Microglia gating pain... and beyond
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KREMBIL FOUNDATION
Inflammation in the spinal cord after nerve lesion

Intact side

Injury side

Microglia (OX42)

Inflammation in the spinal cord after nerve lesion

Ctb central terminals
IB4
Iba1 microglia

Normal pain

Pathological pain

Transmission of pain signal to the brain

Coull, Beggs et al., *Nature* 2005
Can microglia depletion reverse nerve injury-induced pain hypersensitivity?

Ji Zhang

Even 3 months after injury, on-going BDNF secretion is involved. Even 2 weeks after injury, 3 months after injury.
Nociceptors 

Touch afferents

Nociceptive relay channels

Non-nociceptive relay channels

Labrakakis et al In *Inhibitory Synaptic Plasticity* 2011
Brief Communication

Morphine-Induced Chemotaxis and Brain-Derived Neurotrophic Factor Expression in Microglia

Naoko Takayama and Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8521, Japan

Figure 2. Morphine-induced chemotaxis of microglia in the Boyden Chamber. Microglia were stimulated with DMEM or 1µM morphine for 90 min...

Figure 4. Morphine-induced BDNF mRNA expression in microglia.
Adaptation to chronic morphine

Pain Threshold

Time (Days)

1 2 3 4 5 6 7 Baseline

Level of Analgesia

Tolerance

Hyperalgesia

Francesco Ferrini
Chronic morphine treatment: hyperalgesia

Testing every morning, just before injections of morphine

Francesco Ferrini

**Thermal**

![Graph showing thermal withdrawal threshold over time.

**Mechanical**

![Graph showing mechanical withdrawal threshold over time.

**Vocalization**

![Graph showing vocalization over time.

**Licking**

![Graph showing licking time for saline and morphine.

**Rotarod**

![Graph showing max RPM over days.

CTR MS
Chronic morphine causes microglial activation

Francesco Ferrini, Alexandra Mattioli

Fluorescence intensity

CTR MS

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MS+Saporin

MS+mac1-saporin

Withdrawal Threshold

Sap Mac1 sap

% of OX-42-ir area

14 12 10 8 6 4 2

0 1 2 3 4 5

1 6 7 8 9 10

CTR MS

0.6 0.8 1.0 1.2

0 1.0 2.0 3.0

0 1 2 3 4 5

1 6 7 8 9 10
BDNF from microglia; necessary for morphine hyperalgesia?

CD11b-Cre+/LoxBDNF mice do not develop morphine–induced hyperalgesia

Yet, morphine analgesia is intact

Tuan Trang
P2X$_4$ receptors; necessary for morphine hyperalgesia

Reversing established hyperalgesia in vivo

P2X$_4$ -/- mice do not develop hyperalgesia
Morphine causes paradoxical hyperalgesia... by triggering a central inflammatory response... that leads to impaired Cl⁻ homeostasis...

What about the brain?
Disrupted Cl⁻ homeostasis in opiate-dependence

Opiate state controls bi-directional reward signaling via GABA_A receptors in the ventral tegmental area

Steven R Laviolette¹, Roger A Gallegos², Steven J Henriksen² & Derek van der Kooy¹,³

The neural mechanisms that mediate the transition from a drug-naive state to a state of drug dependence and addiction are not yet known. Here we show that a discrete population of GABA_A receptors in the mammalian ventral tegmental area (VTA) serves as a potential addiction switching mechanism by gating reward transmission through one of two neural motivational systems: either a dopamine-independent (opiate-naive) or a dopaminergic (opiate-dependent or opiate-withdrawn) system. Bi-directional transmission of reward signals through this GABA_A receptor substrate is dynamically controlled by the opiate state of the organism and involves a molecular alteration of the GABA_A receptor. After opiate exposure and subsequent withdrawal, the functional conductance properties of the rat VTA GABA_A receptor switch from an inhibitory to an excitatory signaling mode.

Figure 6 Single-unit extracellular recordings of VTA GABAergic neurons shows that a subpopulation of these neurons respond with excitatory depolarization to receptor activation in the opiate-dependent/withdrawn state.
Microglial activation in the ventral tegmental area (VTA) of acute and chronic drug exposure

Anna Taylor
(Cathy Cahill)
Impaired morphine-evoked dopamine release in animals with peripheral nerve injury

Anna Taylor
(Cathy Cahill)
Intra-VTA opiate injection fails to produce place preference in animals with peripheral nerve injury.
Microglia-BDNF-KCC2 cascade in the ventral tegmental area (VTA) in animals with peripheral nerve injury

Anna Taylor (Cathy Cahill)
Neuropathic pain-induced Cl⁻ dysregulation in VTA GABAergic neurons blunts the reward response

Anna Taylor (Cathy Cahill)

What about the ACC?
Time-dependent development of anxio-depressive-like phenotypes

Novelty supressed feeding

Splash

Forced Swimming Test


Ipek Yalcin (Michel Barrot)
Ablation of the ACC, but not the pIC blocks the anxio-depressive-like phenotype following nerve lesion

**Novelty supressed feeding**

**Splash**

**Forced Swimming Test**


Ipek Yalcin
Optogenetic stimulation of the ACC triggers anxio-depressive-like behaviours

Ipek Yalcin

Microglial activation in the ACC...

...Only several weeks after nerve injury


Ipek Yalcin
What about sex?
Microglia respond to peripheral nerve injury in females as well as in males.

Robert Sorge
Josiane Mapplebeck
Simon Beggs
(Jeff Mogil)
(Michael Salter)
Microglia depletion reverses pain hypersensitivity... but only in males...

Sorge et al. unpublished
Pain hypersensitivity prevented by microglia BDNF depletion in males only

Sorge et al. unpublished

**KO = CX3CR1cre X loxP BDNF Tamoxifen treatment 6wks pre-SNI**
Pain hypersensitivity reserved by P2X4 receptor blocker but only in males

Sorge et al. unpublished
What microglia?
The expanding optogenetic toolbox...

- Transsynaptic Labelling (Retrograde)
- Virus injection in thalamus
- Optogenetic Control
- Targeted Double Patch Recording
- Optrode Detection and Recording
- Afferent and Transsynaptic Labeling

Challenge: Functional imaging of cellular dynamics in situ

IPL Device

Imaging light path

Focal plane offset (telescope)

IPL Device

LED

DETECTOR

Feedback loop control

Microscope objective

Z axis piezo nanopositioner

Dichroic mirrors

Fluorescence detection

Laffray, Pages et al. PLoS One 2011

Sophie Laffray & Stéphane Pagès
In vivo functional imaging: dealing with movement...

Sophie Laffray & Stéphane Pagès

Adaptive, real-time movement compensation

Laffray, Pages et al. PLoS One 2011
Challenge: Functional imaging of cellular dynamics in behaving animals

Erik Bélanger
Sophie Laffray

A characterization of microglia based on their dynamic behaviour

Sophie Laffray

In vivo two photon imaging across the dura matter

Normal surveillance mode

Response to a focal laser lesion

After peripheral nerve injury (NI)

After peripheral nerve injury (NI)
Merci!