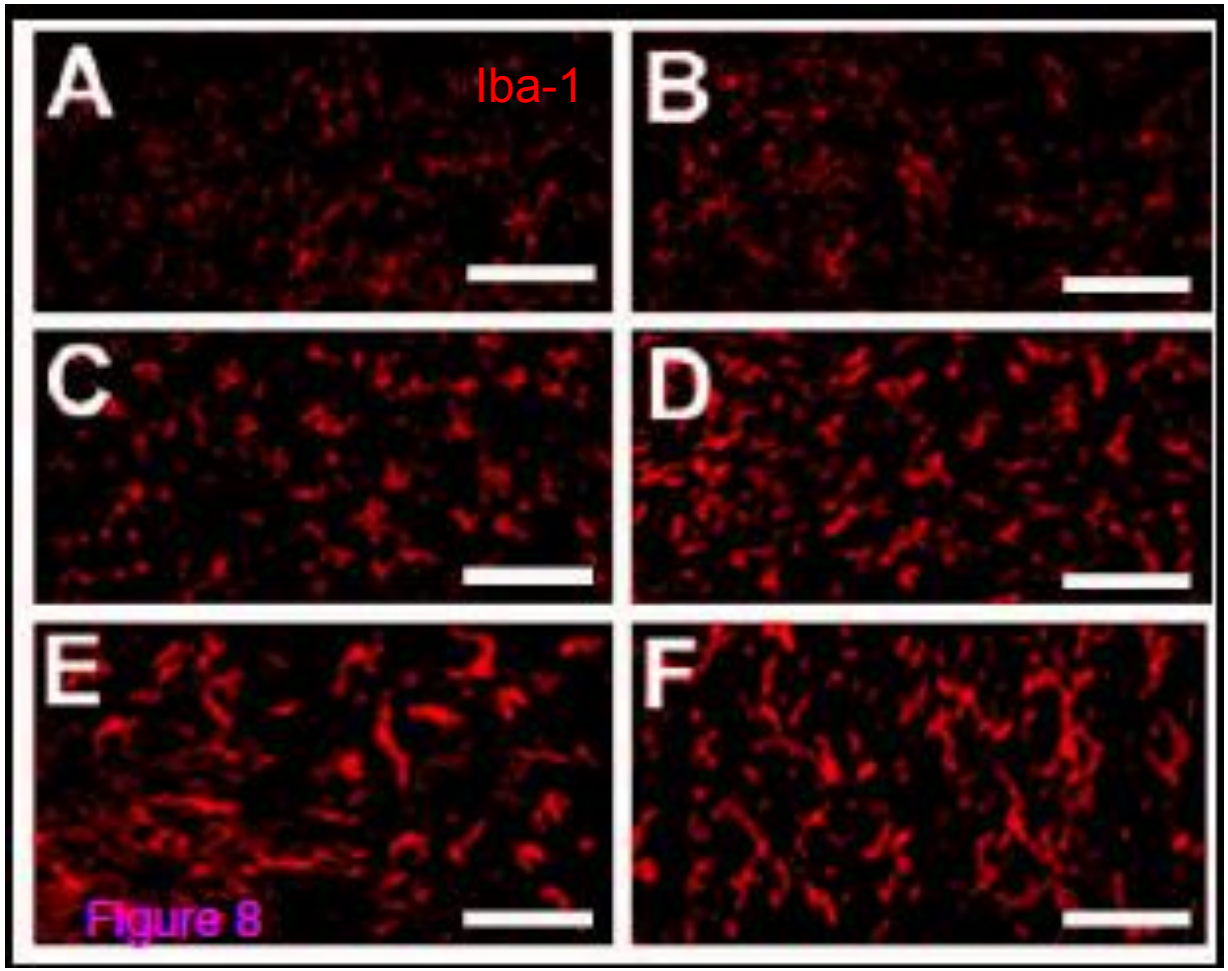
Experiment 2 - CB2 Receptor Agonism/Antagonism
Diabetic (n=72) Mice
Intranasal/Intraperitoneal CB2 Agonist/Antagonist
Discontinue
Prevent
Diabetic (n=12) Mice
Intranasal/Intraperitoneal CB2 Agonist/Antagonist
Discontinue
Rescue
Non-Diabetic (n=8) Mice
Intranasal/Intraperitoneal Placebo
Discontinue
Non-Diabetic (n=8) Mice
Intranasal/Intraperitoneal Placebo
Discontinue
Month 4
Month 5
Experiment 3 - CB1 Receptor Agonism/Antagonism
Diabetic (n=108) Mice
Intranasal/Intraperitoneal CB1 Agonist/Antagonist
Discontinue
Prevent
Diabetic (n=18) Mice
Intranasal/Intraperitoneal CB1 Agonist/Antagonist
Discontinue
Non-Diabetic (n=8) Mice
Intranasal/Intraperitoneal Placebo
Discontinue
Non-Diabetic (n=8) Mice
Intranasal/Intraperitoneal Placebo
Discontinue
Rescue

Results - Behavior





Results - Microglia



Results – Microglial Density

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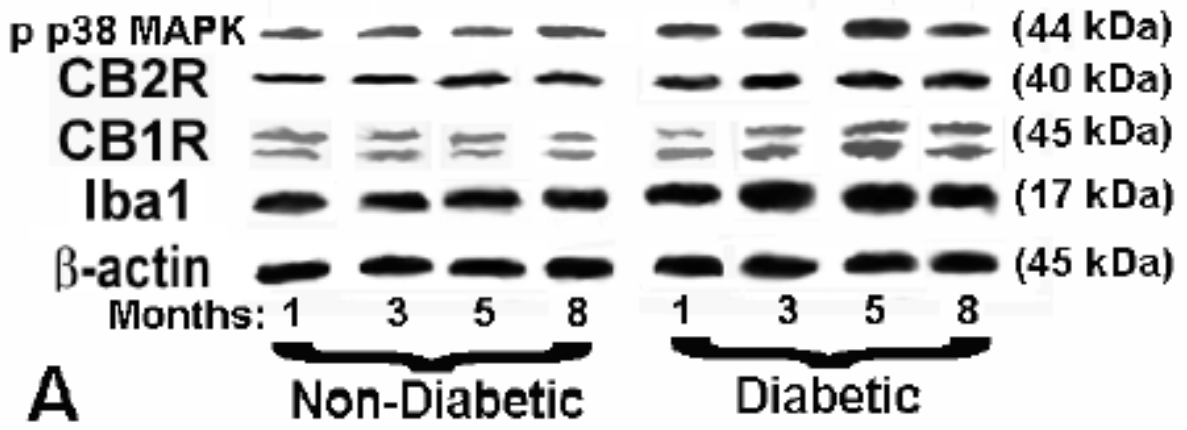
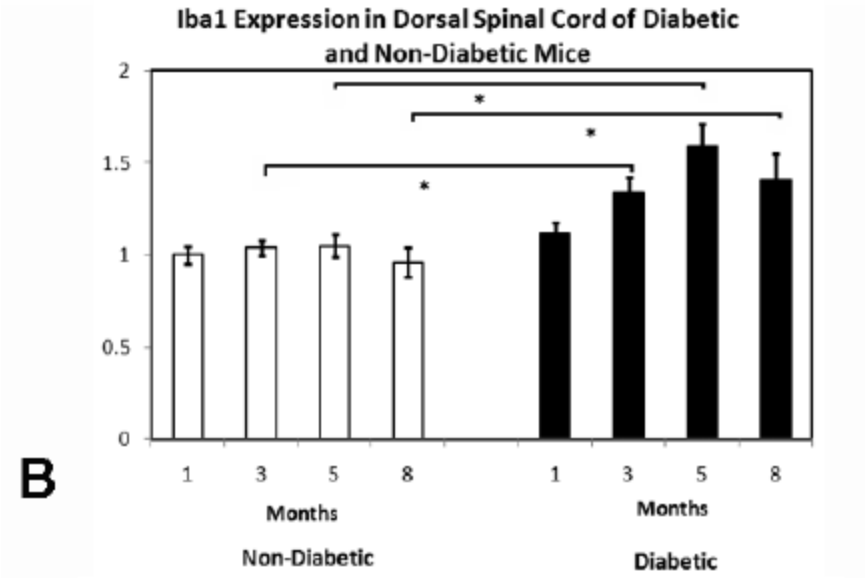
Table 2 - Quantitative and qualitative analysis of microglia density within the dorsal horn and thalamus after indicated time points of diabetes

Mouse Cohort	**Microglial Density in Ventral Lumbar Spinal Cord (number/mm^2)**			
	1 Month	3 Months	5 Months	8 Months
Non-Diabetic	167.3 ± 14.9 (-)	154.1 ± 11.3 (-)	142.1 ± 15.8 (-)	139.2 ± 13.4 (-)
Diabetic	183.8 ± 16.7 (-)	227.5 ± 14.2* (+)	308.4 ± 19.6* (+)	256.4 ± 22.5* (+)
	Microglial Density in Thalamic Nuclei (number/mm^2)			
	1 Month	3 Months	5 Months	8 Months
Non-Diabetic	108.6 ± 10.2(-)	104.3 ± 9.8(-)	101.2 ± 9.6 (-)	99.5 ± 8.4 (-)
Diabetic	113.2 ± 12.4(-)	123.0 ± 11.4 (+/-)	128.3 ± 10.1* (+/-)	116.3 ± 13.7 (-/+)

Results – Microglial Protein Expression





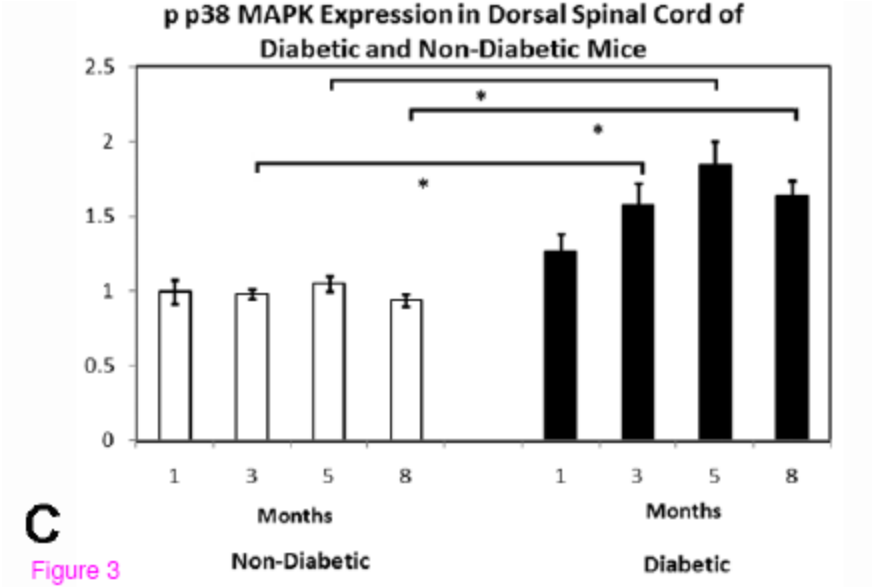


Figure 3

Results – Fluorescent Tagging

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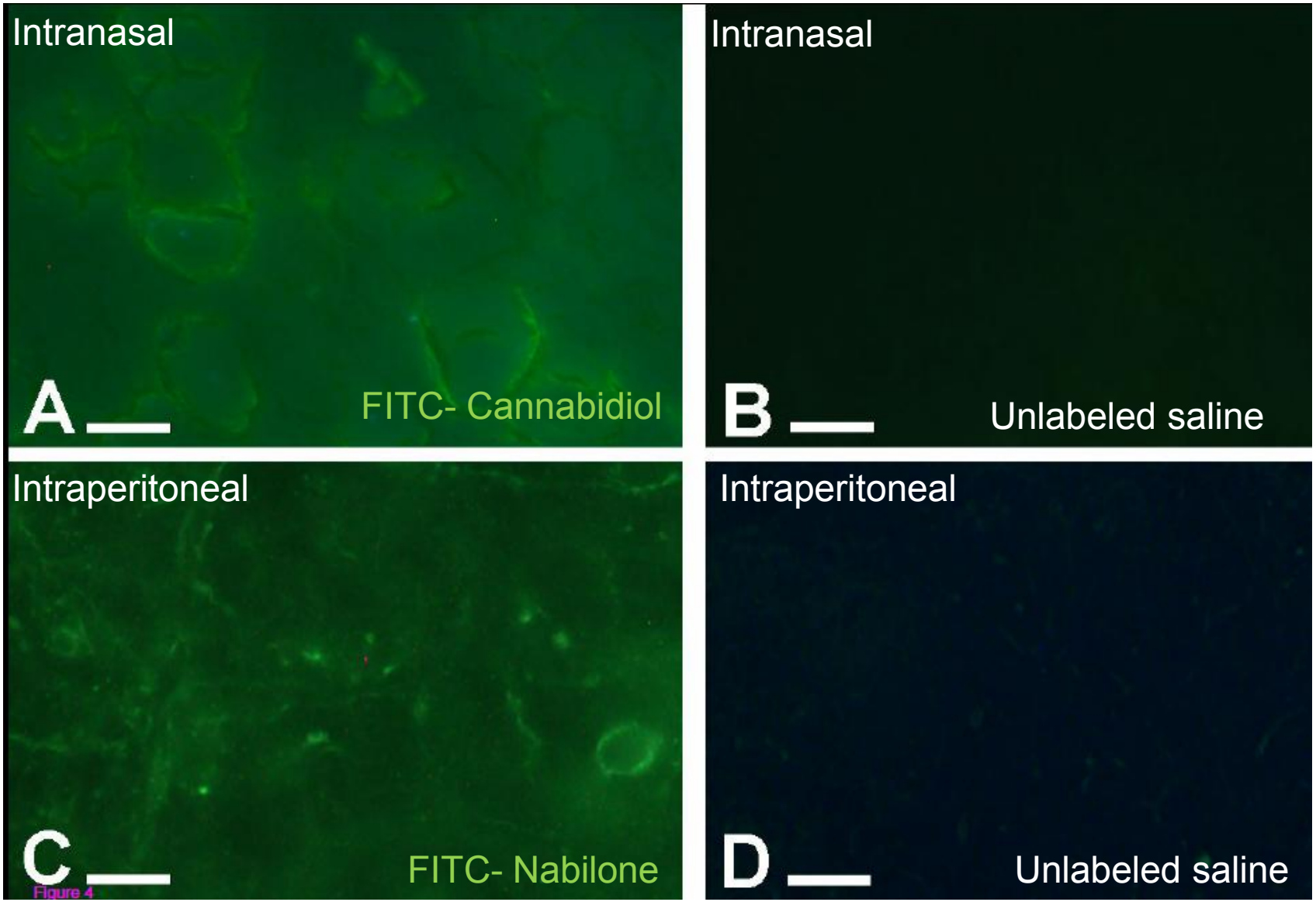

Methods

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• We next examined the role of CB2 receptor activation or blockade in the same model of murine DPN

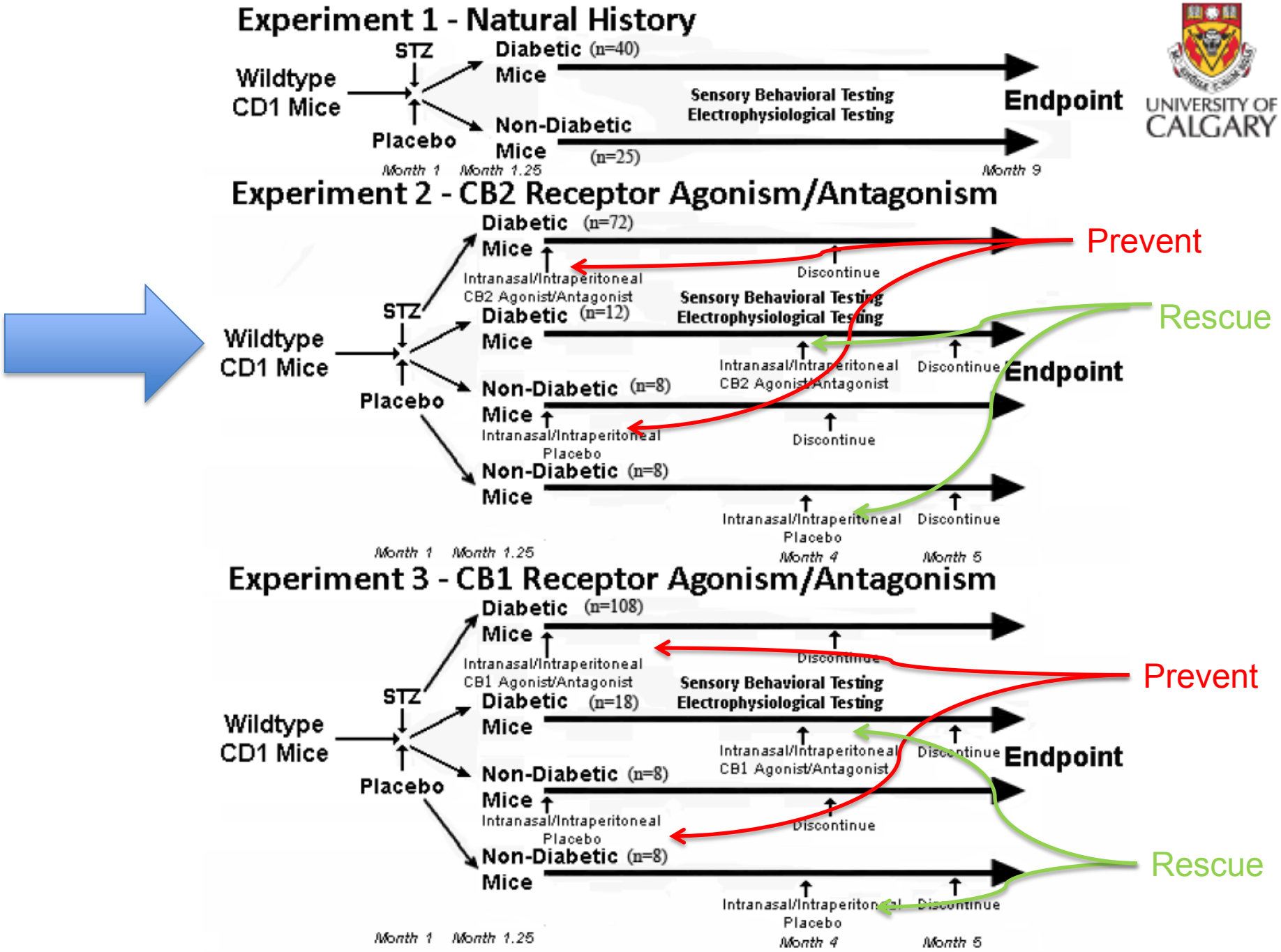
• We provided either: 1) intranasal or I.P. cannabidiol (selective CB2 agonist)

2) intranasal or I.P. SR144528 (selective CB2 antagonist)

starting at the time of confirmation of diabetes at 1 week post-STZ injections for the duration of 3 months (PREVENT)

• Then we provided either: 1) intranasal or I.P. cannabidiol (selective CB2 agonist

2) intranasal or I.P. SR144528 (selective CB2 antagonist) starting after 4 months of diabetes for the duration of 1 month (RESCUE)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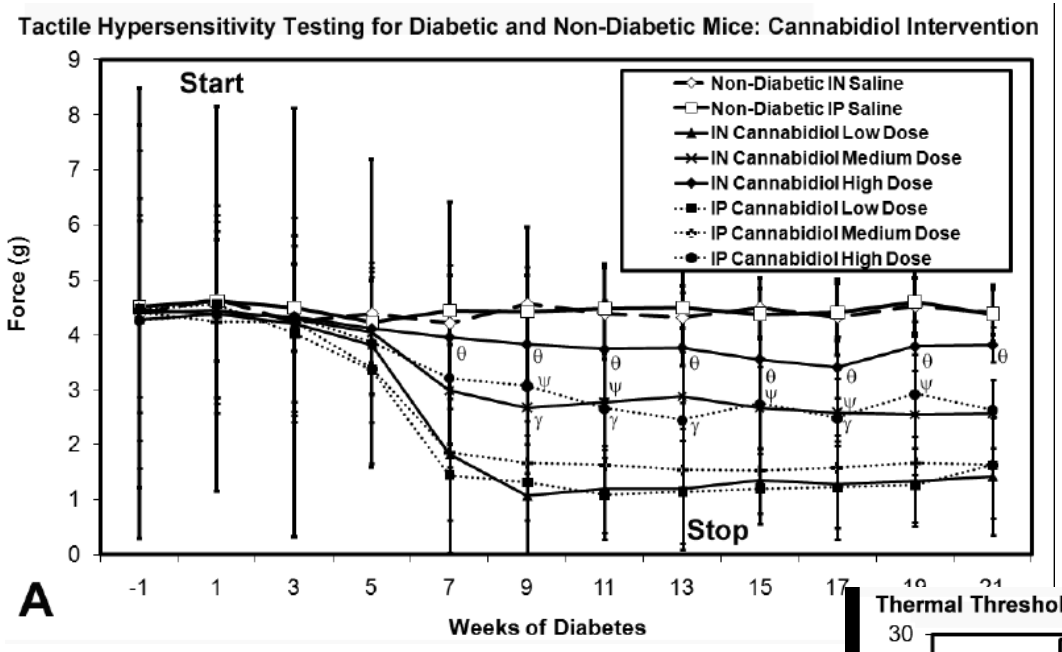
Rescue

Results – CB2 Agonist



selective CB2 agonist delivery (CANNABIDIOL)

PREVENT

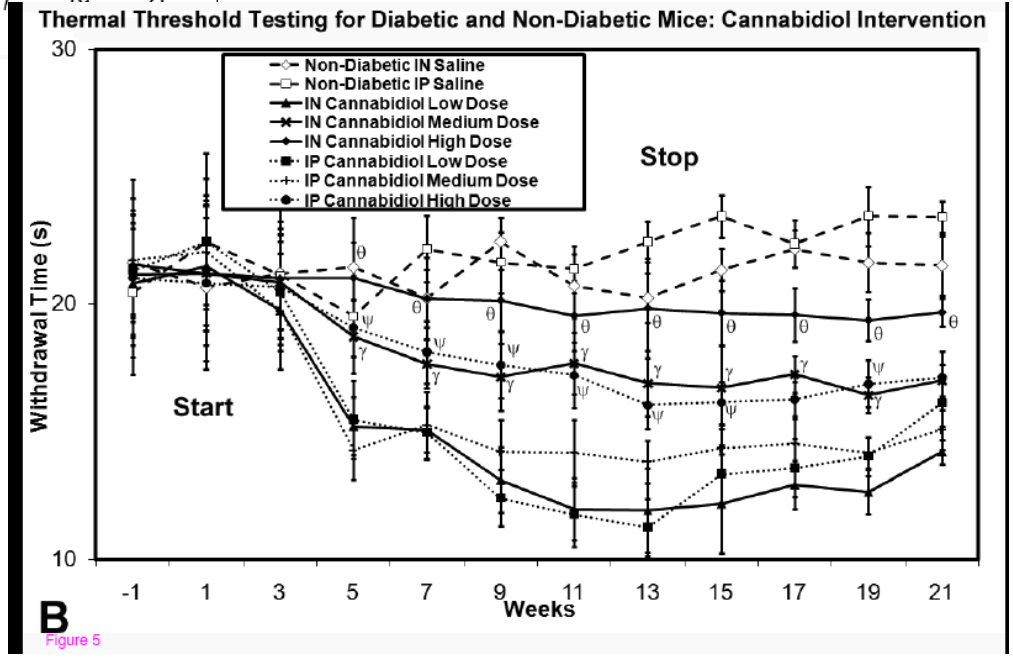


Figure 5

Results – CB2 Antagonist

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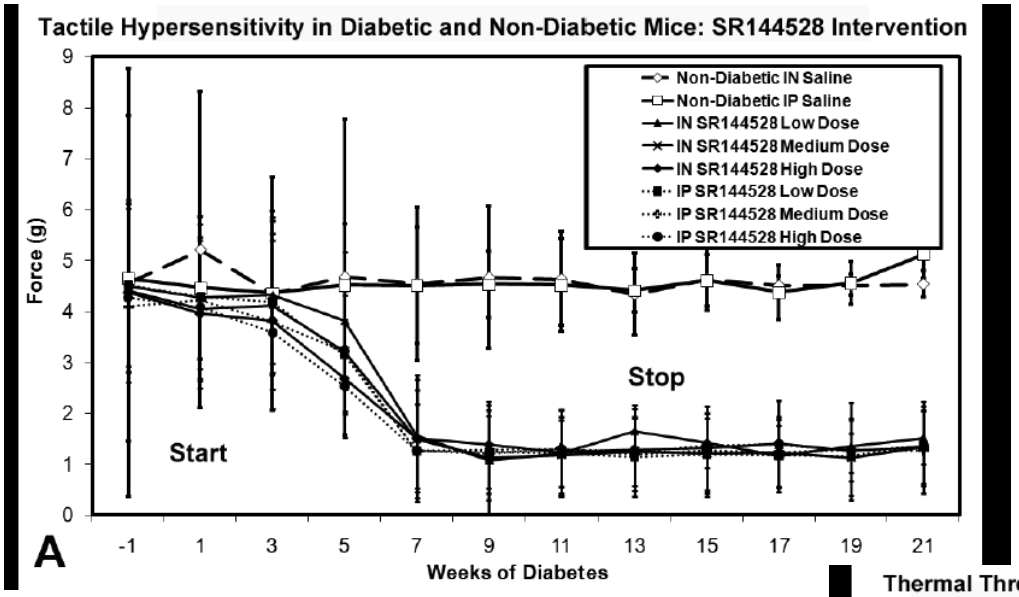

selective CB2 antagonist delivery (SR144528)

PREVENT

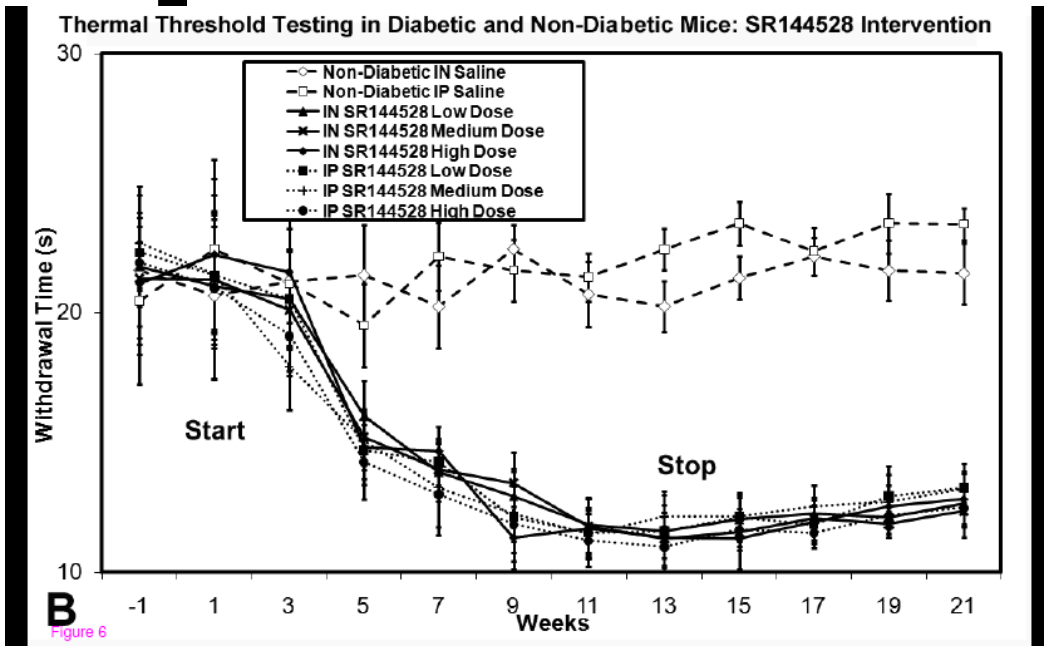

Figure 6

Results – Rescue Protocol

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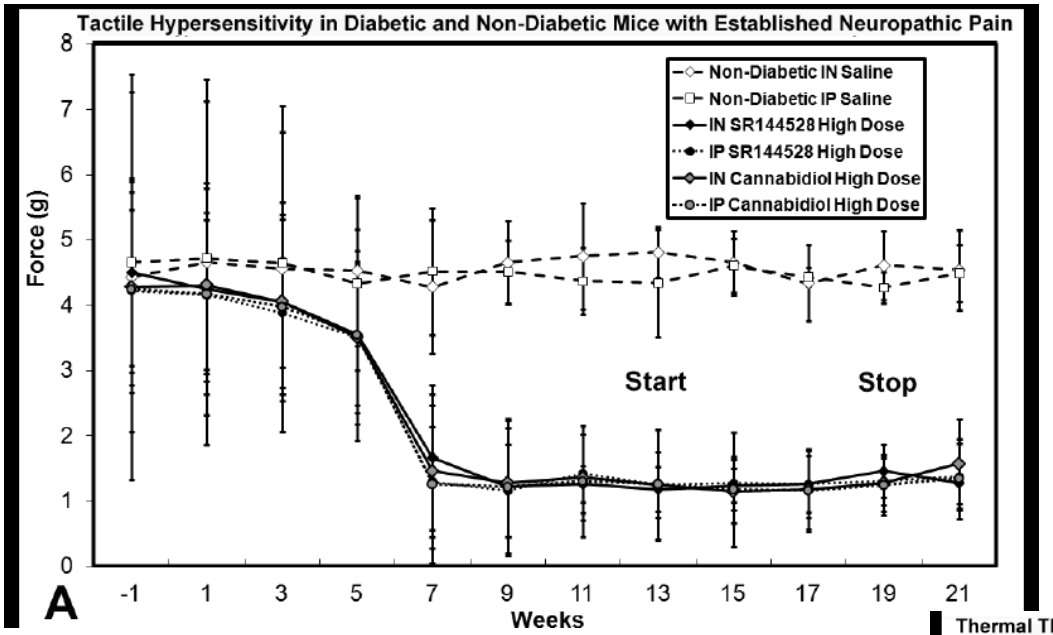
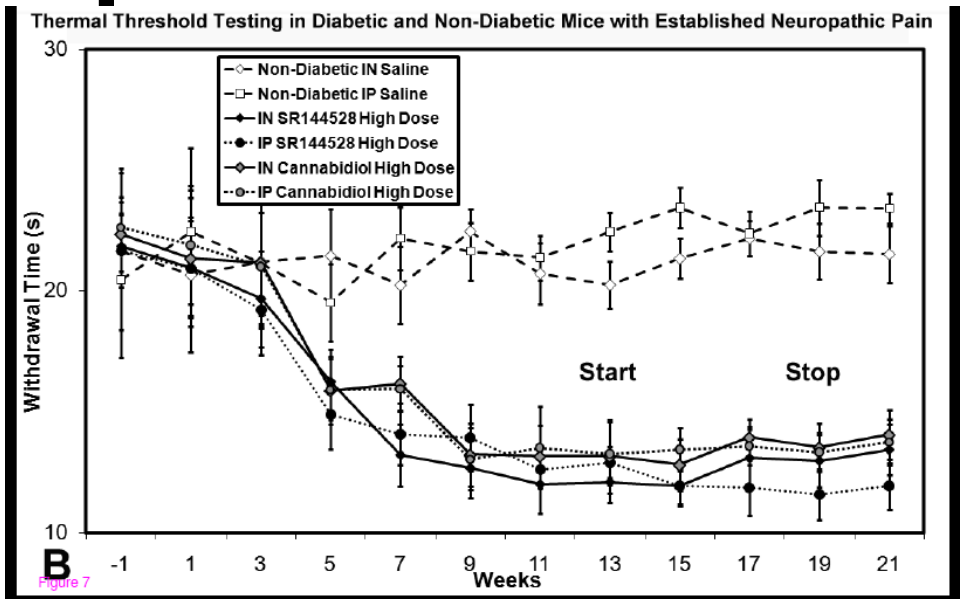


selective CB2 agonist and antagonist delivery after establishment of neuropathic pain (CANNABIDIOL and SR144528)

RESCUE



Methods

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• We next examined the role of CB1 receptor activation or blockade in the same model of murine DPN

• We provided either: 1) intranasal or I.P. nabilone solution (non-selective CB1 and CB2 agonist)
2) intranasal or I.P. WIN55212-2 (selective CB1 agonist)
3) intranasal or I.P. SR141716A (selective CB1 antagonist)
or 4) saline
starting at the time of confirmation of diabetes at 1 week post-STZ injections for the duration of 3 months (PREVENT)

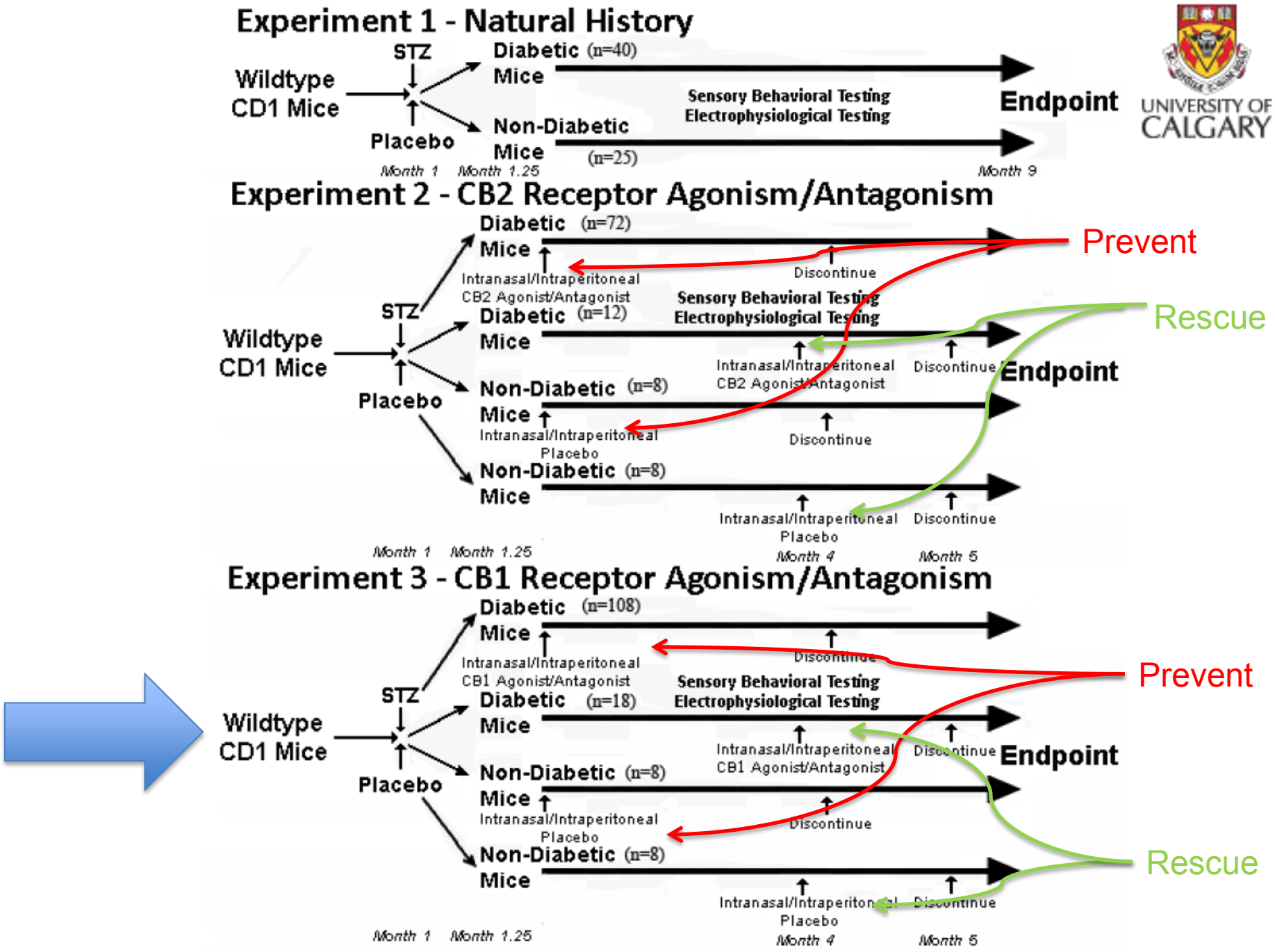
Methods

Then we provided either: 1) intranasal or I.P. nabilone or WIN55212-2 (non-selective or selective CB1 agonist)

2) intranasal or I.P. SR141716A (selective CB2 antagonist)

or 3) saline

starting after 4 months of diabetes for the duration of 1 month (RESCUE)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Non-Diabetic (n=8) Mice

PREVENT Results – CB1 Agonists

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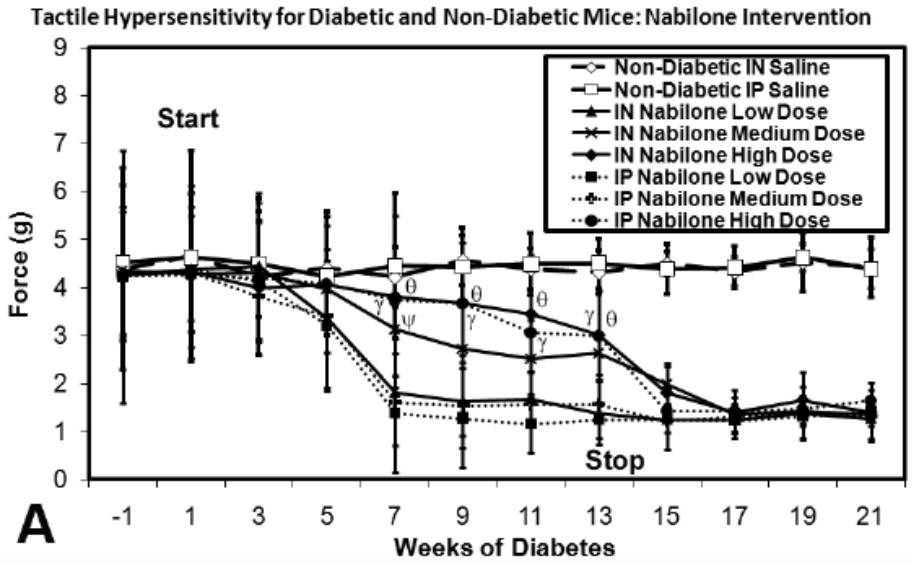

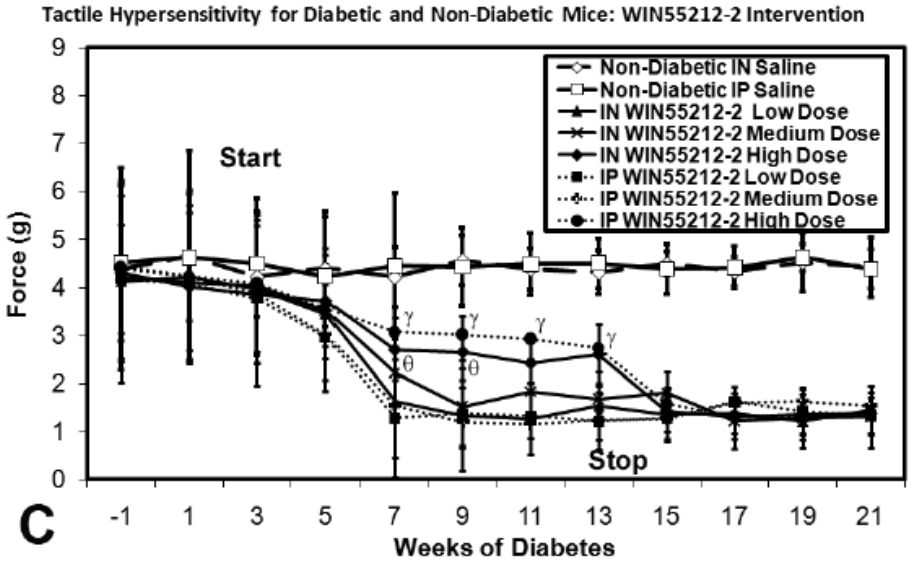

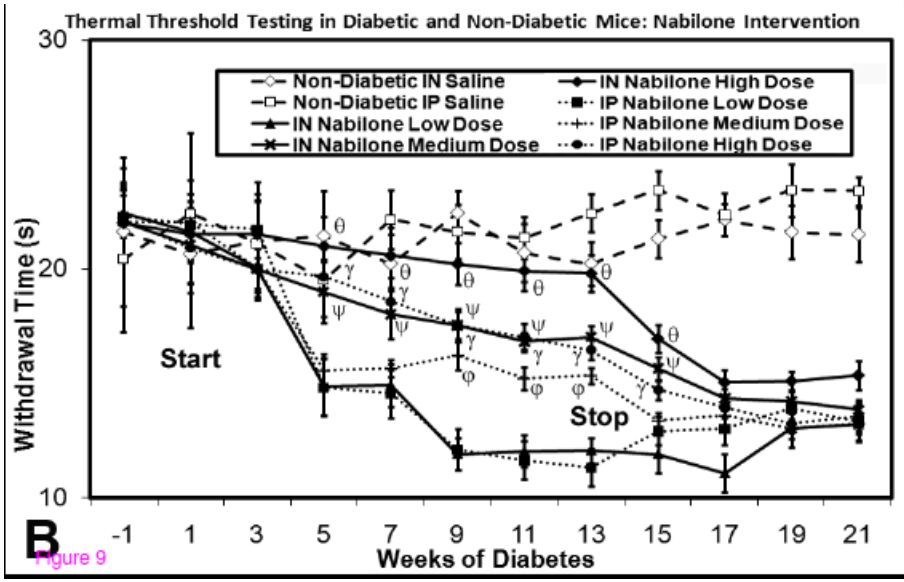

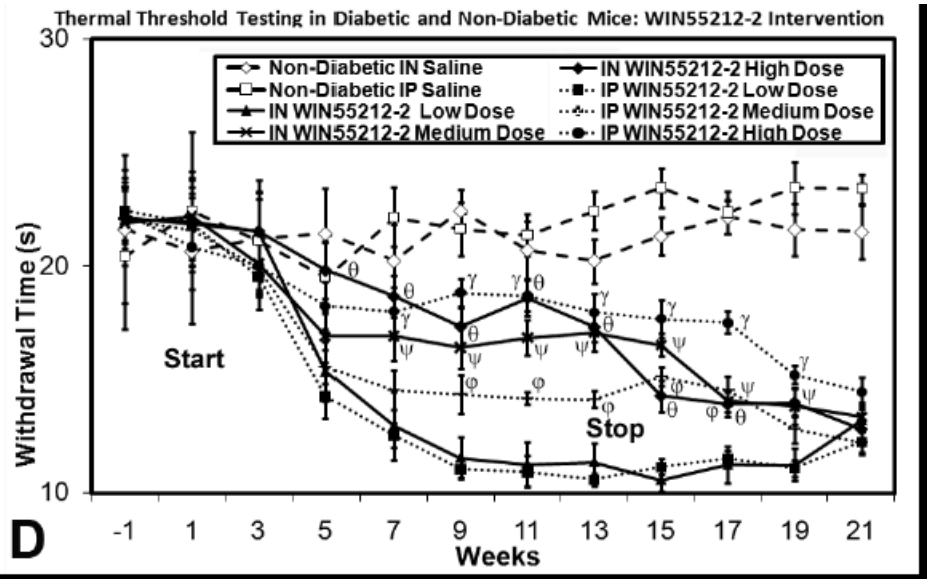

Figure 9

Results – Rescue Protocol

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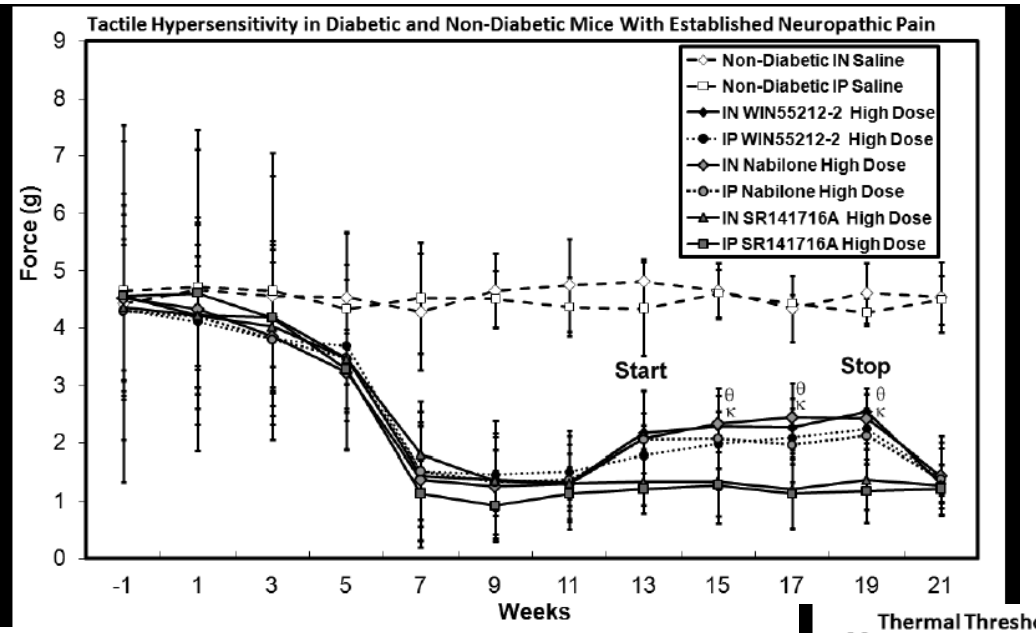


RESCUE

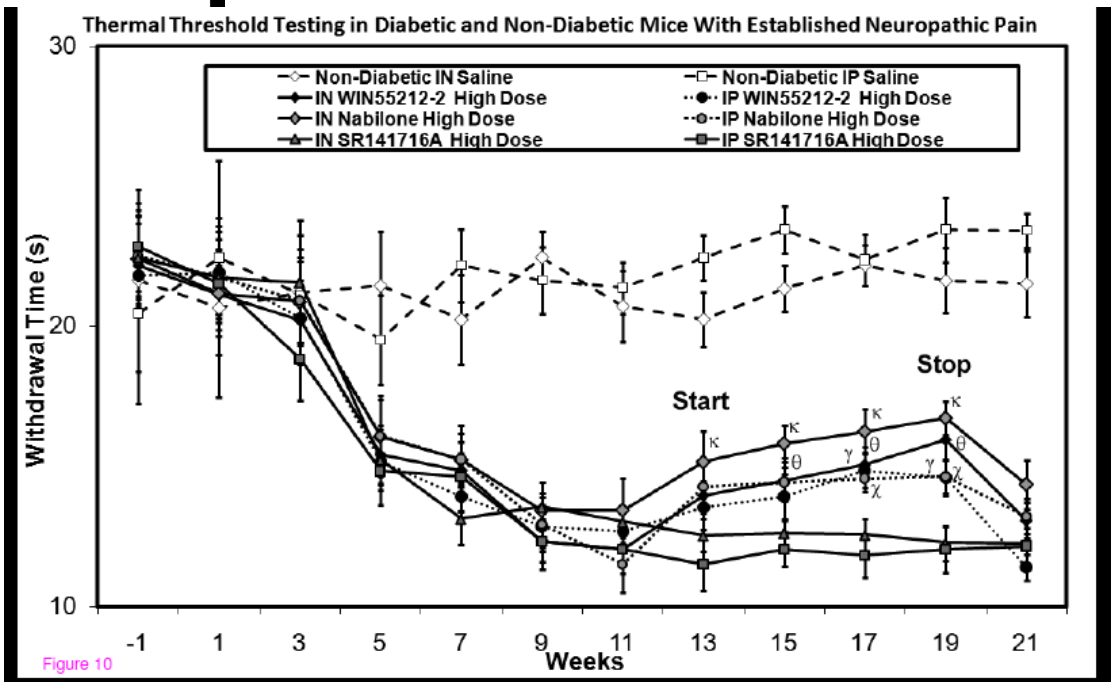
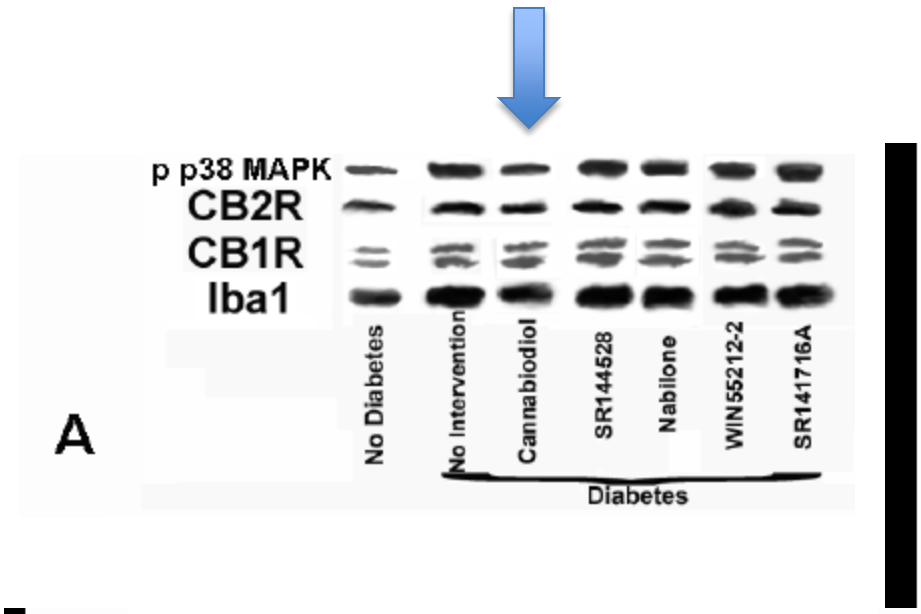
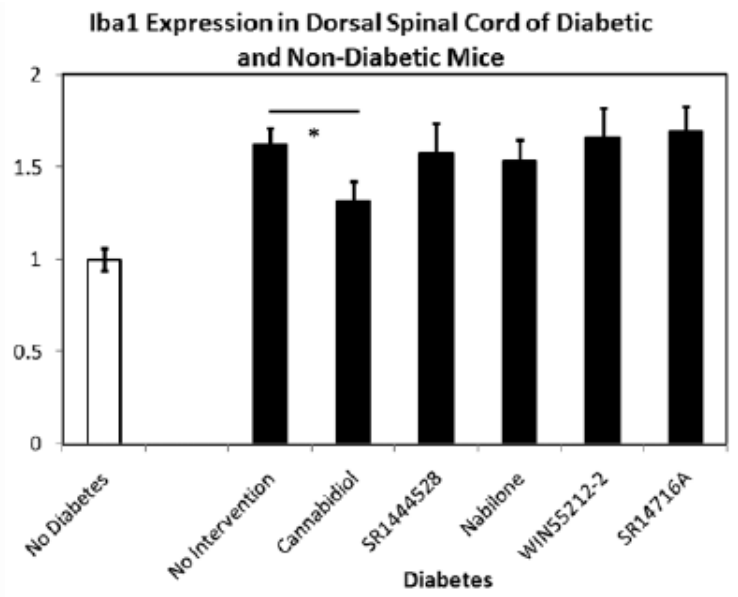
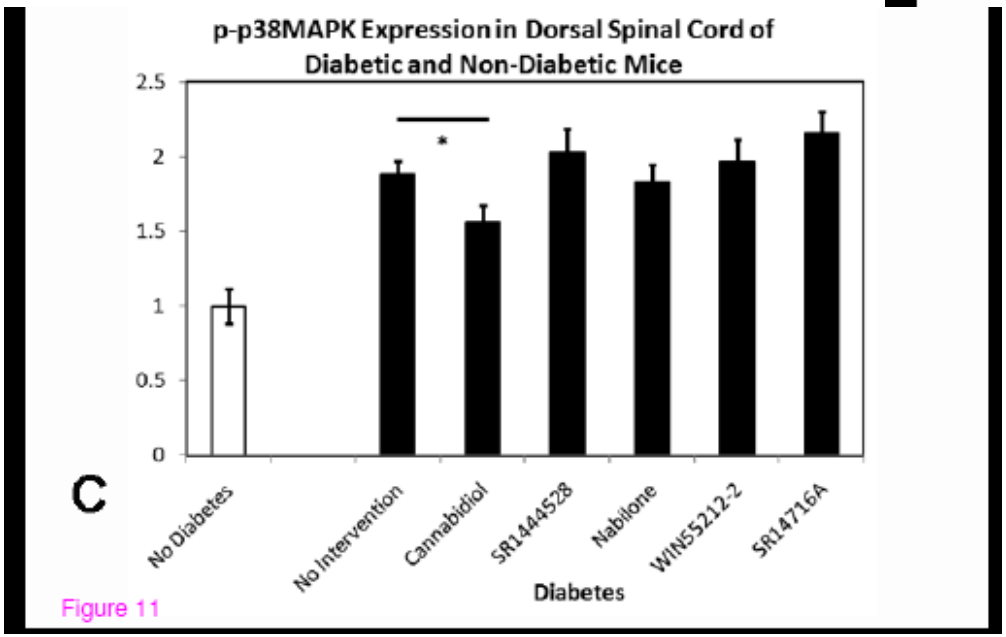


Figure 10

Results







Only Cannabidiol (CB2 agonist) in prevent mode led to lowered expression of Iba-1 (microglia marker) and p-p38MAPK expression (activated microglial marker)

Results

Mouse Cohort	Microglial Density (number/mm^2)	
	Ventral Lumbar Spinal Cord	Thalamic Nuclei
Non-Diabetic		
Intraperitoneal		
Saline	147.3 ± 17.2 (-)	99.2 ± 9.9 (-)
Intranasal		
Saline	136.1 ± 19.2 (-)	105.3 ± 9.3 (-)
Diabetic		
Intraperitoneal		
Early Intervention		
Cannabidiol	228.6 ± 15.3* (+)	116.5 ± 9.4 (+/-)
SR144528	316.6 ± 17.8 (+)	127.5 ± 11.3 (+/-)
Nabilone	309.3 ± 18.2 (+)	125.6 ± 10.5 (+/-)
WIN55212-2	305.7 ± 20.5 (+)	124.4 ± 9.7 (+/-)
SR141716A	314.7 ± 16.9 (+)	130.0 ± 10.4 (+/-)
Late Intervention		
Cannabidiol	314.2 ± 18.8 (+)	118.2 ± 10.8 (+/-)
SR144528	312.5 ± 19.4 (+)	125.6 ± 9.5 (+/-)
Nabilone	319.5 ± 19.9 (+)	124.5 ± 9.2 (+/-)
WIN55212-2	311.3 ± 18.9 (+)	131.2 ± 11.2 (+/-)
SR141716A	325.3 ± 17.7 (+)	129.0 ± 9.0 (+/-)
Intranasal		
Early Intervention		
Cannabidiol	209.5 ± 14.8* (+)	114.7 ± 12.2 (+/-)
SR144528	302.3 ± 18.2 (+)	125.4 ± 11.3 (+/-)
Nabilone	301.1 ± 16.5 (+)	132.5 ± 11.7 (+/-)
WIN55212-2	319.1 ± 18.4 (+)	126.1 ± 8.9 (+/-)
SR141716A	316.3 ± 17.8 (+)	130.5 ± 9.6 (+/-)
Late Intervention		
Cannabidiol	314.5 ± 18.1 (+)	120.0 ± 9.5 (+/-)
SR144528	322.6 ± 20.5 (+)	133.4 ± 11.6 (+/-)
Nabilone	328.1 ± 19.9 (+)	125.6 ± 11.1 (+/-)
WIN55212-2	321.1 ± 18.0 (+)	122.0 ± 9.0 (+/-)
SR141716A	312.6 ± 18.4 (+)	124.6 ± 9.4 (+/-)

Conclusions

- The present data confirm the efficacy of cannabinoid agonists, both for the CB1 and CB2 receptor, in modulation of acute thermal and tactile hypersensitivity as features of neuropathic pain.
- CB2 agonism from the onset of the offending stimulus (diabetes) normally leading to neuropathic pain ameliorated the development of a neuropathic pain state.

Conclusions

- In contrast, we did not demonstrate worsening of the neuropathic pain state with CB1 or CB2 antagonists.
- Such selective targeting with CB2 selective agonists may play a role in the prevention of neuropathic pain states if treatment could be delivered at the time of neural injury or disease, early in the start of illness behavior

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W. Frey provided consultation regarding intranasal delivery