July 9, 2015, Ion Channel Retreat, Vancouver
Ion Channel and Pain Targets

Modulation of TRP channels by resolvins in mouse and human

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Duke Anesthesiology

Omega-3 polyunsaturated fatty acids

Dietary essentially fat

↓

Omega-3 polyunsaturated fatty acids

↓ ↓

Docosahexaenoic acid (DHA)	Eicosapentaenoic acid (EPA)

↓

Powerful metabolic products

(Resolvins)

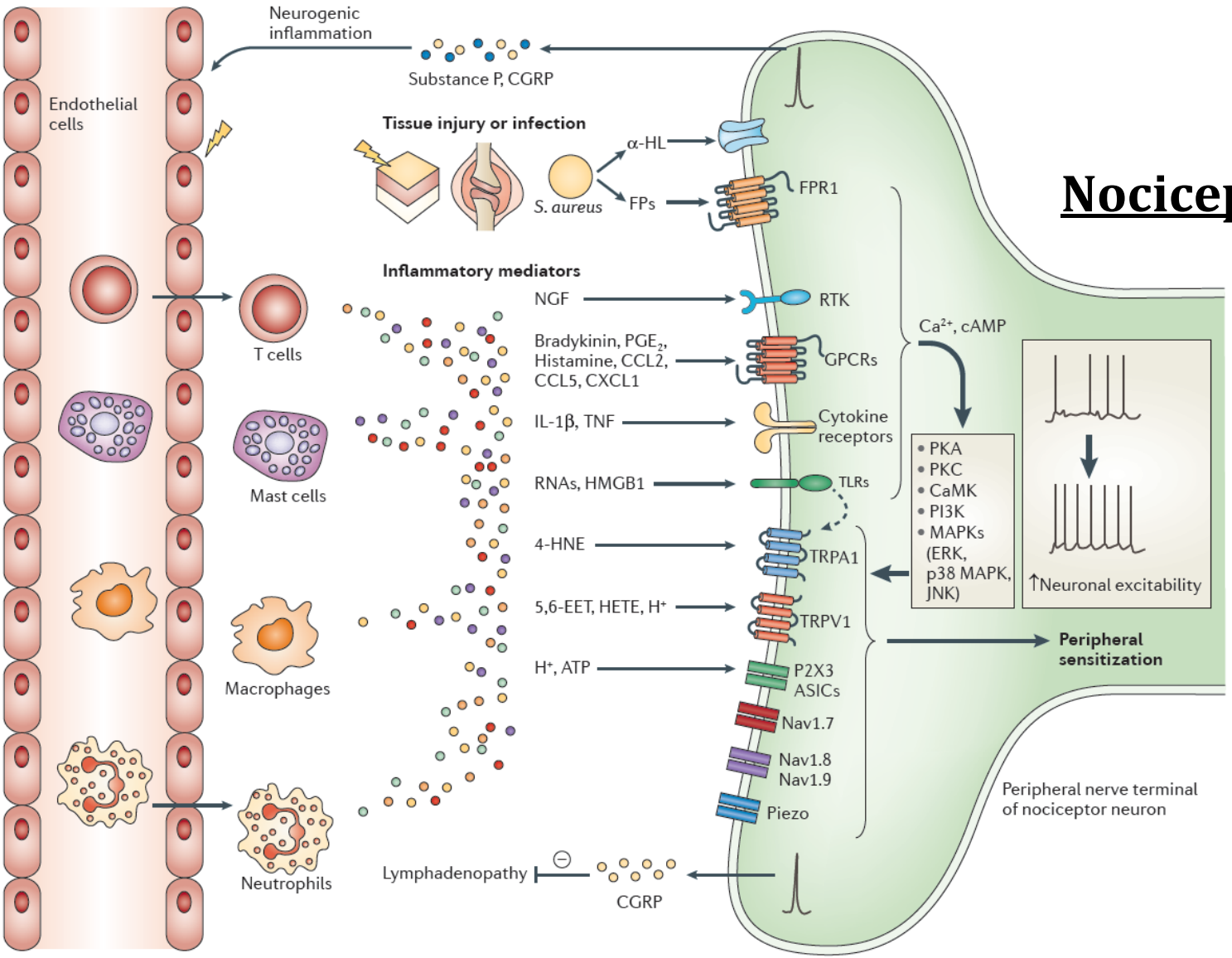
Ji et al., *Trends Neurosci*, 2011

Chronic pain is a major heath problem

- **Over 100 million Americans have chronic pain**
- **The total financial cost of is near 600 billion a year in the US.**
- **Annual US expenditure on pain (medical costs and lost wages) is higher than that for cancer, heart disease, and diabetes combined.**
- **Co-morbidity: Chronic pain is associated with depression, anxiety, insomnia, etc.**
- **Prevention and treatment of this epidemic are equally important.**

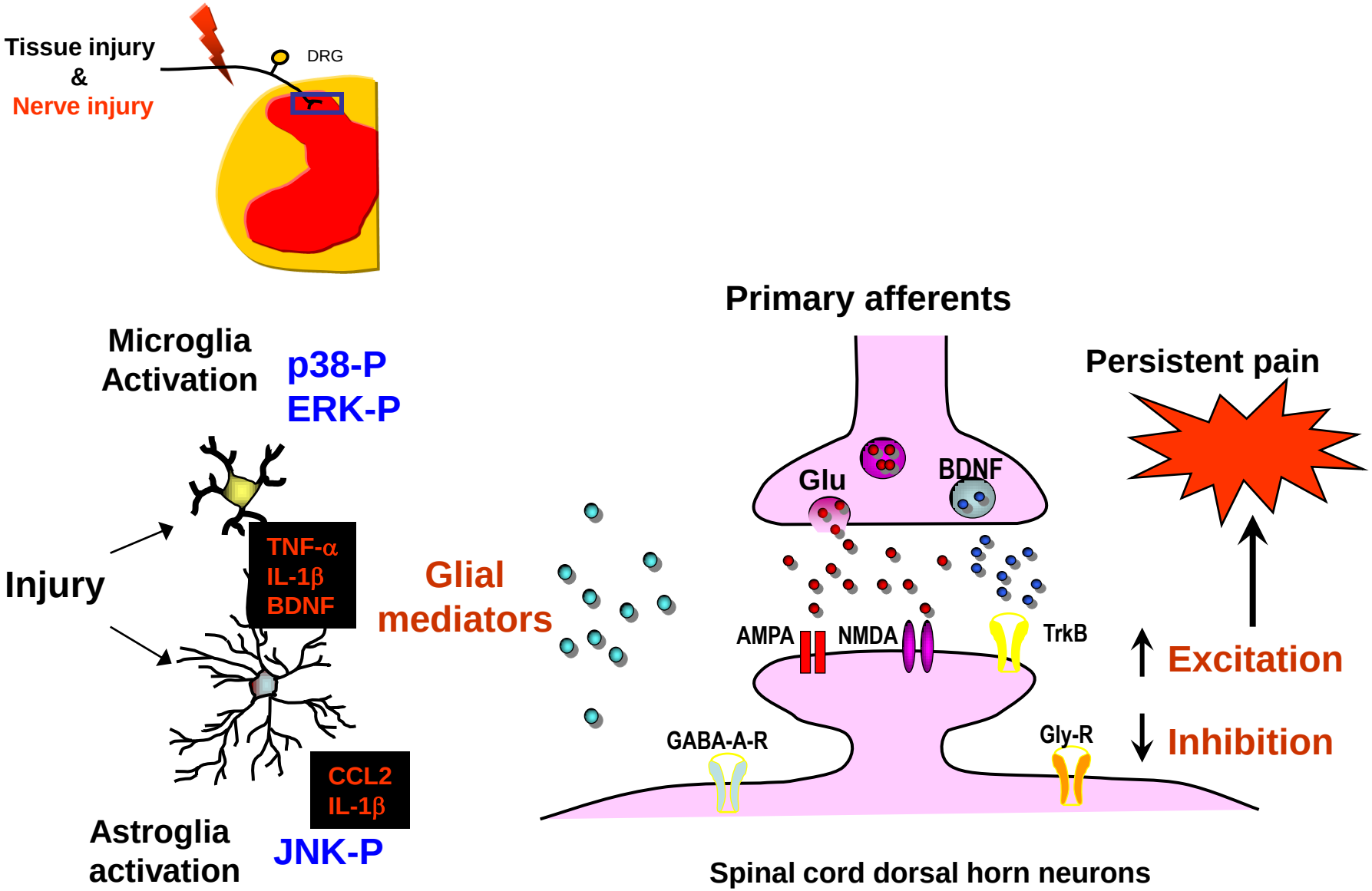
Pizzo & Clark, N Engl J Med., 2012
American Pain Society

Peripheral mechanisms of pain



Ji et al., Nat Rev Drug Disco., 2014

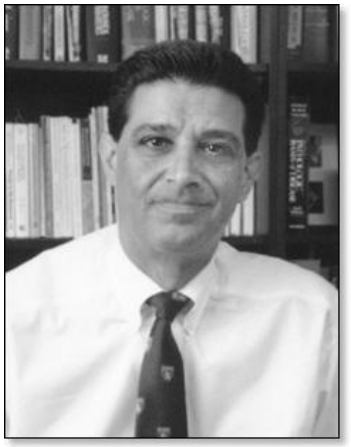
Central mechanisms of pain

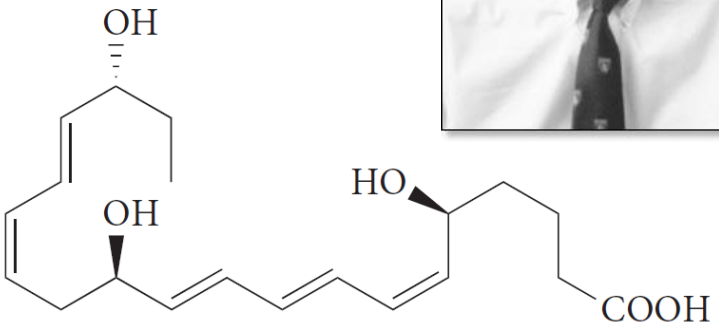


Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators

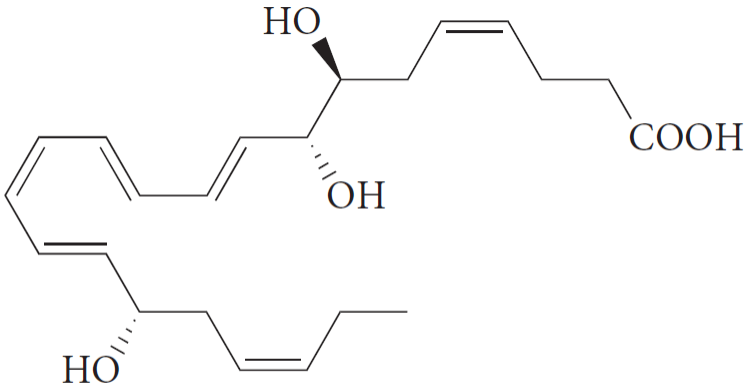
Charles N. Serhan, Nan Chiang* and Thomas E. Van Dyke‡*

Charles Serhan





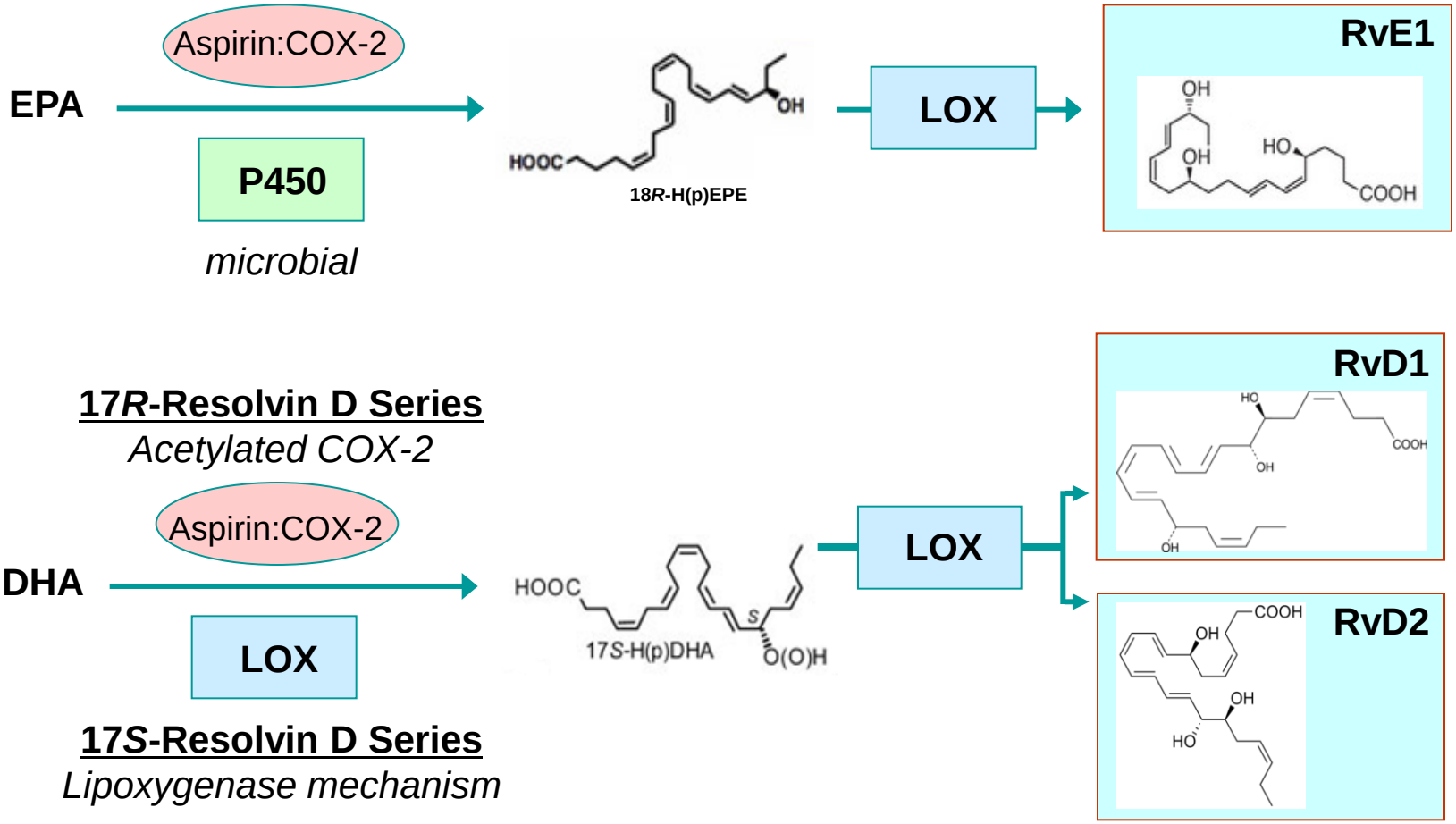
Resolvin E1 **(RvE1)**



Resolvin D1 **(RvD1)**

Serhan et al., 2008; *Nature Reviews Immunology*

Biosynthesis of resolvins from PUFA Precursors



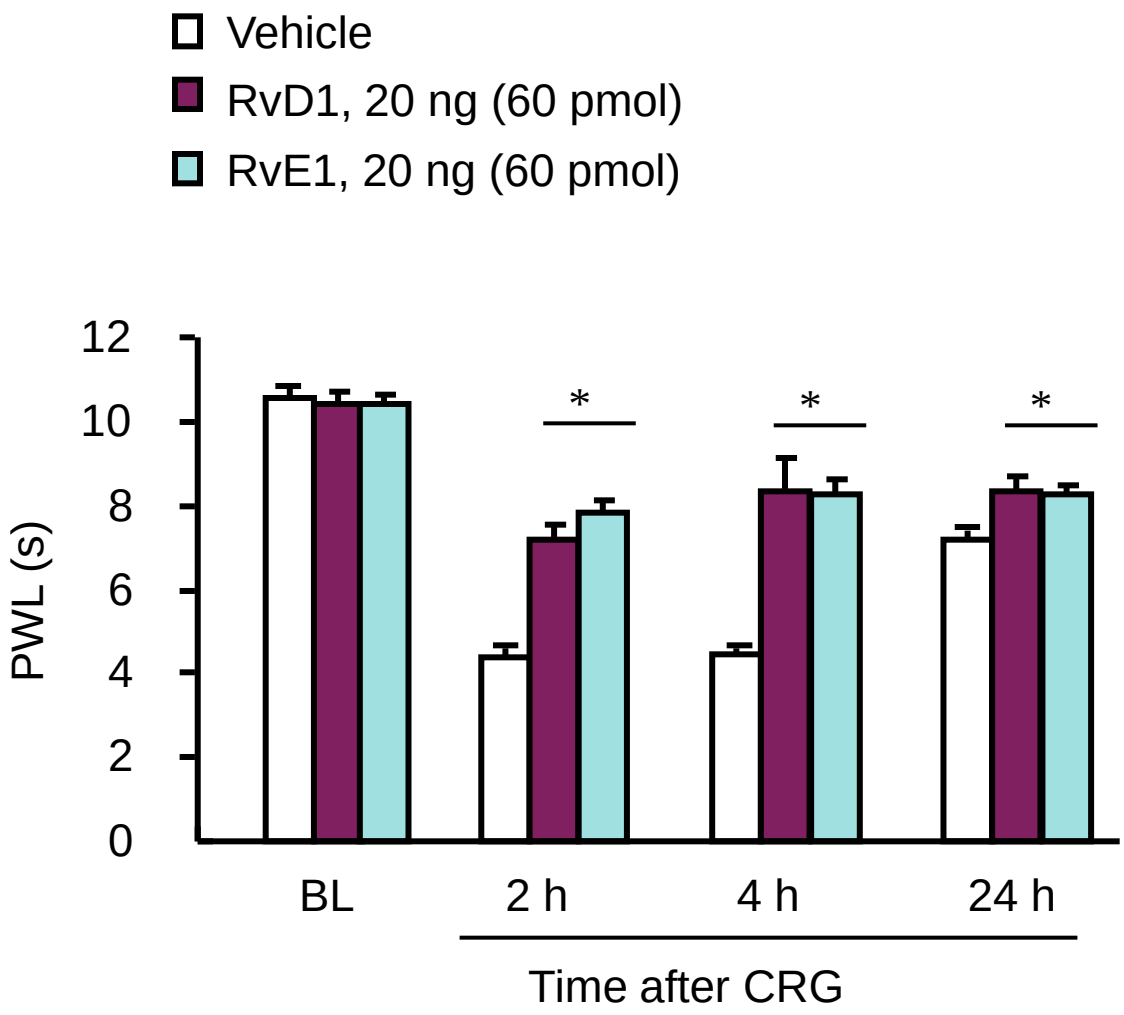
Resolvins are potent inhibitors of inflammation in various animal models

Table 1. Anti-inflammatory actions of resolvins in animal models of inflammation

Resolvin	Animal	Inflammatory condition	Anti-inflammatory effects	Refs
RvE1	Mouse	Dorsal air pouch	Stops neutrophil recruitment	[42]
		Peritonitis	Reduces neutrophil recruitment	[49,59]
			Regulates cytokine and chemokine expression	[49,59]
		Retinopathy	Alleviates neovascularization and reduces TNF-α expression	[61]
		Colitis	Decreases neutrophil recruitment and improves survival	[62]
		Allergy	Resolves allergic airway inflammation	[63,64]
			Regulates NK cell migration	[63,64]
		Ocular lesion	Controls HSV-induced ocular inflammatory lesions	[65]
			Increases IL-10 and decreases IL-6 expression	[65]
		Lung injury	Protects against bacterial pneumonia and acute lung injury	[66]
			Enhances clearance of bacteria	[66]
RvD1	Mouse	Kidney ischemia	Protects from ischemia–reperfusion-induced kidney damage	[67]
			Regulates macrophage function	[67]
		Peritonitis	Stops neutrophil recruitment and modulates miRNAs	[68]
		Dorsal skin air pouch	Stops neutrophil recruitment	[46]
		Oxidative stress	Controls inflammation during oxidative stress	[69]
AT-RvD1	Mouse	TMJ inflammation	Protects inflammation in the TMJ	[70]

Ji et al., Trends Neurosci, 2011

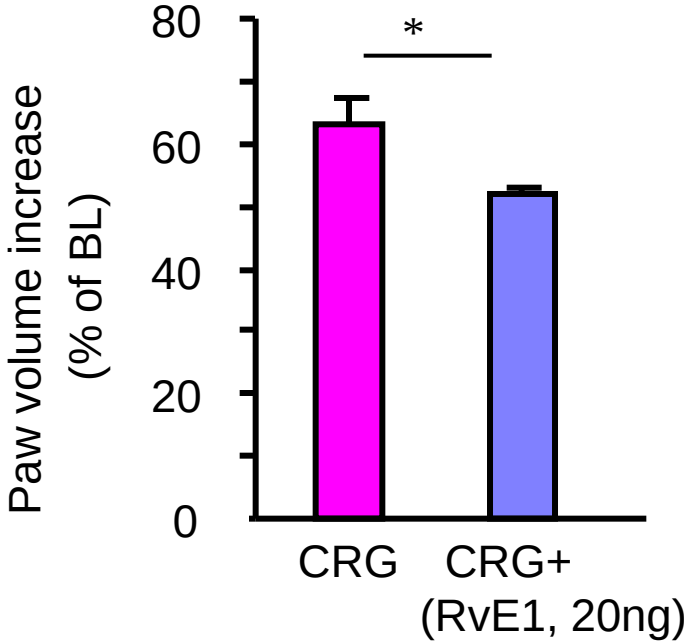
Resolvins prevent CRG-induced heat hyperalgesia



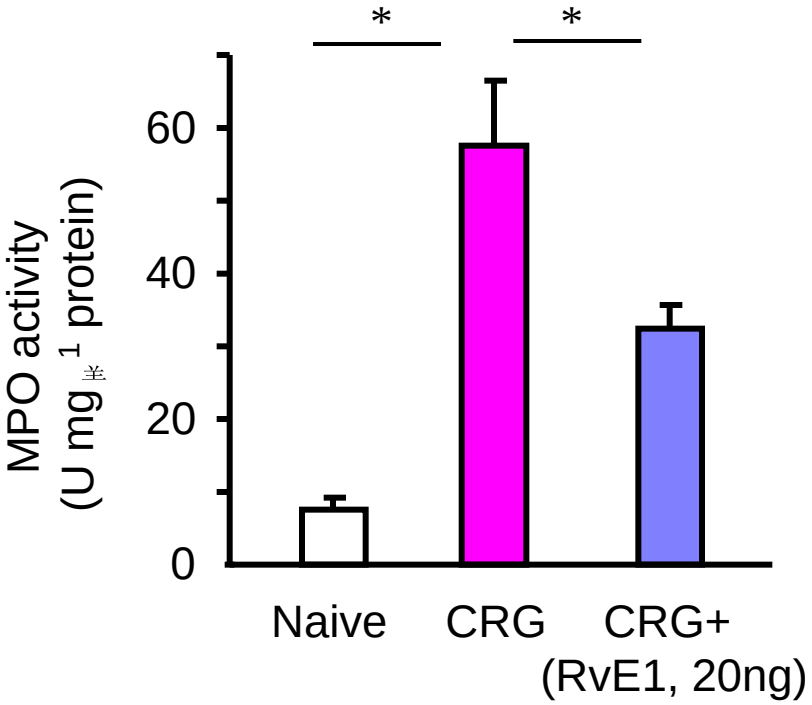
Xu et al., Nat Med, 2010

RvE1 inhibits CRG-induced inflammation

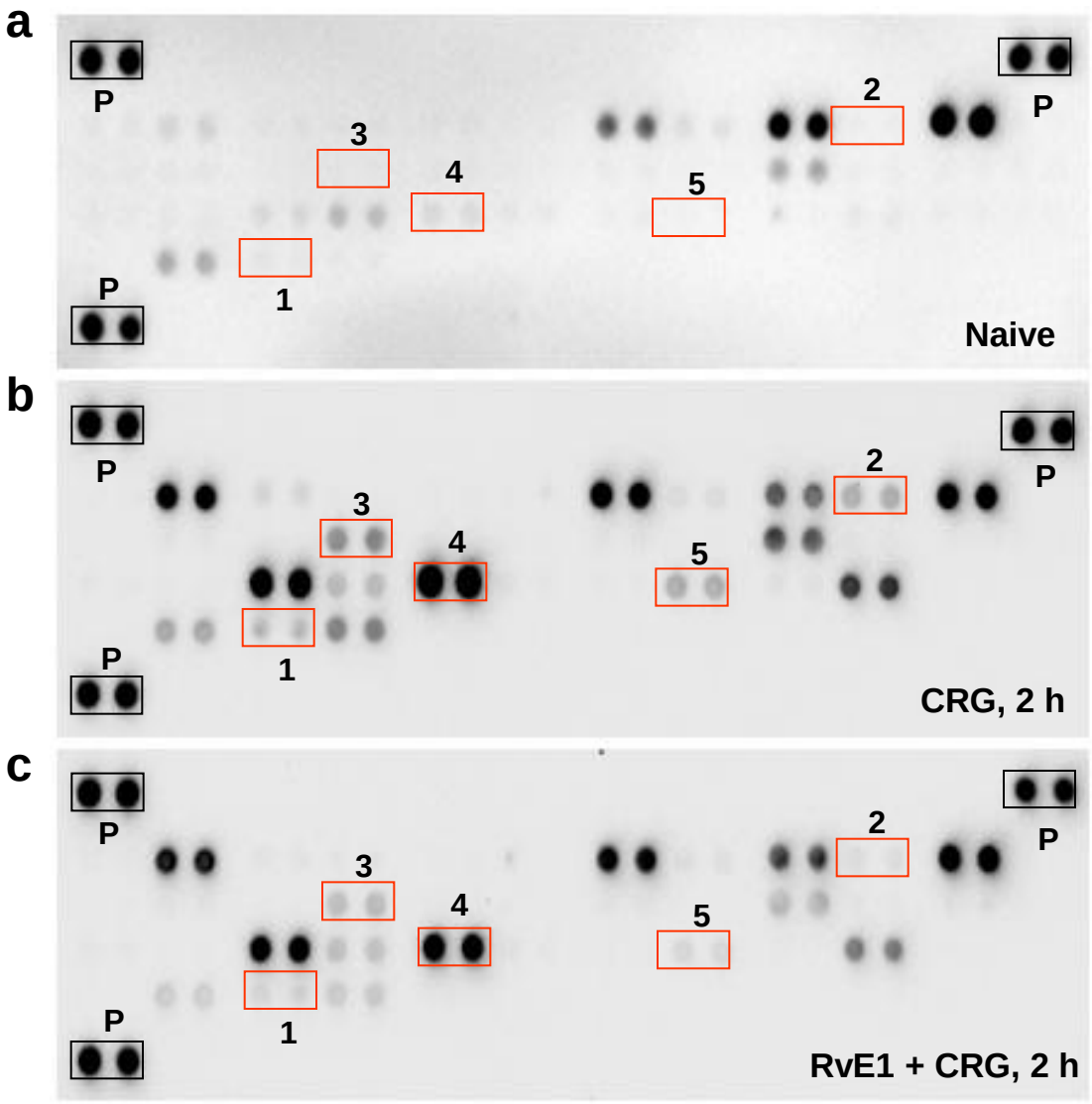
Paw edema



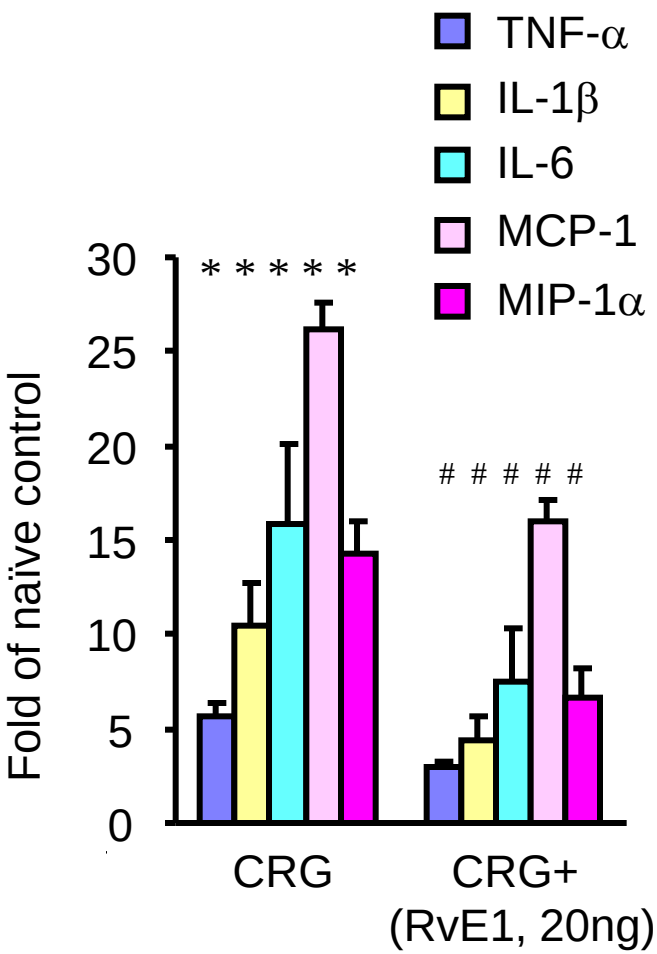
Neutrophil infiltration



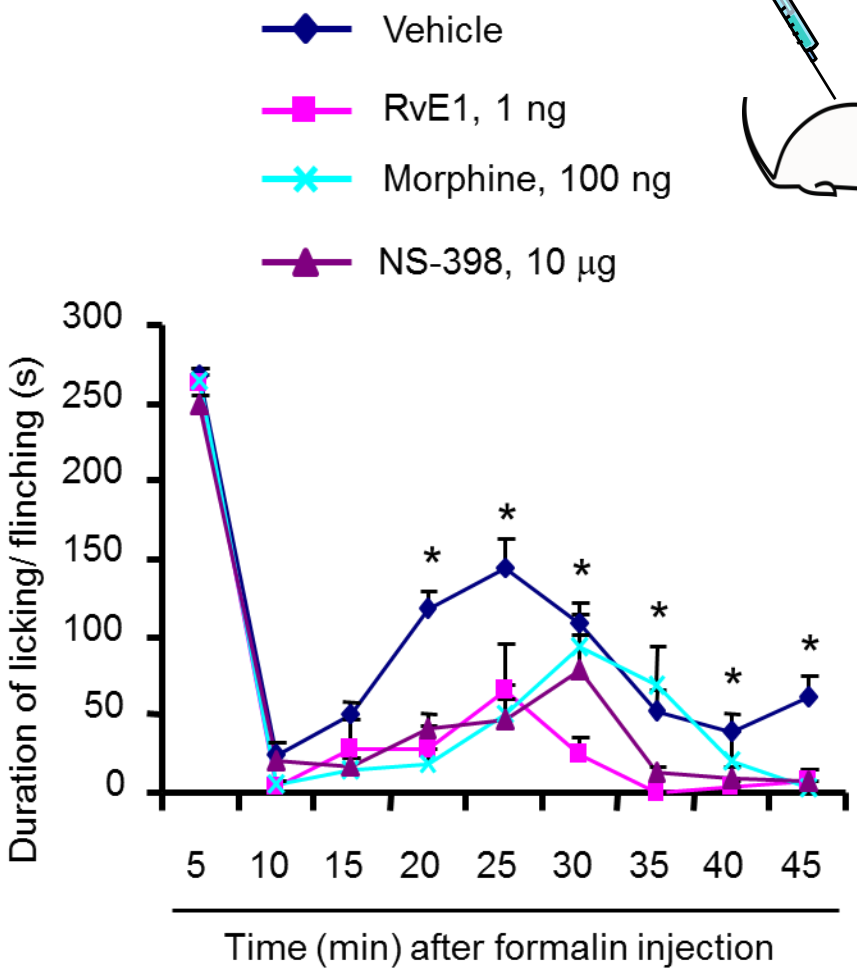
RvE1 inhibits cytokine/chemokine expression after inflammation

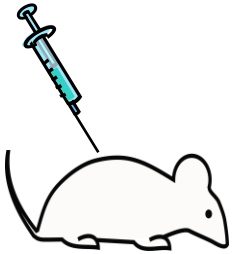


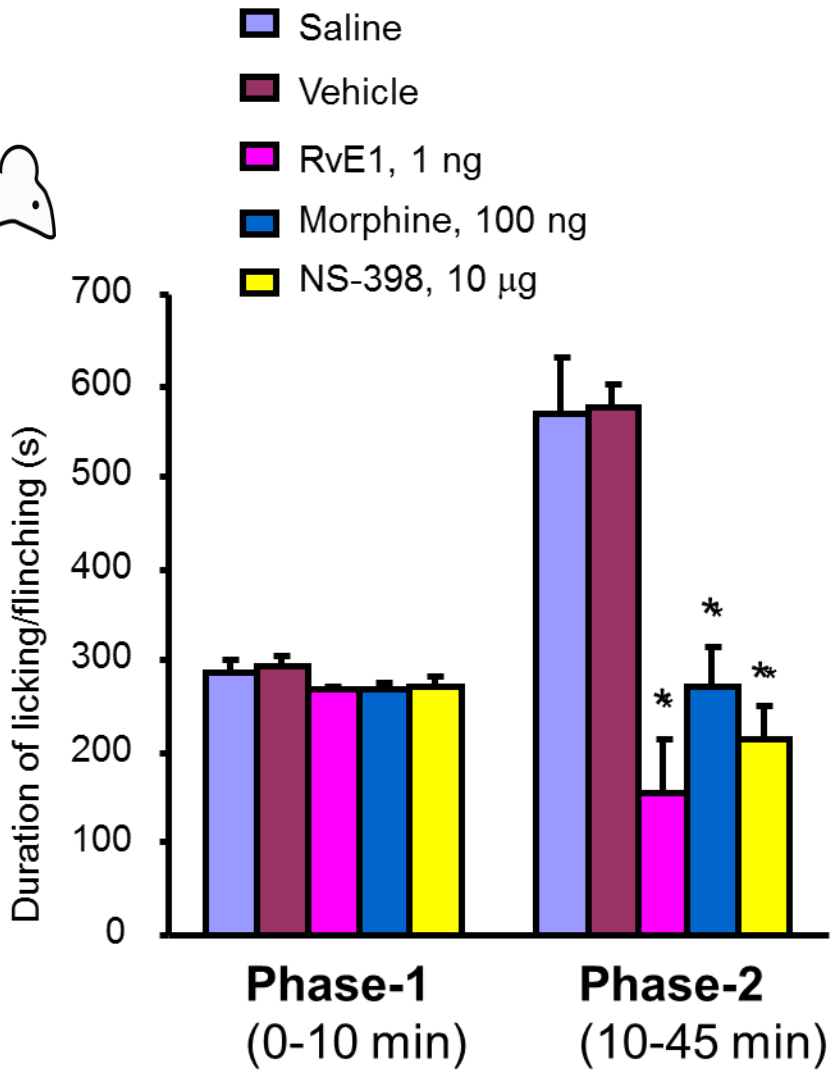
Cytokine array



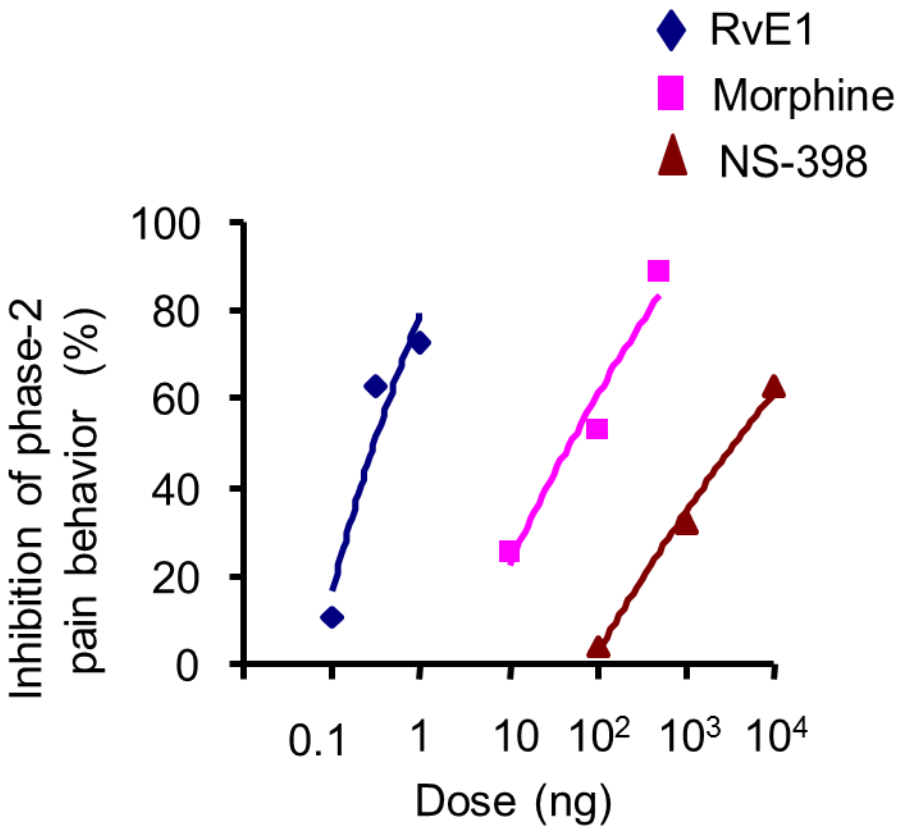
Formalin-induced spontaneous pain



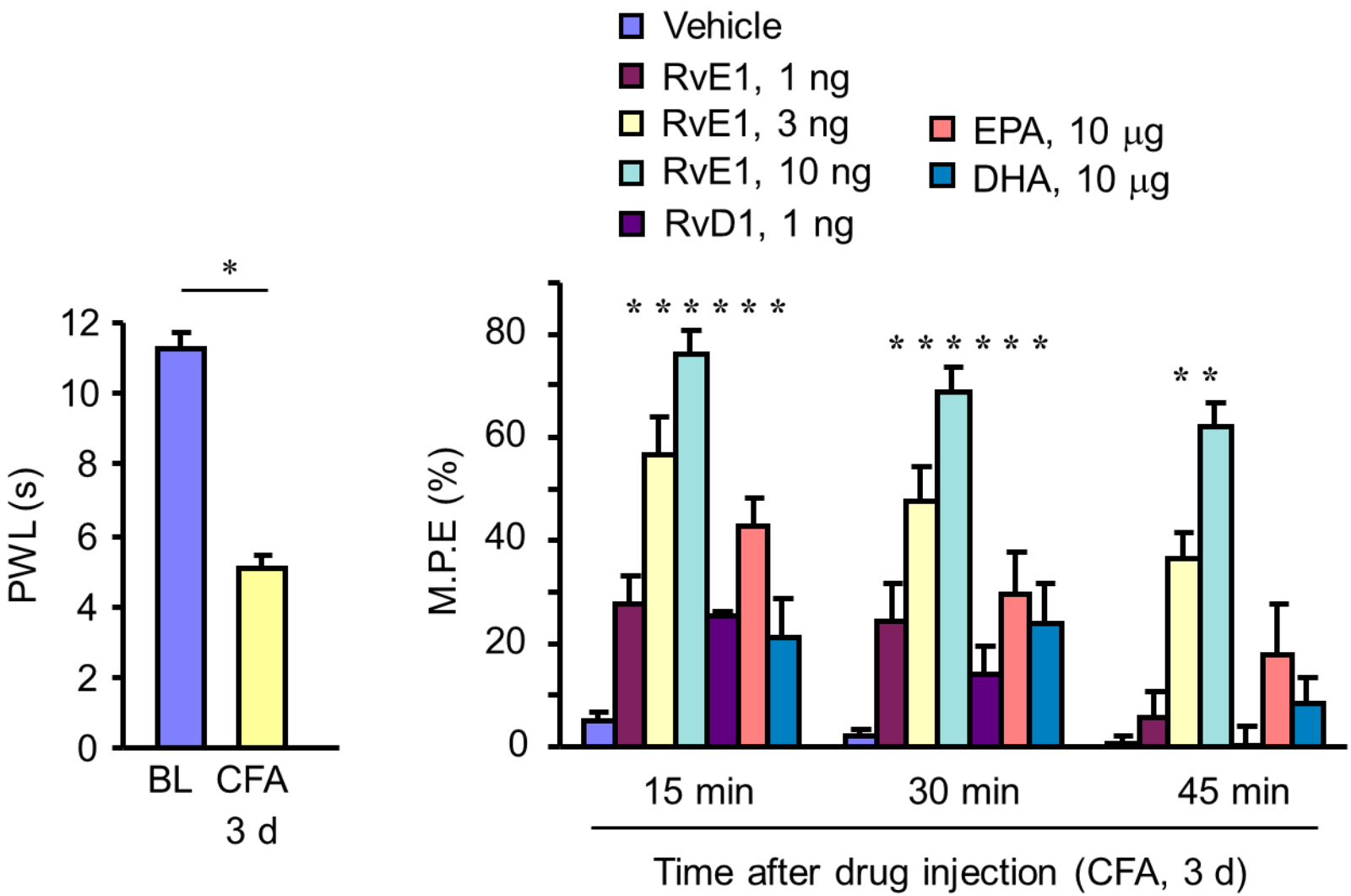




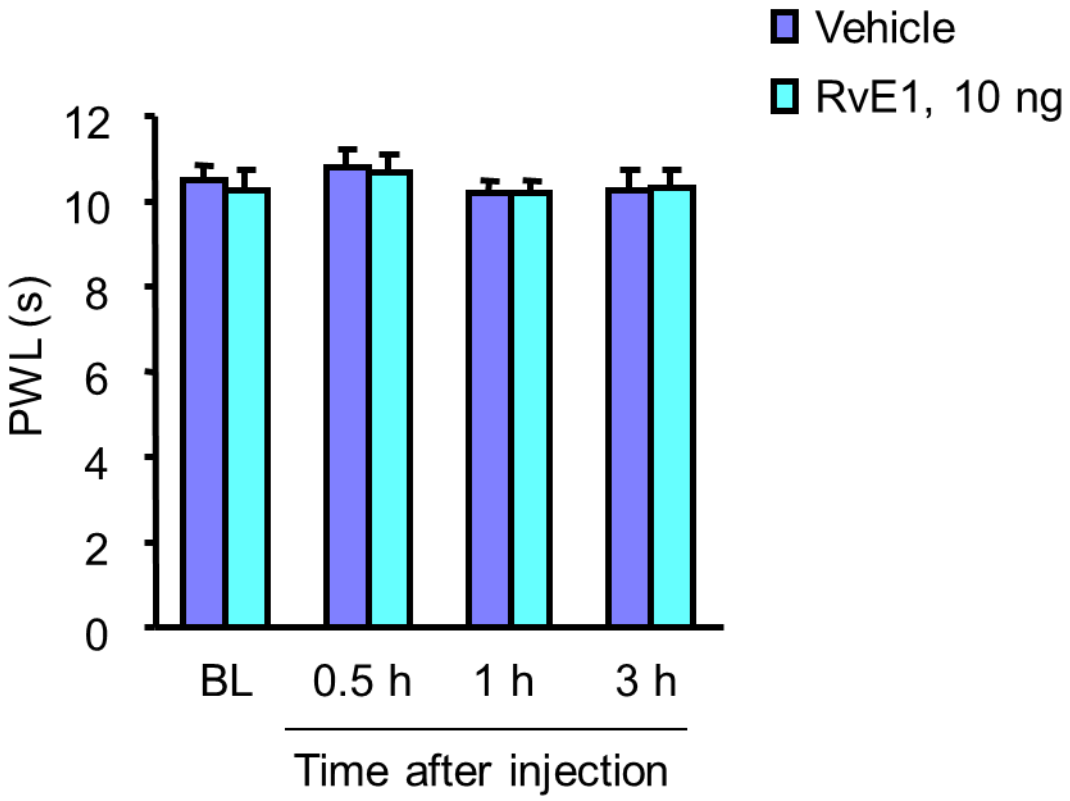
Formalin-induced 2nd-phase pain



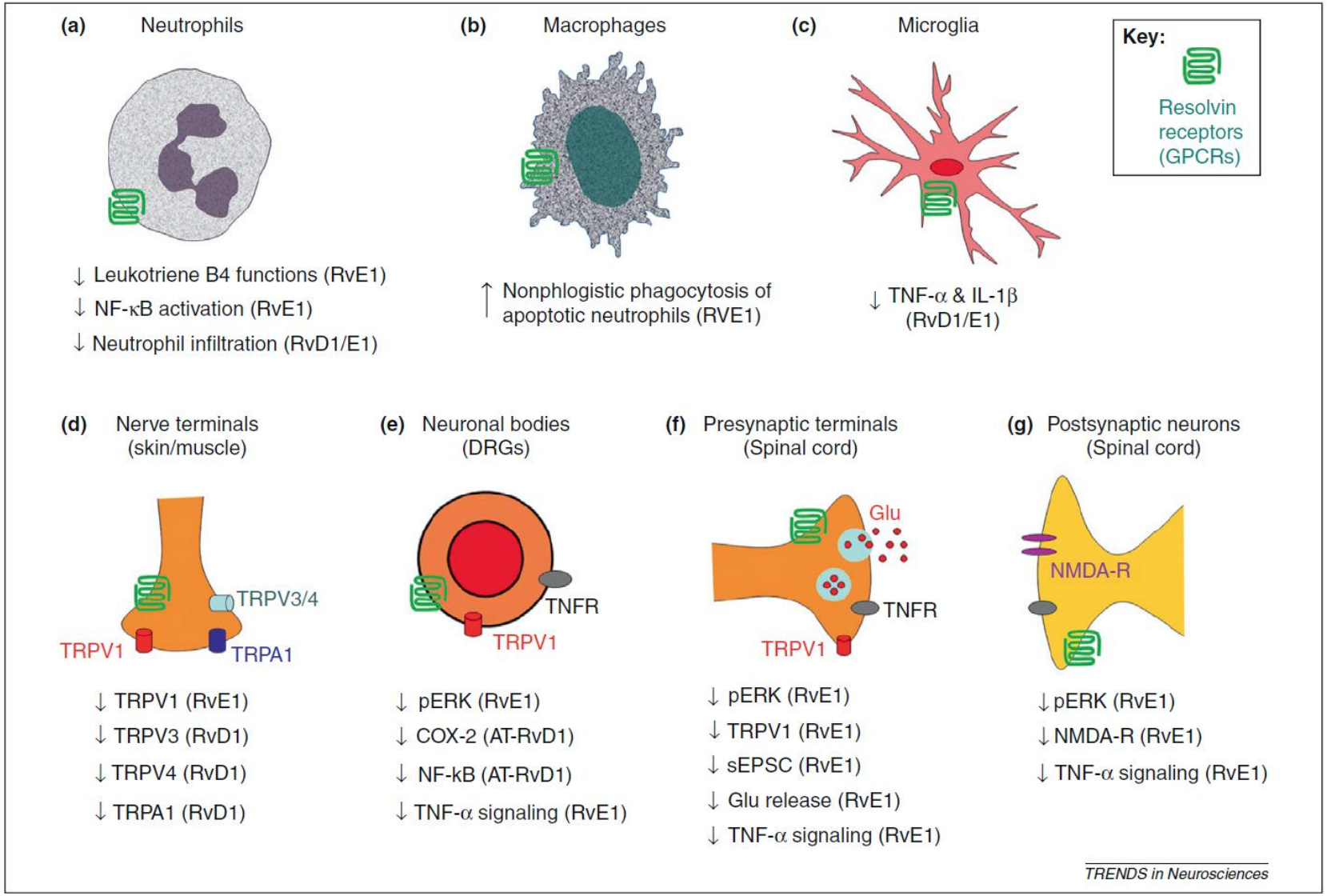
CFA-induced heat hyperalgesia
(Rapid effects: 15-45 min)



No effect of RvE1 on basal pain

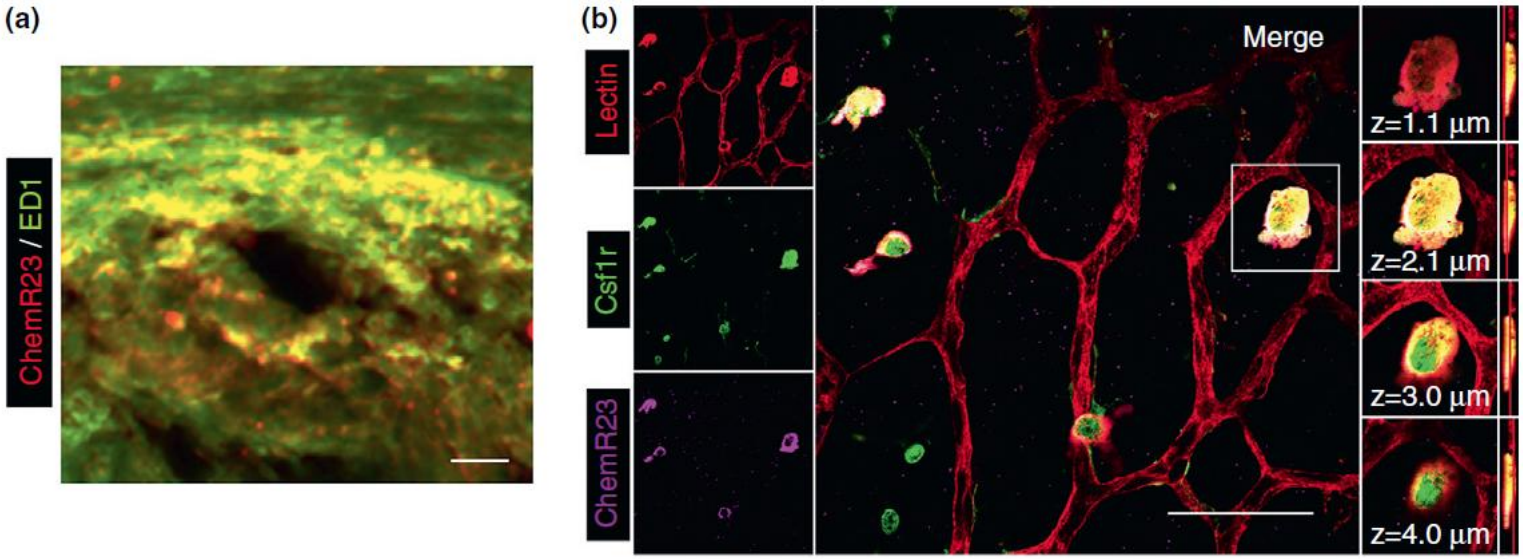


Broad actions of resolvins

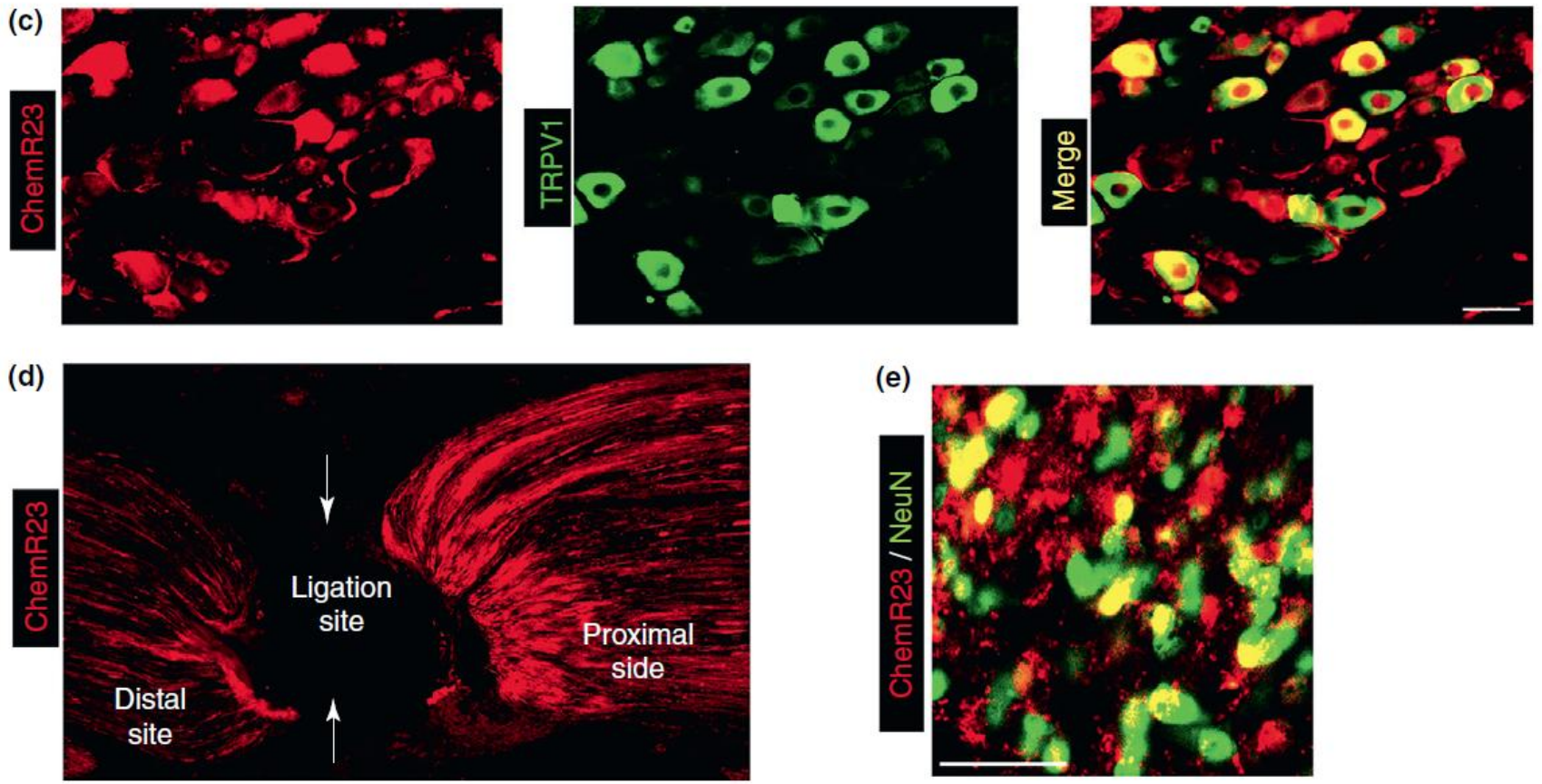


Ji et al., Trends Neurosci, 2011

ChemR23 expression in macrophages and microglia



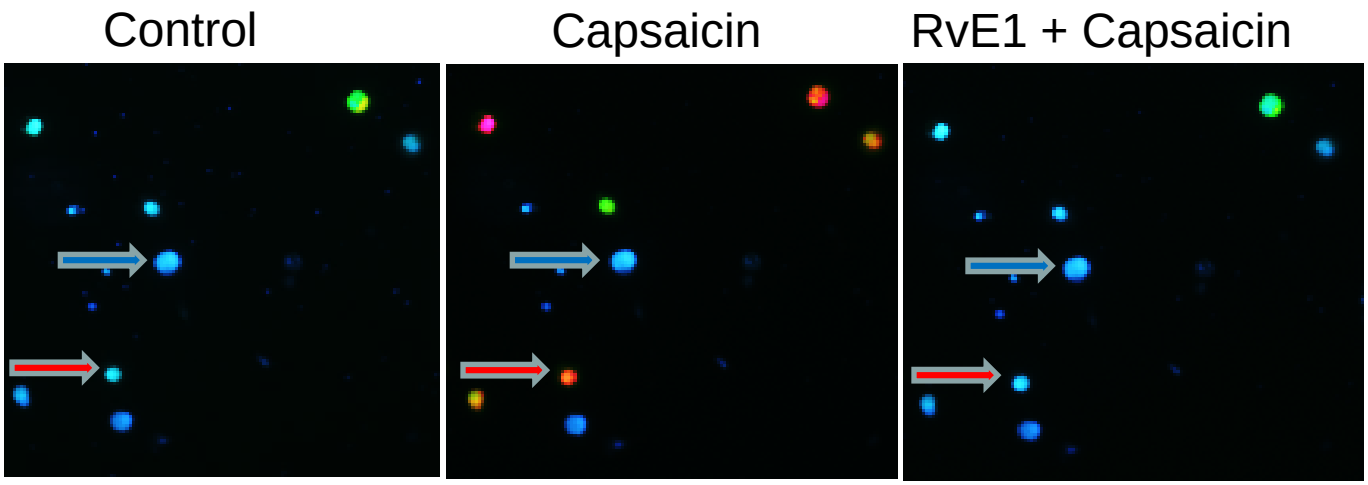
ChemR23 expression in neurons in the PNS and CNS

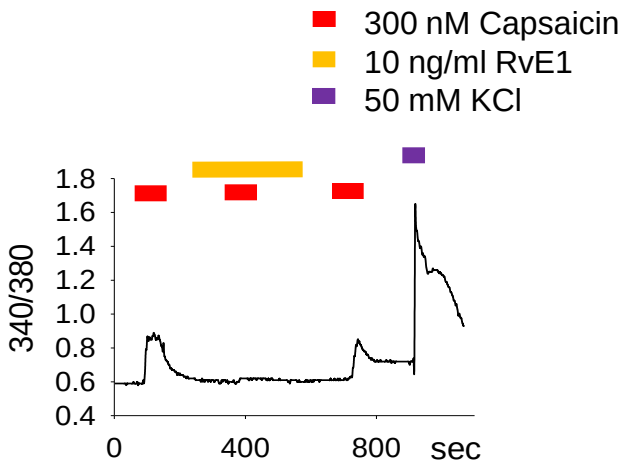


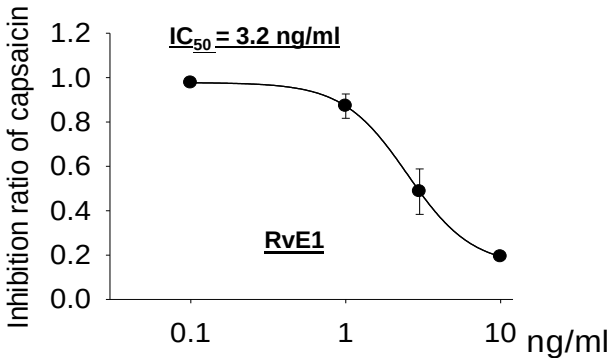
TRENDS in Neurosciences

Ji et al., Trends Neurosci, 2011

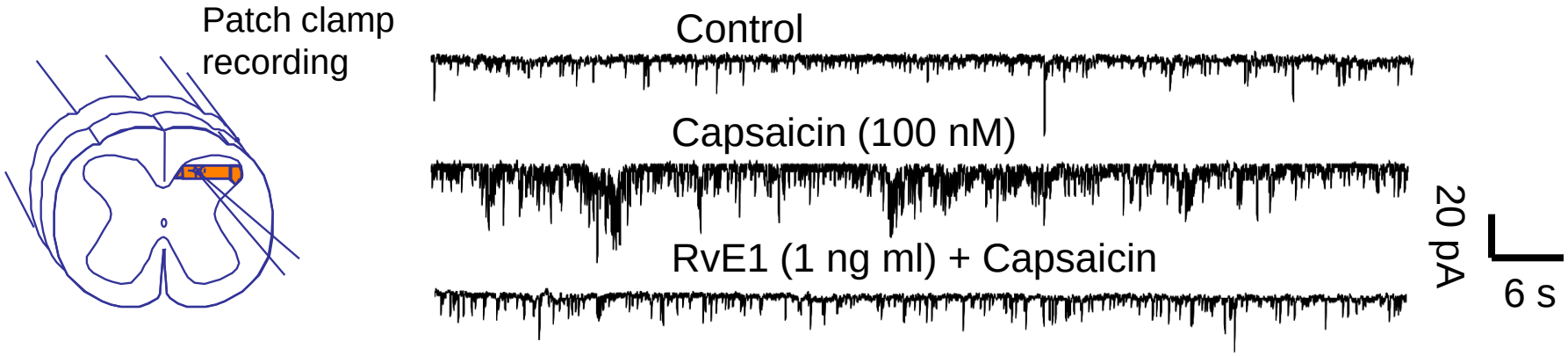
RvE1 blocks capsaicin-induced signaling in mouse DRG neurons

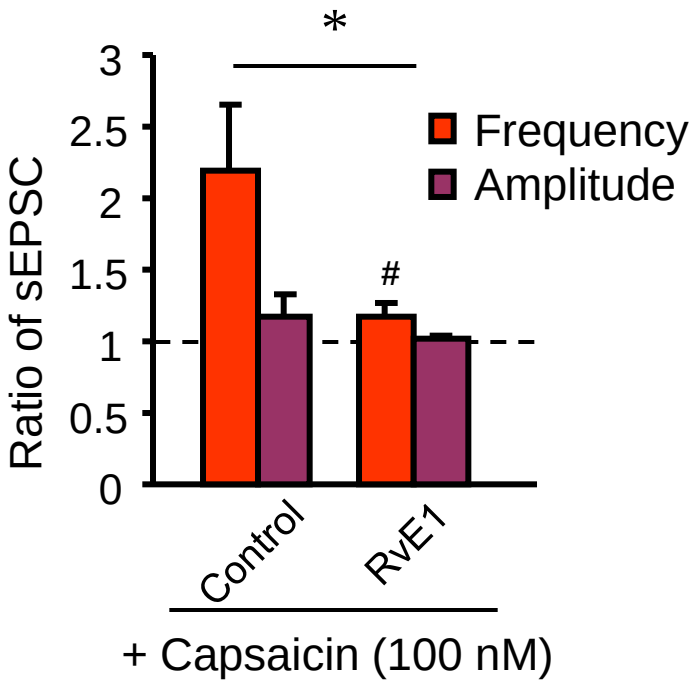


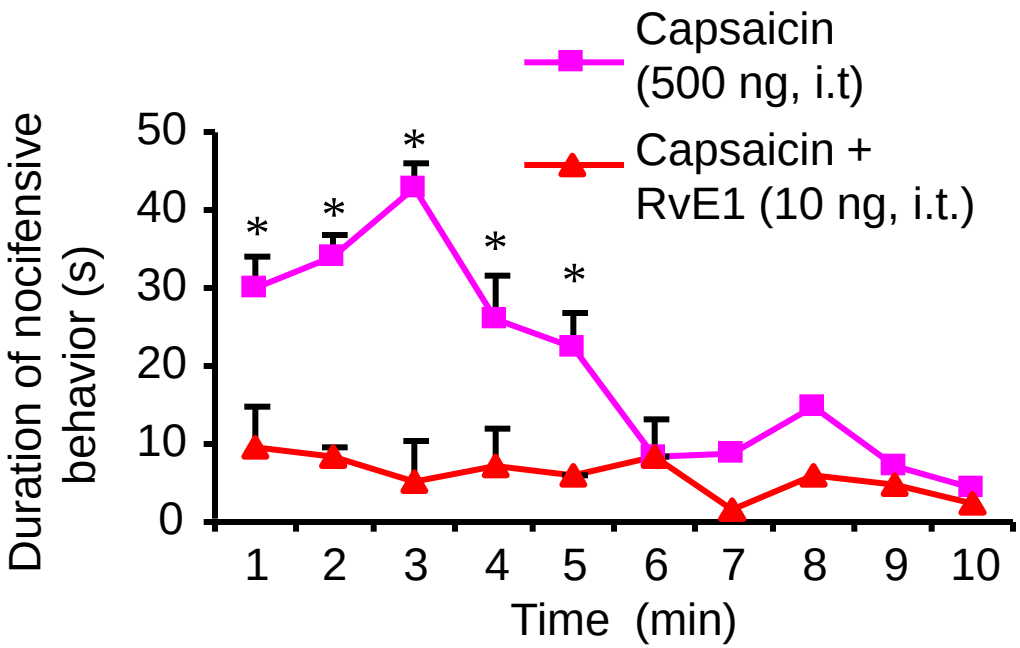


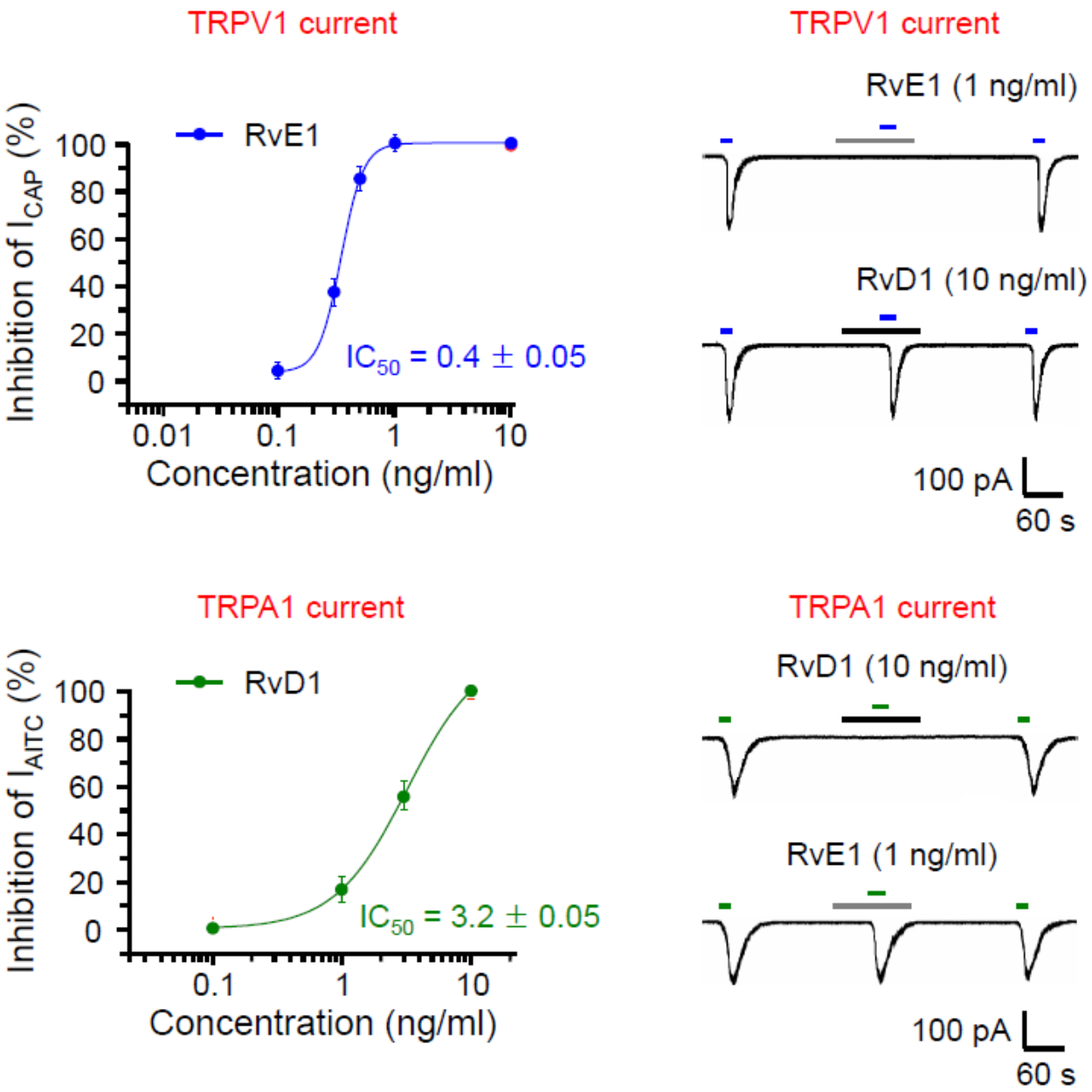


RvE1 blocks TRPV1-mediated spinal synaptic transmission & pain








TRPV1 current
RvE1
Inhibition of I_{CAP} (%)
100
80
60
40
20
0
0.01
0.1
1
10
Concentration (ng/ml)
IC_{50} = 0.4 ± 0.05
TRPV1 current
RvE1 (1 ng/ml)
RvD1 (10 ng/ml)
100 pA
60 s
TRPA1 current
RvD1
Inhibition of I_{AITC} (%)
100
80
60
40
20
0
0.1
1
10
Concentration (ng/ml)
IC_{50} = 3.2 ± 0.05
TRPA1 current
RvD1 (10 ng/ml)
RvE1 (1 ng/ml)
100 pA
60 s

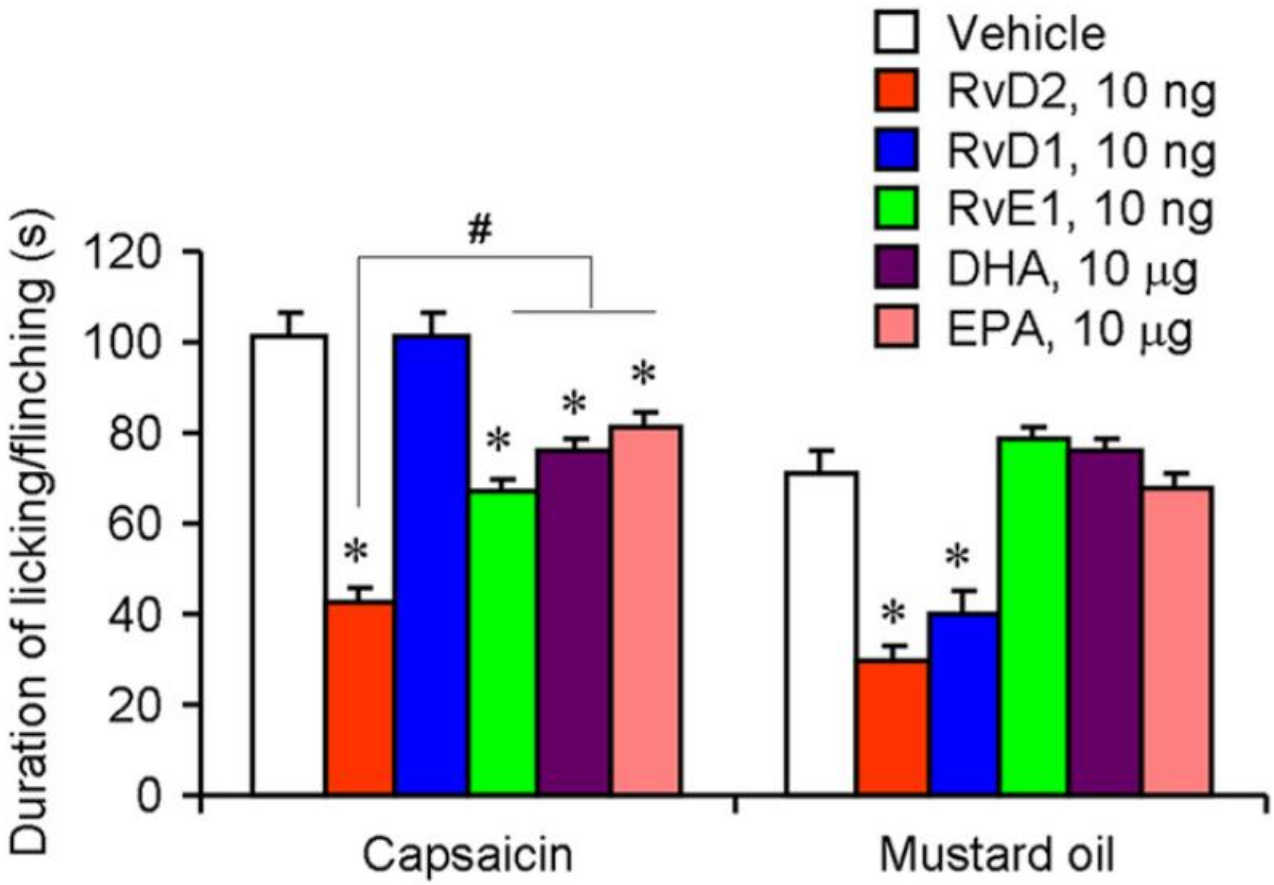
Resolvins are potent endogenous inhibitors of TRPV1/A1

Table 1. IC_{50} for inhibition of TRPV1 and TRPA1 currents by RvD2, RvD1, RvE1, and their fatty acid precursors in DRG neurons

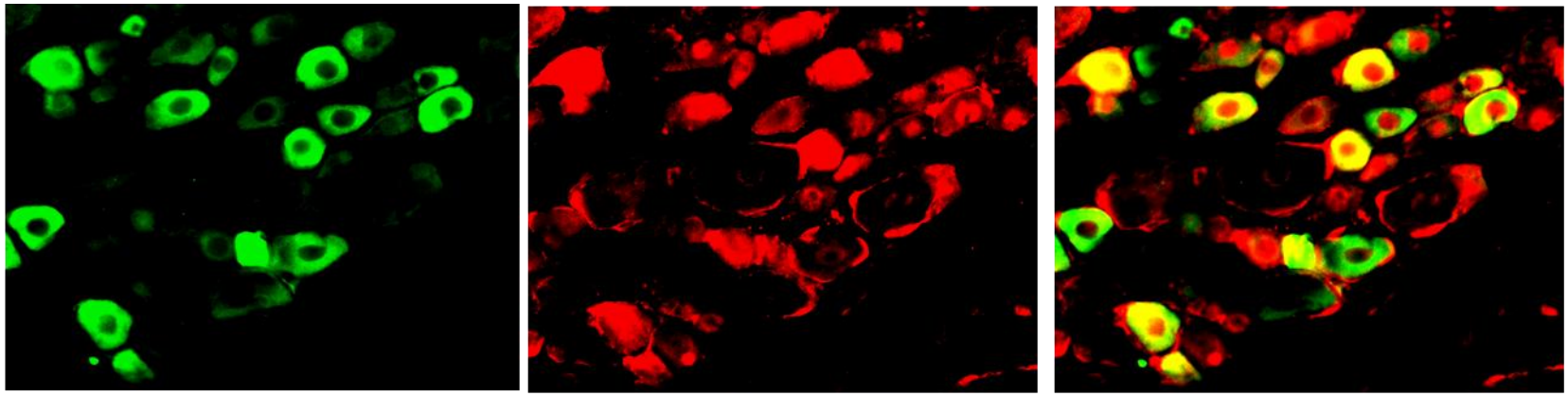
Inhibitors	Molecular weight	TRPV1 IC_{50} (nM)	TRPA1 IC_{50} (nM)
RvE1	350.4	1.0 ± 0.1	>28.5
RvD1	376.5	>26.6	8.5 ± 0.1
RvD2	376.5	0.1 ± 0.01	2.1 ± 0.5
DHA	328.5	1200.0 ± 20.0	>304,000.0
EPA	302.5	224.0 ± 10.0	>330,578.0

TRPV1 and TRPA1 currents were induced by capsaicin (100 nM) and AITC (300 μM), respectively.

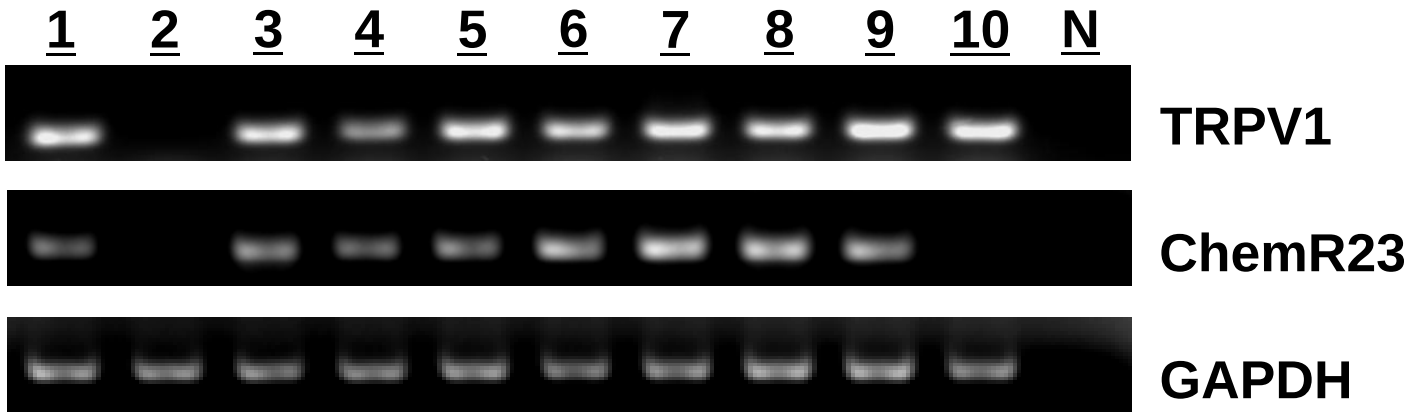
Capsaicin and mustard oil-induced pain



Resolvin E1 (RvE1) blocks TRPV1 current in mouse DRG neurons



TRPV1 ChemR23 Merge

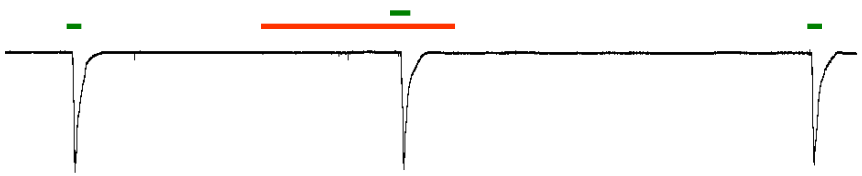


ChemR23 is sufficient to mediate RvE1's effect in HEK 293 cells

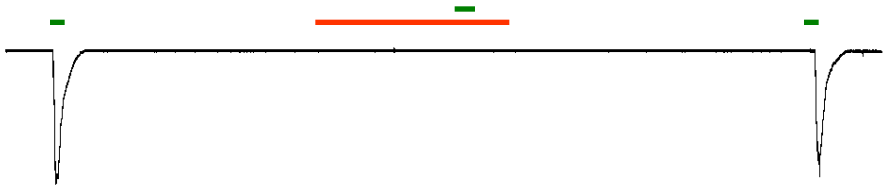
Capsaicin (100 nM)

RvE1 (10 ng/ml)

TRPV1-expressing HEK cells



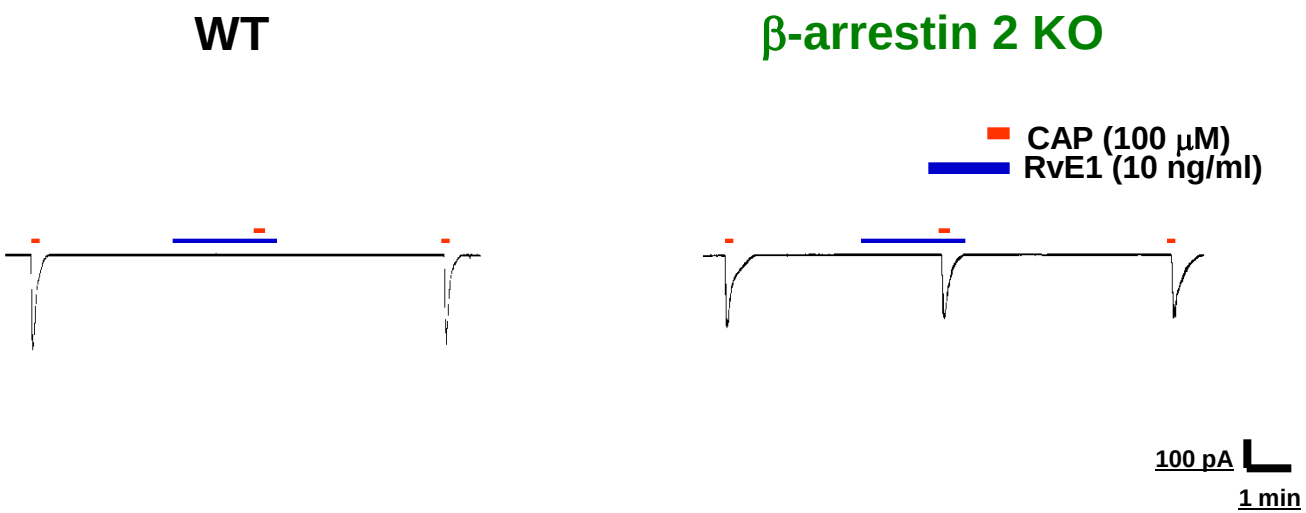
TRPV1 and ChemR23-coexpressing HEK cells

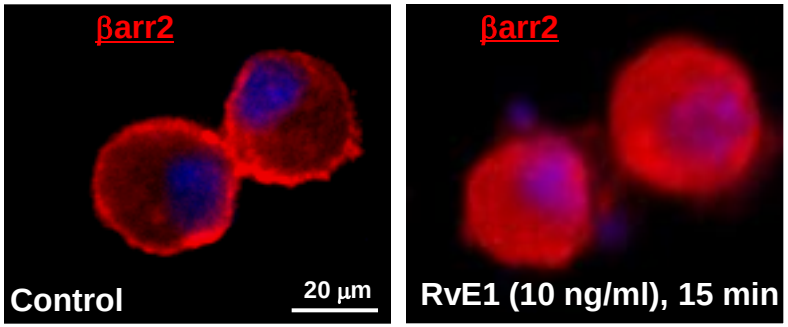


100 pA

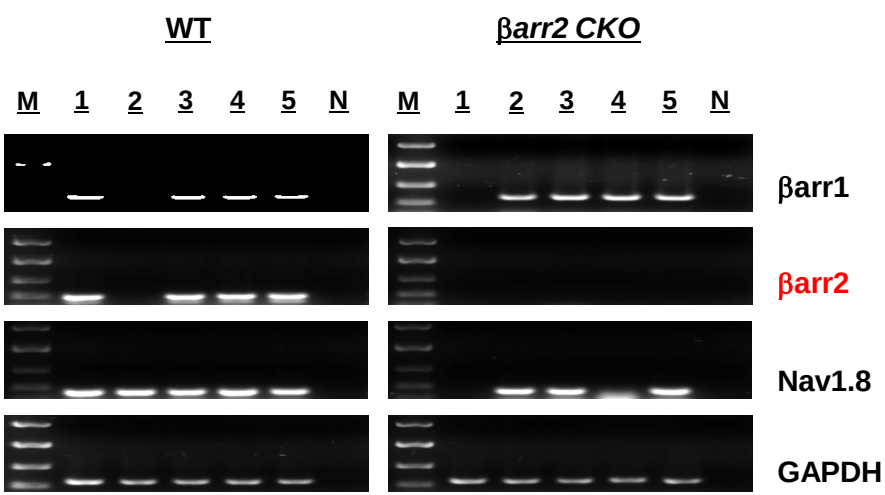
1 min

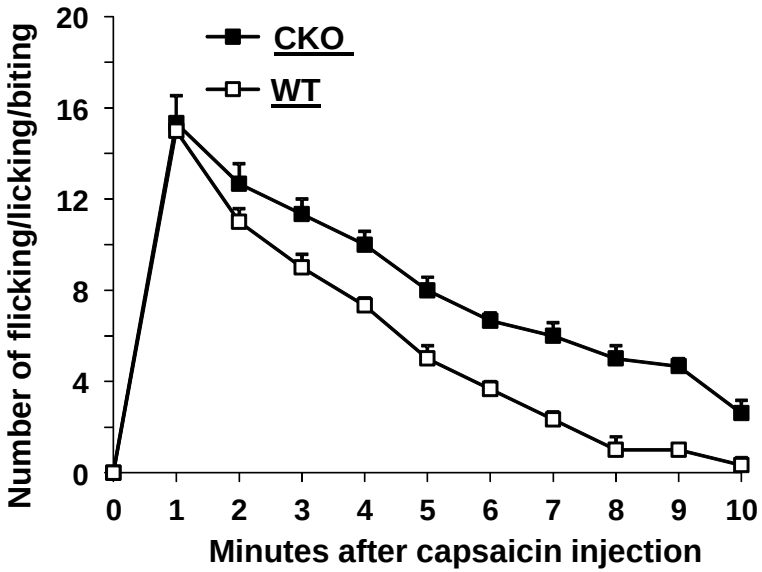
RvE1 fails to inhibit TRPV1 signaling in β-arrestin 2 KO mice



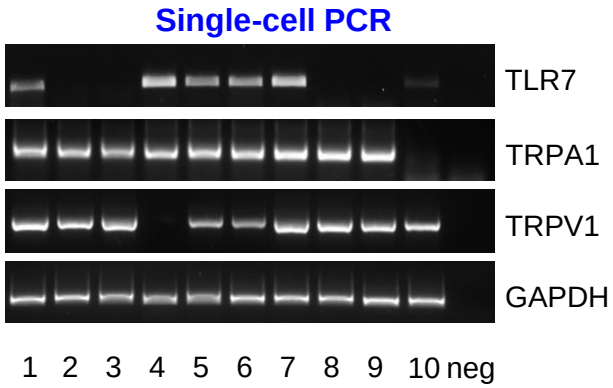


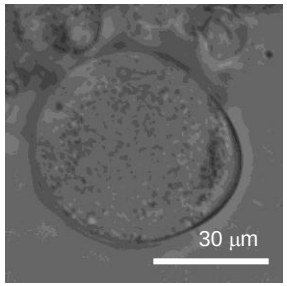
TRPV1 signaling is potentiated in β-arrestin 2 CKO mice

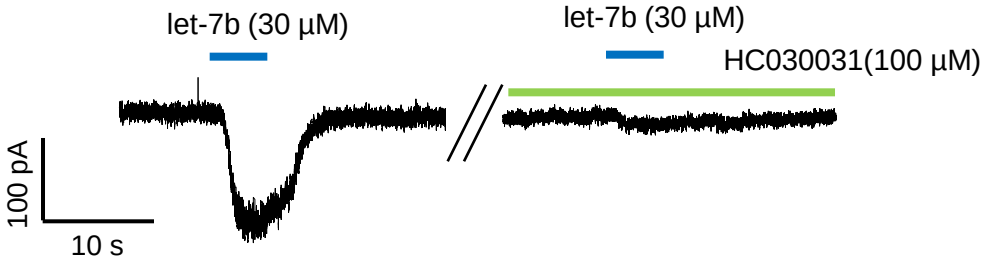




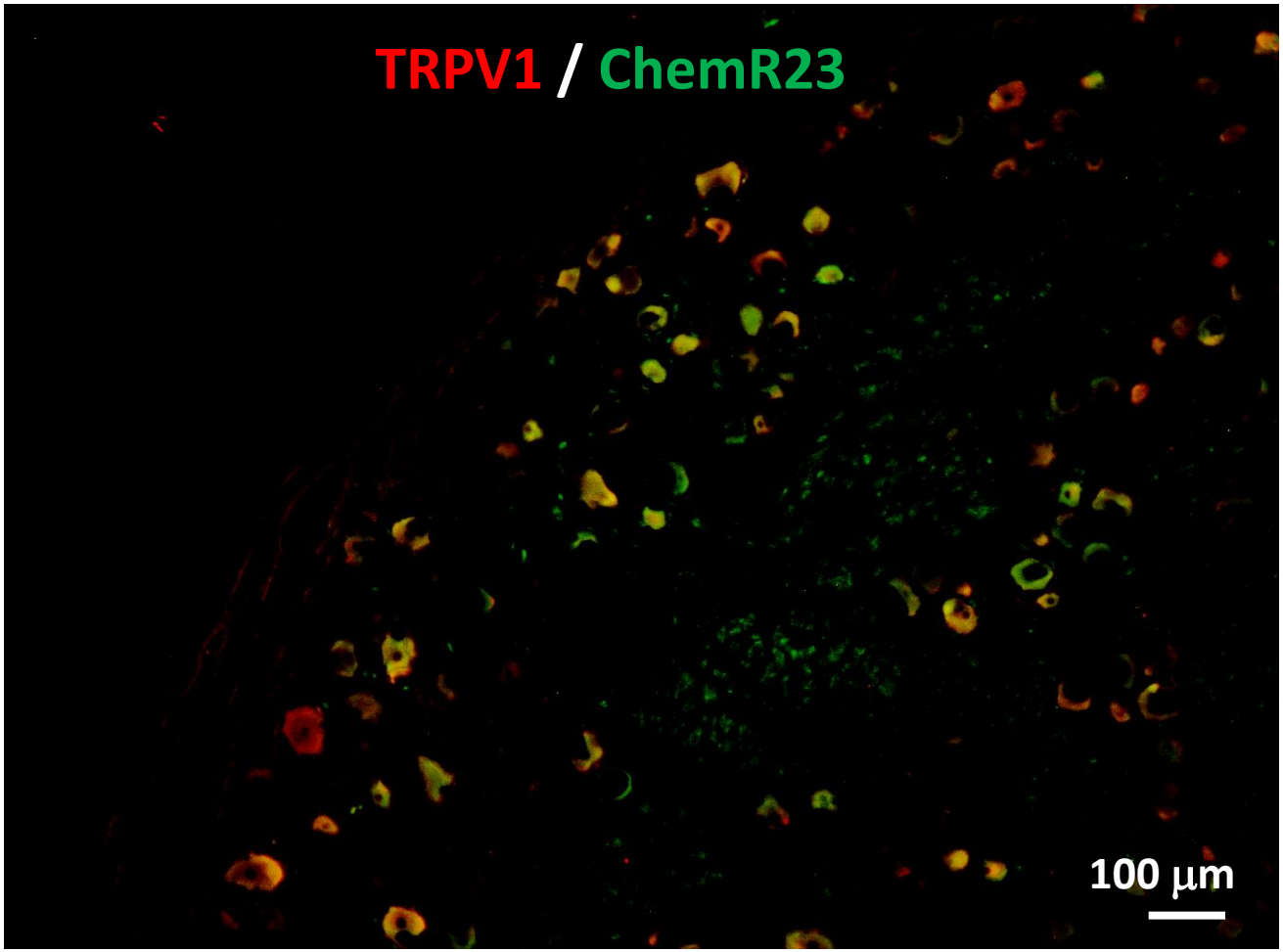
Ion channel research on
Primary sensory neurons of human DRGs



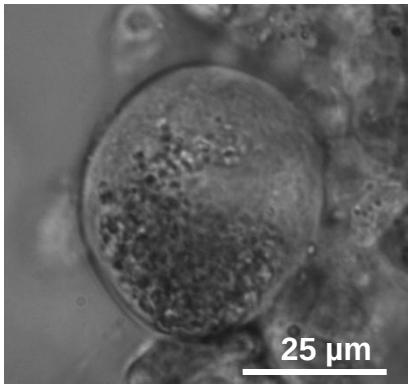


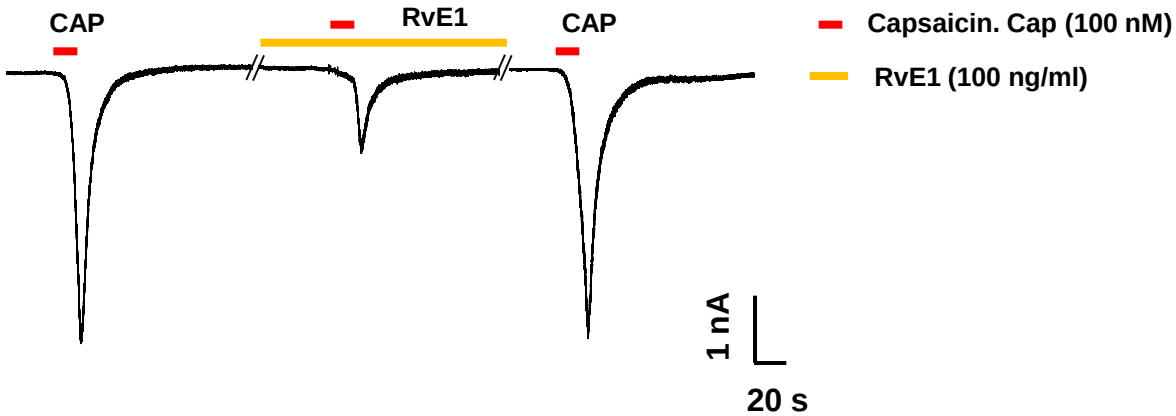


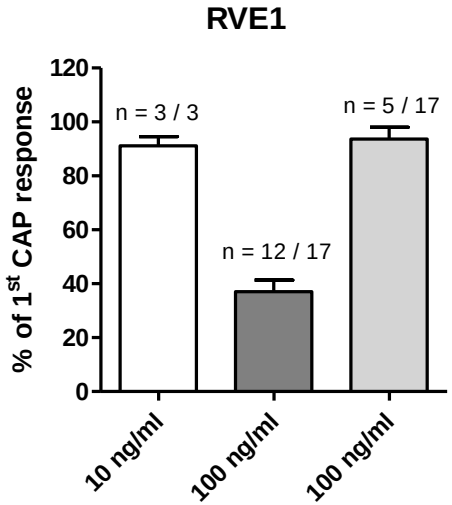
Co-expression of TRPV1 and ChemR23 in human DRG neurons



RvE1 inhibits TRPV1 currents in human DRG neurons







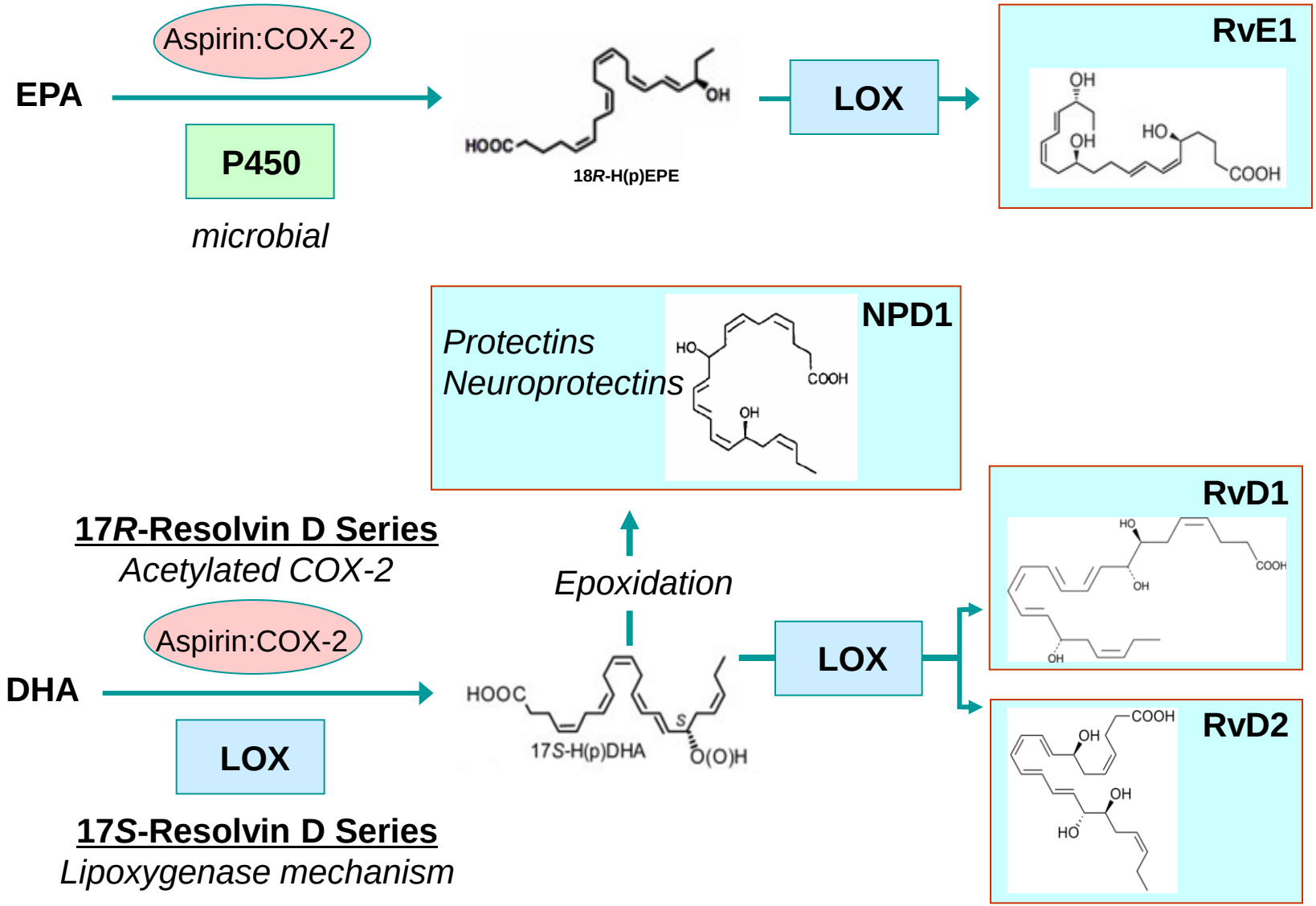
Multiple beneficial effects of resolvins

- **Anti-inflammatory actions**
- **Pro-resolution actions**
- **Block TNF-α synthesis and signaling**
- **Modulate synaptic plasticity**
- **Powerful endogenous inhibitors of TRPV1/A1**
- **No effects on basal pain**
- **Unknown side effects**

Limitations

- Unstable
- Difficult to measure
- Expensive to produce

Biosynthesis of Protectins from PUFA Precursors



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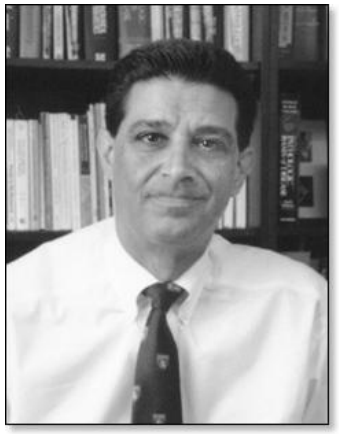
R01: DE17794
R01: NS54932
R01: DE22743
R01: NS89479
Pfizer/Neusentis/Icagen

Charles Serhan



Seok-Yong Lee