Neuroimaging Techniques – I

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Different Neuroimaging Modalities: Hemodynamics, Electrophysiology, Neurochemistry

- **Hemodynamic Response**
  - ↑ Blood Flow
  - ↑ Blood Volume
  - ↑ Blood Oxygenation

- **Brain Structure**
  - Gray matter density, volume, thickness

- **Neurotransmitter and Receptor Activity**
  - Glu., GABA, opioid, etc.

- **Electrical Activity**
  - EEG
  - MEG

- **PET**
  - ¹H-MRS

- **MRI, fMRI, fcMRI**
**Mechanisms of Pain**

*Acute pain*

Peripheral nociceptive input from thermal, chemical or mechanical nociceptors

*Chronic pain*

- Central as well as peripheral factors
- Amount of pathology in periphery does not always match pain

*Stimulus*
Brain Regions Involved in Pain Processing and Modulation

Pronociceptive regions:
- Somatosensory cortices (S1/S2)
- Anterior insula cortex (aIC)
- Posterior insula cortex (pIC)
- Anterior cingulate cortex (ACC)
- Mid cingulate cortex (MCC)
- Posterior cingulate cortex (PCC)
- Thalamus (THAL)
- Amygdala (AMY)

Antinociceptive regions:
- Dorsolateral prefrontal cortex (DLPFC)
- Perigenual anterior cingulate cortex (pgACC)
- Periaqueductal gray (PAG)
- Rostral ventromedial medulla (RVM)
Neurochemical Receptor Imaging

- Positron Emission Tomography (PET)
  - Utilizes radioactive substances.
  - Various tracers for blood flow, metabolism, neurotransmitters and their receptors. Some involved in pain (opioid, dopamine) but more ligands are in development.
  - Has good spatial and molecular resolution, but poor temporal resolution as compared to fMRI.
  - One drawback is that it is somewhat difficult to assign changes in receptor binding to either changes in ligand concentration or receptor number/affinity.
Positron Emission Tomography (PET)
Opioid Receptor Availability

PET Measurements

Tracer Transport (rCBF x Tracer Extraction)

Incorporation to Specific Binding Sites

1 min 2 min 3 min 5 min 10 min 30 min 70 min

Data Analysis

Generation of Parametric Maps

Logan Plots (DVR)

Coregistration with Anatomical MRI

Non-Linear Anatomical Standardization (ICBM Template Space)

Statistic Images for Statistical Parametric Mapping (SPM)
Opioid Receptors and Pain

- Opioid receptors belong to the G protein-coupled receptor superfamily.
- They are located in “pain” neuropathways.
- Opioid receptors increase neuronal inhibition.
  - Reducing calcium influx: presynaptic.
  - Increasing potassium efflux: postsynaptic.
- μ-opioid receptors (MORs) bind endogenous and exogenous opioids.
  - \(^{11}\text{C}\)-carfentanil: Positron Emission Tomography (PET).

Manglik et al. *Nature* 2012
Receptor Positron Emission Tomography

- $[^{11}\text{C}]-\text{carfentanil}$: selective agonist of $\mu$-opioid receptors (MOR).

- **Binding potential** (BP): reflects number of receptors available to bind ligand. This is a measurement of receptor availability.
Binding Potential (BP)

- Occupancy and number of μ-opioid receptors influences BP.

Increased Release of Endogenous Opioids

μ-opioid receptor

- endogenous opioids
- carfentanil

BP ↑

BP ↓

Zubieta et al. Science 2001
FM Patients Have Reduced MOR BP

Harris et al. JNeurosci 2007

<table>
<thead>
<tr>
<th>Region</th>
<th>Z</th>
<th>p-value*</th>
<th>%Δ BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>L NAcc</td>
<td>4.12</td>
<td>&lt;0.05</td>
<td>33.1(7.1)</td>
</tr>
<tr>
<td>lAMY</td>
<td>4.21</td>
<td>&lt;0.05</td>
<td>31.1(7.0)</td>
</tr>
<tr>
<td>L dACC</td>
<td>3.39</td>
<td>&lt;0.05</td>
<td>21.5(6.4)</td>
</tr>
</tbody>
</table>
MOR BP is Associated with Clinical Pain

I. Pain increases release of endogenous opioids

AND/OR

II. Decreased receptors result in increased pain
Possible Explanations

I. Increased Pain

Increased Release of Endogenous Opioids

μ-opioid receptor

endogenous opioids

carfentanil

Baraniuk et al. *BMC Musc Dis* 2004
Implications...

- Regardless of the reason for reduced binding of opioid receptors, we would expect FM patients to respond poorly to opioid medications:
  - Either their receptor numbers are low (due to down regulation)…AND/OR
  - Their receptors are already occupied by higher levels of endogenous opioids.
Endogenous opioidergic dysregulation of pain in fibromyalgia: a PET and fMRI study

Andrew Schrepf, Daniel E. Harper, Steven E. Harte, Heng Wang, Eric Ichesco, Johnson P. Hampson, Jon-Kar Zubieta, Daniel J. Clauw, Richard E. Harris

Schrepf et al. PAIN 2016
Opioid Receptor Binding is Related to the Affective Dimension of Pain

### Table 3

<table>
<thead>
<tr>
<th>Brain region</th>
<th>MNI co-ordinates (X, Y, Z)</th>
<th>Affective/sensory pain ratio, r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFG (BOLD)</td>
<td>−12, 2, 62</td>
<td>−0.518</td>
<td>0.027</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.629</td>
<td>0.005</td>
<td></td>
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<tr>
<td>PCC (BOLD)</td>
<td>−8, −44, 40</td>
<td>−0.593</td>
<td>0.010</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.497</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>pgACC/MFG (BOLD)</td>
<td>−16, 40, 38</td>
<td>−0.581</td>
<td>0.012</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.571</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Precentral (BOLD)</td>
<td>32, −4, 34</td>
<td>−0.436</td>
<td>0.070</td>
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<tr>
<td>MOR BP</td>
<td>−0.507</td>
<td>0.032</td>
<td></td>
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<tr>
<td>DLPFC (BOLD)</td>
<td>−42, 8, 30</td>
<td>−0.331</td>
<td>0.179</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.246</td>
<td>0.324</td>
<td></td>
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<tr>
<td>MTG (BOLD)</td>
<td>−58, −58, 4</td>
<td>−0.408</td>
<td>0.093</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.410</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>sgACC (BOLD)</td>
<td>10, 40, −2</td>
<td>−0.754</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.545</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>NAc (BOLD)</td>
<td>−18, 6, −12</td>
<td>−0.425</td>
<td>0.079</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.301</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>Cerbellum (BOLD)</td>
<td>34, −60, −44</td>
<td>−0.294</td>
<td>0.236</td>
</tr>
<tr>
<td>MOR BP</td>
<td>−0.318</td>
<td>0.198</td>
<td></td>
</tr>
</tbody>
</table>

ROI
- ACC (BOLD) −12, 38, 28 −0.378 0.122
- MOR BP −0.552 0.018
- NAc (BOLD) −18, 6, −12 −0.425 0.079
- MOR BP −0.301 0.224

Bolded regions were significantly associated with clinical pain.

ACC, anterior cingulate cortex; BP, binding potential; BOLD, blood oxygen level-dependent; DLPFC, dorsolateral prefrontal cortex; MFG, medial frontal gyrus; MOR, μ opioid receptor; MTG, middle temporal gyrus; NAc, nucleus accumbens; PCC, posterior cingulate cortex; pgACC, perigenual anterior cingulate cortex; ROI, region of interest; sgACC, subgenual anterior cingulate cortex.

Schrepf et al. PAIN 2016
Model of Altered Endogenous Opioid Dysregulation in FM

MOR downregulation

Loss of GABA Interneuron Inhibition

1.) Schrepf et al. *PAIN* 2016
2.) Akaishi et al. *Neurosci Res* 2000
3.) Chen et al. *Mol Pain* 2008
4.) Fischer et al. *Regul Pept* 2000;
5.) Unterwald et al. *Mol Brain Res* 1995
Implications...

- Not only are endogenous opioids not helping pain in FM...maybe they are making it worse.
  - Activation of opioid receptors by endogenous opioids may *paradoxically* exacerbate pain.
  - ...endogenous opioid induced hyperalgesia

- If so we might expect FM patients to have an analgesic response to agents that block opioid receptors.
Opioid Receptor Antagonist (Low Dose Naltrexone) Reduces FM Pain

Younger et al. *Pain Medicine* 2009

Younger et al. *Arthritis and Rheum* 2013
Insula Displays Augmented Neural Activity in Fibromyalgia (FM)

Pressure Pain
Gracely et al. *Arth Rheum* 2002

Heat Pain
Cook et al. *J Rheum* 2004
Proton Magnetic Resonance Spectroscopy (1H-MRS)

- Externally applied radio frequency pulse alters proton procession.

- Fourier transformation of the Free Induced Decay results in Spectra that is MOLECULE SPECIFIC.

- Measure relative concentrations of specific molecules within the brain.
**1H-MRS Spectra**

- **Metabolites**
  - GABA
  - Glx (glutamate + glutamine)
  - N-acetyl aspartate (NAA)
  - Creatine (Cr)
  - Choline containing compounds (Cho)

- Absolute concentrations in tissue are estimated in arbitrary units (AIU) or ratios are taken to other metabolites (Cr).
Glutamate (Glu) is an Excitatory Neurotransmitter

- Glutamate is used by over 90% of neurons in the brain.
- Fast action relates to binding of Glu to NMDA and AMPA channels and excites neurons.
- These receptors are known to play a role in long-term synaptic plasticity.

Fibromyalgia (FM) Patients have Increased Levels of Glutamate (Glu) within the CSF

- 20 Fibromyalgia (FM) patients and 20 healthy pain free controls (HC).
- Cerebrospinal fluid sampled and assessed for levels of Glutamate (Glu).

Hypothesis

- FM patients have elevated Glu levels in the brain (insula) and these levels are associated with pain report.
FM Patients display Elevated Insular Glx and this is Associated with Lower Pain Threshold

Harris et al. Arth Rheum 2009
Functional Connectivity Is Associated With Altered Brain Chemistry in Women With Endometriosis-Associated Chronic Pelvic Pain

Sawsan As-Sanie, * Jieun Kim, †,1 Tobias Schmidt-Wilcke, †,2 Pia C. Sundgren, § Daniel J. Clauw, † Vitaly Napadow, † and Richard E. Harris †

![Graph showing data on Anterior Insula Glx Concentration (AU) with different groups and p-values.](image URL)
Increased Glx is Associated with Increased Insula – mPFC Connectivity

- ENDO@CPP
- HC

- ENDO@CPP > HC
- ENDO@CPP < HC

- $r = 0.87$

- $z = 10\text{mm}$
- $x = -10\text{mm}$

- $z = 22\text{mm}$
- $x = 0\text{mm}$
Glutamate is a Neurotransmitter and Metabolite

Inhibitory Pain Neurotransmission and Modulation

- GABA (γ-amino-butyric acid)
- Opioid

Excitatory Glutamate
Gamma-Aminobutyric Acid (GABA)

- Brain’s major inhibitory neurotransmitter.
- Binds to metabotropic and ionotropic receptors in brain.
- Lowers or shunts membrane potential thereby inhibiting formation of action potentials.

Akabas et al. *IntRevNeurobiol* 2004
http://en.wikipedia.org/wiki/GABAA_receptor
Hypothesis

- Chronic pain patients (FM) have reduced GABA in the insula and these levels are inversely associated with pain report.
FM Patients Display Reduced GABA in Insula

Higher GABA levels are also associated with higher pain thresholds.

Foerster et al. *ArthRheum* 2011
GABAergic Medication Reduces Pain in Fibromyalgia

- **Gamma-Hydroxybutyrate**
  - Metabolite of GABA
  - Approved by US FDA for narcolepsy

Russell et al. *Pain* 2011
Does $^1$H-MRS have good test-retest reliability?

- Glx and GABA: CV=6-7%

  O’ Gorman et al. *JMagnResonImaging* 2011

What magnitude of change is able to be perceived with $^1$H-MRS?

- 6% Glx and 12% GABA

  Waschkies et al. *Neuropsychopharm* 2014
Preclinical Assessment of $^1$H-MRS Utility in Drug Development

- Vigabatrin inhibits GABA catabolism and is associated with a dose dependent increase in $^1$H-MRS derived GABA levels.
  - Waschkies et al. *Neuropsychopharmacology* 2014
In vivo $^1$H-MRS and ex vivo Levels of Neurotransmitters During Vigabatrin are Similar

- Liquid chromatography-mass spect for assessment of neurotransmitter concentrations ex vivo.
- Consistent off set of 1.2 to 1.3 across doses of vigabatrin.
- Pharmaco$^1$H-MRS may be a viable tool for preclinical drug development.

Table 1: Comparison of In vivo $^1$H pharmacoMRS and Ex vivo LC–MS/MS Readouts of GABA and Glutamate Levels in Striatum and Prefrontal Cortex of Sprague–Dawley Rats Subjected to Pharmacological Interventions with Vigabatrin

<table>
<thead>
<tr>
<th>Vigabatrin dose</th>
<th>0 mg/kg</th>
<th>30 mg/kg</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PharmacoMRS (µmol/ml)</td>
<td>$2.01 \pm 0.05$</td>
<td>$2.06 \pm 0.06$</td>
<td>$2.24 \pm 0.07$</td>
<td>$3.02 \pm 0.22$</td>
</tr>
<tr>
<td>LC–MS/MS (µmol/g)</td>
<td>$2.60 \pm 0.17$</td>
<td>$2.38 \pm 0.17$</td>
<td>$2.55 \pm 0.10$</td>
<td>$4.08 \pm 0.34$</td>
</tr>
<tr>
<td>PFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PharmacoMRS (µmol/ml)</td>
<td>$1.84 \pm 0.05$</td>
<td>$1.82 \pm 0.04$</td>
<td>$2.15 \pm 0.07$</td>
<td>$3.89 \pm 0.27$</td>
</tr>
<tr>
<td>LC–MS/MS (µmol/g)</td>
<td>$1.81 \pm 0.08$</td>
<td>$1.91 \pm 0.03$</td>
<td>$2.33 \pm 0.06$</td>
<td>$3.89 \pm 0.27$</td>
</tr>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PharmacoMRS (µmol/ml)</td>
<td>$7.17 \pm 0.09$</td>
<td>$7.11 \pm 0.12$</td>
<td>$7.27 \pm 0.16$</td>
<td>$7.33 \pm 0.13$</td>
</tr>
<tr>
<td>LC–MS/MS (µmol/g)</td>
<td>$9.05 \pm 0.25$</td>
<td>$8.85 \pm 0.20$</td>
<td>$8.68 \pm 0.16$</td>
<td>$8.50 \pm 0.34$</td>
</tr>
<tr>
<td>PFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PharmacoMRS (µmol/ml)</td>
<td>$8.89 \pm 0.06$</td>
<td>$8.78 \pm 0.16$</td>
<td>$8.75 \pm 0.15$</td>
<td>$8.45 \pm 0.11$</td>
</tr>
<tr>
<td>LC–MS/MS (µmol/g)</td>
<td>$10.95 \pm 0.12$</td>
<td>$10.67 \pm 0.28$</td>
<td>$10.62 \pm 0.15$</td>
<td>$9.77 \pm 0.32$</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.

Waschkies et al. Neuropsychopharm 2014
Do Pharmacologic Interventions Alter Neurotransmitter Levels in Humans?

- Pregabalin was the first FDA-approved medication for the treatment of FM.
  - Reduces pain and other symptoms
    - Crofford et al. *Arthritis and Rheumatism* 2005
- It’s pre-clinical mechanism of action is to bind to the alpha-2-delta calcium channel subunit.
  - Reduces glutamate release at the synapse.
    - Gee et al. *JBiolChem* 1996
- It’s clinical mechanism of action is largely unknown.
Pregabalin Reduces Posterior Insula Glx

This was not seen or the anterior insula.

Harris et al. *Anesthesiology* 2013
Pre-Treatment Levels of Glx “Predict” Subsequent Anti-hyperalgesia

Harris et al. Anesthesiology 2013

![Graph showing the relationship between pre-treatment levels of Glx and subsequent anti-hyperalgesia. The correlation coefficient is r = -0.54, p = 0.026.](image)
Implications for Therapy and Drug Development?

- Personalized analgesia could be possible.
  - Patients with higher Glx might respond better to drugs with glutamatergic mechanism of action (pregabalin).

- Drug development might be improved if...
  - Novel agents were available that are thought to act on glutamate receptors, and...
  - Patients with higher Glx were enrolled in early proof of concept or Phase II drug trials.
  - This would enrich patient pool for specific pathology.
Are Glx and GABA Levels within the Insula Necessary and Sufficient to Cause Pain?

Is the imbalance in neurotransmitter level driving pain or is it simply a response to having pain?

Does one need peripheral factors (inflammation, small fiber neuropathy etc.) to cause the pain or are neurotransmitter imbalances enough?
Are Neurotransmitter Imbalances a Cause or and Effect of Pain?

- Reverse translation of $^1$H-MRS findings is needed.
- Chronic constriction injury (CCI) in rats increases insular glutamate.

![Graph showing glutamate levels in different conditions.](image-url)

Watson *Pain* 2016
Decreasing Glutamate Receptor Activity or Increasing [GABA], Reverses Hyperalgesia.
Altering Insular Levels of Glutamate and GABA in \textit{Naïve} Rats Results in Pain

Inhibit Glutamate Reuptake

Block GABA Synthesis

Watson \textit{Pain} 2016
Balance of Insular Neurotransmitters Influences Pain

Imbalance is Necessary (blocking action of receptors reduces pain) AND Sufficient (altering neurotransmitter levels in pain free rats) for Causing Pain.
Summary

- Brain neuroimaging can provide us with information about chronic pain pathology
  - Increased glutamate, decreased GABA
  - Reduced opioid receptor binding
- This information may allow personalized therapies for pain and could improve clinical trial design.
The Team

- Daniel Clauw MD, George Mashour MD PhD, UnCheol Lee PhD
- Jon-Kar Zubieta, MD PhD: PET
- Johnson Hampson, Eric Ichescos, Anson Kairys: fMRI
- Andrew Schrepf PhD, Daniel Harper PhD, Steve Harte PhD
- Vitaly Napadow PhD: fMRI; Dave Scott PhD: fMRI + PET
- Chelsea Kaplan, Tony Larkin, Ishtiaq Mawla: graduate students
- Rachel Harrison, Laura Mayo-Bond, John Romond, Greta Naylor, Craig Urwin: study coordinators

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  - NIH R01 AT 001415 and DA 016423: J. K. Zubieta
  - NIH K12 DE023574-03 to D.J.C.
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Thank You for Listening