

The 10th Annual NIH Pain Consortium Symposium

Looking Back and to the Future: Advances in Pain Research in Brain Imaging, Neural-glial Mechanisms, Genetics and Epigenetics, Novel Therapy Development, and Cognitive and Emotional Influences

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Summary Report

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Acronym Definitions

Acronym	Acronym Definition
AC	anterior cingulate
ACT	acceptance and commitment therapy
ADAPT	“Addressing Depression and Pain Together” Trial
APS	American Pain Society
BDNF	brain-derived neurotrophic factor
BMS	Burning Mouth Syndrome
BRAIN	Brain Research through Advancing Innovative Neurotechnologies
CaRE	Cancer pain Relief for Everyone
CB ₁	cannabinoid 1 receptor
CB ₂	cannabinoid 2 receptor
CBT	cognitive behavioral therapy
CFA	Complete Freund’s Adjuvant
CLBP	chronic low back pain
CNS	central nervous system
Cx43	connexin 43
CXCL1	chemokine (C-X-C motif) ligand 1
CXCR2	interleukin-8 receptor, beta (also known as IL8RB)
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
DNMTs	DNA methyltransferases
DRG	dorsal root ganglia
fMRI	functional magnetic resonance imaging
GR	glucocorticoid receptor, also known as NR3C1
HAT	histone acetyltransferase
HDAC	histone deacetylase
HPA	hypothalamic-pituitary-adrenal
IBS-D	irritable bowel syndrome with diarrhea
IPRCC	Interagency Pain Research Coordinating Committee
MAP	mitogen-activated protein
MBSR	mindfulness-based stress reduction
MEF	murine embryonic fibroblasts
MEMSCap™	Medication Event Monitoring System Cap
mir-29	a microRNA precursor
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NAc	nucleus accumbens

NCCIH	National Center for Complementary and Integrative Health
NIDCR	National Institute of Dental and Craniofacial Research
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NPD1	neuroprotectin D1
NR3C1	nuclear receptor subfamily 3, group C, member 1, also known as the glucocorticoid receptor
NRS	Numeric Pain Rating Scale
P2X ₄	purinoceptor
PFC	prefrontal cortex
PLGA	poly(lactic-co-glycolic acid)
PST-DP	problem-solving therapy for depression and pain
RNA	ribonucleic acid
SCN9A	gene that encodes the Nav1.7 sodium ion channel
SFN	small fiber neuropathy
SNRI	serotonin-norepinephrine reuptake inhibitor
SPARC	secreted protein, acidic, rich in cysteine
SSRI	selective serotonin reuptake inhibitor
TNF- α	tumor necrosis factor-alpha
TRPA1	transient receptor potential cation channel, subfamily A, member 1
TRPV1	transient receptor potential cation channel subfamily V member 1

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Executive Summary

Introduction

The National Institutes of Health (NIH) established the NIH Pain Consortium in 2003 to foster pain research at the agency's Institutes and Centers and to promote collaboration among researchers across the many NIH Institutes and Centers that have programs and activities addressing pain. The Pain Consortium held its 10th annual Symposium on May 26 and 27, 2015, to reflect on the advances in pain research in the past decade and to highlight the most productive paths forward for pain research and treatment.

The Symposium featured a keynote presentation by Clifford Woolf, which highlighted recent technological advances that allow researchers to study mechanisms of pain and pain disorders in cultures of human nerve cells. These advances may improve and accelerate drug development and enable a more personalized approach to the treatment of chronic pain.

The meeting brought together panels of basic, translational, and clinical researchers to discuss past and future efforts in five areas of pain research:

1. Cognitive and emotional aspects of pain
2. Genetics and epigenetics of pain
3. Pain signatures and predictors from imaging research
4. Neuron-glia mechanisms of chronic pain
5. Novel treatments for pain

Each panel consisted of scientific presentations and discussion. An American Pain Society (APS) representative provided information on the organization's research, education, and advocacy activities. The meeting also included presentations by three junior investigators, selected by the Pain Consortium based on outstanding poster abstracts submitted for consideration. Mark Pitcher, one of the junior investigators, received the 2015 Mitchell Max Award for Research Excellence for his research on the effects of voluntary exercise on chronic inflammation in mice.

Panel Session Highlights

Cognitive and emotional aspects of pain

Research over the past decade has demonstrated that there are neural correlates of the cognitive aspects of chronic pain. Brain regions and neural networks are both structurally and functionally disrupted in chronic pain, resulting in cognitive and physical disabilities. Treatments that address the cognitive and emotional aspects of pain can reverse these abnormalities and reduce pain.

For example, the Addressing Depression and Pain Together (ADAPT) trial investigated whether simultaneous treatment of pain and depression could improve outcomes for patients with chronic low back pain (CLBP). In this study, researchers assessed the ability of antidepressants and cognitive therapies to improve pain and depression in CLBP patients. Results indicate that these treatments improved outcomes for the majority of the patients, although the addition of

a cognitive therapy has not yet demonstrated superior efficacy to antidepressant therapy alone.

Future research on the cognitive and emotional aspects of pain will continue to address the contributions of cognitive therapy in treating pain and depression. Researchers also seek to better understand the shift from acute to chronic pain and to identify strategies to prevent this shift, to break the cycle of pain, stress, and depression, and to individualize pain treatments.

Genetics and epigenetics of pain

Rare genetic gain- or loss-of-function mutations in peripheral neuronal sodium channels result in either extreme hypersensitivity or a complete inability to feel pain. The identification of many of these mutations has informed the mechanisms behind more prevalent pain conditions. For example, patients with small fiber neuropathy (SFN) often have loss-of-function mutations that result in a more subtle pain phenotype. New therapies that target these channels would represent a new class of pain medications with minimal central nervous system (CNS) effects and minimal addictive potential.

Mechanisms of epigenetic regulation, such as the acetylation of histones and the methylation of promoter DNA, result in increased or decreased gene expression. The study of epigenetic regulation has greatly increased in the past decade and will likely lead to a greater understanding of chronic pain. For example, researchers have discovered substantial differences in the methylation status of the entire genome of animals with or without chronic pain. Determining which genes are up- or down-regulated in subjects with chronic pain can help scientists better understand the processes that cause and maintain chronic pain.

Researchers have assessed the epigenetic regulation of genes involved in visceral pain pathways using an animal stress model of chronic water avoidance. Stressed animals have been shown to harbor increased levels of methylation in the promoter regions of key regulatory genes in pain pathways, resulting in decreased production of tight junction proteins in visceral epithelial cells and increased permeability of the intestinal epithelium. Data from the NIH Roadmap Epigenome project indicate that epigenetics is likely to predict more human traits and disease states than DNA coding variants.

Pain signatures and predictors from imaging research

Advances in neural imaging techniques, especially functional magnetic resonance imaging (fMRI), have allowed researchers to better understand the processes of pain and recovery. For example, fMRI has increased the understanding of the neurobiology of pain, the role of individual differences in pain pathways, mechanisms of the placebo effect, and gray matter changes in chronic pain conditions. Imaging studies are also increasingly used in clinical trials to assess the effects of pain medications and to help determine whether further drug development should proceed.

Research has examined the brain changes that occur when a patient transitions from acute to chronic pain. In one longitudinal, observational study, researchers conducted fMRI scans on patients with acute back pain and observed brain activity as some patients progressed to

chronic pain. Using this approach, researchers identified that the limbic system's white matter and connectivity as well as the size of the amygdala and hippocampus are predictors of chronic pain. Understanding the processes involved in the transition to chronic pain may help researchers develop therapies to intervene and halt this process.

There is a need for specific and sensitive fMRI biomarkers of chronic pain. fMRI activity is often implicitly used as a biomarker for pain; however, the inability to replicate studies and the lack of specificity have hindered the development of fMRI patterns as biomarkers. To address these problems, researchers developed a machine learning technique to establish stable fMRI signatures that accurately predict pain, emotional patterns, or other disorders. Such fMRI techniques can help determine whether patients have a CNS component to their pain and need medication to address this aspect of their condition.

Neuro-glia mechanisms of chronic pain

There has been increased interest in glial cells, such as microglia and astrocytes, in the regulation of pain over the past 10 years. Microglia respond to the site of injury and express pro-inflammatory cytokines and chemokines; selective deletion of microglia results in decreased pain. Astrocytes provide structural support for neurons and also release gliotransmitters like chemokines. Persistent activation of astrocytes results in a chemical cascade that maintains pain for longer periods of time.

Signaling pathways between glial cells and neurons contribute to pain. These glial-neural pathways account for how morphine initially reduces pain, while after prolonged use paradoxically increases sensitivity to pain. Inhibition of these glial pathways through depletion of microglia, can reverse pain hypersensitivity. This effect appears to be sex-specific, and the pain pathways utilized by microglia in females have yet to be determined.

Future research on the neuro-glia mechanisms of chronic pain aims to gain a better understanding of the interactions between glia and neurons and how these interactions differ between individuals. Researchers hope to use this knowledge to develop therapeutics that target glial cells. New imaging techniques that allow researchers to examine microglial activation in awake, behaving animals will help with these efforts.

Novel treatments for pain

Existing pain treatments are insufficient—sometimes even detrimental—for many who suffer from chronic pain. For example, extended morphine use in an animal model for breast cancer-induced bone pain led to increased pain and increased bone loss and fractures, indicating the need for new, more effective treatments. Novel approaches to pain treatment include targeting endogenous receptor systems and psychosocial interventions.

Cannabinoid receptors are thought to play a role in pain modulation. Some receptors, such as CB₂, do not have psychotropic effects and therefore may be useful as pain medications. In the breast cancer animal model mentioned above, CB₂ agonists reduced pain, preserved bone density and function, and even decreased tumor burden. Research to develop a commercial CB₂ agonist is under way.

Researchers are also investigating interleukin-10 (IL-10) for the treatment of chronic pain. IL-10 is an anti-inflammatory protein that normalizes glial activity and binds to a single receptor with high affinity. Scientists now understand the negative feedback loop that shuts off IL-10 signaling, enabling the development of a plasmid-based delivery system that provides the correct therapeutic dose of IL-10. Toxicology and efficacy studies conducted by Xalud Therapeutics, the company that developed this new system, have demonstrated that IL-10 can prevent pain and improve function in animal models; clinical trials are expected to begin in 2016.

Many psychosocial therapies are used to treat chronic pain; however, their benefits may be modest. New efforts are needed to understand the mechanisms by which these therapies work to develop better psychosocial treatments. Some evidence supports the Shared Mechanisms Model, which postulates that psychosocial therapies work through a set of common mechanisms that result in reduced pain. Proponents of the Shared Mechanisms Model support future research that focuses on understanding and amplifying the beneficial components of existing psychosocial therapies.

Conclusions

Chronic pain is a significant problem in the United States, resulting in morbidity and mortality, health care costs, and decreased productivity. The HHS, NIH Pain Consortium, APS, and other groups have brought the issue of pain research and treatment to the nation's attention. As a result of cumulative efforts of the pain research and care community, new national and strategic efforts, including the National Pain Strategy and the Federal Pain Research Strategy, are under way to improve the lives of patients with chronic pain to and identify the gaps and opportunities in the chronic pain research portfolio. The 10th Annual NIH Pain Consortium Symposium contributed to the research effort by highlighting advances in pain research and treatment the progress of the past decade.

Meeting Summary

Introduction: Looking Back and to the Future of Pain Research

Walter Koroshetz, MD, Acting Director, NINDS; Chair, NIH Pain Consortium Executive Committee

Background

The NIH Pain Consortium was established in 2003 to align NIH wide efforts to stimulate and support pain research. The mission of the NIH Pain Consortium is to “enhance pain research and promote collaboration among researchers across the NIH Institutes and Centers that have programs and activities addressing pain.” The Pain Consortium currently includes 26 NIH Institutes, Centers, and Offices (see Appendix 2: Meeting Participants for a list of current members). Walter Koroshetz (Director, NINDS) chairs the consortium’s executive committee; Patricia Grady (Director, NINR), Nora Volkow (Director, NIDA), Josephine Briggs (Director, NCCIH) and Martha Somerman (Director, NIDCR)The newly formed Office of Pain Policy, headed by Linda Porter, manages and facilitates the Pain Consortium’s activities and programs and coordinates the activities of the Interagency Pain Research Coordinating Committee (IPRCC), a federally mandated advisory committee tasked with developing the National Pain Strategy. NIH funding for chronic pain research has significantly increased since the inception of the Pain Consortium, from \$173 million in 2002 to \$402 million in 2014.

Dr. Koroshetz welcomed all participants to the 10th Annual Pain Consortium Symposium, titled “Looking Back and to the Future: Advances in pain research in brain imaging, neural-glial mechanisms, genetics and epigenetics, novel therapy development, and cognitive and emotional influences.” The goal of this meeting was to reflect both upon advances in the past decade and identify how pain research should proceed in the future.

Progress on the goals of the Pain Consortium (2003-2015)

The NIH Pain Consortium has focused on fulfilling the goals set forth in 2003. In the following list, major bullets indicate the initial goals, and sub-bullets indicate progress to date.

- **Leverage the NIH Roadmap for pain research:**
 - The NIH Collaboratory: The NIH established the NIH Roadmap in 2004 to address major gaps and opportunities in biomedical research that require the collective attention and resources of the agency. The NIH Collaboratory, which focuses on collaborative research within health care systems, funded two large pragmatic trials for chronic pain.
- **Leverage the NIH Blueprint for Neuroscience Research for pain research:**
 - The NIH Blueprint Grand Challenge: The NIH Pain Consortium developed a Grand Challenge funding opportunity, funded by the Blueprint initiative and titled “The Transition from Acute to Chronic Neuropathic Pain.” Thus far, the consortium has awarded nine grants focusing on neurobiological changes in the transition from acute to chronic neuropathic pain.

- The Brain Research through Advancing Innovative Neurotechnologies (BRAIN) InitiativeSM: The BRAIN Initiative is a broad public-private partnership focused on neural networks and the development of tools to measure electrical and chemical changes in brain circuitry.
- **Develop collaborative workshops:**
 - Crucial issues in pain research: Many workshops have been supported by the consortium, including the September 2014 workshop titled “The Role of Opioids in the Treatment of Chronic Pain” which assessed the effectiveness and risks of opioids for the treatment of chronic pain.
- **Develop collaborative initiatives:**
 - Education: The NIH Pain Consortium funds Centers of Excellence in Pain Education to develop pain management resources for patients, doctors, and other relevant stakeholders.
 - Shared funding: The NIH Pain Consortium has developed a number of funding opportunities using various award mechanisms.
- **Develop collaborative research resources:**
 - NIH Task Force on Research Standards for Chronic Low Back Pain: This task force developed consistent terminology, data collection methods, and outcome assessments to guide low back pain research.
 - The Pain Registry: The Pain Registry is the result of collaboration between the NIH Pain Consortium and Stanford University that collects self-reported outcomes from chronic pain patients.
- **Conduct administrative activities to support the NIH Pain Consortium’s efforts:**
 - NIH Pain Consortium website: The NIH Pain Consortium website¹ contains information on pain-related funding opportunities, NIH pain programs, workshops, and the NIH Pain Research Twitter feed.
 - Pain portfolio database: The Interagency Pain Research Portfolio database provides information on pain research and training activities supported by the Federal Government².
- **Develop and implement national public health and research initiatives:**
 - The National Pain Strategy: In response to an Institute of Medicine report on pain as a public health problem, the IPRCC developed The National Pain Strategy to address primary population needs for pain prevention, care, and education.
 - The Federal Pain Research Strategy: The IPRCC also will lead the development of the Federal Pain Research Strategy, which will identify opportunities and gaps in pain research. The NIH Pain Consortium plans to issue a Request for Information (RFI) related to the goals of the Federal Pain Research Strategy in the summer of 2015.

¹ The NIH Pain Consortium website can be found at: <http://painconsortium.nih.gov>

² The Interagency Pain Research Portfolio database can be found at: <http://paindatabase.nih.gov>

In conclusion, the NIH Pain Consortium has made great progress in achieving the goals set in its inaugural year and is well poised to advance pain research in the coming years.

Keynote Presentation: Pain in a Dish

Clifford Woolf, MB, BCh, PhD, Director, F.M. Kirby Neurobiology Center and Program in Neurobiology, Boston Children's Hospital; Professor of Neurology and Neurobiology, Harvard Medical School

The drug development paradox: more money, but less innovation

Despite advances in science and increases in research and development investments, the number of drugs approved per year has steadily declined over the past several decades.³ Bringing a single new drug to market now requires spending more than \$1 billion. Drug development is a linear process that begins with target identification and validation followed by further testing in preclinical and clinical models of disease.

Dr. Woolf highlighted problems with the current model of drug development; for example, if a poor target is chosen, then all downstream efforts are futile. Furthermore, once a target is selected, it is usually expressed in heterologous systems for high throughput screening of a large number of chemical compounds. Drug developers often claim that targets will retain their normal function under these conditions; however, Dr. Woolf emphasized that targets work normally only in their native molecular and cellular environments; post-translational modifications and cellular neighbors contribute significantly to the function of a biological molecule.

Changing the strategy: screening the phenotype instead of the target

Technological advances now allow researchers to study targets and screen compounds against these targets in their native environment. Researchers can use stem cell technology to study targets in their native environments by inducing the differentiation of cells in vitro. This allows scientists to create in vitro disease models. Instead of screening compounds against a single target in a non-native state, it is now possible to screen compounds against a particular phenotype, across many targets.

There are several strategies to create particular types of differentiated cells. In **differentiation**, embryonic stem cells are treated with factors to move the cells from a pluripotent state to a defined cell state. This method has been successfully used in some cases; however, the use of embryonic stem cells comes with significant ethical and political concerns. It is now possible to avoid using embryonic cells by making induce pluripotent stem cells from any subject or patient and also by starting with mature cells from an adult and transforming them into a mature cell type through a process known as transdifferentiation.

³ Jack W. Scannell et al., "Diagnosing the Decline in Pharmaceutical R&D Efficiency," *Nature Reviews Drug Discovery* 11, no. 3 (March 2012): 191–200.

The ability to turn fibroblasts from a patient with chronic pain into sensory neurons allows researchers to study “pain in a dish.” Scientists can now study the properties of nociceptors, the primary sensory neurons that detect noxious stimuli, by:

- **Screening for analgesic drugs** that work effectively against a particular patient’s nociceptors, facilitating a precision medicine approach that can improve patient outcomes
- **Understanding rare pain diseases**, such as channelopathies, which have profound pain phenotypes, such as congenital insensitivity or oversensitivity to pain (primary erythromelalgia)
- **Identifying cellular factors** that put an individual at increased risk of developing persistent pain

Cellular alchemy: turning murine fibroblasts into nociceptors

Developing a protocol to create neurons from pluripotent precursor cells was difficult. Initial efforts to direct the differentiation of embryonic stem cells into nociceptors were unsuccessful. Later approaches involved trans-differentiation of murine embryonic fibroblasts (MEFs) and used reporter genes to indicate successful transition to a nociceptor. However, the application of transcription factors known to be necessary for nociceptor development did not transform MEFs to nociceptors. At this point, Dr. Woolf correctly hypothesized that additional, unidentified transcription factors were needed to complete the transformation. RNA profiling of nociceptors identified new transcription factors⁴ that, when added to MEFs, successfully transformed them into functional nociceptors.

This transformation process did not result in a clonal population of nociceptors as expected; instead, the transformation resulted in a heterogeneous population of nociceptors, with different levels of myelination and molecular markers. Interestingly, the distribution of nociceptor subtypes was similar to that seen in the native murine dorsal root ganglia (DRG).

Researchers compared the transformed nociceptors with primary neurons to ensure that all properties of the nociceptor were retained. Electrophysiology of the nociceptors indicated appropriate responses to capsaicin, and the cells expressed the nociceptor marker, the sodium channel Na_v1.7. The nociceptors also expressed nociceptor-specific mRNA and no longer expressed MEF-specific mRNA; they also exhibited a broad action potential as well as the tendency to fire in a repetitive fashion, which are both characteristic of nociceptors. Woolf noted that, in essence, the nociceptor cells in the dish said the equivalent of “Ouch!” upon addition of capsaicin or mustard oil.

Creating human nociceptors to study pain disorders

Creating nociceptors from human fibroblasts is more complex than in the mouse, because there is no analogous reporter gene system to indicate a successful transformation. However,

⁴ Isaac M. Chiu et al., “Transcriptional Profiling at Whole Population and Single Cell Levels Reveals Somatosensory Neuron Molecular Diversity,” *eLife* 3 (2014).

addition of the five transcription factors used in the murine model resulted in neuron-like cells, some of which expressed nociceptor molecular markers. These human-induced neurons have been useful for the study of human pain and neuropathy disorders:⁵

- Outgrowths of induced neurons from patients with familial dysautonomia, which is characterized by dysfunction of the autonomic nervous system and insensitivity to pain, are reduced compared to induced neurons from healthy controls.
- Induced neurons from cancer patients can be used to screen for the potentially neurotoxic effects of various chemotherapeutic agents, such as oxaliplatin.
- Genome editing techniques allow researchers to introduce or correct mutations in the induced nociceptors' sodium channels and examine the resulting phenotypes.

Dr. Woolf's group is now in the process of obtaining purer populations of human nociceptors to perform further molecular and genetic analyses.

Update from the American Pain Society

Robert Gereau, PhD, Director, Washington University Pain Center, Department of Anesthesiology, Washington University School of Medicine and APS Board Chair for Research

Organization and mission of the APS

Gereau reviewed the mission, organization, and activities of the American Pain Society (APS) and described how the APS has collaborated with the NIH to increase recognition of and funding for pain research. The APS is a chapter of the International Association for the Study of Pain, whose members include scientists, clinicians, and other professionals. The mission of the APS is to increase the knowledge of pain and transform public policy and clinical practice to reduce pain-related suffering. The APS is the only professional organization in the United States whose primary aim is the promotion of pain research.

The APS is diverse, with members from medicine, psychology, basic science, nursing, and pharmacy. APS members are also clinicians, researchers, and educators, and the board reflects this diversity. The current president of the APS is Dr. Gregory Terman.

The four key activities of the APS

The APS mission involves four key activities: research, education, treatment, and advocacy, which are in turn aided by four administrative divisions: finance, membership, governance, and communications. Further details on each of the four key activities are provided on the website.⁶

1. **Research:** APS's goal is to encourage the NIH and other funders to recognize pain as a distinct and high-priority health care problem, deserving increased resources for research. Dr. Robert Gereau is currently the APS Board Chair for Research.

⁵ Brian J. Wainger et al., "Modeling Pain in Vitro Using Nociceptor Neurons Reprogrammed from Fibroblasts," *Nature Neuroscience* 18, no. 1 (January 2015): 17–24.

⁶ The APS website can be found at: <http://americanpainsociety.org>

2. **Treatment:** APS's goal is to improve the prevention and treatment of pain in diverse clinical settings by applying APS standards of cost-effective, interdisciplinary, evidence-based care. Dr. Mark Wallace is the APS Board's Clinical Chair.
3. **Education:** APS's goal is to be the primary educational source for the acquisition and dissemination of the latest scientifically based information on pain and its interdisciplinary treatment. The APS Board co-chairs are Tonya Palermo and Timothy Ness.
4. **Advocacy:** APS aims to effectively influence the evolution of public and private regulations, policies, and practices in a manner that supports the development of optimal research, education, and interdisciplinary treatment of pain for all people. The APS Board Advocacy Chair is Edward Michna.

Dr. Gereau noted that the APS envisions a world where pain prevention and relief are available to all individuals. He invited all meeting participants to join the APS.

Panel on Cognitive and Emotional Aspects of Pain

Moderator: **Wendy B. Smith, MA, PhD, BCB**, Senior Scientific Advisor for Research Development and Outreach, NIH Office of Behavioral Science and Social Research

Cognitive aspects of acute and chronic pain: 10 advances in 10 years

David Seminowicz, PhD, Assistant Professor, Department of Neural and Pain Sciences, University of Maryland School of Dentistry

Dr. Seminowicz highlighted advances in a key area of research on pain and cognition for each year from 2006 to 2015, then provided a glimpse into potentially fruitful research areas for the future.

Ten years of advances in pain and cognition

- **2006: What are the neural correlates of pain catastrophizing, or the tendency for an exaggerated emotional response to pain, in healthy subjects?**

In response to a mild or moderate noxious stimulus, most healthy individuals report similar levels of pain intensity but a wide range of catastrophizing scores. Functional magnetic resonance imaging (fMRI) reveals that deactivation of the dorsolateral prefrontal cortex (DLPFC) is correlated with greater catastrophizing.⁷

- **2007: Is pain a cognitive load?**

Researchers used fMRI to demonstrate that two brain networks must work in opposition for cognitive tasks.⁸ Specifically, task-positive networks must be activated and task-negative networks must be deactivated for good performance on a cognitive

⁷ David A. Seminowicz and Karen D. Davis, "Cortical Responses to Pain in Healthy Individuals Depends on Pain Catastrophizing," *Pain* 120, no. 3 (February 2006): 297–306.

⁸ David A. Seminowicz and Karen D. Davis, "Pain Enhances Functional Connectivity of a Brain Network Evoked by Performance of a Cognitive Task," *Journal of Neurophysiology* 97, no. 5 (May 2007): 3651–59.

task. Mild to moderate pain affects the task-positive networks required for cognitive tasks, potentially explaining how pain can disrupt cognitive abilities.

- **2008: Are cognitive networks disrupted in chronic pain?**

The default mode network (DMN) includes cortical areas of the brain that are active at rest. fMRI of patients with chronic back pain compared to healthy controls indicates that, while both groups can effectively complete a task, those with chronic back pain have reduced DMN activation compared to controls.⁹ This disruption of cognitive networks has been replicated in other chronic pain disorders, including migraine and fibromyalgia.

- **2009: What drives attention-related pain modulation?**

Researchers examined the role of expectations or anticipation in the perception of pain intensity using fMRI on individuals in a hypnotic state.¹⁰ Increased activation of the DLPFC positively correlated with increased perceptions of pain intensity, suggesting that the DLPFC may have a role in the anticipation of pain.

- **2010: What brain regions mediate the pain experience?**

Researchers examined the effects of cues on the expectations of pain using fMRI.¹¹ Increased activity in the DLPFC was positively correlated with increased expectations of pain, identifying this region as a key component of the cognitive effects of pain.

- **2011: Can treatment reverse abnormal cognitive-related activity in chronic pain?**

Chronic pain sufferers have decreased thickness of the DLPFC as well as other areas of the brain. Researchers examined the MRIs of patients with chronic low back pain (CLBP) before and after surgery or spinal injections.¹² Treatment of CLBP increased the thickness of the DLPFC, which was in turn positively correlated with decreased subjective pain. Previous research also demonstrated that the DLPFC is not deactivated to the same extent in individuals with CLBP. fMRI studies indicate that after treatment, deactivation of the DLPFC occurred to the same extent as healthy controls.

- **2012: Can we modulate activity in the left DLPFC and affect pain?**

Two studies in 2012 demonstrated that modulation of the left DLPFC could induce analgesia. The first study showed that repetitive transcranial magnetic stimulation of the DLPFC induced an analgesic effect.¹³ This analgesia was blocked if the individual was first given the opioid antagonist, naloxone, indicating the involvement of endogenous

⁹ Marwan N. Baliki et al., "Beyond Feeling: Chronic Pain Hurts the Brain, Disrupting the Default-Mode Network Dynamics," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 28, no. 6 (February 6, 2008): 1398–1403.

¹⁰ Tuukka T. Raij et al., "Strength of Prefrontal Activation Predicts Intensity of Suggestion-Induced Pain," *Human Brain Mapping* 30, no. 9 (September 2009): 2890–97.

¹¹ Lauren Y. Atlas et al., "Brain Mediators of Predictive Cue Effects on Perceived Pain," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 30, no. 39 (September 29, 2010): 12964–77.

¹² David A. Seminowicz et al., "Effective Treatment of Chronic Low Back Pain in Humans Reverses Abnormal Brain Anatomy and Function," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31, no. 20 (May 18, 2011): 7540–50.

¹³ Joseph J. Taylor, Jeffrey J. Borckardt, and Mark S. George, "Endogenous Opioids Mediate Left Dorsolateral Prefrontal Cortex rTMS-Induced Analgesia," *Pain* 153, no. 6 (June 2012): 1219–25.

opioids in DLPFC-mediated analgesic effects. The second study demonstrated a positive relationship between mindfulness meditation and increased activation of the DLPFC.¹⁴

- **2013: Are there catastrophizing-related treatment effects?**

Researchers demonstrated that cognitive behavioral therapy (CBT) increases the gray matter volume of the DLPFC and also reduces catastrophizing in patients with chronic pain.¹⁵

- **2014: Does catastrophizing (rumination) disrupt cognitive networks in chronic pain patients?**

Moving beyond individual brain regions to cognitive networks, researchers examined the role of rumination in chronic pain patients with idiopathic temporomandibular disorder.¹⁶ Compared to healthy controls, patients with temporomandibular disorder had increased functional connectivity of the DMN as assessed by fMRI. Furthermore, the amount of rumination was associated with increases in functional connectivity in other areas of the brain, such as the periaqueductal gray and thalamus. These results confirm that cognitive networks are disrupted during rumination in chronic pain.

- **2015: Can we restore cognitive network function with treatment?**

Researchers used fMRI to examine patients with CLBP before and after treatment as well as healthy controls to determine whether treatment could restore the imbalance in the task-positive and task-negative cognitive networks.¹⁷ Treatment partially restored the connectivity to the task networks, demonstrating that treatment can restore some degree of the network imbalance induced by chronic pain.

In summary, research has shown that catastrophizing affects pain-related activity. Furthermore, pain has a cognitive load that affects network function. Finally, the left DLPFC is involved in catastrophizing and may be a good target for intervention.

Pain and cognition research in the future

Looking ahead, Dr. Seminowicz noted three areas of pain and cognition that are likely to yield promising results in the future.

1. **Shift to limbic circuits:** Dr. Seminowicz is interested in the processes of how a healthy brain changes from an acute injury, transitions to chronic pain, and recovers in response to an intervention. Animal models are particularly useful in the study of brain changes

¹⁴ Micah Allen et al., "Cognitive-Affective Neural Plasticity Following Active-Controlled Mindfulness Intervention," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32, no. 44 (October 31, 2012): 15601–10.

¹⁵ David A. Seminowicz et al., "Cognitive-Behavioral Therapy Increases Prefrontal Cortex Gray Matter in Patients with Chronic Pain," *The Journal of Pain: Official Journal of the American Pain Society* 14, no. 12 (December 2013): 1573–84.

¹⁶ Aaron Kucyi et al., "Enhanced Medial Prefrontal-Default Mode Network Functional Connectivity in Chronic Pain and Its Association with Pain Rumination," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 34, no. 11 (March 12, 2014): 3969–75.

¹⁷ Marta Čeko et al., "Partial Recovery of Abnormal Insula and Dorsolateral Prefrontal Connectivity to Cognitive Networks in Chronic Low Back Pain after Treatment," *Human Brain Mapping* 36, no. 6 (June 2015): 2075–92.

from chronic pain. For example, rats demonstrate gray matter changes in sensory and affective regions of the brain following acute injury, with greater loss of gray matter as the severity of pain increases.¹⁸ At later time points following injury, however, decreases in prefrontal cortical volume are associated with increases in anxiety. Two other studies also support the idea that changes in the brain after acute pain at first involve sensory circuits and later transition to changes in limbic circuits during chronic pain.^{19,20}

2. **Maladaptive stress:** Several studies have demonstrated the role of the hippocampus and amygdala in the stress of chronic pain. For example, patients with Burning Mouth Syndrome (BMS) are typically pain-free in the morning, with pain worsening throughout the day. Compared to control individuals, patients with BMS had decreased gray matter volume in the medial PFC (mPFC) and increased gray matter volume in the hippocampus.²¹ This syndrome provides an opportunity to study the connectivity of brain regions with and without pain in the same patient. For example, there is increased connectivity between the mPFC and hippocampus and amygdala in the afternoon compared to the morning. Furthermore, the extent of connectivity between these regions was positively correlated with depression scores. The hippocampus has also been shown to be involved in the stress of CLBP.²² These and other studies are starting to unravel the relationships between pain, stress, and depression.
3. **Refinement of treatment targets:** Dr. Seminowicz noted that a good target for pain intervention should have three components: (1) it does something when you hit it, (2) it does not move, and (3) it might not always do what you expect. For example, if the DLPFC is truly involved in pain pathways, then it should be involved in more than a single pain syndrome. In fact, the DLPFC has been implicated in BMS, migraine, CLBP, and pain catastrophizing. Mindfulness has also been shown to decrease pain and catastrophizing, as well as reduce pain-related activity in the anterior and posterior insula. In the future, it may be possible to analyze whether an individual with pain has a disruption of the DLPFC versus dysfunction of the limbic system, allowing for a personalized strategy for pain relief.

These three future research areas will hopefully address the following questions: (1) Can we prevent the shift to chronic pain? (2) Can we break the cycle of maladaptive stress, reduce

¹⁸ David A. Seminowicz et al., "MRI Structural Brain Changes Associated with Sensory and Emotional Function in a Rat Model of Long-Term Neuropathic Pain," *NeuroImage* 47, no. 3 (September 2009): 1007–14.

¹⁹ Catherine S. Hubbard et al., "Behavioral, Metabolic and Functional Brain Changes in a Rat Model of Chronic Neuropathic Pain: A Longitudinal MRI Study," *NeuroImage* 107 (February 15, 2015): 333–44.

²⁰ David A. Seminowicz et al., "Thalamocortical Asynchrony in Conditions of Spinal Cord Injury Pain in Rats," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32, no. 45 (November 7, 2012): 15843–48.

²¹ Shariq A. Khan et al., "Altered Structure and Function in the Hippocampus and Medial Prefrontal Cortex in Patients with Burning Mouth Syndrome," *Pain* 155, no. 8 (August 2014): 1472–80.

²² Etienne Vachon-Presseau et al., "The Stress Model of Chronic Pain: Evidence from Basal Cortisol and Hippocampal Structure and Function in Humans," *Brain: A Journal of Neurology* 136, no. Pt 3 (March 2013): 815–27.

stress, and decrease depression?, and (3) Can we refine treatment targets for individualized interventions for pain?

Depression and low back pain in older adults: results of the ADAPT trial

Jordan F. Karp, MD, Associate Professor of Psychiatry, Anesthesiology, and Clinical and Translational Science; Medical Director for Psychiatry, UPMC Pain Medicine at Centre Commons, University of Pittsburgh School of Medicine

Background and rationale for the study

Dr. Karp described the rationale for the recently completed Addressing Depression and Pain Together trial (ADAPT), which assessed interventions for the treatment of depression and CLBP in older adults. Late-life depression is of great public health significance, resulting in decreased quality of life, increased health care costs, and increased morbidity and mortality.²³ Between 55 and 81 percent of patients with late-life depression fail to recover with selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs). There is insufficient scientific evidence to guide either pharmacotherapy or psychotherapy for these patients. Moderators of late-life depression include medical comorbidities, anxiety, executive dysfunction, a history of non-response to antidepressants, and pain.

Pain in late life also is prevalent, affecting 25 to 50 percent of community-dwelling older adults and 49 to 83 percent of nursing home residents. Chronic pain in late life is associated with anxiety and depression as well as functional and cognitive impairment, and opioid use for pain has been shown to worsen memory. CLBP is the most common reason for referral to the pain clinic.

Both pain and depression contribute to homeostenosis, or an inability to maintain homeostasis, in late life. Pain and depression have overlapping symptoms, such as disability, cognitive impairment, insomnia, and suicide and have a shared neurobiology and psychology.

Study design

The ADAPT trial attempted to replicate the treatment patients receive in primary care. The patient population included older adults with depression and CLBP who failed previous treatment for their back pain. The primary aim of the study was to compare high-dose venlafaxine with problem-solving therapy for depression and pain (PST-DP) and high-dose venlafaxine with supportive management. The study design included enrollment of 250 individuals aged 60 years or older with depression and CLBP. In phase I, all patients were treated with up to 150 mg of venlafaxine. Responders exited the study, while non-responders were randomized to 300 mg of venlafaxine with or without PST-DP in phase II of the study.

Recruitment for the study was from the primary care setting. If the patient met the eligibility criteria, then a pop-up on the electronic health record screen informed the physician that the

²³ E. J. Lenze et al., "Comorbid Anxiety Disorders in Depressed Elderly Patients," *The American Journal of Psychiatry* 157, no. 5 (May 2000): 722–28.

patient was eligible for a study. Dr. Karp noted that this method of recruitment was highly effective and low-cost; in fact, 27 percent of enrollees were identified using this method. The outcomes measured included depression, pain, and disability.²⁴

Study interventions

Venlafaxine is an SNRI with unusual pharmacodynamics properties. Up to 150 mg per day, venlafaxine is primarily an SSRI; while at doses between 150 and 300 mg per day, venlafaxine also inhibits the reuptake of norepinephrine, thereby acting as an SNRI. Dr. Karp and his colleagues hypothesized that these higher doses may enhance the antidepressant and analgesic effect.

The PST-DP intervention is a seven-step process that assesses the patient's problem-solving orientation, defines a problem, generates solutions, and reviews progress. PST-DP has a broad evidence base in older adults and is relatively easy to implement in primary care. PST-DP has the additional advantage in that non-mental health interventionists can be trained to deliver this therapy. The active control group received venlafaxine and supportive medication management, but not PST-DP.

Study results

The recruitment for this study was successful, with 263 patients consenting. Of consenting patients, 92 percent completed phase I. Of the initial population, 164 patients were non-responders, yielding a non-response rate of 75 percent. Researchers randomized one hundred thirty-nine patients in phase II. Blinded analysis of phase II is ongoing.

The demographics of the responder and non-responder populations were similar. Non-responders however, had higher rates of cumulative illness, depression, non-response to antidepressant therapy, more pain in more regions of the body, and higher rates of disability. Both populations were obese on average and had similar rates of fibromyalgia and spine surgery.

The researchers investigated predictors of response or non-response to 150 mg of venlafaxine and found that a 2-week change in the Numeric Pain Rating Scale (NRS) was predictive of response. Further analysis demonstrated that pain could be an effective predictor for fibromyalgia. A positive response to the question, "Do you often feel like you hurt all over?" in conjunction with a specific pain map had good specificity for identifying a patient with fibromyalgia.

For phase II of the trial, the median dose of venlafaxine was 244 mg per day. Response to the phase II intervention was defined as two sequential doctor visits yielding a Patient Health Questionnaire-9 score less than or equal to 5, indicating decreased depression, and a greater or equal to 30 percent reduction in the NRS, indicating decreased pain. Each intervention arm in phase II had an approximately 40 percent response rate, including reductions in pain and

²⁴ Jordan F. Karp et al., "Addressing Both Depression and Pain in Late Life: The Methodology of the ADAPT Study," *Pain Medicine (Malden, Mass.)* 13, no. 3 (March 2012): 405–18.

disability. Dr. Karp noted that this response rate is good, given the difficulty of effectively treating this patient population. There was no significant difference in outcomes between the two arms.

In conclusion, an additional 40 percent of phase I non-responders improved in phase II. Evidence from the study did not support the use of PST-DP in addition to venlafaxine.

Discussion

Others have documented higher rates of depression and pain in daughters of depressed women or women with chronic pain. Dr. Karp noted that the ADAPT trial does collect patient histories of pain and acknowledged that parents' attitudes about stress and pain have important effects on how children deal with stress later in life.

When asked whether a study arm with psychotherapy only, without the use of venlafaxine, was considered, Karp responded that the ADAPT trial was intended to reproduce primary care with a simple second line therapy of PST-DP; however, he agreed that studying the effects of psychotherapy alone on this patient population would be worthwhile.

Panel on Genetics and Epigenetics of Pain

Moderator: **Gayle Lester, PhD**, Program Director, Clinical Research & Diagnostic Imaging Tools for Osteoarthritis and Bone Quality, the National Institute of Arthritis and Musculoskeletal and Skin Diseases

From genes to pain: lessons from rare inherited diseases and extrapolation to the rest of us
Steve Waxman, MD, PhD, Director, Center for Neuroscience and Regeneration and Neurorehabilitation Research, Yale University School of Medicine

Waxman emphasized that treatment of neuropathic pain is an unmet need that requires more research. His research focuses on voltage-gated ion channels and the lessons that can be learned from individuals with rare mutations in genes involved in pain.

Background

Voltage-gated sodium (Na) channels are large transmembrane proteins (approximately 1,800 amino acids) that regulate the influx of sodium into neurons during action potentials. The voltage-gated Na channel family is diverse; a total of nine genes express proteins with different properties. These proteins are selectively expressed in different types of neurons. The electrophysiology of Na channels also varies. Some examples include Na_v1.7, which is expressed in sympathetic ganglia and DRG and amplifies small depolarizations, and Na_v1.8, which works in tandem with Na_v1.7. Na_v1.8 has a higher threshold than Na_v1.7, and when activated, produces most of the transmembrane current responsible for repetitive action potentials.

Mutations that increase or change Na channel expression or function can lead to both rare and common pain disorders. As a result, some members of the Na channel family of proteins are possible therapeutic targets for the treatment of pain. Patch-clamp recordings from a single injured axon demonstrate repetitive firing, suggesting that an inappropriate mixture of Na

channels contributes to the hyper-excitability of pain signaling nerve cells. Dr. Waxman posited that there might be subtypes of Na channels that are only expressed in the peripheral nerves and are essential for pain signaling. If this were true, then these Na channels would make excellent therapeutic targets, because treatments for these targets would theoretically not cause central nervous system (CNS) or cardiac side effects.

Rare genetic mutations in voltage-gated Na channels

Rare genetic disorders can help scientists understand molecular mechanisms in humans and identify potential therapeutic targets that may be relevant to more common disorders. For example, primary erythromelalgia, also known as the Man on Fire syndrome, demonstrates the importance of Na_v1.7 (encoded by the gene *SCN9A*) in pain. Patients experience severe burning pain triggered by mild warmth. The disorder is dominantly inherited and always expressed in carriers of the gene; unfortunately, in most families the disorder is refractory to all existing pharmacotherapies.

Patients with primary erythromelalgia have gain-of-function mutations in the *SCN9A* gene encoding the Na_v1.7 protein. For example, two Chinese families have mutations that cause the Na_v1.7 protein to have enhanced responses to small, slow depolarizations compared to the wild-type protein.^{25,26} To date, researchers have identified more than a dozen mutations in the *SCN9A* gene in families all over the world. Another example is the F1449V mutation, identified in a large family with primary erythromelalgia. Structural modeling of this mutation demonstrates destabilization of the closed state of the protein, and electrophysiology studies reveal that the mutant protein supports an increased frequency of firing in DRG neurons.^{27,28} Researchers have also discovered loss-of-function mutations in the *SCN9A* gene in individuals who have no ability to feel pain including in response to fractures, burns, childbirth, and tooth extractions.²⁹

The role of Na_v1.8 in regulating the effects of mutations in Na_v1.7

Cell lineage is important in determining the effects of mutations in the Na_v1.7 protein. For example, a gain-of-function mutation results in hyper-excitability, or increased firing, in DRG neurons, but hypo-excitability in sympathetic ganglia. One difference between these two cell types is the presence of Na_v1.8; DRG express the Na_v1.8 protein, while sympathetic ganglia do

²⁵ Y. Yang et al., "Mutations in *SCN9A*, Encoding a Sodium Channel Alpha Subunit, in Patients with Primary Erythromelalgia," *Journal of Medical Genetics* 41, no. 3 (March 2004): 171–74.

²⁶ Theodore R. Cummins, Sulayman D. Dib-Hajj, and Stephen G. Waxman, "Electrophysiological Properties of Mutant Nav1.7 Sodium Channels in a Painful Inherited Neuropathy," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24, no. 38 (September 22, 2004): 8232–36.

²⁷ Angelika Lampert et al., "A Pore-Blocking Hydrophobic Motif at the Cytoplasmic Aperture of the Closed-State Nav1.7 Channel Is Disrupted by the Erythromelalgia-Associated F1449V Mutation," *The Journal of Biological Chemistry* 283, no. 35 (August 29, 2008): 24118–27.

²⁸ S. D. Dib-Hajj et al., "Gain-of-Function Mutation in Nav1.7 in Familial Erythromelalgia Induces Bursting of Sensory Neurons," *Brain: A Journal of Neurology* 128, no. Pt 8 (August 2005): 1847–54.

²⁹ James J. Cox et al., "An *SCN9A* Channelopathy Causes Congenital Inability to Experience Pain," *Nature* 444, no. 7121 (December 14, 2006): 894–98.

not. Researchers demonstrated that expression of the Na_v1.8 protein in sympathetic ganglia also expressing mutant Na_v1.7 reverses the effect of the mutation.³⁰ Na_v1.8 acts as a molecular switch, determining by its presence or absence whether the Na_v1.7 gain-of-function mutations produce hyper- or hypo-excitability.

Research into mutations resulting in pain disorders is paving the way for personalized pain treatment. For example, most patients with primary erythromelalgia do not respond to any pain medication. However, there is one family with primary erythromelalgia that carries a mutation in Na_v1.7, which sensitizes the protein to the effects of the anti-epileptic drug carbamazepine.³¹ The atomic-level structure of this mutant protein indicates that other amino acids nearby within the folded protein may also sensitize the channel to this drug. Indeed, this pharmacogenetic approach successfully predicts the response of channel variants to carbamazepine.³²

Extrapolation of lessons learned from rare Na_v1.7 mutations to more common pain disorders

There are several examples of mutations in Na_v1.7 and Na_v1.8 in more common pain disorders. For example, researchers have demonstrated that Na_v1.7 and Na_v1.8 accumulate in the damaged axon tips in painful neuromas, indicating that these proteins may be appropriate targets for drug therapy.³³

Small fiber neuropathy (SFN) is another more common pain disorder, which presents as burning in the hands and feet. Approximately half of all SFN cases are idiopathic; known causes of SFN include chemotherapy, diabetes, and amyloidosis. Researchers have identified mutations in the *SCN9A* gene in 8 of 28 patients diagnosed with SFN. These mutations resulted in amino acid changes in the loops and linkers between the transmembrane portions of the Na_v1.7 protein, consistent with the more subtle phenotype of SFN compared to primary erythromelalgia.³⁴ These mutant Na_v1.7 proteins had relatively subtle changes in gating including impaired fast-and/or slow-inactivation, resulting in inappropriate and spontaneous firing of DRG neurons. Researchers have also identified mutations in the genes encoding Na_v1.8 and Na_v1.9 in patients with severe itch and SFN. For example, approximately 1 percent of patients with SFN who do not have mutations in Na_v1.7 or Na_v1.8 have gain-of-function mutations in Na_v1.9.³⁵

³⁰ Anthony M. Rush et al., "A Single Sodium Channel Mutation Produces Hyper- or Hypoexcitability in Different Types of Neurons," *Proceedings of the National Academy of Sciences of the United States of America* 103, no. 21 (May 23, 2006): 8245–50.

³¹ Tanya Z. Fischer et al., "A Novel Nav1.7 Mutation Producing Carbamazepine-Responsive Erythromelalgia," *Annals of Neurology* 65, no. 6 (June 2009): 733–41.

³² Yang Yang et al., "Structural Modelling and Mutant Cycle Analysis Predict Pharmacoresponsiveness of a Nav1.7 Mutant Channel," *Nature Communications* 3 (November 13, 2012): 1186.

³³ Joel A. Black et al., "Multiple Sodium Channel Isoforms and Mitogen-Activated Protein Kinases Are Present in Painful Human Neuromas," *Annals of Neurology* 64, no. 6 (December 2008): 644–53.

³⁴ Catharina G. Faber et al., "Gain of Function Nav1.7 Mutations in Idiopathic Small Fiber Neuropathy," *Annals of Neurology* 71, no. 1 (January 2012): 26–39.

³⁵ Jianying Huang et al., "Gain-of-Function Mutations in Sodium Channel Na(v)1.9 in Painful Neuropathy," *Brain: A Journal of Neurology* 137, no. Pt 6 (June 2014): 1627–42.

Future directions for Na channel neuropathies

Early clinical studies targeting Na channels show promising results. The next step is to replicate these early results with larger clinical trials and broader pain diagnoses, with the ultimate goal of a new, more effective class of pain medications with minimal central side effects and addictive potential.

Epigenetic regulation of pain: what we know so far

Laura Stone, PhD, Associate Professor, Alan Edwards Centre for Research on Pain, McGill University

Background

Epigenetic modifications regulate gene expression and play a significant role in the regulation of pain. There is a growing literature on both the inducers and effects of epigenetic changes. Life itself is an “epigenetic disease,” in that every aspect of the environment is a potential inducer of epigenetic changes, including socioeconomic influences, diet, exercise, hormones, and toxins. Epigenetic changes can, in turn, affect the development of numerous human diseases, including cancer, diabetes, cardiovascular disease, and pain. The sum of our experiences creates either risks or protections in the form of the epigenetic regulation of our genomes.

Epigenetic modifications can occur by several molecular mechanisms. Examples include:

- **Histone modifications:** Human DNA is wrapped around histone proteins, which help structure DNA into chromosomes. Modification of histones can result in compaction or unraveling of DNA, affecting the ability of genes to be transcribed into RNA. For example, histone acetyltransferases (HATs) place acetyl groups on histones, which relax the chromatin and allow genes to be transcribed. Histone deacetylases (HDACs) quiet the expression of genes by removing the acetyl group from histones. Deacetylation of histones results in the compaction of DNA, making genes inaccessible for transcription.
- **DNA methylation:** In order for transcription to occur, transcription factors must bind to the promoter of a gene. However, if methyl groups are attached to the promoter of a gene by DNA methyltransferases (DNMTs), transcription factors cannot bind to the promoter and transcription is inhibited. In an alternative epigenetic mechanism, methyl groups on the promoter can attract a protein that sits on the methylated promoter and blocks transcription.

An example of epigenetic regulation: the glucocorticoid receptor

Researchers have shown that early maternal care can affect the epigenome and results in certain behaviors as an adult. For example, rat pups that are well cared for by their mothers tend to have decreased stress responses as adults. Alternatively, pups that are neglected have increased stress responses. Researchers have demonstrated that the RNA levels of the glucocorticoid receptor (GR/NR3C1) are significantly decreased in animals that were neglected

as pups. This decrease in GR RNA levels alters the functioning of the hypothalamic-pituitary-adrenal (HPA) axis and decreases the resiliency of the animal.³⁶

Further research demonstrated that in animals receiving maternal care, there was an increase in histone acetylation, allowing transcription factors to bind to the promoter of the GR gene. In contrast, animals with poor maternal care had increased DNA methylation of the promoter, effectively blocking transcription of the GR gene. Stone noted that the enzymes responsible for acetylation and deacetylation of histones, as well as those that methylate or demethylate DNA, are potential targets for changing the epigenetic state of a particular gene.

The epigenetic regulation of the GR gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) can be translated to a similar phenomenon in humans. Researchers have shown that epigenetic regulation of *NR3C1* in the human brain is associated with childhood abuse.³⁷ Abused individuals had significantly decreased levels of the GR and increased methylation of the *NR3C1* promoter. These examples have implications for the epigenetic regulation of pain. For example, socioeconomic factors such as maternal care and abuse may alter the physiological response to an initial injury and result in psychosocial risk factors for chronic pain, including decreased resiliency and increased anxiety.

Genome-wide epigenetic regulation

Epigenetic regulation is a genome-wide phenomenon; the sum of the epigenetic state of all genes can be defined as the “epigenome.” It is now possible to evaluate the extent of methylation for all genes in a genome. For example, there are significant differences in genome-wide methylation of DNA from the prefrontal cortex (PFC) and T-cells of monkeys that were reared by their mothers versus a surrogate.³⁸ The presence of methylation differences in both the PFC and T-cells suggests that changes to the epigenome are widespread and not limited to just one tissue.

Epigenetic regulation of pain

Epigenetic regulation of pain occurs at every step in the pain transmission pathway, including detection of pain by nociceptors, transduction and transmission of pain signals to the brain, perception of pain, and descending modulation of pain. As we grow older, our cumulative experiences create either a risk or protective epigenetic environment. Upon injury, this susceptibility influences whether pain progresses to a chronic state.

For example, researchers examined twins who were differentially sensitive to pain from heat. Twins are genetically identical, so any differences in heat sensitivity between the two

³⁶ D. Francis et al., “Nongenomic Transmission across Generations of Maternal Behavior and Stress Responses in the Rat,” *Science (New York, N.Y.)* 286, no. 5442 (November 5, 1999): 1155–58.

³⁷ Patrick O. McGowan et al., “Epigenetic Regulation of the Glucocorticoid Receptor in Human Brain Associates with Childhood Abuse,” *Nature Neuroscience* 12, no. 3 (March 2009): 342–48.

³⁸ Nadine Provençal et al., “The Signature of Maternal Rearing in the Methylome in Rhesus Macaque Prefrontal Cortex and T Cells,” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32, no. 44 (October 31, 2012): 15626–42.

individuals should be a result of epigenetic regulation. In fact, there was an association between the amount of methylation on the transient receptor potential cation channel, subfamily A, member 1 (*TRPA1*) promoter and sensitivity to heat.³⁹

Researchers have also studied the epigenetics of mice deficient in a protein called “secreted protein, acidic, rich in cysteine,” or SPARC. SPARC deficiency leads to progressive and accelerated disc degeneration. SPARC-deficient mice have both back and leg pain and are a model of accelerated aging in the mouse. Scientists have shown that the *SPARC* promoter has significantly more methylation in mice, resulting in decreased transcription of the *SPARC* gene.⁴⁰ Methylation of the *SPARC* promoter is also increased in painful human discs compared to healthy controls. Stone noted that epigenetic regulation of *SPARC* might also play a role in osteoarthritis, rheumatoid arthritis, and other pain disorders.

Chronic pain changes the epigenome

Chronic pain induces long-lasting, yet reversible changes in the brain as well as global changes to the epigenome. For example, the amount of methylation was decreased significantly in the PFC of animals that sustained a nerve injury 6 months prior. Researchers hypothesized that an environmental enrichment could reverse the methylation deficiency of these animals and decrease pain. Injured animals that had an enriched environment, including playmates and exercise equipment, had restored levels of methylation in the PFC as well as significantly decreased pain.⁴¹ Although researchers have noted increases in total amounts of methylation during recovery, the particular genes that, when remethylated, contribute to reductions in pain remain unknown.

Massart and Gregoire et al. (unpublished data) conducted a genome-wide analysis of how much individual genes are methylated in the PFC of rats 9 months post injury compared to controls. Peripheral nerve injury resulted in the differential methylation of thousands of genes, a dramatic difference usually seen between two different tissues or species. The epigenetic machinery itself was also different between the two groups of animals; injured animals had increased levels of DNMT1 and HDAC1. In another study by Bai et al., inflammation of the spinal cord could be reversed in animals treated with HDAC inhibitors, further implicating these enzymes in the regulation of pain.⁴² The search continues for genes that are differentially methylated in normal versus chronic pain conditions.

Conclusions

Epigenetics may contribute to risk factors for developing chronic pain as well as to the induction and maintenance of chronic pain. Epigenetics can be studied at the individual gene,

³⁹ J. T. Bell et al., “Differential Methylation of the TRPA1 Promoter in Pain Sensitivity,” *Nature Communications* 5 (2014): 2978.

⁴⁰ Maral Tajerian et al., “DNA Methylation of SPARC and Chronic Low Back Pain,” *Molecular Pain* 7 (2011): 65.

⁴¹ Maral Tajerian et al., “Peripheral Nerve Injury Is Associated with Chronic, Reversible Changes in Global DNA Methylation in the Mouse Prefrontal Cortex,” *PLoS One* 8, no. 1 (2013): e55259.

⁴² Guang Bai et al., “Inhibition of Class II Histone Deacetylases in the Spinal Cord Attenuates Inflammatory Hyperalgesia,” *Molecular Pain* 6 (2010): 51.

genome-wide, or systems level. Advances in treating pain will involve new target identification and the targeting of epigenetic machinery.

Epigenetic regulation in chronic stress-induced visceral pain

John Wiley, MD, Professor Internal Medicine, University of Michigan

Background

Wiley defined epigenetics as “the inheritable, potentially reversible processes that regulate gene activity and expression and that are independent of actual changes in DNA sequence.”⁴³ Mechanisms of epigenetic regulation include DNA methylation, histone modification, and microRNA regulation.

Epigenetics of chronic stress-induced visceral pain

Wiley hypothesized that the epigenetic mechanisms of methylation and acetylation have an important role in the regulation of chronic stress-induced visceral pain. His previous research demonstrated that chronic, stress-induced, visceral pain is correlated positively with a decrease in the anti-nociceptive endocannabinoid (CB₁) receptor and an increase in the pro-nociceptive transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor in DRG neurons.⁴⁴ In an animal model of stress-induced visceral pain, chronic psychological stress activates the HPA axis and stimulates the production of corticosterone, inducing production of the GR. This transcription factor increases production of HATs that result in histone acetylation and increased transcription of the *TRPV1* gene. In addition, DNMT1 is up-regulated, providing negative feedback control of the GR as well as inducing DNA methylation and suppression of CB₁ expression.

To measure chronic visceral pain, researchers used a rat model of chronic water avoidance stress. Rats were placed on a platform surrounded by water to induce stress, while control animals were placed in the same apparatus without water. Researchers then measured the animals' visceral sensitivity to colorectal distension and analyzed the animals' epigenetics. In order to test the validity of the stress model described above, researchers examined the effect of a GR antagonist on pain levels following the water avoidance stressor. Animals treated with the GR antagonist had significantly diminished visceral pain following the water avoidance stress, indicating the importance of the HPA axis and the GR in eliciting a pain response.

Chronic water avoidance stress increased levels of DNMT1, methylation of the *GR* and *CB₁* promoters, and visceral pain perception.⁴⁵ Blockage of DNMT1 by small, interfering RNA, reduced the pain in stressed animals. Chromatin immunoprecipitation studies also

⁴³ Tom Misteli, “The Cell Biology of Genomes: Bringing the Double Helix to Life,” *Cell* 152, no. 6 (March 14, 2013): 1209–12.

⁴⁴ Shuangsong Hong et al., “Corticosterone Mediates Reciprocal Changes in CB₁ and TRPV1 Receptors in Primary Sensory Neurons in the Chronically Stressed Rat,” *Gastroenterology* 140, no. 2 (February 2011): 627–37.

⁴⁵ Shuangsong Hong, Gen Zheng, and John W. Wiley, “Epigenetic Regulation of Genes That Modulate Chronic Stress-Induced Visceral Pain in the Peripheral Nervous System,” *Gastroenterology* 148, no. 1 (January 2015): 148–57.e7.

demonstrated an increase in HAT, resulting in an increase in acetylation of the histones surrounding the *TRPV1* gene. These epigenetic changes occurred in DRG neurons that innervate the colon.

Effects of chronic stress on epithelial tight junction protein expression and function

Irritable bowel syndrome with diarrhea (IBS-D) is associated with an increased permeability of the bowel epithelium that is correlated with increased pain.⁴⁶ Researchers assessed whether the intestinal barrier is altered during chronic stress. The junctions of intestinal epithelial cells are regulated by a host of proteins that interact with neighboring cells. Chronic stress changes the epithelial profile of junction proteins, resulting in increased permeability of intestinal epithelia. For example, researchers demonstrated that IBS-D patients have an increase in the mir-29 microRNA precursor, which results in decreased expression of the epithelial tight junction protein claudin-1 and increased intestinal permeability.⁴⁷

Conclusion

In conclusion, the chronic stress rat model of visceral pain shows increased methylation of the *GR* and *CB₁* promoters and decreased expression of *GR* and *CB₁* as well as increased histone acetylation of the *TRPV1* promoter and increased expression of *TRPV1*. There is an overall decrease in the anti-nociceptive pathway and increase in the pro-nociceptive pathway, resulting in increased pain and epigenetic changes that are cell specific, involving nociceptive DRG neurons that innervate the colon.

Future directions in the study of epigenetics in visceral pain include understanding ethnic, gender, and individual differences in epigenetic regulation, the role of epigenetics in the breakdown of intestinal barriers, and the mechanisms behind generational epigenetic memory. Wiley highlighted the efforts of the NIH Roadmap Epigenome project, whose initial data provide growing evidence for the role of epigenetics in human traits and disease. Initial data further demonstrate that epigenetic regulation is likely to be a more significant predictor of traits and disease than variation in the genetic code.⁴⁸

Discussion

When asked about a possible role of epigenetics in pain disorders such as fibromyalgia, Waxman posited that epigenetics may be a fruitful avenue of future research for all pain disorders and noted that existing mutations in coding regions do not account for all cases of idiopathic erythromelalgia.

Another participant enquired whether early-life epigenetic changes were modifiable later in life. Stone remarked that studies that provide enrichment and physical activity demonstrate that positive environmental change can reverse epigenetic changes and improve chronic pain.

⁴⁶ QiQi Zhou, Buyi Zhang, and G. Nicholas Verne, "Intestinal Membrane Permeability and Hypersensitivity in the Irritable Bowel Syndrome," *Pain* 146, no. 1–2 (November 2009): 41–46.

⁴⁷ QiQi Zhou et al., "MicroRNA 29 Targets Nuclear Factor- κ B-Repressing Factor and Claudin 1 to Increase Intestinal Permeability," *Gastroenterology* 148, no. 1 (January 2015): 158–69.

⁴⁸ Information on the NIH Roadmap Epigenomics Mapping Consortium can be found at: <http://www.roadmapepigenomics.org>

Wiley also commented that CBT can produce brain changes on the magnitude of that seen for psychotropic drugs, further supporting the notion that these changes are reversible. It may be that stress is retained in a more durable fashion in early life. Stone emphasized the importance of also studying epigenetic contributions from the germline and examining the effects on children of parents who have experienced trauma.

Panel on Pain Signatures and Predictors from Imaging Research

Moderator: **Catherine Bushnell, PhD**, Scientific Director, Division of Intramural Research, National Center for Complementary and Integrative Health

A retrospective of advances through imaging and signatures of pain disease and recovery

David Borsook, MD, PhD, Director, Pain and Imaging Neuroscience Group, Boston Children's Hospital, Massachusetts General, and McLean Hospitals

Brain imaging techniques have greatly advanced over the past decades and will play a significant role in the evaluation and treatment of individuals with chronic pain. Evidence for the involvement of CNS pathways in pain began to emerge in 1965.⁴⁹ Brain imaging researchers have since taken fMRI studies beyond the initial development and trial phases and are now applying this technique to the study of pain. Interestingly, fMRI studies of animals have lagged behind those of humans in a reversal of the usual experimental paradigm.

The brain exhibits characteristic changes in chronic pain, including altered brain chemistry, altered brain network connectivity, a decrease in the gray matter volume of the DLPFC, and structural changes in nerve tracts. These physiological changes manifest as pain behaviors, such as experiencing pain at rest, anxiety, depression, decreased attention, and an inability to seek or feel rewards. Structural imaging of the brain using fMRI has increased the understanding of the structural and behavioral changes in patients with chronic pain.

Borsook noted that researchers should use imaging to find new ways to improve the lives of chronic pain patients. He divided fMRI's contribution to the understanding of chronic brain changes into four domains: CNS neurobiology, applied biology and pharmacology, CNS disease processes, and therapies. A summary of findings in each of these areas is outlined below.

1. CNS neurobiology:

- Identifying sensory and affective components of pain: Researchers first began to identify the sensory and affective components of pain with imaging techniques in the 1990s.⁵⁰ This research identified the role of the anterior cingulate (AC) in pain affect. A review of advances in 2013 highlighted that attentional and emotional

⁴⁹ R. Melzack and P. D. Wall, "Pain Mechanisms: A New Theory," *Science (New York, N.Y.)* 150, no. 3699 (November 19, 1965): 971–79.

⁵⁰ J. D. Talbot et al., "Multiple Representations of Pain in Human Cerebral Cortex," *Science (New York, N.Y.)* 251, no. 4999 (March 15, 1991): 1355–58.

factors affect the perception of pain through different pathways that are disrupted in chronic pain.⁵¹

- Mapping a sensory pathway: Beginning in 2000, researchers have used imaging techniques to map changes in sensory pathways during pain.⁵² Subsequent research showed that these changes can predict the onset of a migraine attack.⁵³
- Reward circuitry and the accumbens: Scientists discovered the importance of the nucleus accumbens (NAc) and its connection to the PFC in predicting pain persistence. For example, Baliki et al. demonstrated that increased connectivity between the NAc and the mPFC predicted pain persistence in chronic back pain.⁵⁴
- Pain modulation: Improvements in imaging techniques allowed scientists to image subregions of the periaqueductal gray. Subsequent research demonstrated that each of these subregions has a discrete role in pain modulation.⁵⁵ Research on the periaqueductal gray in animals has illuminated how this region may modulate pain intensity via spinal cord activity.⁵⁶ This research points to a new understanding of ascending pain perception and descending pain modulation.
- Dissecting circuits: Advances in imaging have allowed researchers to dissect the exact circuits involved in pain. For example, researchers were recently able to map both afferent and efferent connections to the habenula, determine their role in pain, and examine how these connections change in various disease states.⁵⁷
- Inter-individual differences: Researchers have used imaging to examine differences in the brain of individuals with low or high levels of pain or in individuals with mutations in genes responsible for pain responses.⁵⁸ They further identified a brain signature that identifies a priori patients who will experience a placebo response to treatment.⁵⁹

⁵¹ M. Catherine Bushnell, Marta Ceko, and Lucie A. Low, "Cognitive and Emotional Control of Pain and Its Disruption in Chronic Pain," *Nature Reviews. Neuroscience* 14, no. 7 (July 2013): 502–11.

⁵² Alex F. M. DaSilva et al., "Somatotopic Activation in the Human Trigeminal Pain Pathway," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 22, no. 18 (September 15, 2002): 8183–92.

⁵³ Anne Stankewitz et al., "Trigeminal Nociceptive Transmission in Migraineurs Predicts Migraine Attacks," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31, no. 6 (February 9, 2011): 1937–43.

⁵⁴ Marwan N. Baliki et al., "Corticostriatal Functional Connectivity Predicts Transition to Chronic Back Pain," *Nature Neuroscience* 15, no. 8 (August 2012): 1117–19.

⁵⁵ Ajay B. Satpute et al., "Identification of Discrete Functional Subregions of the Human Periaqueductal Gray," *Proceedings of the National Academy of Sciences of the United States of America* 110, no. 42 (October 15, 2013): 17101–6.

⁵⁶ Christian Sprenger, Jürgen Finsterbusch, and Christian Büchel, "Spinal Cord-Midbrain Functional Connectivity Is Related to Perceived Pain Intensity: A Combined Spino-Cortical fMRI Study," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35, no. 10 (March 11, 2015): 4248–57.

⁵⁷ L. Shelton et al., "Mapping Pain Activation and Connectivity of the Human Habenula," *Journal of Neurophysiology* 107, no. 10 (May 2012): 2633–48.

⁵⁸ Robert C. Coghill, John G. McHaffie, and Ye-Fen Yen, "Neural Correlates of Interindividual Differences in the Subjective Experience of Pain," *Proceedings of the National Academy of Sciences of the United States of America* 100, no. 14 (July 8, 2003): 8538–42.

⁵⁹ Javeria A. Hashmi et al., "Brain Networks Predicting Placebo Analgesia in a Clinical Trial for Chronic Back Pain," *Pain* 153, no. 12 (December 2012): 2393–2402.

- Sex differences: Imaging studies have demonstrated significant differences in the brains of men versus women. For example, men and women have different responses to activation of the μ -opioid receptor.⁶⁰ Imaging studies have also shown that menstruation is a stressor, implying that a woman's cycle should be a consideration in designing and analyzing clinical trials.⁶¹ There are also sex differences in brain activity during migraine: women's brains show more activation of brain regions involved in emotion compared to men.⁶²
- Pain at the extremes of age: Imaging studies have demonstrated that Alzheimer's patients show increased pain sensitivity and pain-related brain activity compared to individuals without disease.⁶³ Pain-related brain activity is also similar between infants and the elderly; these two populations may have less connectivity between regions of the brain involved in pain regulation than others.⁶⁴

2. Applied biology and pharmacology:

- Pain anticipation, anxiety, and fear: Imaging studies have shown that the anticipation of pain results in similar brain activation patterns as pain itself. Furthermore, exercises to reduce fear and anxiety in children with pain are effective in reducing pain and result in less pain-related brain activity.⁶⁵
- The placebo effect: Researchers have shown that patients who experience a placebo response activate the same regions of the brain as patients who are treated with opioids.⁶⁶ The placebo effect also results in spinal cord changes that are associated with diminished pain.⁶⁷ The challenge for the future is to find ways to extend the duration of the placebo effect.
- Pain catastrophizing: A patient's tendency to catastrophize is an excellent predictor of post-surgical pain, implying that an individual who catastrophizes is predisposed to enter a chronic pain state. Imaging studies have provided visual correlates of catastrophizing in patients with chronic pain.⁶⁸

⁶⁰ Jon-Kar Zubieta et al., "Mu-Opioid Receptor-Mediated Antinociceptive Responses Differ in Men and Women," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 22, no. 12 (June 15, 2002): 5100–5107.

⁶¹ Jae Chan Choi et al., "Different Brain Activation Patterns to Pain and Pain-Related Unpleasantness during the Menstrual Cycle," *Anesthesiology* 105, no. 1 (July 2006): 120–27.

⁶² Nasim Maleki et al., "Her versus His Migraine: Multiple Sex Differences in Brain Function and Structure," *Brain: A Journal of Neurology* 135, no. Pt 8 (August 2012): 2546–59.

⁶³ Leonie J. Cole et al., "Pain Sensitivity and fMRI Pain-Related Brain Activity in Alzheimer's Disease," *Brain: A Journal of Neurology* 129, no. Pt 11 (November 2006): 2957–65.

⁶⁴ Sezgi Goksan et al., "fMRI Reveals Neural Activity Overlap between Adult and Infant Pain," *eLife* 4 (2015).

⁶⁵ L. E. Simons et al., "The Responsive Amygdala: Treatment-Induced Alterations in Functional Connectivity in Pediatric Complex Regional Pain Syndrome," *Pain* 155, no. 9 (September 2014): 1727–42.

⁶⁶ Predrag Petrovic et al., "Placebo and Opioid Analgesia—Imaging a Shared Neuronal Network," *Science (New York, N.Y.)* 295, no. 5560 (March 1, 2002): 1737–40.

⁶⁷ Falk Eippert et al., "Direct Evidence for Spinal Cord Involvement in Placebo Analgesia," *Science (New York, N.Y.)* 326, no. 5951 (October 16, 2009): 404.

⁶⁸ Catherine S. Hubbard et al., "Altered Brain Structure and Function Correlate with Disease Severity and Pain Catastrophizing in Migraine Patients," *eNeuro* 1, no. 1 (December 30, 2014): e20.14.

- Empathy and pain: Research has revealed interesting correlations between empathy and pain. For example, imaging studies have demonstrated that similar regions of the brain are involved in one's own pain as well as in empathy for the pain of others. Furthermore, regions of the brain involved in empathy are activated even in individuals who have never felt pain, indicating that empathy is an innate biological process.⁶⁹

3. CNS disease processes:

- Phantom limb pain: Imaging studies demonstrate that patients who lose a limb have altered circuitry in pain-related brain regions; mirror therapy for phantom limb pain, in which patients can look at their remaining limb in a mirror to visualize the lost limb, can restore normal circuitry and reduce pain.⁷⁰
- Glia and neuroinflammation: Researchers have demonstrated that glial cells are activated in patients with chronic pain, indicating a role for these cells in the establishment of the chronic cycle of pain in CLBP.⁷¹
- Gray matter changes: Imaging studies have established that decreases in brain gray matter occur in a number of chronic pain conditions, including CLBP and tension headache.⁷² This loss of gray matter results from a loss of dendritic processes, thereby reducing the number of neuronal connections in the brain. Therapies that reduce pain have also been shown to restore gray matter volume.⁷³
- Other neuropathic pain states: Scientists have used imaging to better understand altered brain structure and function in conditions such as diabetic neuropathy, post-herpetic neuralgia, and small fiber neuropathy.⁷⁴
- Pain in children: Children with chronic pain develop abnormal connectivity in pain-related brain regions that persists into adulthood. Therapy can both decrease pain and as well as reverse these brain abnormalities.⁷⁵
- Psychiatric disease and pain: There are almost no psychiatric disorders without a pain component. For example, the majority of patients with major depressive

⁶⁹ Nicolas Danziger, Isabelle Faillenot, and Roland Peyron, "Can We Share a Pain We Never Felt? Neural Correlates of Empathy in Patients with Congenital Insensitivity to Pain," *Neuron* 61, no. 2 (January 29, 2009): 203–12.

⁷⁰ J. Foell et al., "Mirror Therapy for Phantom Limb Pain: Brain Changes and the Role of Body Representation," *European Journal of Pain (London, England)* 18, no. 5 (May 2014): 729–39.

⁷¹ Marco L. Loggia et al., "Evidence for Brain Glial Activation in Chronic Pain Patients," *Brain: A Journal of Neurology* 138, no. Pt 3 (March 2015): 604–15.

⁷² T. Schmidt-Wilcke et al., "Gray Matter Decrease in Patients with Chronic Tension Type Headache," *Neurology* 65, no. 9 (November 8, 2005): 1483–86.

⁷³ David A. Seminowicz et al., "Effective Treatment of Chronic Low Back Pain in Humans Reverses Abnormal Brain Anatomy and Function," *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31, no. 20 (May 18, 2011): 7540–50.

⁷⁴ Paul-Chen Hsieh et al., "Imaging Signatures of Altered Brain Responses in Small-Fiber Neuropathy: Reduced Functional Connectivity of the Limbic System after Peripheral Nerve Degeneration," *Pain* 156, no. 5 (May 2015): 904–16.

⁷⁵ Nathalie Erpelding et al., "Rapid Treatment-Induced Brain Changes in Pediatric CRPS," *Brain Structure & Function*, December 17, 2014.

disorder also develop generalized pain syndromes. Imaging research has demonstrated altered pain responses in individuals with depression.⁷⁶

- Stress and pain: Stress and pain both increase the allostatic load and result in changes to the hippocampus across a spectrum of pain disorders.⁷⁷
- Chemical markers: Researchers are examining the chemical changes in the brain that occur in chronic pain and how drugs may change the chemistry of the brain in responders and non-responders.⁷⁸
- Pain measures in the operating room: Scientists are imaging pain in the operating room in an attempt to predict pain levels post surgery.
- Opioids change the brain: Imaging studies reveal that patients who become dependent on opioids have altered white matter tracts and changes in endogenous opioid function.⁷⁹

4. Therapies:

- Translational imaging: Imaging studies provide an opportunity to understand brain processes in disease and highlight potential therapeutic strategies. For example, imaging techniques identified brain changes during the aura phase of a migraine; potential interventions can be evaluated for their ability to prevent these changes.⁸⁰
- Drug development: Imaging can optimize drugs that affect in the CNS.⁸¹ The use of imaging during phase I trials is increasing, demonstrating an increasing recognition of their utility in the drug development process.

Chronic pain disrupts healthy, adaptive processes, resulting in maladaptive processes, such as increased pain intensity and unpleasantness, autonomic disturbances, decreased sex drive and appetite, sleep disturbances, depression, anxiety, and cognitive and motor dysfunction. Imaging techniques can provide an individual fingerprint of the state of these processes, highlight altered brain regions and circuitry, and chart a way forward for personalized treatment.

⁷⁶ Irina A. Strigo et al., "Association of Major Depressive Disorder with Altered Functional Brain Response during Anticipation and Processing of Heat Pain," *Archives of General Psychiatry* 65, no. 11 (November 2008): 1275–84.

⁷⁷ Vachon-Preseau et al., "The Stress Model of Chronic Pain," *Brain: A Journal of Neurology* 136, Pt 3 (March 2013): 815-827.

⁷⁸ I. D. Grachev, B. E. Fredrickson, and A. V. Apkarian, "Abnormal Brain Chemistry in Chronic Back Pain: An in Vivo Proton Magnetic Resonance Spectroscopy Study," *Pain* 89, no. 1 (December 15, 2000): 7–18.

⁷⁹ Jaymin Upadhyay et al., "Alterations in Brain Structure and Functional Connectivity in Prescription Opioid-Dependent Patients," *Brain: A Journal of Neurology* 133, no. Pt 7 (July 2010): 2098–2114.

⁸⁰ N. Hadjikhani et al., "Mechanisms of Migraine Aura Revealed by Functional MRI in Human Visual Cortex," *Proceedings of the National Academy of Sciences of the United States of America* 98, no. 8 (April 10, 2001): 4687–92.

⁸¹ David Borsook et al., "Decision-Making Using fMRI in Clinical Drug Development: Revisiting NK-1 Receptor Antagonists for Pain," *Drug Discovery Today* 17, no. 17–18 (September 2012): 964–73.

Predictors and consequences of chronic pain

A. Vania Apkarian, PhD, Professor of Physiology, Anesthesiology, and Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University

Background

The process of pain perception and transduction was conceptualized as early as 1644. The process involves firing of peripheral nerves in response to detectable painful stimuli, which is followed by signaling through the spinal cord and brain. Over time, functional and anatomical changes may develop in the brain leading to a state of chronic pain. The entire nervous system is plastic and reorganizes in a way that creates chronic pain.

Apkarian noted that only a small proportion of subjects with similar injuries develop chronic pain; therefore, injury itself does not explain the outcome. Furthermore, factors identified in MRI studies so far predict only 1 percent of chronic back pain cases. As a result, it remains unclear why some individuals transition to chronic pain and how this process occurs.

Studying the transition from acute to chronic pain

Dr. Apkarian described his work on the brain changes during the transition from acute to chronic pain. The goal of the study was to assess the role of the brain as back pain patients transitioned either to recovery or to chronic pain and to identify parameters that are predictive of chronic pain. In this longitudinal, cross-sectional, observational study, researchers recruited patients with moderate to severe back pain and monitored brain parameters for 1 to 3 years. Patients whose pain persisted received a total of five brain scans over the course of 3 years.

Approximately half of the patients in the study recovered, experiencing about a 50 percent reduction in pain levels. Pain levels remained roughly constant in patients who developed chronic pain during the 3-year follow-up period. Imaging identified consistent differences between the two groups in the functional connectivity between the NAc and mPFC throughout the first year. There were also differences in white matter, with decreased baseline myelination in patients who develop chronic pain compared to those who do not.

An analysis of a subset of patients tracked longitudinally for 3 years found that pain levels for the chronic pain patients were approximately the same at the 1- and 3-year time points. Imaging studies in this cohort demonstrated that limbic white matter contains three network clusters. Of these three clusters, the white matter network and functional connectivity of the mPFC-NAc-Amygdala cluster was different in recovered compared to chronic pain patients. The hippocampus and amygdala were smaller and had an altered shape in patients with chronic pain compared to patients who have recovered.

Using risk factors to develop a model to predict individuals who will progress to chronic pain

The researchers developed a model for predicting the transition to chronic pain at 1 year based on brain scans within several weeks after the start of back pain. The model includes several components:

1. White matter connectivity of the mPFC-NAc-amygdala cluster

2. Functional connectivity of the mPFC-NAc-amygdala cluster
3. Right amygdala volume
4. Contribution of risk alleles from the μ -opiate gene

Predictions with this model are approximately 90 percent accurate. However, the model is not predictive of the intensity of back pain at 1 year. Instead, pain intensity at 1 year is mainly related to the pain intensity at the entry into the study. Furthermore, pain intensity does not predict which patients will eventually develop chronic back pain.

Conclusion

Apkarian summarized the stages of the development of chronic pain. There is a structural and functional brain predisposition that explains approximately 90 percent of the risk for developing CLBP. Having this risk factor and experiencing an acute injury leads to brain reorganization and, eventually, to a chronic pain state. These alterations in brain structure and function are maintained in chronic pain patients.

Using fMRI to assess and understand pain

Tor Wager, PhD, Director, Cognitive and Affective Neuroscience Laboratory, University of Colorado

Background

Wager noted that a plethora of afferent and efferent pain pathways are involved in the detection, perception, and modulation of pain. It is difficult to know which pathways should be the focus of research efforts. Researchers would like to understand the mental representations of pain and to have biomarkers that objectively measure the pain experience. Although it is not possible to measure an individual's mental experience of pain, it is possible to measure a biomarker. For a biomarker to be valid, it must be specific and sensitive for the process it is measuring. Biomarkers for pain with established sensitivity and specificity are just beginning to be developed.

Blobs

fMRI activity is used frequently as an implicit biomarker for many psychological processes, including reward, memory, and pain. However, fMRI results, or "blobs" are not true biomarkers of these processes for several reasons. First, researchers use inconsistent definitions to interpret results and are unable to replicate other studies. Furthermore, neuroimaging results are usually for a group and are not applicable to individuals. Current neuroimaging studies lack specificity and sensitivity to provide a sound basis for the use of fMRI as a biomarker. For example, activation of the AC and insula frequently occur in numerous studies and are not specific for pain or any other type of affect.

Biomarkers

The problems of replication and sensitivity are addressable. Machine learning tools can maximize the sensitivity, specificity, and interpretability of fMRI results by optimizing markers of brain patterns, analyzing their generalizability across multiple studies, and characterizing the patterns across other conditions. It is important to develop a pain biomarker using highly

accurate and reliable pain ratings; after doing so, the biomarker can be tested when pain ratings are less reliable.

Dr. Wager described the development and initial validation of a biomarker for acute experimental pain. His team subjected 20 healthy individuals to thermal pain during 12 trials at four temperatures each, corresponding to increasing levels of pain. Machine learning considered more than 200,000 predictors to stabilize the fMRI maps for true biomarker patterns of pain. This trained biomarker was tested using the fMRI results from four studies, including a study in which romantically rejected individuals viewed pictures of ex-partners or experienced physical pain. The trained biomarker was 90 percent sensitive for detecting physical pain and was not able to detect romantic rejection better than chance. This biomarker was subsequently tested on multiple additional fMRI datasets, yielding a 90 to 100 percent sensitivity and specificity rate. It is possible to rapidly test the validity of this biomarker on any given fMRI dataset.

Ongoing research demonstrates that there are different types of brain patterns for different types of affect, and that neuroimaging can distinguish between emotion- and pain-related signals. This distinction is possible because, while activation of similar regions occurs with certain emotions and pain, the pattern for each type of affect is unique.

Using the same machine-learning paradigm described above, Wager established a romantic rejection fMRI-based biomarker. This trained biomarker was successful at detecting romantic rejection and was unable to detect pain better than chance. The machine learning system detects separate populations of neurons and tracks their activity in the condition under study.

Wager is now examining multiple cerebral pathways to pain, including “bottom-up” nociceptive pathways and “top-down” prefrontal-striatal pathways. For example, in one study, researchers asked study participants to cognitively reappraise pain by imagining their skin as burning or as comfortably warm, and assessed their pain rating while subjecting the participants to different temperatures. Pain reappraisal significantly changed perceptions of pain and brain activity in a medial prefrontal-striatal pathway, which mediated reappraisal effects on pain reports. However, pain reappraisal did not affect the brain pattern biomarker. This, coupled with strong positive findings that the brain pattern biomarker did mediate the effects of exposure to different temperatures, indicated that reappraisal of pain may work at a post-nociceptive level on circuits involved in pain decision-making and valuation.

Translation of fMRI results into therapies

Wager argued that researchers should consider events in the brain even when developing peripheral pain therapies. fMRI studies can identify patients that have a CNS component to their pain disorder; for these patients, treatment of both the central and peripheral nervous systems may be necessary.

Wager used machine learning technique to develop a brain signature for patients with fibromyalgia; increases in this signature correlated with increases in actual pain reports. These brain responses were predictive of fibromyalgia in previously untested patients with a

specificity and sensitivity of 86 and 89 percent, respectively. Furthermore, nuances in the fibromyalgia signature were predictive of specific patient symptoms including pain and depression.

In summary, Wager argued for the need to develop, validate, and test sensitive and specific biomarkers that can track different aspects of pain and affective responses.

Discussion

Observed brain patterns are associative and do not necessarily imply causal pathways. Wager clarified that causality is important and difficult to infer; however, stronger associations suggest likely targets for intervention. Apkarian commented that imaging studies generate testable hypotheses; for example, studies that alleviate pain by activating the brain with light could not have been done if we had not first discovered the importance of these regions with imaging. Borsook also noted that whenever a patient has a reversal of pain or other metric, the study adds to the body of evidence that the targeted areas are indeed related to pain. He also agreed that scientific papers using imaging should be careful in their statements regarding causality versus association.

Brain imaging would be an even more powerful tool if it were possible to discriminate between subsets of neurons that have different chemical profiles. Wager explained that molecular tagging of neuronal subtypes is an active field of study in animal models but is more difficult in humans.

In response to a question about the contribution of imaging to the study of non-disease states, such as memory and learning, the panel agreed that imaging studies of these processes could improve our understanding of pain-related behaviors.

The panel also discussed the practicality of brain imaging as a biomarker for pain. The development of a blood test based on the information learned from imaging studies would be desirable. Such a test might have more predictive and diagnostic value without requiring a patient to undergo an MRI. Time will tell whether the development of a blood biomarker test for pain is possible.

Panel on Neuro-glia Mechanisms of Chronic Pain

Moderator: **Michael Oshinsky, PhD**, Program Officer, National Institute of Neurological Disorders and Stroke

A retrospective and the role of microglia and astrocytes in chronic pain mechanisms

Ru-Rong Ji, PhD, Professor, Department of Anesthesiology and Neurobiology, Duke University Medical Center

Background

Interest in glial cells, such as microglia and astrocytes, and their involvement in the mechanisms of pain has increased over the past 10 years. Previous research has demonstrated that glial cells

have different activation states that contribute to chronic pain,⁸² including changes in glial markers and morphology, production of glial mediators, interaction of mediators with neurons, and sensitization of the CNS. Ji reviewed the role of microglia and astrocytes in chronic pain and described how pathologies in glial cells drive chronic pain.

Microglia and chronic pain

Microglia act as macrophages for the CNS; they are the major effectors of the CNS innate immune response and are the predominant source of CNS cytokines, including Tumor Necrosis Factor- α (TNF- α), interleukin-1 beta, and interleukin-18. Microglia express specific markers and produce brain-derived neurotrophic factor (BDNF). Microglia react to nerve injury and contribute to the pathogenesis of neuropathic pain.

By labeling a microglia-specific marker with green fluorescent protein, it is possible to see time-dependent microglial activation following nerve injury. Activation peaks a few days after injury and recedes over time. Activation can further be observed by monitoring the phosphorylation of the p38 mitogen activated protein (MAP) kinase following spinal nerve injury. Researchers have replicated this observation in numerous animal studies using different models of nerve injury.

Intracellular signaling in microglia can be stimulated by tissue injury, surgery, cancer, nerve injury, or chronic opioid use. These triggers increase in a number of activators that act on microglial receptors to stimulate the release of microglial mediators. Researchers recently isolated single microglial cells and determined which mediators are expressed within each individual cell. This research demonstrated that microglial cells are the primary source of TNF- α within the spinal cord.⁸³ TNF- α released from microglial cells modulates synaptic transmission in neighboring neurons. In fact, application of low levels of TNF- α can impact synaptic transmission to the same extent as capsaicin, indicating that cytokines can modulate neuronal activity in addition to immune function.

Astrocytes and chronic pain

Astrocytes are the most abundant cells in the CNS and express a variety of cell-specific markers. There is evidence for different types of spinal cord astrocytes, each with its own molecular signature. Astrocytes have diverse functions, including providing structural support for neurons, clearing toxins, maintaining ion and water homeostasis, and releasing gliotransmitters such as chemokines and cytokines.

There is persistent activation of astrocytes in neuropathic pain, and the activation remains at the peak 21 days post injury. For example, the astrocyte gap junction protein connexin 43 (Cx43) is up-regulated in late-phase neuropathic pain; notably, peptide inhibitors of Cx43 have

⁸² Ru-Rong Ji, Temugin Berta, and Maiken Nedergaard, "Glia and Pain: Is Chronic Pain a Gliopathy?," *Pain* 154 Suppl 1 (December 2013): S10–28.

⁸³ Temugin Berta et al., "Extracellular Caspase-6 Drives Murine Inflammatory Pain via Microglial TNF- α Secretion," *The Journal of Clinical Investigation* 124, no. 3 (March 2014): 1173–86.

been shown to block pain.⁸⁴ Cx43 modulates synaptic transmission after injury through secretion of chemokine (C-X-C motif) ligand 1 (CXCL1) from astrocytes, which binds to its target on neurons, the interleukin-8 receptor beta, also known as CXCR2. Inhibitors of CXCR2 can block pain at late times post injury.

Astrocytes express many chemokines, which are up-regulated through the c-Jun N-terminal kinase. These chemokines bind to targets on neurons and contribute to pain at later times post injury. Microglia contribute to this process in part by activating astrocytes via secretion of TNF- α .

Gliopathy in chronic pain: astrocyte dysregulation

Dr. Ji described various ways in which astrocyte dysfunction could result in some of the hallmarks of pain. For example, disruption of glutamate and potassium homeostasis could result in neuronal hyper-excitability, and alteration of water homeostasis could result in edema. Secretion of chemokines by astrocytes can promote pain, and Cx43 dysfunction might affect normal long-range signaling and switch the function of Cx43 to paracrine signaling.

Ji posited that chronic pain is a “gliopathy” and emphasized that microglia and astrocytes are important in the establishment and maintenance of chronic symptoms. Specifically, glia modulate pain via neuron-glia interactions by producing cytokines and chemokines, which can affect synaptic transmission. Therapeutic strategies include interventions at various points along the glial pain pathways. Some potential therapies include cytokine inhibitors, anti-inflammatory cytokines, chemokine inhibitors, and MAP kinase inhibitors.

Clinical significance and future directions

Significant advances in cancer treatments have occurred by combining chemotherapies and immune therapies. Ji noted that a similar strategy might also be successful in the treatment of chronic pain, by combining neuron-targeting therapies with glia-targeting therapies. Researchers could target both types of cells either by developing multiple drugs that target each cell type individually or by developing one drug that affects both neurons and glia.

For example, resolvins and neuroprotectins are anti-inflammatory compounds that reduce pain. Neuroprotectin D1 (NPD1) can provide relief of neuropathic pain by modulating both neuronal and glial activation post injury.⁸⁵ NPD1 has a good safety profile, has analgesic and anti-inflammatory properties, inhibits glial activity, and provides neuronal protection. NPD1 is particularly suitable for the prevention of chronic pain development after surgery, trauma, and chemotherapy. However, NPD1 is unstable and its pharmacodynamic and pharmacokinetic properties are not ideal. Researchers have identified the receptor for NPD1 and are currently developing small molecule agonists that can deliver more consistent pain relief.

⁸⁴ Gang Chen et al., “Connexin-43 Induces Chemokine Release from Spinal Cord Astrocytes to Maintain Late-Phase Neuropathic Pain in Mice,” *Brain: A Journal of Neurology* 137, no. Pt 8 (August 2014): 2193–2209.

⁸⁵ Zhen-Zhong Xu et al., “Neuroprotectin/protectin D1 Protects against Neuropathic Pain in Mice after Nerve Trauma,” *Annals of Neurology* 74, no. 3 (September 2013): 490–95.

Future research directions include the development of new molecular markers for astrocytes and microglia and identification of mediators produced by these cells. It will also be important to better understand the interactions between glia and neurons, as well as how these interactions might differ in individuals of different age or sex.

Neuro-glial mechanisms of pain

Yves De Koninck, PhD, Professor of Psychiatry & Neuroscience, Laval University; Scientific Director of the Quebec Mental Health Institute, Quebec, QC (Canada)

Background on interactions between neurons and glia

When an afferent nerve is damaged, microglia activate and migrate to the damaged side of the spinal cord. These microglia express the purinoceptor, P2X₄. Researchers have shown microglial cells also secrete BDNF, which targets neighboring neurons and results in the loss of the potassium-chloride transporter 2 (KCC2). This transporter normally maintains low chloride levels within the neuron; however, BDNF causes the loss of this transporter and degradation of the ion gradient.⁸⁶

Selective destruction of microglial cells reverses hypersensitivity for up to 3 months following injury. De Koninck emphasized that there is likely interaction between nociceptive and non-nociceptive pathways in pain; lifting inhibition between these pathways may be a way to unmask the contributions of non-nociceptive circuits to pain.

Understanding the mechanisms of morphine-induced hyperalgesia

A growing literature suggests that chronic morphine use causes inflammation in the CNS. Morphine use initially, causes analgesia; however, with prolonged use, chronic morphine results in tolerance and hyperalgesia. It is difficult to study how morphine produces hyperalgesia in an injured animal, because morphine's analgesic effect is potentially confounding. To address this issue, researchers gave morphine to animals every day but measured pain and inflammation in the morning just prior to the morphine dose. By using this experimental design, the researchers could examine the chronic effects of morphine without confounding from the immediate effects of morphine administration.

Results from the study showed that chronic morphine use produces hyperalgesia as measured by decreases in the thermal threshold, increased vocalization, and increased licking behaviors. This hyperalgesia is coupled with an increase in microglial activation; selective destruction of microglia reverses the hyperalgesic effect. Researchers further demonstrated that microglial BDNF is required for morphine-mediated hyperalgesia, because animals without this gene in microglia show no hyperalgesic effects from chronic morphine use. P2X₄ receptor knockout mice also do not develop hyperalgesia from long-term morphine administration. The researchers therefore concluded that morphine causes a paradoxical hyperalgesia by triggering a CNS inflammatory response that leads to impaired chloride homeostasis and altered neuronal

⁸⁶ Jeffrey A. M. Coull et al., "BDNF from Microglia Causes the Shift in Neuronal Anion Gradient Underlying Neuropathic Pain," *Nature* 438, no. 7070 (December 15, 2005): 1017–21.

function. Researchers are currently investigating KCC2 as a potential drug target for the treatment of pain.

Morphine's effects on the brain

In 2004, researchers demonstrated that chronic morphine use blunted animals' hedonic response to morphine.⁸⁷ Examination of the neurons in the ventral tegmental area showed that opiate dependence causes these neurons to lose their ability to inhibit signaling and remain in an excitatory state. DeKoninck hypothesized that microglia might be involved in the perturbation of chloride regulation in these neurons. In fact, microglia migrate to the ventral tegmental area during chronic morphine use, a phenomenon that can be reversed with the addition of minocycline, which preferentially kills microglial cells. Researchers have shown that there is impaired morphine-induced dopamine release in animals that have a peripheral nerve injury, demonstrating that the reward response is blunted. This blunted reward response is due to a similar cascade in the CNS that involves microglial secretion of BDNF and targeting of KCC2 on inhibitory neurons.

The role of the anterior cingulate cortex in pain-related depression and anxiety

Previous research has established the role of the ACC in the development of depression and anxiety in patients with chronic pain. Ablation of the ACC, but not other brain regions, blocked anxiodepressive behaviors without affecting pain behaviors.⁸⁸ Conversely, optogenetic stimulation of the AC triggered depression and anxiety. De Koninck's research team is assessing whether the same microglial-mediated inflammatory pathways are involved in the development of depression and anxiety through the ACC.

Evidence for sex as a mediator of microglial-mediated inflammation following injury

Microglia respond to nerve injury in both males and females; however, depletion of microglia reverses pain hypersensitivity in males only. This sex-specific effect is correlated with depletion of BDNF, and only male BDNF knockout animals experience a reversal of pain hypersensitivity. Blockage of the P2X₄ receptor also alleviates pain following injury in males only.

Refining which microglial cells are involved in inflammatory pathways

There is significant diversity in microglial signaling following injury. For example, females and males both activate microglial cells after injury but through different pathways. New tools are needed to refine the characterization of microglial cells in behaving animals. Scientists have developed new techniques that allow functional imaging in the brain and spinal cord of

⁸⁷ Steven R. Laviolette et al., "Opiate State Controls Bi-Directional Reward Signaling via GABAA Receptors in the Ventral Tegmental Area," *Nature Neuroscience* 7, no. 2 (February 2004): 160–69.

⁸⁸ Florent Barthas et al., "The Anterior Cingulate Cortex Is a Critical Hub for Pain-Induced Depression," *Biological Psychiatry* 77, no. 3 (February 1, 2015): 236–45.

behaving animals.^{89,90} These innovations are allowing scientists to examine microglial activation following injury in real time.

Discussion

Different subsets of microglia and astrocytes are likely more important than others in the regulation of pain. The panelists agreed that characterization of glial subtypes is a critical area of study; sex differences in microglial activation are a good example of the roles of different glial subtypes in pain regulation. Ji noted that these glial subtypes likely have different phenotypes and different roles in pain, some of which could be beneficial and others detrimental. For therapeutic purposes, it will be necessary to identify those microglial cells that reduce pain and to gain a better understanding of the mechanisms behind their analgesic responses.

Glial cells might play a role in other disorders that cause generalized pain, such as illness or fibromyalgia. De Koninck noted that immune processes might be involved in a general sensitization of an individual to pain. He also highlighted the importance of studying why pain subsides for the majority of individuals but persists in others.

Microglia might respond to morphine through other pathways than the opiate μ -receptor, because naloxone, a μ -receptor antagonist, prevented some, but not all, of the microglial-induced response, implying that another pathway could be utilized. The panel acknowledged that the effect of morphine on microglial opiate μ -receptors deserves further study in males versus females.

Panel Session on Novel Treatments

Moderator: **Ann O'Mara, PhD, RN**, Head Palliative Care Research, National Cancer Institute

Causes of bone cancer pain and disease modification by a CB₂ cannabinoid

Todd W. Vanderah, PhD, Professor and Head, Department of Pharmacology, University of Arizona

Background on breast cancer–induced bone pain

Breast cancer is the most frequent malignancy in women across the globe, and more than 70 percent of women with advanced breast cancer have bone metastases—more than any other cancer type with pain being the most frequent complaint.⁹¹ Therapies for bone pain include radiation, opiates, and bisphosphonates. However, these therapies have limited efficacy and

⁸⁹ Steven A. Prescott, Qiufu Ma, and Yves De Koninck, "Normal and Abnormal Coding of Somatosensory Stimuli Causing Pain," *Nature Neuroscience* 17, no. 2 (February 2014): 183–91.

⁹⁰ Sophie Laffray et al., "Adaptive Movement Compensation for in Vivo Imaging of Fast Cellular Dynamics within a Moving Tissue," *PLoS One* 6, no. 5 (2011): e19928.

⁹¹ Robert E. Coleman, "Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity," *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 12, no. 20 Pt 2 (October 15, 2006): 6243s–6249s.

numerous side effects. As a result, there is an urgent need for novel therapies for cancer-related bone pain.

Animal models of bone pain and limitations of morphine treatments

Dr. Vanderah described the development of an animal model for breast cancer–induced metastasis and bone pain. Using a syngeneic breast cancer model, researchers placed mammary adenocarcinoma cells inside the femur of mice and sealed the cancer cells inside the bone. They then analyzed pain behaviors by observing limb guarding and flinching. Within 7 days after injection, the animals began to exhibit spontaneous behaviors indicating pain in the injected limb. The animals injected with breast cancer cells also experienced increased bone loss and femur fractures.

Morphine is often used to treat bone pain from breast cancer metastases. At first, the patient usually responds with reduced pain; however, higher doses are soon needed to maintain the therapeutic effect. Furthermore, there is evidence that chronic morphine use may be linked to bone degeneration. Previous research has demonstrated that chronic morphine use increases the likelihood of spontaneous fracture in patients and is a risk factor for osteoporosis.^{92,93}

Mice injected with breast cancer cells in the femur received either extended morphine or placebo, and researchers assessed pain behaviors over a 2-week period. At day 10, pain levels in mice receiving morphine were significantly lower than control animals. However, by day 13, the situation reversed, and animals receiving morphine experienced more pain than control animals. Dr. Vanderah hypothesized that tolerance or sensitization may be the mechanisms by which animals experience hyperalgesia during chronic morphine use. Researchers have also demonstrated that morphine use in these animals results in demineralization of the bone. Morphine can accelerate the development of osteoclasts, which may be a mechanism of morphine-induced bone loss.

The effects of cannabinoids on bone pain, tumor burden, and metastasis

Cannabinoid use has expanded in the United States over the past decade, and several states have approved cannabinoids for medical purposes. The cannabinoid 2 receptor (CB₂), unlike the cannabinoid 1 receptor (CB₁), does not produce psychotropic effects. CB₂ receptors, which are found in the spleen, immune cells, glia, and osteoclasts, inhibit inflammation and neuropathic pain and decrease the number of osteoclasts, thus maintaining bone density.^{94,95} Both acute and chronic administration of CB₂ receptor agonists inhibit pain in the animal model of cancer-induced bone pain; this effect can be reversed by administration of a CB₂ receptor antagonist.

⁹² P. Vestergaard, L. Rejnmark, and L. Mosekilde, "Fracture Risk Associated with the Use of Morphine and Opiates," *Journal of Internal Medicine* 260, no. 1 (July 2006): 76–87.

⁹³ Consalvo Mattia, Eleonora Di Bussolo, and Flaminia Coluzzi, "Non-Analgesic Effects of Opioids: The Interaction of Opioids with Bone and Joints," *Current Pharmaceutical Design* 18, no. 37 (2012): 6005–9.

⁹⁴ Mohab M. Ibrahim et al., "CB₂ Cannabinoid Receptor Activation Produces Antinociception by Stimulating Peripheral Release of Endogenous Opioids," *Proceedings of the National Academy of Sciences of the United States of America* 102, no. 8 (February 22, 2005): 3093–98.

⁹⁵ Orr Ofek et al., "Peripheral Cannabinoid Receptor, CB₂, Regulates Bone Mass," *Proceedings of the National Academy of Sciences of the United States of America* 103, no. 3 (January 17, 2006): 696–701.

Increasing the levels of endogenous cannabinoids also significantly decreases pain. CB₂ receptor agonists significantly decrease cancer-induced bone wasting by inhibiting the number and activation of osteoclasts. CB₂ receptor agonists may prevent pain by inhibiting inflammatory cytokines/chemokines, preventing bone loss and remodeling, and reducing the number of fractures.

CB₂ receptor agonists also have effects on tumor burden. Breast cancer cells from both mice and humans express CB₂ receptors. Activation of CB₂ receptors triggers apoptosis in cultured breast cancer cells. Researchers in Dr. Vanderah's lab can visualize metastasis in vivo by labeling cancer cells with a luciferase reporter and observe the spread of cancer cells. Using this approach, they demonstrated that CB₂ receptor agonists decrease the number of breast cancer metastases and decrease the overall tumor burden.

Conclusion

In summary, CB₂ receptor agonists inhibit pain and bone wasting in an animal model of cancer-induced bone pain by decreasing inflammation, decreasing bone loss, and lessening the tumor burden. Future research includes clinical trials with Eli Lilly's CB₂ receptor agonist, LY2828360, and examination of the potential analgesic synergies with opioid and CB₂ receptor agonists. Researchers are also examining the mechanisms behind CB₂ receptor agonist-mediated anti-proliferative effects.

Targeting glia for pain therapy

John Forsayeth, PhD, Co-Chair Scientific Advisory Board, Xalud Therapeutics, Inc.

Background

Glial cells are activated in every clinically relevant model of chronic pain, including peripheral nerve injury, bone cancer, and spinal cord injury. Inflammatory processes normally, are protective and result in the elimination of invading pathogens, but they are hyperactive in chronic pain. Suppressing glial activation and pro-inflammatory cytokines results in decreases in pain and can return pain sensitivity to normal levels.

Multiple mediators can lead to spinal glial activation and chronic pain, including pro-inflammatory cytokines, TNF- α , nitric oxide, heat shock proteins, and opioids. Activation causes glia to release pain-enhancing substances, such as pro-inflammatory cytokines and prostaglandins. These substances amplify pain signaling to the spinal cord and pain transmission to the brain. Several anti-inflammatory drugs are available to treat chronic pain, including TNF- α receptor antagonists, calcium channel inhibitors, and SNRIs.

Interleukin-10 and its utility as an anti-inflammatory mediator

Inflammatory mediators are numerous and redundant, ensuring that the brain is alerted when something is wrong in the body. Anti-inflammatory molecules, such as interleukin-10 (IL-10), regulate the activity of inflammatory molecules and modulate pro-inflammatory processes. Endogenous IL-10, however, is not produced in sufficient quantities to affect chronic pain. IL-10 is an attractive therapeutic agent because of its anti-inflammatory properties, its ability to normalize microglial activity, and the fact that it binds to a single receptor with high specificity.

Previous research supports the use of IL-10 as a potential therapy for pain in humans. For example, pro-inflammatory cytokines are elevated in the cerebrospinal fluid of pain patients, while IL-10 levels are inversely correlated to the severity of pain. Furthermore, researchers have discovered polymorphisms in the *IL-10* gene that are associated with pain.

It has been difficult to unlock the therapeutic potential of IL-10 and develop a molecule that is stable and effective. Recombinant IL-10 has a short half-life and is unable to penetrate the CNS. While PEGylated IL-10 has a longer half-life, it has not been developed for clinical use. Delivery of IL-10 via an adenovirus or similar vector resulted in long-lasting expression of IL-10, but did not inhibit pain for longer than 1 week. Some viruses carry their own versions of IL-10 to reduce inflammation and prevent the immune system from recognizing their presence.

Developing a new IL-10 delivery system

Research on the IL-10 signaling pathway explained why previous attempts to express IL-10 for clinical use failed. Binding of IL-10 to its target receptor results in the activation of a signaling pathway with anti-inflammatory activities. Increased binding of IL-10 to its receptor however, initiates an inhibitory feedback loop that shuts off the signaling cascade. The relationship between the amount of IL-10 and its therapeutic activities is a bell-shaped curve: just enough IL-10 is therapeutic while too much or too little IL-10 provides no protective effects. The previous IL-10 delivery systems described above produced far too much IL-10, activating the negative feedback loop and inhibiting the therapeutic activities of IL-10.

Based on these data, Xalud Therapeutics developed a plasmid-based gene therapy system, wherein plasmids expressing IL-10 are encapsulated with poly(lactic-co-glycolic acid) (PLGA) and injected intrathecally or intra-articularly. This therapy (XT-101) delivered non-narcotic, non-addictive pain relief for a total of 12 weeks. Scientists further improved this system by introducing a point mutation in *IL-10* that resulted in superior duration of activity up to 90 days in an animal model of chronic pain. Application of an IL-10 antibody reversed the analgesic effect, demonstrating that pain relief was moderated by the activities of IL-10.

Toxicology studies in mice demonstrated efficacy of XT-101 over a broad range of doses. XT-101 also reversed paclitaxel-induced neuropathic pain in rats. Researchers tested XT-101 in a dog model of neuropathic pain and osteoarthritis pain. Pain assessments demonstrated significant positive changes in pain and function that lasted for weeks. Development of an aqueous delivery system extended these effects.

Summary and Next Steps

Dr. Forsayeth noted that the excellent toxicology results coupled with efficacy studies in rats and dogs will enable the company to move forward with clinical trials. IL-10 plasmid therapy was able to suppress neuropathic pain for 12 weeks and improve joint function and reduce pain in animals with osteoarthritis. Clinical trials in neuropathic pain and osteoarthritis are planned for 2016.

Understanding mechanisms of psychosocial treatments for chronic pain

John Burns, PhD, Professor, Department of Behavioral Sciences, Rush University Medical Center

Background

Psychosocial therapies, such as behavioral therapy, cognitive therapy, CBT, and biofeedback, have similar and modest efficacy in reducing pain. Newer psychosocial approaches such as mindfulness-based stress reduction (MBSR) and acceptance and commitment therapy (ACT) are also modestly effective. Comparative effectiveness studies have demonstrated no significant differences between two or more psychosocial therapies on the primary outcomes.^{96,97} Furthermore, studies have demonstrated no significant differences between single interventions and combination interventions.⁹⁸ Dr. Burns suggested that researchers should focus on understanding the mechanisms that contribute to the efficacy of psychosocial therapies instead of developing new adequate, but non-superior, treatments.

Identifying mechanisms of psychosocial chronic pain treatments

Dr. Burns defined “mechanisms” of psychosocial treatments as “the thoughts, emotions, and behaviors targeted for change through therapeutic procedures, which in turn impact pain and function.” For example, cognitive therapy acts to decrease pain catastrophizing, which in turn decreases pain. Until now, research into the mechanisms of psychosocial pain treatments has operated under the Specific Mechanism Model, where all treatments achieve the same goal by distinct therapeutic pathways. For example, cognitive therapy works to reduce catastrophizing to reduce pain, whereas MBSR increases mindfulness to reduce pain.

An alternative concept, the Shared Mechanism Model, suggests that all psychosocial chronic pain treatments might work through mechanisms shared by all approaches. The observation that different psychosocial therapies produce similar effects supports this model. If the Shared Mechanism Model is correct, then research should aim to uncover the shared mechanisms that result in the observed efficacy of extant psychosocial treatments.

Dr. Burns noted that answering the question “Does a therapy work?” is easier than answering the question “How does the therapy work?” Understanding how a psychosocial therapy works requires that researchers categorize and measure mechanisms and develop trial designs and statistics to distinguish mechanism effects.

⁹⁶ Rob J. E. M. Smeets et al., “Active Rehabilitation for Chronic Low Back Pain: Cognitive-Behavioral, Physical, or Both? First Direct Post-Treatment Results from a Randomized Controlled Trial [ISRCTN22714229],” *BMC Musculoskeletal Disorders* 7 (2006): 5.

⁹⁷ Samuel Yeung-Shan Wong et al., “Comparing the Effectiveness of Mindfulness-Based Stress Reduction and Multidisciplinary Intervention Programs for Chronic Pain: A Randomized Comparative Trial,” *The Clinical Journal of Pain* 27, no. 8 (October 2011): 724–34.

⁹⁸ Robert D. Kerns et al., “Can We Improve Cognitive-Behavioral Therapy for Chronic Back Pain Treatment Engagement and Adherence? A Controlled Trial of Tailored versus Standard Therapy,” *Health Psychology: Official Journal of the Division of Health Psychology, American Psychological Association* 33, no. 9 (September 2014): 938–47.

Research that addresses the mechanisms of psychosocial pain treatments

Some recent research has addressed therapeutic mechanisms, including secondary analyses of controlled and uncontrolled clinical trials. Secondary analyses were necessary because the host studies were not designed to address mechanistic questions. The designs and quality of mechanistic studies vary widely. Some of these study designs and their results are listed below:

- **Co-variation between mechanisms and outcomes:** These studies have focused on pre- and post-changes in treatment mechanisms and correlations with pre- and post-changes in treatment outcomes to answer whether a cause precedes the treatment effect.⁹⁹
- **Early changes in treatment mechanisms that correlate with early changes in treatment outcomes:** Researchers have shown that early treatment changes in pain catastrophizing result in early treatment changes in outcomes.¹⁰⁰
- **Cross-lagged panel designs:** These studies examine early treatment mechanisms and their correlation to late treatment outcomes and vice versa. For example, researchers demonstrated that early treatment changes in pain catastrophizing predicted late treatment outcomes but not vice versa.¹⁰¹
- **Testing multiple mechanisms:** These types of studies have the potential to provide the best evidence of mechanistic specificity. For example, Vowles et al. noted significant pre- and post-changes in acceptance and pain catastrophizing with CBT and demonstrated that these changes predicted most of the variance in pre- and post-outcomes.¹⁰²
- **Testing multiple treatments:** Smeets et al. compared CBT, exercise, and CBT plus exercise to wait list controls for the management of pain.¹⁰³ The three treatments did not differ in pre- and post-changes in pain catastrophizing, and all three treatments accurately predicted changes in outcomes over the study period.
- **Analysis of non-specific mechanisms:** This line of research addresses whether components such as having a supportive therapist or interventionist is responsible for changes in outcomes.

Conclusion

The studies described above provide evidence that treatment mechanisms are shared by multiple psychosocial therapies. There may be several potent mechanisms involved in these

⁹⁹ Kevin E. Vowles and Lance M. McCracken, "Comparing the Role of Psychological Flexibility and Traditional Pain Management Coping Strategies in Chronic Pain Treatment Outcomes," *Behaviour Research and Therapy* 48, no. 2 (February 2010): 141–46.

¹⁰⁰ J. W. Burns et al., "Cognitive Factors Influence Outcome Following Multidisciplinary Chronic Pain Treatment: A Replication and Extension of a Cross-Lagged Panel Analysis," *Behaviour Research and Therapy* 41, no. 10 (October 2003): 1163–82.

¹⁰¹ Ibid.

¹⁰² Kevin E. Vowles, Lance M. McCracken, and Christopher Eccleston, "Processes of Change in Treatment for Chronic Pain: The Contributions of Pain, Acceptance, and Catastrophizing," *European Journal of Pain (London, England)* 11, no. 7 (October 2007): 779–87.

¹⁰³ Rob J. E. M. Smeets et al., "Reduction of Pain Catastrophizing Mediates the Outcome of Both Physical and Cognitive-Behavioral Treatment in Chronic Low Back Pain," *The Journal of Pain: Official Journal of the American Pain Society* 7, no. 4 (April 2006): 261–71.

therapies, including reducing pain catastrophizing and increasing mindfulness and pain self-management. Non-specific factors may also play a role in the success of these therapies.

In summary, there is evidence for the Shared Mechanism Model; however, more work remains to be done. Burns suggested that it is time to revise our approach to randomized controlled trials and embrace a new paradigm addressing how psychosocial therapies for pain work. Research should identify and amplify the successful aspects of therapies and identify and eliminate ineffective components of treatment. This goal can be achieved in two steps:

1. Identify key mechanisms in existing psychosocial treatments for chronic pain to identify active, beneficial mechanisms
2. Search for new mechanisms and treatments to activate them with the overarching goal of increasing efficacy over existing therapies

Discussion

The panelists agreed that understanding the basis for non-response to therapies is an important area of research. Dr. Forsayeth noted that it is impossible to extrapolate whether a therapy will be successful in humans purely based on the results of animal data; however, this research is necessary to provide a rationale for studying compounds in humans. Burns commented that the understanding of moderators to psychosocial treatments is completely undeveloped.

Responding to a question about translating the success of IL-10 therapy in dogs to humans, Dr. Forsayeth acknowledged that IL-10 does appear to have species-specific effects. For example, human IL-10 has anti-inflammatory properties in mice, but not in rats. He noted that identification of the best patient population to test the IL-10 therapy in humans might be a challenge.

Dr. Vanderah clarified the relationship between CB₂ agonists and marijuana. People are growing different strains of marijuana that have non-psychoactive properties. Families of patients who benefit from medical marijuana use are moving to states where the use of these compounds is legal. Dr. Vanderah noted that he focused on breast cancer–induced bone pain and that other researchers were actively investigating other bone pain conditions such as sarcoma. He also commented that there might be synergies between CB₂ agonists and other drug classes that treat pain. For example, there is some evidence that CB₂ agonists can inhibit the side effects of opiates.

In response to a question about the potential role of neural imaging in detangling the mechanisms of psychosocial treatments for pain, Dr. Burns commented that imaging could provide information on brain changes during treatment; however, he cautioned that these studies would provide only associations, not causality. Dr. Burns also noted that most pain patients eventually seek some kind of psychosocial pain management approach and emphasized that patient preference is an important consideration. Patient expectations for the success of CBT overlap with patient outcomes, highlighting the importance of patient expectations for treatment success.

The Mitchell Max Award and Oral Presentations by Junior Investigators

Martha Somerman, DDS, PhD, Director, NIDCR; NIH Pain Consortium Executive Committee

Dr. Somerman introduced the Mitchell Max Award for junior investigators, which honors the lifetime contributions of Dr. Mitchell Max to pain research. The symposium organizers chose three abstracts for oral presentations based on their quality, relevance, and significance, authored by Drs. Mark Pitcher, Kate Yeager, and Lucie Low. Dr. Pitcher received this year's Mitchell Max Award for the best poster presentation for his work developing a rat model of voluntary exercise for pain relief.

Effect of voluntary exercise in a rat model of persistent inflammatory pain

Mark Pitcher, PhD, Postdoctoral Fellow, NCCIH

Background

Exercise can ameliorate the symptoms of depression and pain; the mechanisms involved in exercise-induced analgesia however, are unknown.¹⁰⁴ A common model for studying exercise in animals involves forcing animals to run on a treadmill. The benefit of this approach is that researchers can control the exercise parameters, such as how far and how fast the animals run. However, inducements such as electric shocks are needed to force the animals to exercise. Forced exercise might increase in stress and anxiety, which are known to affect endocrine and immune function. Thus, forced exercise may have a significant impact on study results.

A voluntary animal model of exercise-induced analgesia

Rodent species voluntarily exercise in the wild and enjoy exercising on a running wheel. Pitcher asked whether a rodent with hind leg pain would voluntarily exercise, and whether this exercise would result in an analgesic effect. Researchers injected rats in the ankle with Complete Freund's Adjuvant (CFA) to produce inflammation and pain. A portion of the injured and control rodents had voluntary access to a running wheel for 2 hours per day. Researchers measured hypersensitivity, inflammation, and stress 24 hours post exercise.

Results

1. **Running amount and distance:** Injured animals ran as much, if not more, than control animals, although this difference was not statistically significant. There was also inter-group variability in the running distance, but again, no difference between the two groups.
2. **Inflammation:** Animals injected with CFA experienced persistent swelling. Exercise did not change the level of inflammation; however, exercise did improve the range of motion.
3. **Hypersensitivity:** Sedentary, injured animals had a persistent deficit in the amount of weight they were willing to place on their injured hindpaws. Exercise improved the animal's ability to bear more weight on the injured limb. For sedentary, injured animals, the thermal latency time was much shorter compared to injured animals that exercise; in fact, thermal latency times in exercising rats did not significantly vary from times prior to their injury.
4. **Stress:** Exercise decreased levels of corticosterone in injured animals.

¹⁰⁴ Heather R. Kroll, "Exercise Therapy for Chronic Pain," *Physical Medicine and Rehabilitation Clinics of North America* 26, no. 2 (May 2015): 263–81.

5. Correlations between running distance and measured outcomes: Dr. Pitcher hypothesized that more exercise might correlate with improved outcomes. However, running distance did not correlate with improvements in weight bearing or decreased corticosterone levels, implying that access to exercise matters more than the amount of exercise.

Conclusion

Dr. Pitcher's research demonstrated that persistent inflammation is both painful and stressful. This research showed that rats exercise voluntarily, even when injured. Results indicated that voluntary exercise does not improve inflammation but does improve hypersensitivity and stress. Regular access to exercise, not the amount of exercise, provides these beneficial effects.

Questions

Dr. Pitcher explained that his data did not support the idea that a minimum amount of exercise is necessary to decrease hypersensitivity and stress and noted that it is difficult to evaluate whether exercise may be a form of environmental enrichment, because enrichment is hard to define and quantify. His experiment was primarily designed to be an alternative to forced exercise.

He further clarified that control animals had a needle inserted into their hindpaw, but did not receive an injection of CFA. Injured animals were moved to another cage to have access to a running wheel, while control animals remained in their home cage. He acknowledged that moving both groups to another cage to examine the effects of moving to another environment would be a good future control to include.

Adherence in African Americans being treated for cancer pain

Kate A. Yeager, PhD, RN, Assistant Professor, Emory University

Background

Pain is a significant problem for those with cancer; approximately 40 percent of individuals with cancer pain do not get adequate pain relief. African Americans with cancer experience worse pain and are less adherent to pain medications than patients of other ethnicities. They are less likely to receive pain medications, and pharmacies in lower income areas are less likely to carry pain drugs. However, prior research has not adequately explored the factors behind the lack of adherence in this cancer population.

Study design

The aims of the Cancer pain Relief for Everyone (CaRE) study are to:

1. Examine individual and interpersonal factors on adherence to around-the-clock opiate treatment
2. Determine whether neighborhood factors moderate adherence
3. Use qualitative methods to explore unique factors affecting adherence

These aims are based on a social-ecological model, wherein families, friends, and neighborhood components moderate individual behaviors; however, most of these hypothesized interpersonal and neighborhood factors remain unidentified.

To be included in the study, patients must be 21 years of age or older, be of African American descent, and have lived in the United States for at least 10 years. They must have a cancer diagnosis and be in possession of a prescription for an around-the-clock opiate. Participants must be mentally competent and have lived in their residence in the Atlanta metro area for at least 6 months. Exclusion criteria include surgery in the past month and the use of a pillbox for opiates. Researchers recruited patients from oncology and palliative care clinics at three hospitals in the study area.

To measure adherence to around-the-clock opiate treatment, researchers used a self-report adherence questionnaire as well as a Medication Event Monitoring System Cap (MEMSCap™), which records every time the patient opens a medication bottle. At the first visit, the questionnaire is completed and the MEMSCap is set up. Information on demographics, pain, perceptions of neighborhood safety, social supports, and other information is also collected at this baseline visit. At the second visit, questionnaires are again completed, the MEMSCap is collected, and researchers conduct qualitative interviews with a subset of patients.

To measure neighborhood factors involved in medication adherence in this population, researchers will examine neighborhood socioeconomic status, segregation, and drug-related crime rates.

Preliminary results

So far, researchers have recruited 64 of the needed 100 study participants. The number of male and female participants is approximately equal, and most participants have a high school education or less. The average Brief Pain Inventory score for study participants is moderate, at 4.3, even with pain medication. Symptoms among participants include pain, tiredness, and drowsiness. Seventy-three percent of individuals had chemotherapy or radiation in the past month. Self-report questionnaires demonstrated a 54 percent adherence rate to pain medication. The most common reason for non-adherence was falling asleep or thinking that they do not need the medication. Data from MEMSCap provide an adherence rate of 53 percent, which is in good agreement with self-reports. The researchers plan to continue study enrollment, complete qualitative interviews, and begin the neighborhood analyses.

Questions

In response to a question about the difficulties in obtaining pain medication in certain neighborhoods, Yeager responded that the study would be able to provide evidence that pain medications are more difficult to fill in some neighborhoods, if that is the case.

The stressed rat: out of sight but not out of mind

Lucie Low, PhD, Postdoctoral Fellow, NCCIH

Background

Rodents experience intense stressors while undergoing MRI. The animals are physically restrained and hear loud noises throughout the procedure. They are anesthetized and wake up under restrained conditions. Previous research has demonstrated that both restraint and noise can contribute to stress-induced analgesia as well as stress-induced hyperalgesia.

Study design

Dr. Low examined the effects of these stressors by exposing three groups of rodents to various levels of restraint and noise for 3 consecutive days as described below:

1. **Restrained rodents:** Researchers lightly anesthetized, restrained, and exposed the animals to noise at 90 decibels.
2. **Non-restrained rodents:** Researchers lightly anesthetized and exposed the animals to the restraint apparatus, but let them roam freely and exposed them to noise at 18 decibels.
3. **Non-exposed animals:** Researchers tested these animals in a fashion similar to the non-restrained rodents, but on a different day.

Note that for control purposes, researchers restrained all animals on day 3 of the study. Outcome measures included corticosterone levels and thermal sensitivity.

Results

Restrained animals showed a stress response as evidenced by increased levels of corticosterone. Non-exposed animals had relatively low levels of corticosterone except for a brief increase on day 3, when they too were restrained. However, the non-restrained animals also had increased levels of corticosterone, suggesting a possible effect from testing of the restrained animals that stresses the non-restrained animals.

Restrained rats showed no difference to non-exposed animals in responses to a heat challenge. However, non-restrained rats tested on the same day as restrained rats were hypersensitive to heat.

Conclusion

In summary, this study demonstrated increased stress hormones and pain behaviors in non-restrained control animals. Researchers tested non-restrained animals in the same room where they had previously restrained other rats. These results suggest that the non-restrained rats were possibly exposed to an olfactory trigger, resulting in stress-induced hyperalgesia. Finally, this study indicates that MRI restraint training causes increases in stress hormones but no changes in thermal pain behaviors. These results are important in interpreting the results of MRI studies for other conditions; for example, increases in stress hormones may be due to restraint during the MRI procedure rather than an effect of the condition under study.

Questions

In response to a question about other rodent markers of anxiety, such as pellet output, Low explained that her study used a nine-point stress score that included fecal output. However, she also noted that fecal output is difficult to measure while the animal is restrained within the MRI apparatus. She noted that it would be interesting to examine other behavioral measures of stress.

Closing Remarks

Martha Somerman, DDS, PhD, Director, NIDCR; NIH Pain Consortium Executive Committee

Dr. Somerman thanked all participants for an exciting and productive meeting that highlighted significant advances in pain research and acknowledged Dr. Linda Porter for her efforts in bringing together this year's Pain Consortium Symposium.

She concluded the meeting by expressing optimism that the National Pain Strategy will provide a nationwide transformation of the prevention and treatment of pain. The NIH and other agencies will continue to work together to enhance the federal pain research agenda and develop and disseminate important resources to achieve these goals.

Appendix 1: Agenda

Tuesday, May 26, 2015

- 12:30 p.m. **Introduction: Looking Back and to the Future of Pain Research**
Walter Koroshetz
- 12:50 p.m. **Keynote Presentation: Pain in a Dish**
Clifford Woolf
- 1:30 p.m. Question and Answer Session
- 1:40 p.m. **Panel on Cognitive and Emotional Aspects of Pain**
Moderator: *Wendy B. Smith*
- Cognitive Aspects of Acute and Chronic Pain: 10 Advances in 10 Years**
David Seminowicz
- Depression and Low Back Pain in Older Adults: Results of the ADAPT Trial**
Jordan F. Karp
- 2:40 p.m. Question and Answer Session
- 2:50 p.m. Break and Poster Session
- 3:20 p.m. **Panel on Genetics and Epigenetics of Pain**
Moderator: *Gayle Lester*
- From Genes to Pain: Lessons from Rare Inherited Disorders and Extrapolation to the Rest of Us**
Steve Waxman
- Epigenetic Regulation of Pain: What We Know so Far**
Laura Stone
- Epigenetic Regulation in Chronic Stress-Induced Visceral Pain**
John Wiley
- 5:00 p.m. Question and Answer Session
- 5:10 p.m. Poster Session

Wednesday, May 27, 2015

8:30 a.m. **Update from the American Pain Society**
Rob Gereau

8:50 a.m. **Panel on Pain Signatures and Predictors from Imaging Research**
Moderator: *Catherine Bushnell*

**A Retrospective of Advances through Imaging and Signatures of Pain Disease
and Recovery**
David Borsook

Predictors and Consequences of Chronic Pain
A. Vania Apkarian

Using fMRI to Assess and Understand Pain
Tor Wager

10:10 a.m. Question and Answer Session

10:30 a.m. Break and Poster Session

11:00 a.m. **Patient Perspective**
Christin Veasley

11:20 a.m. **Introduction to Mitchell Max Award**
Martha Somerman

11:30 a.m. **Oral Presentations by Junior Investigators**

Effect of Voluntary Exercise in a Rat Model of Persistent Inflammatory Pain
Mark Pitcher

Adherence for African Americans Being Treated for Cancer Pain
Kate A. Yeager

The Stressed Rat: Out of Sight but Not Out of Mind
Lucie Low

12:15 p.m. Lunch

- 1:15 p.m. **Panel on Neuro-glia Mechanisms of Chronic Pain**
Moderator: *Michael Oshinsky*
- A Retrospective and the Role of Microglia and Astrocytes in Chronic Pain Mechanisms**
Ru-Rong Ji
- Neuro-glial Mechanisms of Pain**
Yves DeKoninck
- 2:15 p.m. Question and Answer Session
- 2:25 p.m. Break
- 2:45 p.m. **Panel Session on Novel Treatments**
Moderator: *Ann O'Mara*
- Causes of Bone Cancer Pain and Disease Modification by a CB₂ Cannabinoid**
Todd W. Vanderah
- Targeting Glia for Pain Therapy**
John Forsayeth
- Understanding Mechanisms of Psychosocial Treatments for Chronic Pain**
John Burns
- 3:55 p.m. Question and Answer Session
- 4:15 p.m. **Poster Award Presentation**
Martha Somerman
- 4:25 p.m. **Closing Remarks**
Martha Somerman
- 4:40 p.m. Adjourn

Appendix 2: Meeting Participants

Executive Committee

Walter Koroshetz (Chair), Director, National Institute of Neurological Disorders and Stroke

Josephine Briggs, Director, National Center for Complementary and Integrative Health

Patricia Grady, Director, National Institute of Nursing Research

Martha Somerman, Director, National Institute of Dental and Craniofacial Research

Nora Volkow, Director, National Institute on Drug Abuse

Staff: NINDS Office of Pain Policy: Linda Porter (Director)

Speakers and Moderators

A. Vania Apkarian, PhD

Professor of Physiology, Anesthesiology, and Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University

David Borsook, MD, PhD

Director, Pain and Imaging Neuroscience Group, Boston Children's Hospital, Massachusetts General, and McLean Hospitals

John Burns, PhD

Professor, Department of Behavioral Sciences, Rush University Medical Center

Yves DeKoninck, PhD

Professor of Psychiatry & Neuroscience, Laval University; Scientific Director of the Quebec Mental Health Institute Research Center, Montreal, Quebec

John Forsayeth, PhD

Co-Chair Scientific Advisory Board, Xalud Therapeutics, Inc.

Rob Gereau, PhD

Director, Washington University Pain Center, Department of Anesthesiology, Washington University School of Medicine

Ru-Rong Ji, PhD

Professor, Department of Anesthesiology and Neurobiology, Duke University Medical Center

Jordan F. Karp, MD

Associate Professor of Psychiatry, Anesthesiology, and Clinical and Translational Science; Medical Director for Psychiatry, UPMC Pain Medicine at Centre Commons, University of Pittsburgh School of Medicine

Walter Koroshetz, MD

Acting Director, NINDS; Chair, NIH Pain Consortium Executive Committee

Gayle Lester, PhD

Program Director, Clinical Research & Diagnostic Imaging Tools for Osteoarthritis and Bone Quality, National Institute of Arthritis and Musculoskeletal and Skin Diseases

Lucie Low, PhD

Postdoctoral Fellow, NCCIH

Ann O'Mara, PhD, RN

Head Palliative Care Research, National Cancer Institute

Michael Oshinsky, PhD

Program Officer, National Institute of Neurological Disorders and Stroke

Mark Pitcher, PhD

Postdoctoral Fellow, NCCIH

David Seminowicz, PhD

Assistant Professor, Department of Neural and Pain Sciences, University of Maryland School of Dentistry

Wendy B. Smith, MA, PhD, BCB

Senior Scientific Advisor for Research Development and Outreach, NIH Office of Behavioral Science and Social Research

Martha Somerman, DDS

Director, NIDCR; NIH Pain Consortium Executive Committee

Laura Stone, PhD

Associate Professor, Alan Edwards Centre for Research on Pain, McGill University

Todd W. Vanderah, PhD

Professor and Head, Department of Pharmacology, University of Arizona

Christin Veasley, BS

Founder, Chronic Pain Research Association

Tor Wager, PhD

Director, Cognitive and Affective Neuroscience Laboratory, University of Colorado

Steve Waxman, MD, PhD

Director, Center for Neuroscience and Regeneration and Neurorehabilitation Research, Yale University School of Medicine

John Wiley, MD

Professor Internal Medicine, University of Michigan

Clifford Woolf, MB, BCh, PhD

Director, F.M. Kirby Neurobiology Center and Program in Neurobiology, Boston Children's Hospital; Professor of Neurology and Neurobiology, Harvard Medical School

Kate A. Yeager, PhD, RN

Assistant Professor, Emory University

NIH Pain Consortium Members

National Cancer Institute

National Eye Institute

National Heart, Lung, and Blood Institute

National Institute of Arthritis and Musculoskeletal and Skin Diseases

National Institute of Biomedical Imaging and Bioengineering

Eunice Kennedy Shriver National Institute of Child Health and Human Development

National Institute of Dental and Craniofacial Research

National Institute of Diabetes and Digestive and Kidney Disorders

National Institute of General Medical Sciences

National Institute of Mental Health

National Institute of Neurological Disorders and Stroke

National Institute of Nursing Research

National Institute on Aging

National Institute on Alcohol Abuse and Alcoholism

National Institute on Deafness and Other Communication Disorders

National Institute on Drug Abuse

National Institute on Minority Health and Health Disparities

John E. Fogarty International Center

National Center for Advancing Translational Sciences

National Center for Complementary and Integrative Health

Warren Grant Magnuson Clinical Center

Office of the Director

- Office of Behavioral and Social Sciences Research
- Office of Technology Transfer
- Office of Rare Diseases
- Office of Research on Women's Health