Translational Role Models of Pain: The transgenic sickle mice

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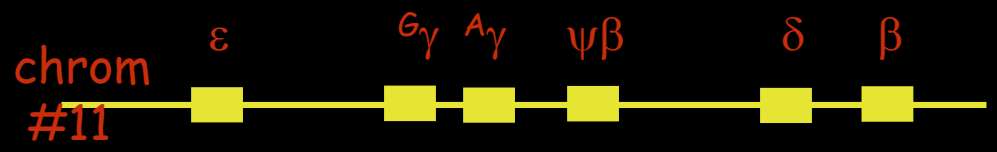
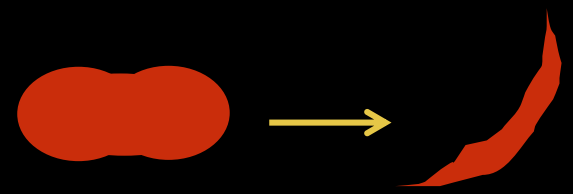
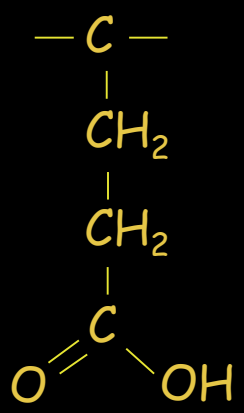
Sickle Cell Anemia: Molecular Basis

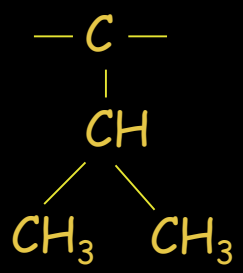


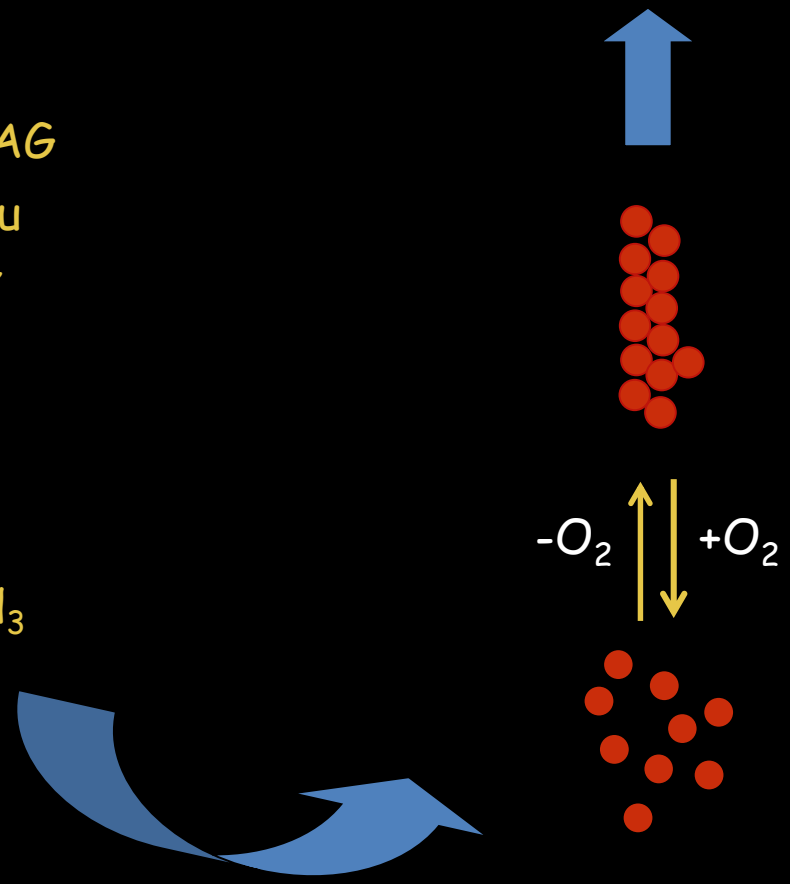


CCT GAG GAG
Pro Glu Glu
5 6 7

CCT GTG GAG
Pro Val Glu
5 6 7





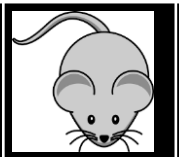
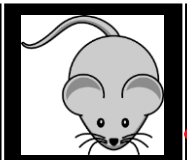


Courtesy, Robert P Hebbel MD

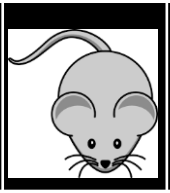
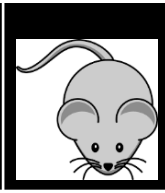
Pain in sickle cell disease is unique

- Sickle RBCs adhere and occlude blood vessels leading to “vasoocclusive crises” resulting in, inflammation, ischemia/reperfusion injury, impaired tissue oxygenation, organ damage and severe pain.
- Pain can start during infancy.
- Recurrent episodes of “crises” and acute pain can be superimposed on chronic pain.
- Relatively high dose of opioids is required often for long periods of time.

Transgenic mice expressing mouse and human sickle hemoglobins

Name								Genetic Strain
	α	β^{major}	β^{minor}	α	β^{A}	β^{S}	$\beta^{S\text{-}Antilles}$	C57BL/6
Normal	+	+	+/-					C57BL/6
Thal	+		+/-					C57BL/6
New York	+		+/-			+		C57BL/6
Hybrid	+		+/-			+	+	C57BL/6
Antilles	+		+/-				+	C57BL/6

Transgenic Townes and BERK mice express human α and β globins but no mouse α and β globins

Name								Genetic Strain
	α	β^{major}	β^{minor}	α	β^{A}	β^{S}	$\beta^{S\text{-}Antilles}$	
HbAA-control				+	+			Mixed
HbAS-hemi				+	+	+		Mixed
HbSS-homo				+		+		Mixed

BERK: Paszty et al, Science 1997 and Townes: Ryan et al., Science 1997

Can mouse models be used to study human disease?

"--- to boost the quality of animal studies by better characterizing and understanding how mouse models correspond to human disease,"

Perrin S. Nature 507, 423-25, 2014

How to <u>select a specific model</u> and how to <u>design</u> a study to study pain in sickle cell anemia?

Characteristic features of pain in SCD

[i] Increased sensitivity to wind speed

[ii] Pain evoked by temperature extremes, hot and cold

[iii] Deep pain in joints and body parts

[iv] Headaches

[v] Visceral

Transgenic mouse model presenting features of human sickle cell anemia

HbSS-BERK: Homozygous for knockout of both murine alpha and beta globins, and carry linked transgenes for human α and β-S globins. These mice have severe disease that simulates human SCA (hemolysis, reticulocytosis, anemia, extensive organ damage, forshortened lifespan and pain). *Paszty C et al., 1994, Science*

HbAA-BERK: Genetic background similar to HbSS-BERK, but express normal human hemoglobin A.

Pain characteristics in HbSS-BERK sickle mice are similar to human SCD.
Kohli DR, Li Y, Khasabov SG, Gupta V, Kehl LJ, Ericson ME, Nguyen J, Gupta P, Hebbel RP, Simone DA, Gupta K. Pain related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: Modulation by cannabinoids. Blood 2010, 116, 456-65. Editorial commentary, "New era dawns on sickle pain" pages 311-12.

BERK sickle mice share characteristics and mechanisms of human sickle pain

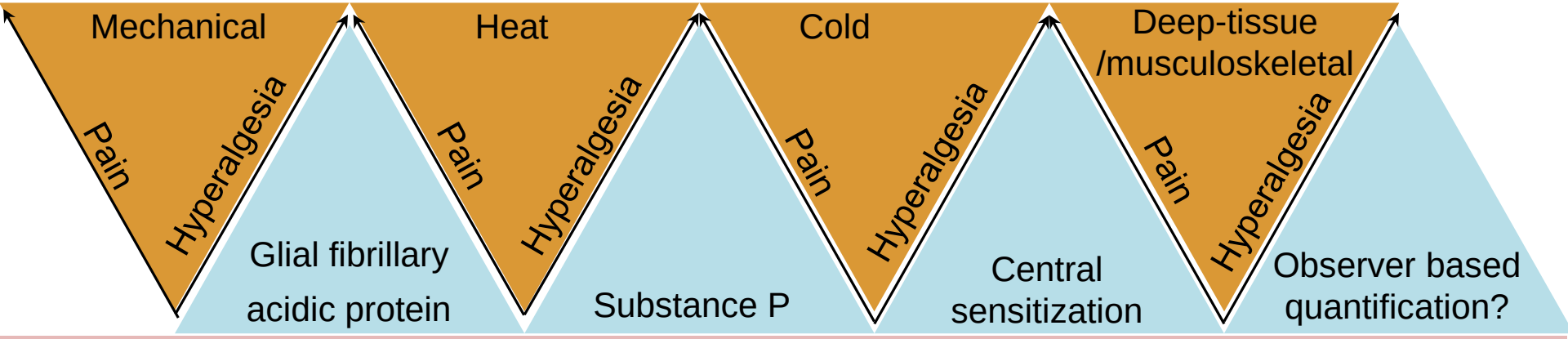
Pain increases with age

Females show more pain than males

Kohli et al., Blood 2010; Hillery et al., Blood 2011; Cain et al., 2012;

Facial features show more pain, Mittal et al., 2015

Central Sensitization: Cataldo et al., Pain 2016



Quantitative Sensory Testing in patients with SCA

Brandow et al., Am J Hematol 2013

Campbell et al., J Pain 2016

Ezenwa et al., Pain Practice 2016

Jacob et al., J Ped Hematol Oncol 2016

Central Sensitization

Darbari et al., 2015

Substance P and CGRP, mediators of pain are upregulated in the skin of sickle mice.

Magenta, SP/CGRP

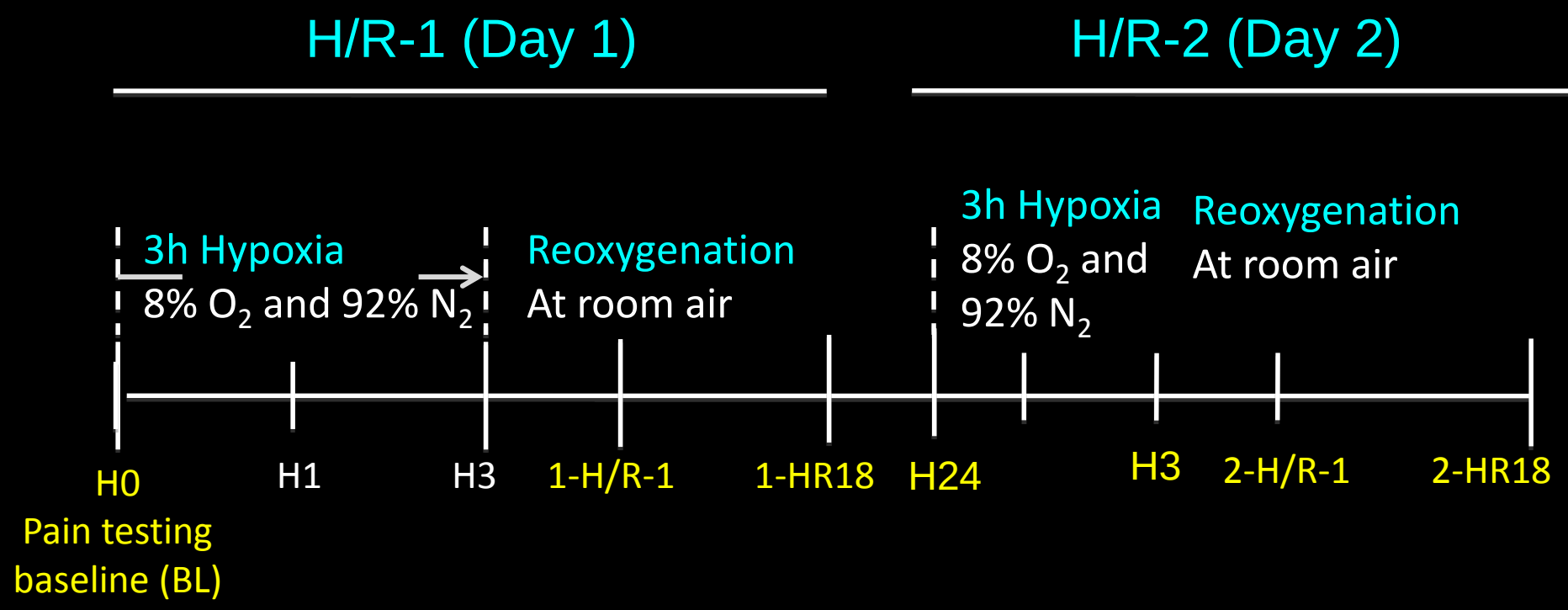
Red, SP

Blue, CGRP

Green, blood vessels

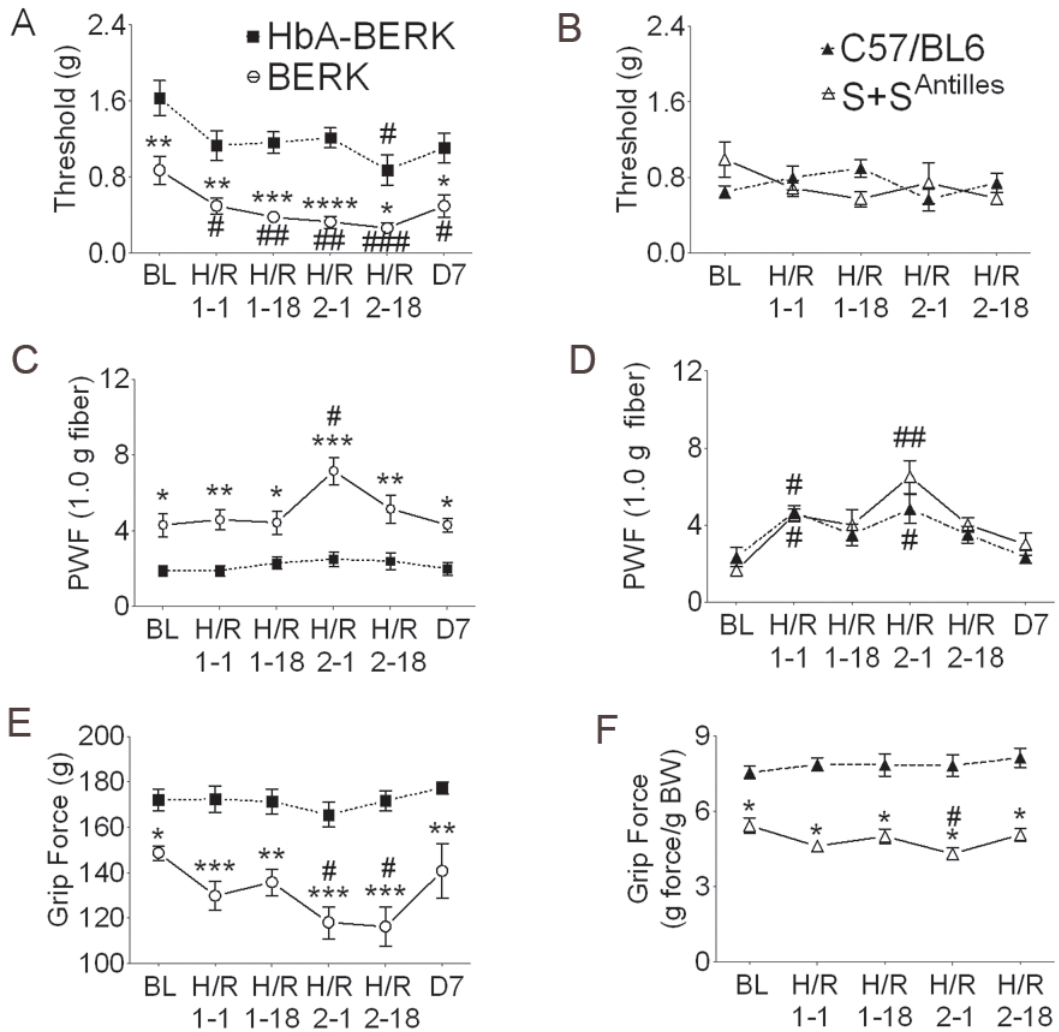
Kohli DR, Li Y, Khasabov SG, Gupta V, Kehl LJ, Ericson ME, Nguyen J, Gupta P, Hebbel RP, Simone DA, Gupta K. Pain related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: Modulation by cannabinoids. Blood 2010, July 22, 116(3), 456-65 Editorial commentary, "New era dawns on sickle pain" pages 311-12.

Model for acute pain to simulate vasoocclusive criris evoked by hypoxia/reoxygenation



(Cain et al., Br J Haematol 2012)

Hypoxia/reoxygenation-evoked hyperalgesia is higher in homozygous BERK sickle mice as compared to S+S^{Antilles}.



Cain et al., Mouse models for studying pain in sickle disease: effects of strain, age, and acuteness. Br J Haematol 2012

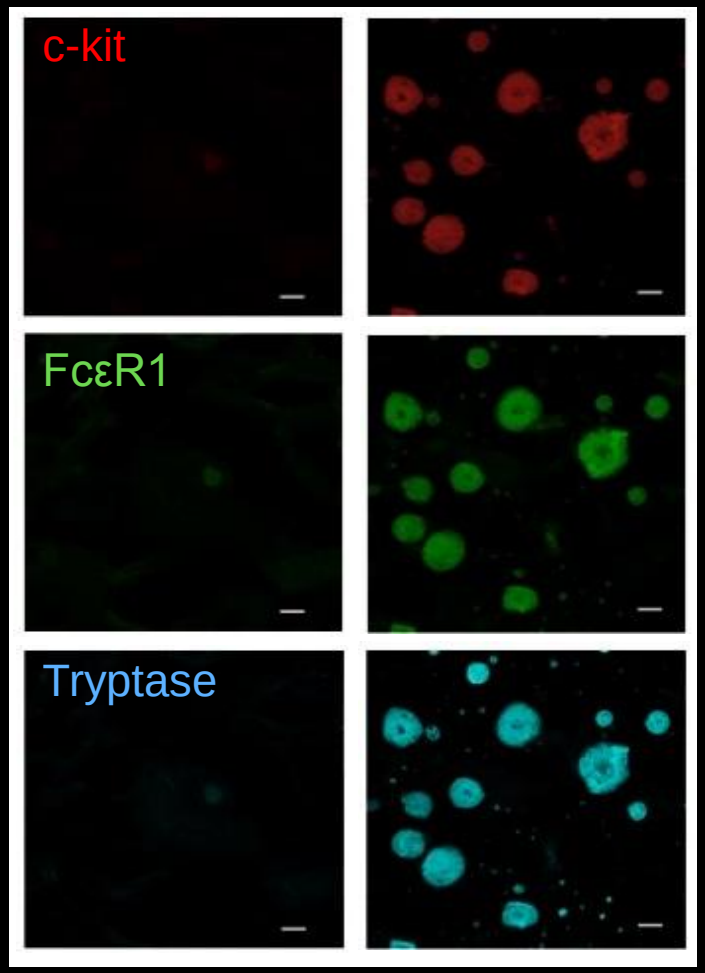
Mast cells

- Role in allergic response and anaphylaxis.
- Close proximity to vasculature and nerve fibers
- Act in an autocrine as well as paracrine manner
- Release cytokines, proteases, neuropeptides including CGRP and substance P, prostaglandins, from pre-formed granules.
- Morphine activates mast cells.

Mast cell degranulation occurs in Sickle Mice

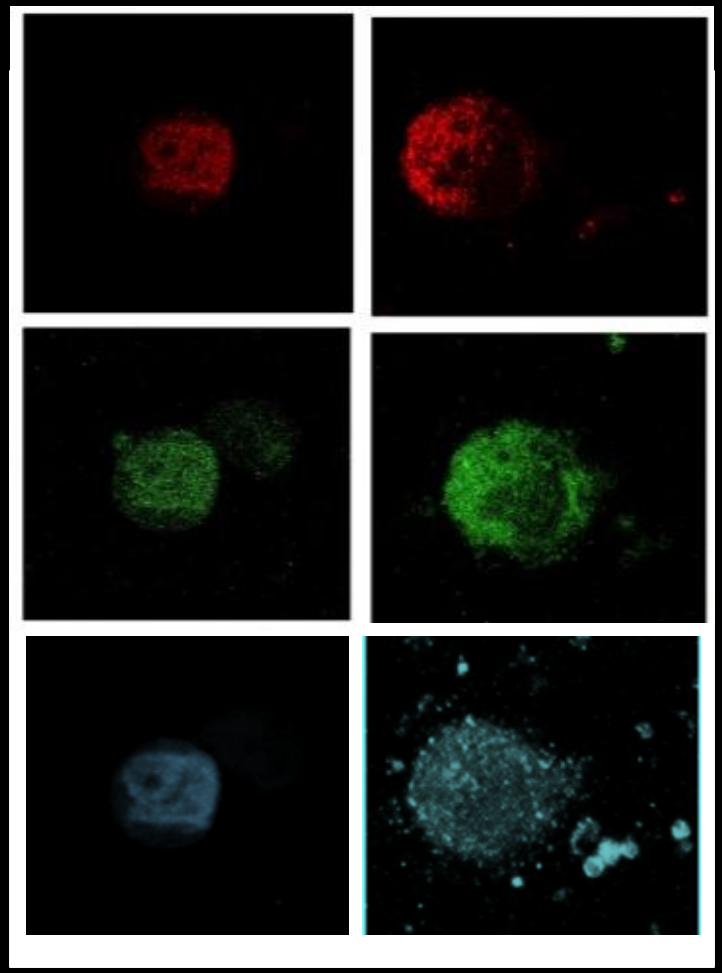
Skin

HbAA-BERK HbSS-BERK

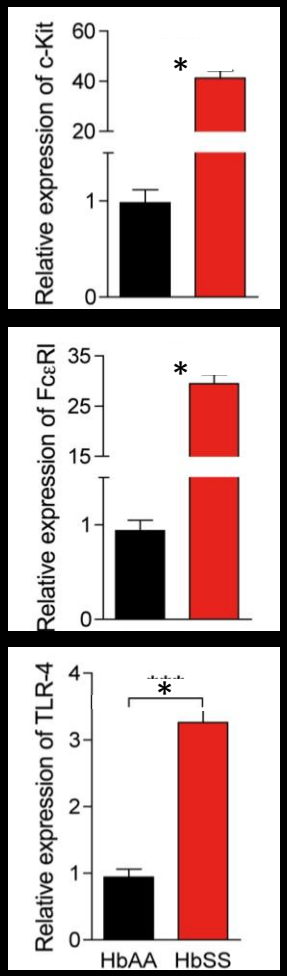


Mast cells in culture

HbAA-BERK HbSS-BERK



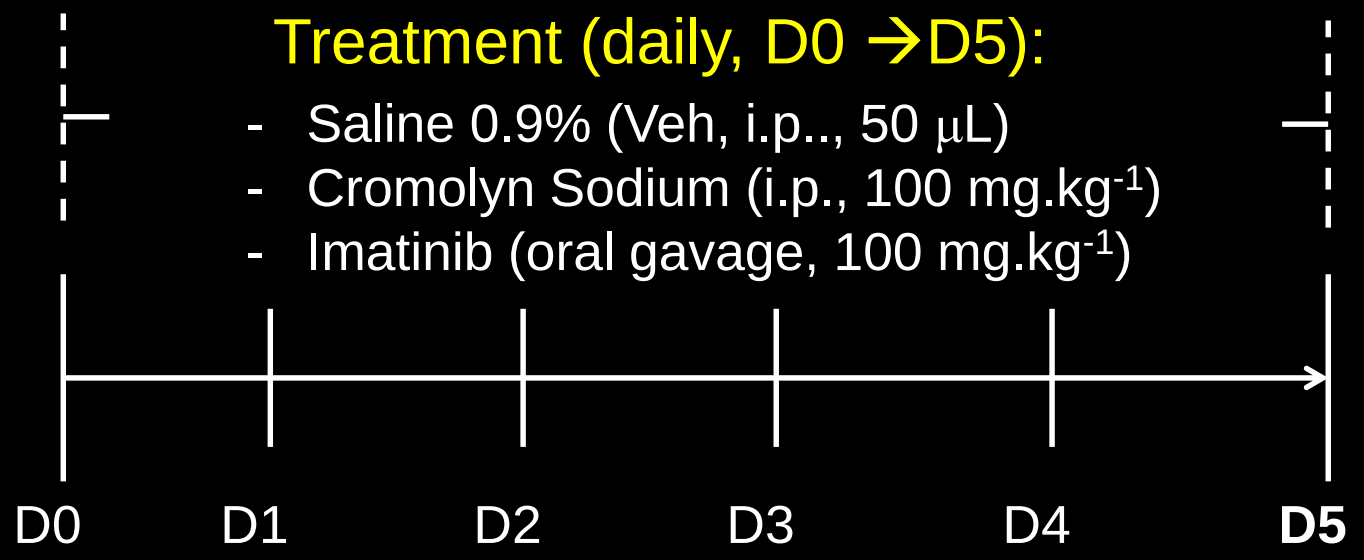
Rt-qPCR



* $p < 0.001$

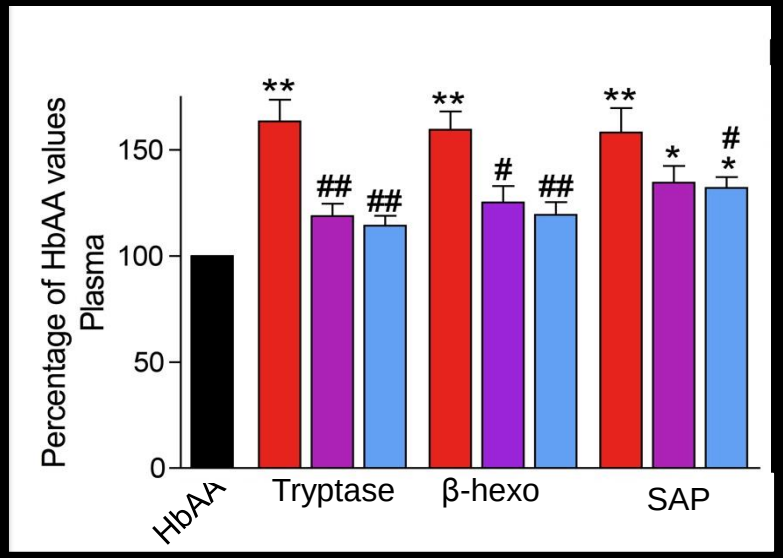
TLR-4 – Toll Like Receptor-4

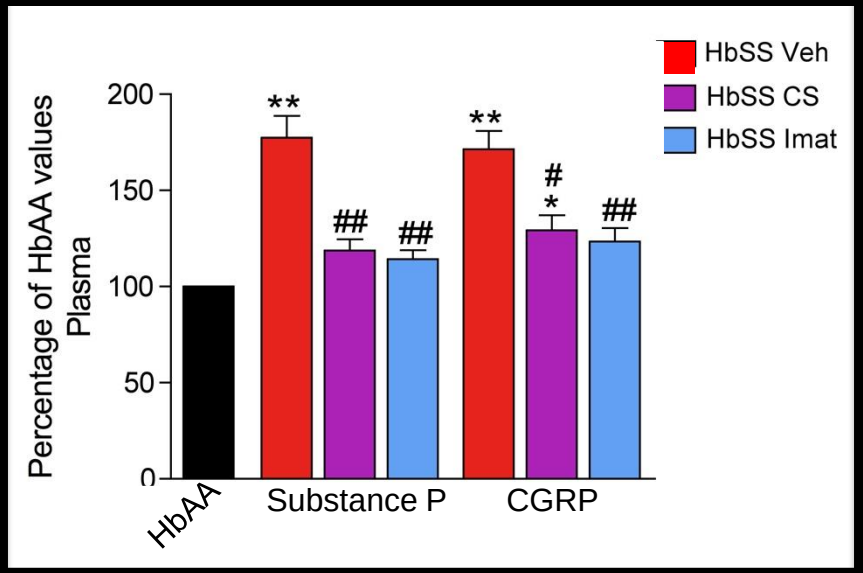
Treatment of mice with two different mast cell inhibitors



Mast cell degranulation is accompanied by increased circulating neuropeptides

Plasma levels





Abbreviations:

β-hexo: β-hexosaminidase

SAP: Serum Amyloid-protein

* $p < 0.05$ vs HbAA
** $p < 0.01$ vs HbAA

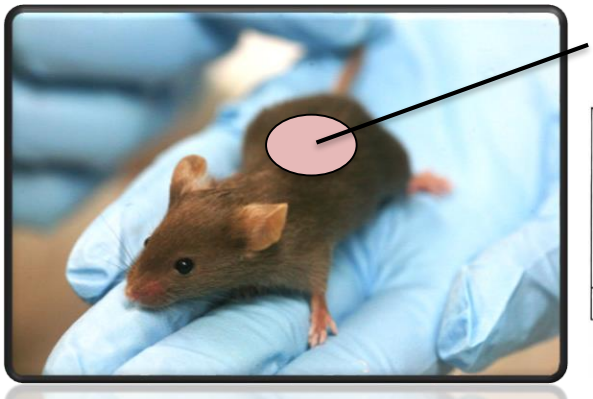
Abbreviations:

CGRP – Calcitonin Gene-related Peptide

\# $p < 0.05$ vs. HbSS Veh
\#\# $p < 0.01$ vs. HbSS Veh

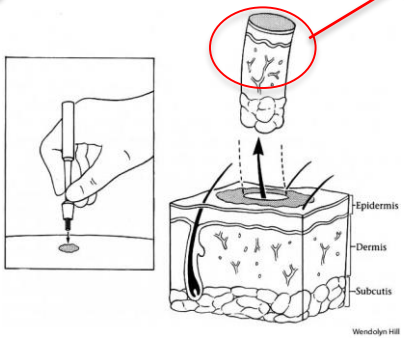
Functional assay to examine the release of products in the skin and DRG

Mice were shaved one day prior to tissue collection

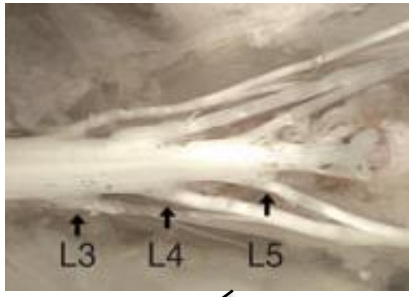


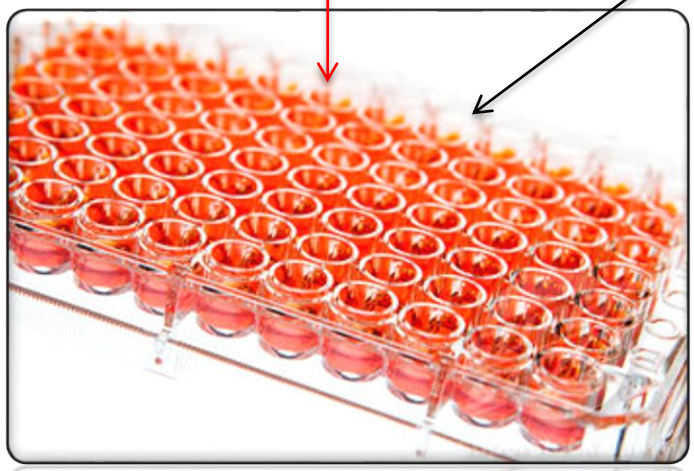
DORSAL SKIN

4 mm biopsy



DRG





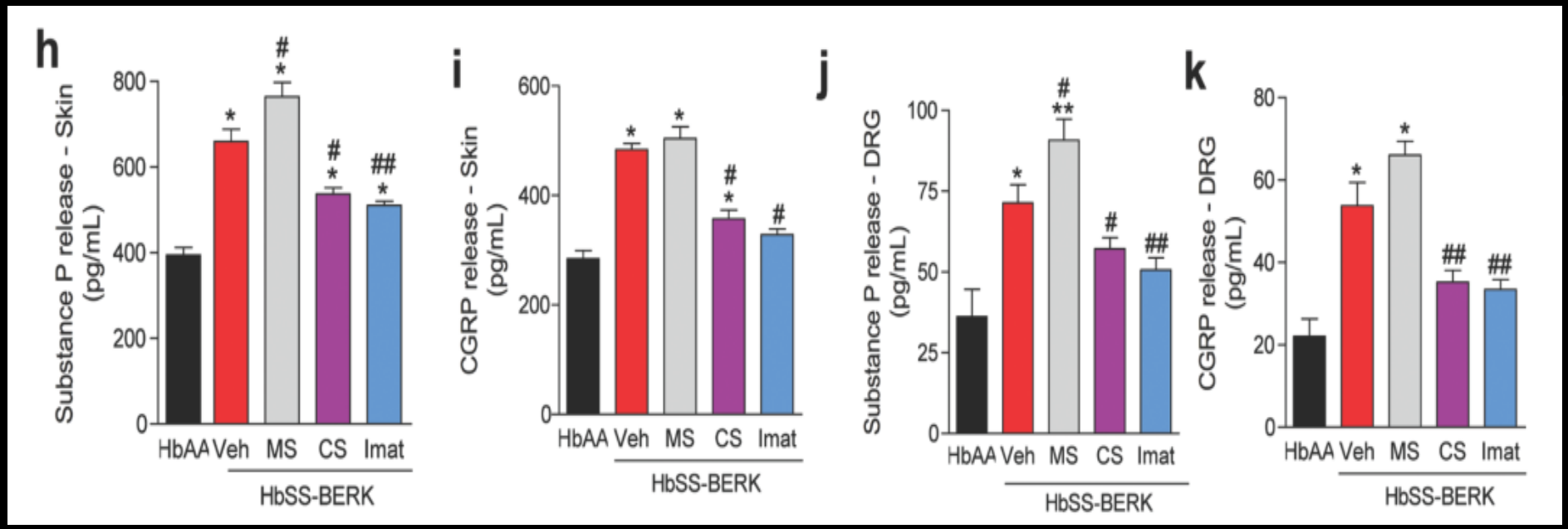
24 h incubation at 37˚C

↓

Releasate (supernatant)
Cytokines, neuropeptides, enzymes

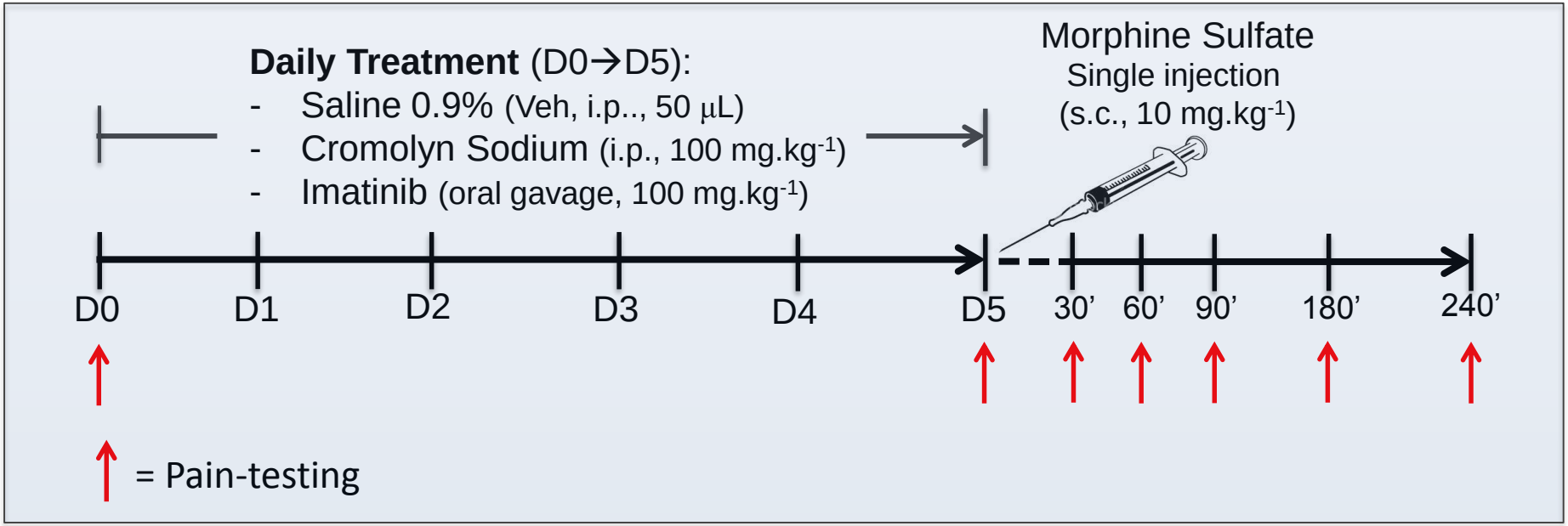
Mast cell inhibitors ameliorate but morphine stimulates neuropeptide release in the skin and DRG

Skin DRG



Does morphine-induced mast cell activation increase hyperalgesia while promoting analgesia via the CNS?

Mast cell inhibition will improve morphine analgesia?



Genetic approach to validate the pharmacologic approach for the contribution of mast cell degranulation in sickle pain

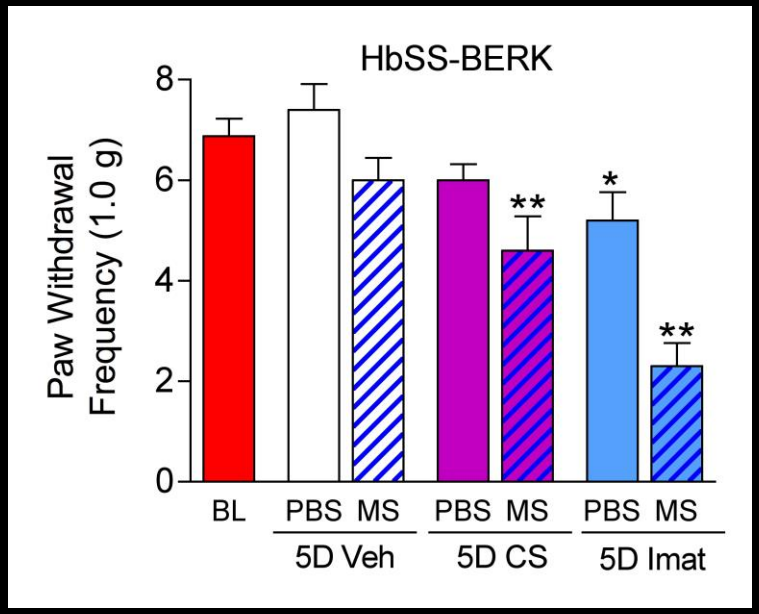
Sickle (HbSS-BERK) mice expressing human sickle hemoglobin and *Control (HbAA-BERK) mice* expressing normal human hemoglobin
(Paszty et al., 1997)

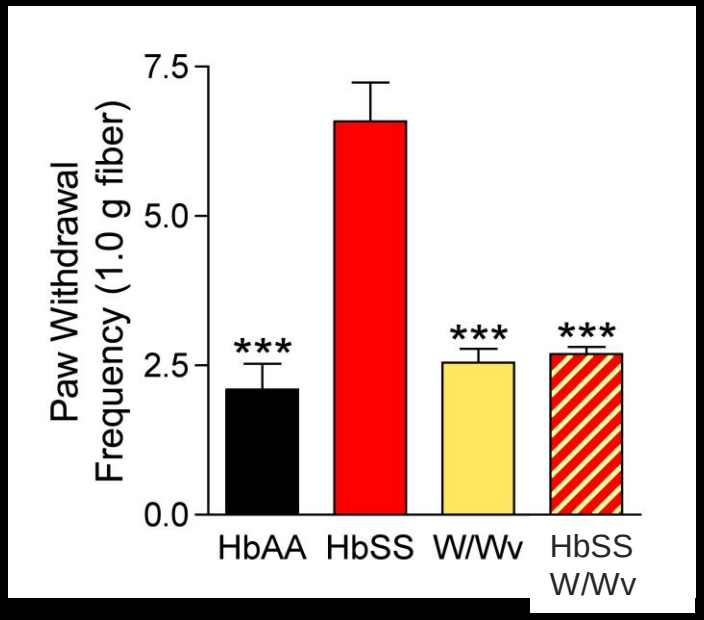
- These mice show pain and inflammation and higher expression of neuropeptides. *(Kohli et al, 2010; Cain et al., 2011; Belcher et al, 2003).*

W/Wv mice: Mast cell deficient mice with a mutation in the tyrosine kinase domain of c-kit/CD117. c-kit is encoded at white spotting (W) locus of mouse chromosome 5. Point mutation causes exon skipping producing truncated *c-kit* (*W* mutation), and point mutation in the tyrosine kinase domain of *c-kit* (*Wv*, W-viable mutation). White, anemic, decrease in the number of bone marrow and blood neutrophils.

HbSS-*W/Wv*: Sickle mice deficent in mast cells produced by backcrossing BERK sickle mice with *W/Wv* mice.

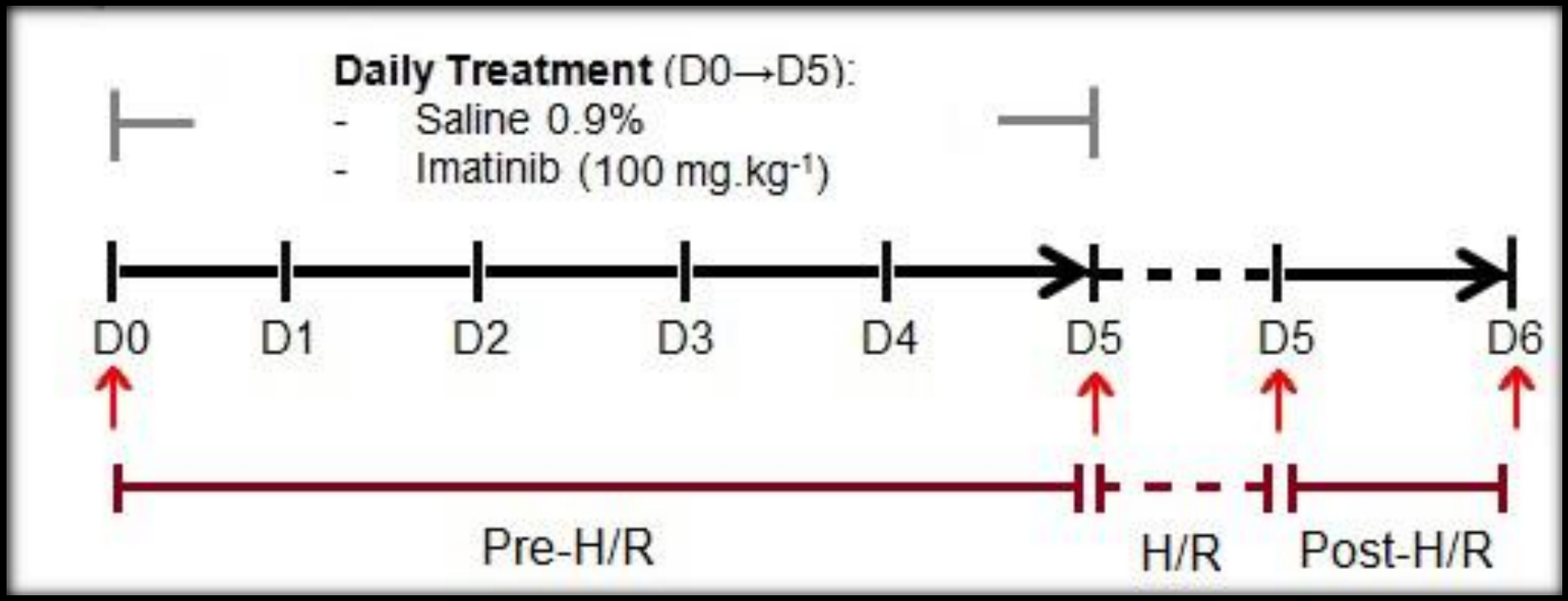
Mast cell inhibition or lack of mast cells reduces pain in sickle mice





Mast cell inhibition may reduce nociceptor activation peripherally and/or reduce the inflammatory response and sickling, thus influencing the pathobiology of SCA.

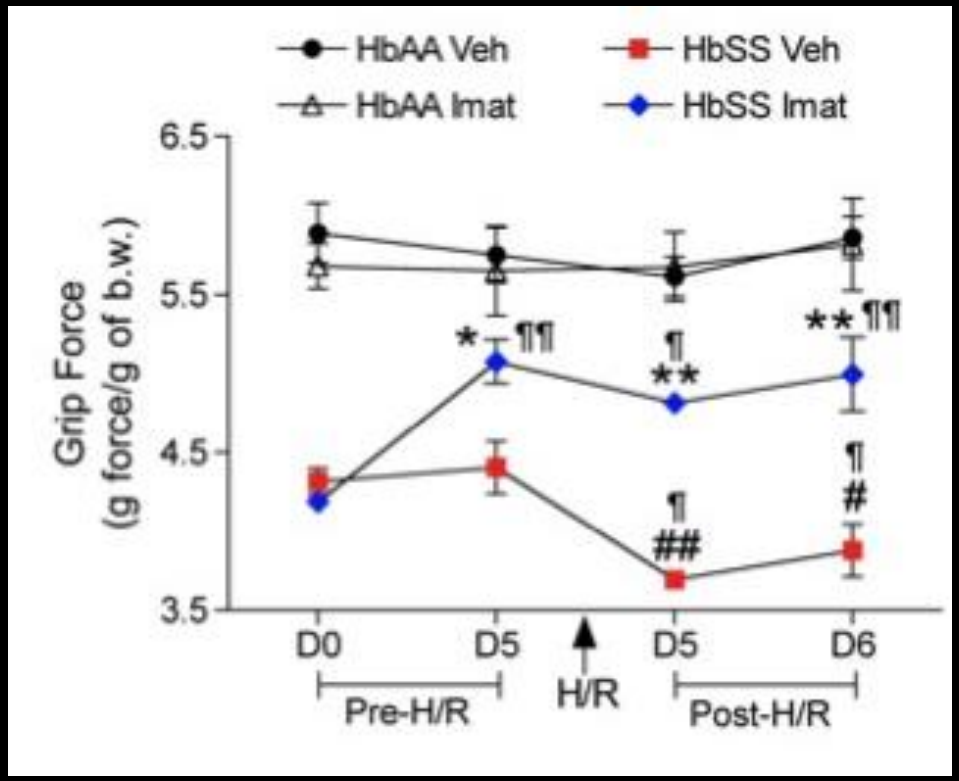
Protocol
Sensory testing following hypoxia/reoxygenation



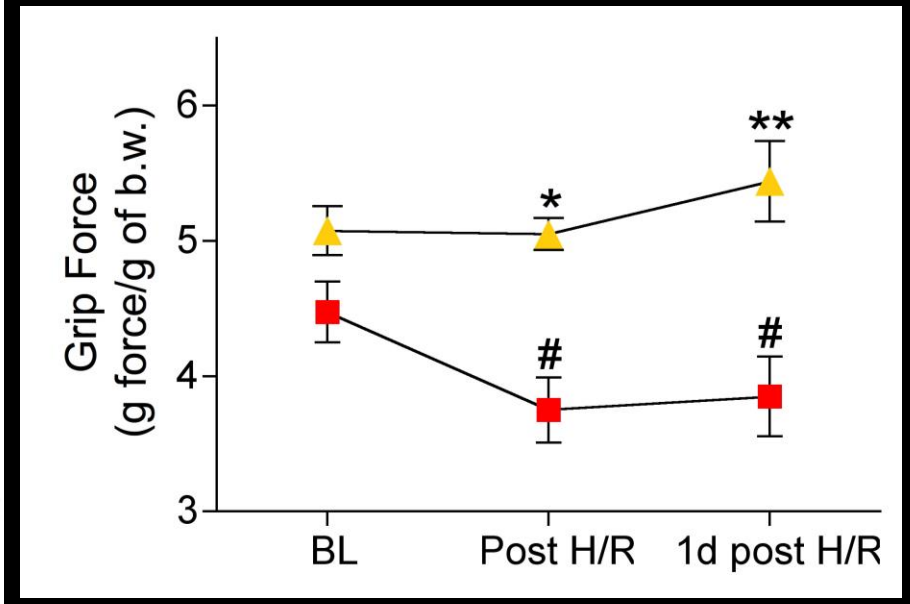
Hypoxia/reoxygenation (H/R)
3h of hypoxia with 8% Oxygen followed by 1h of oxygenation at room air

Imatinib and genetic mast cell deletion attenuates hypoxia/reoxygenation-evoked pain in sickle mice

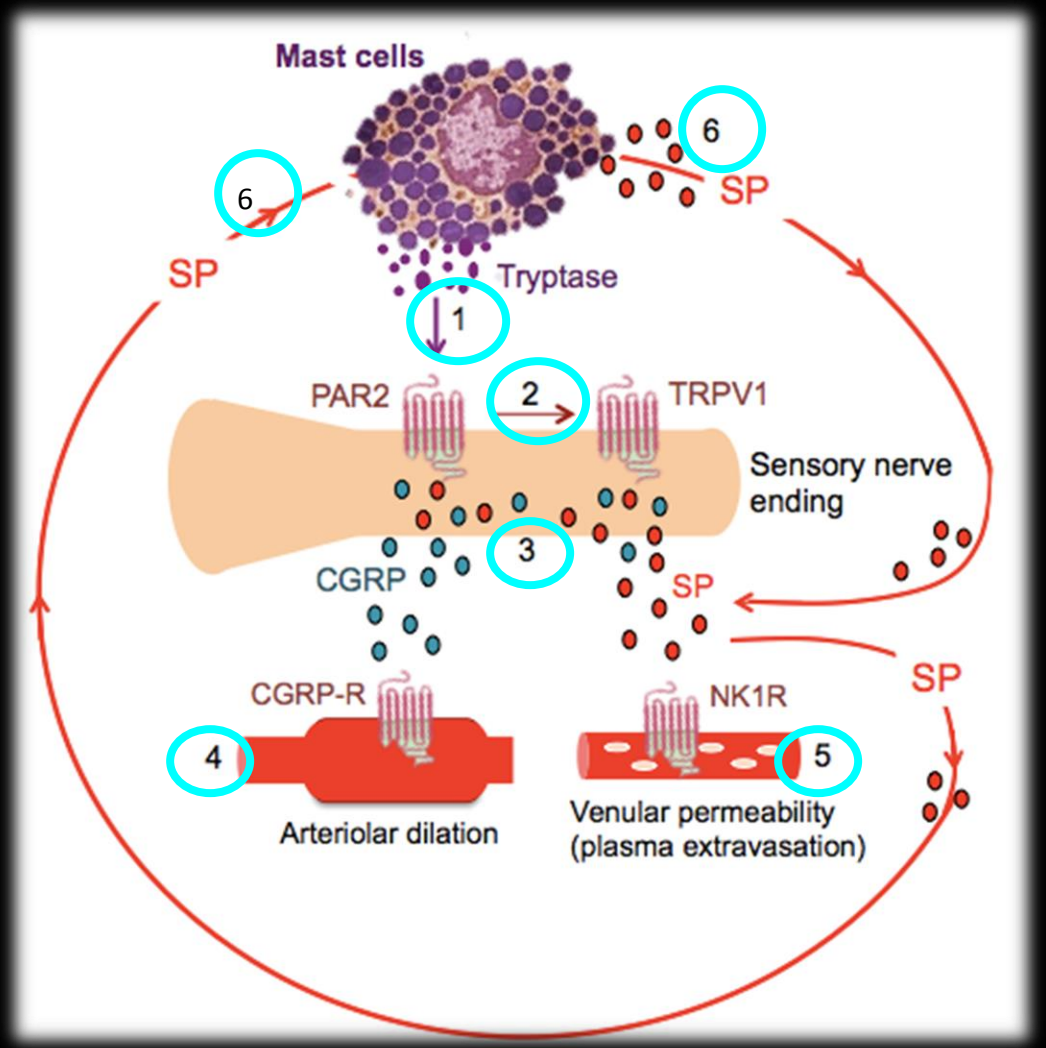
Imatinib treated mice



Mast cell lacking sickle mice (yellow)



A vicious cycle of mast cell degranulation and nociceptor activation leads to pain in SCA



(1) Mast cell-tryptase cleaves PAR2 on peripheral nociceptors

(2) Activation of PAR2 sensitizes TRPV1 channels on nerve endings

(3) Activation of PAR2 and TRPV1 leads to release of CGRP and SP from the sensory nerve endings

(4) CGRP may stimulate arteriolar dilatation.

(5) SP stimulates plasma extravasation leading to neurogenic inflammation

(6) SP acts in a paracrine and autocrine manner by activating mast cells thus promoting a vicious cycle of mast cell degranulation and nocicepor activation.

Vincent et al. "Plenary Paper" Blood 2013;122:1853-62.
Editorial Commentary by Kutlar A "GLEE-ful for sickle pain?"

Do studies on mice translate into clinically relevant outcomes? LIT or FIT?

Chronic myeloid leukemia and sickle cell disease: could Imatinib prevent vaso-occlusive crisis? Stojanovic et al., Br J Hematol 2011

Homozygous SCD with CML; 5 VOCs in one year; Imatinib Tx led to rapid decrease in WBC counts and NO VOC occurred

"Each time that the patient forgot to take imatinib for several days, VOC recurred after 2 or 3 days, and stopped immediately when the patient took imatinib again." …."The recurrence of VOC after discontinuation of imatinib was not explained by the recurrence of increased WBC counts, nor by changes in haemoglobin concentration or HbF level, or haemolysis tests, which suggests that another mechanism was involved in the prevention of acute VOC due to sickle cell disease. Thus, tyrosine kinase inhibitors should be added to the list of potential treatments for SCD.."

CASE REPORT

Fatal Overdose Due to Prescription Fentanyl Patches in a Patient With Sickle Cell/-Thalassemia and Acute Chest Syndrome

A Case Report and Review of the Literature

*Olaf J. Biedrzycki, BSc, MBBS, MRC(Path), DMJ(Path), MFFLM,**
*David Bevan, MRCP,† and Sebastian Lucas, FRC(Path)**
Am J Forensic Med Pathol 2009

- Regular Fentanyl analgesia for >18 mo after developing myoclonic jerks while on pethidine

Sickle crises type pain for 2 days

Itchy

Acute chest syndrome and respiratory depression secondary to fentanyl overdose

- Blood mast cell tryptase = 76 ug/L (normal 2-14 ug/L)

Acute sickling of RBCs in lung arterioles and capillaries

- Cause of death: Acute anaphylaxis shock due to opioid-induced mast cell release of histamine, etc? Fentanyl is a weak stimulant but due to high levels of fentanyl might have caused mast cell degranulation.

FIT

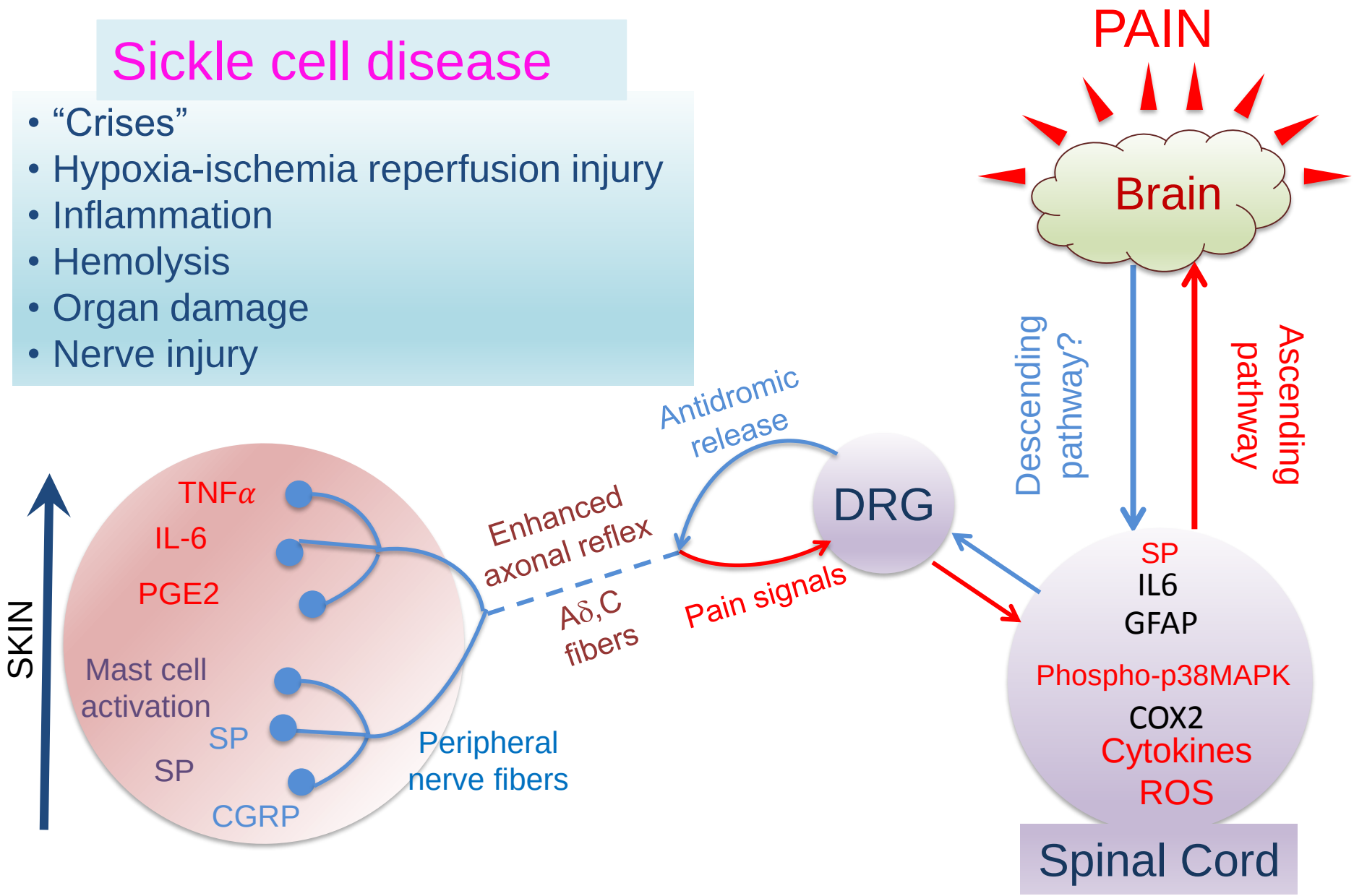
- Cromolyn sodium nasal spray and hydroxyurea for 3 months <u>significantly reduced pain and sickling.</u> Karimi M et al., Clinical Response of Patients with Sickle Cell Anemia to Cromolyn Sodium Nasal Spray. Am J Hematology 81: 809-16, 2006

Therefore, targeting mast cells in SCA may improve morphine analgesia and/or have disease modifying effects leading to reduced pain.

Peripheral & central mechanisms of nociception

Sickle cell disease

- "Crises"
- Hypoxia-ischemia reperfusion injury
- Inflammation
- Hemolysis
- Organ damage
- Nerve injury



Nociceptor sensitization

Aich et al., 2016

Amplification and central sensitization

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Lab Members
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Fei Peng

U of M facilities
EM facility
UIC

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Thank You