Dorsal Horn Plasticity

“A key feature of synaptic processing in the dorsal horn is that it is not fixed or hard-wired, but instead is subject to diverse forms modifiability or plasticity.” -- C. Woolf, Textbook of Pain

The CNS is a very dynamic system – The response to any given input is affected by what has happened prior to the input.

The nature of these effects are dependent on the timing of the events. (i.e. short term and long term effects).
Persistent Pain States

Peripheral Injury

- Inflammatory Pain
- Neuropathic Pain

Mechanical and Thermal Hyperalgesia and/or Allodynia

Mecanisms? Peripheral and/or Central sensitization

Central sensitization

Two basic ways to get central sensitization

1) Increase excitatory drive
2) Decrease inhibition (disinhibition)
Presynaptic inhibition
monosynaptic
polysynaptic
hyperpolarization
monosynaptic

C/Aδ nociceptor
GABA/Gly interneuron

Aβ/Aδ LTM

Excitatory interneuron

projection neuron
Structural/Anatomical Changes in the Dorsal Horn Associated with Hypersensitivity

• Although persistent pain states usually begin with changes in the periphery, most investigators believe that the peripheral changes lead to central changes in the CNS (e.g. spinal cord, brainstem, thalamus, cortex).

• Structural changes may include sprouting, cell death, changes in expression, changes in location of receptors (membrane insertion), etc.

• Once again the timing of the events (e.g. neonate vs adult) is critical.

• These changes determine what types of treatments will be effective.
Beta-Cholera Toxin- HRP labeling

rat  mouse

EPSCs evoked in lamina II cells in response to dorsal root stimulation.

Immature rat (P21)

Mature rat (P56)

Injury Early in Development can Alter Dorsal Horn Structure/Function in Adults.

A) Adult L6 spinal segment from control rat.

B) Adult L5 spinal segment in rat injected with CFA on P14. Note symmetrical WGA-HRP labeling. Compared to CFA injection on P1 where asymmetry is obvious.

C) CGRP staining in adult rat injected with CFA on P1 - note increase in center of DH.

D) No change in IB4 labeling

E-F) L5 and S1 segment for adult rat injected with CFA on P1 but labeled with β-Choleratoxin to label myelinated afferents; i.e. no effect on myelinated afferents

Ruda et al. Science 289:628
Dorsal horn neurons (unidentified) were more sensitive

1) Lots of cells were recorded - <120 in 5 rats, treated and untreated.
2) Spontaneous activity was greater

Ruda et al. Science 289:628
Structural changes are associated with hypersensitivity upon reinflammation only*

1) Note slight difference in B and difference in y-axis.

2) Others investigators do report changes at baseline with neonatal insult (Lidow et al 2000). Some human studies suggest baseline changes.

Ruda et al. Science 289:628
Second phase of nociceptive behaviors in formalin test occurs earlier in treated rats.

Ruda et al. Science 289:628
Summary

If the injury occurs during this critical period during development it can significantly change the response to a subsequent injury in the adult.
They demonstrated in chronic pain patients that input from myelinated fibers was required to evoke mechanical allodynia.
Hair follicle afferents in rats with regenerated sciatic nerves DO enter Lamina II (and even I)

Woolf et al., Nature 1992, 355:76
Beta-Cholera Toxin – HRP Labeling
Adult Mouse

Woodbury et al., JCN, 2008
Maybe sprouting of A-fibers doesn’t happen after all

1) Tong et al 1999 found that following injury most sensory neurons (including C-fibers) transport CTB (they looked in the DRG).
2) Bao et al 2002 found that if you labeled sciatic nerve with CTB and then cut, central labeling looked normal - i.e. prelabeled A-fibers did not show sprouting.

PKCγ ICC to demarcate SG/LIII border

Hughes et al., J. Neurosci, 2003, 23:9491
Intact A-fibers flirt with SG but don’t go beyond PKC labeling
7 weeks after sciatic nerve transection A-fibers have not moved any further dorsal

Hughes et al., J. Neurosci, 2003, 23:9491
Sagittal view of Aβ hair follicle afferent in cat

Sagittal view of Aβ slowly adapting type 1 mechanoreceptor
Adult cat regenerated Aβ-fiber responsive to brushing the skin.

Koerber et al., 1989
Aβ Myelinated nociceptors project throughout superficial dorsal horn in naïve adults

Woodbury et al., 2008
Low threshold mechanoreceptors do not project into the superficial dorsal horn after nerve injury

Woodbury et al., 2008
Myelinated nociceptors project throughout superficial dorsal horn following nerve injury.
A-fiber nociceptors have decreased mechanical and thermal thresholds after nerve regeneration.

Jankowski et al., J. Neurosci, 2009
Low threshold fibers have decreased sensitivity after regeneration.
These findings suggest that in adults there seems to be little structural change following nerve injury.

These findings also suggest that the population of myelinated nociceptors could be responsible for mediating mechanical allodynia.

However, alteration in spinal networks could still alter the way low threshold inputs are processed, leading to the activation of pain circuits.
Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain

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immature

Na-K-2Cl

GABA<sub>A</sub>R

high [Cl<sup>-</sup>]<sub>i</sub>

depolarizing excitatory

mature

KCC2

low [Cl<sup>-</sup>]<sub>i</sub>

hyperpolarizing inhibitory

Lee et al., 2003
By altering the intracellular chloride concentration. Inhibition can be reduced or possibly switched to excitation.
Dorsal Horn Cells

Primary Sensory Neurons

Na-K-2Cl

high $[\text{Cl}^-]_i$

depolarizing excitatory

Dorsal Horn Cells

GABA$_A$R

KCC2

low $[\text{Cl}^-]_i$

hypermñpolarizing inhibitory

Lee et al., 2003
Presynaptic inhibition

Presynaptic terminal
- action potential/depolarization
- Ca^{++} influx
- release transmitter vesicles

Synaptic cleft- diffusion

↓ Postsynaptic excitatory potential

Presynaptic Inhibition in pain control

decreases transmitter (glutamate, substance P) release

less pain
Recent studies have shown that Na-K-2Cl co-transporter is upregulated in DRGs following peripheral nerve injury.

It is thought that this increase expression could lead to GABA-induced action potential generation in afferent terminals in the spinal cord. These action potentials could be conducted into the peripheral terminals of afferent fibers where they could release peptides etc…
Changes in glia associated with persistent pain

1) The spinal cord is immune privileged and therefore has it cells that deal with damage.

2) Nerve injury is associate with loss of synaptic contacts and remodeling of the dorsal horn. This means that bits of tissue will be getting chewed up and recycled.

3) There are two main cells types resident in the spinal cord that can function in a manner similar to leukocytes; Astrocytes and microglia.

4) Astrocytes have many functions in normal spinal maintaining homeostasis and synaptic function. Some investigators even suggest that they can act almost like “interneurons”.

5) Microglia are thought to be derived from the same lineage as leukocytes but some investigators have proposed that they do come from neuroectoderm.
Changes in glia associated with persistent pain (cont)

6) Both microglia and astrocytes can proliferate in response to injury and both get activated. This activation includes changes in shape, function, and gene expression.

7) Microglia tend to be activated first and then they activate astrocytes.

8) Activated microglia upregulate major histocompatibility complex (MCH) classes I and II and cellular adhesion molecules CD4 and CD45.

9) Astrocytes increase expression of glial fibrillary acidic protein (GFAP).

10) Microglia also express toll-like receptors (e.g. TLR-4) which are responsible for responding to bacterial components like the endotoxin lipopolysaccharide (LPS). (TLR-4 was recently found in peptidergic sensory neurons).
Nerve injury set off cascade of events that can contribute to chronic pain

1) Nerve injury produces stressors that can activate TLR4.
2) Once microglia get activated they release cytokines and growth factors that activate astrocytes.
3) Activated astrocytes are lousy at regulating homeostasis (like glutamate reuptake).
4) This leads to central sensitization.

Deleo et al., Neuroscientist, 2004, 10:40
Why are opioids less effective in treating neuropathic pain than other types of persistent pain?
Opioid receptors are expressed in DRG and spinal cord

1) There are three different opioid receptors, μ (MOR), δ (DOR) and κ (KOR).

2) All three are expressed in DRG and spinal cord.

3) All three are Gi-coupled GPCR.

4) MOR binds morphine, enkephalin (but DOR is better), endomorphins, and β-endorphin.

5) DOR binds enkephalin and β-endorphin.

6) KOR binds dynorphin.

7) They can be coexpressed in the DRG and spinal cord neurons.
1) The goal of these studies was to determine why opiates don’t work great on neuropathic pain patients.

2) Below, transection of sciatic nerve decreases percent of cell expressing µ opioid receptor (MOR).

3) Panel on rt shows that MOR is expressed on small afferents before and after injury.

Zhang et al Neuroscience 1998, 82: 223
MOR is expressed on dorsal horn neurons in Lamina I and II.

Zhang et al Neuroscience 1998, 82: 223
14 days post sciatic nerve transection MOR disappears in the dorsal horn (c)
MOR is expressed both pre- and post-synaptically

a) Shows single MOR-positive (indicated by squares) afferent terminal making synapse with multiple dendrites (D). Note that MOR is often outside synaptic zone.

b) More common pattern, afferent is MOR-negative, dendrites MOR-positive.

c) Axosomatic MOR-positive synapses.

d) MOR-positive interneuron making local synapse.

Zhang et al., Neuroscience 1998, 82: 223
The number of MOR-positive neurons increase with inflammation but DOR- and KOR positive neurons decrease

1) Potency of morphine to inhibit C-fiber activity is increased 30-fold by carrageenan-induced inflammation (Stanfa et al 1993)
Inhibition of calcium currents via high-voltage-activated channels containing the α2δ-1 subunit, leading in turn to reduced neurotransmitter release and attenuation of postsynaptic excitability, is a biologically plausible mechanism and one which is consistently observed at therapeutically relevant concentrations in pre-clinical studies of GBP and PGB.
1. An action potential arrives, causing Ca\textsuperscript{2+} to diffuse into the axon bulb.

2. Ca\textsuperscript{2+} causes vesicles containing neurotransmitter to fuse with the cell membrane, releasing neurotransmitter.

3. Neurotransmitter binds to receptors on chemically-gated sodium channels, causing the channels to open.

4. Diffusion of sodium produces a graded potential in the local region of the synapse.
Questions