The 11th Annual NIH Pain Consortium Symposium on Advances in Pain Research:

Innovative Models and Methods

WORKSHOP SUMMARY

Natcher Conference Center
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Table of Contents

Executive Summary ii
Introduction ii
Panel Session Highlights ii
Conclusion v
Workshop Summary 1
Welcome and Opening Remarks 1
Keynote Address: Challenges of Translational Pain Research 1
Panel Session: Lessons Learned in Translational Research 3
Does My Mouse Have a Headache? 3
Beyond Traditional Assessments of Pain 4
Introduction to Junior Investigator Presentations 7
Haploinsufficiency of the BDNF Gene and Reduced Sensitivity 8
Opioid Sparing Analgesic Effects in Mice 8
Nicotine Deprivation 9
Panel Session: Bridging the Gap Between Models and the Clinic 10
Translational Role Models of Pain: The Transgenic Sickle Mice 10
Pain Begets Pain: Modeling Chronic Overlapping Pain Conditions 11
The Painful Consequences of Stress 12
A Patient's Perspective on Pain Research 13
Mitchell Max Award for Best Junior Investigator Presentation 14
Update from the American Pain Society 15
Enhancing the Reproducibility and Transparency of Research Findings 15
New Technologies in Translational Pain Research 17
Panel Session: Applying New Approaches to Pain Research 19
Visualizing Touch and Pain in the Periphery 19
Imaging Glial Activation in Human Pain Disorders 20
Developing Models for New Treatment Technologies 22
Closing Remarks 24

Appendix 1: Agenda 25
Appendix 2: Meeting Participants 28

EXECUTIVE SUMMARY

INTRODUCTION

The National Institutes of Health (NIH) established the NIH Pain Consortium to foster pain research at the agency's Institutes and Centers and to promote collaboration among researchers both inside and outside the many NIH Institutes and Centers that have programs and activities addressing pain.

The Pain Consortium held its 11th Annual Symposium to discuss existing and future models and methods to better understand pain mechanisms that could lead to the development of improved treatments.

The Symposium featured two keynote presentations by prominent pain researchers. The presentation by David Clark discussed the strengths and weaknesses of some existing animal models. He also made useful recommendations to better support future translational studies. The presentation by Robert Gereau discussed the role of optogenitics, a technology under research which uses lights of various wavelengths to inhibit neuronal activity in genetically modified neurons.

The meeting brought together panels of basic, translational, and clinical researchers to discuss past and future efforts surrounding three broad topics:

1. Lessons learned in translational research
2. Bridging the gap between models and the clinic
3. Applying new approaches to pain research

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. A summary of the panel presentations is presented below.

PANEL SESSION HIGHLIGHTS

Lessons Learned in Translational Research

Presenters in the first panel discussed models for migraine and analgesic efficacy. Migraine is often identified by the typical acute phase where individuals can experience pain, nausea, vomiting, sweating, photophobia, phonophobia, and allodynia. But in about 30 percent of individuals that suffer from migraines, the attack can be preceded by an aura which can include visual and somatosensory hallucinations. Similarly, following the attack patients can experience a "migraine hangover" or the persistence of pain and sensory amplifications.

Due to these and other complexities, an appropriate model is needed to better understand this condition. The casein kinase 1 delta mutation mouse (CK1δ) model was tested to determine if it could be a suitable migraine model.

Studies found that CK1δ mice with induced migraines showed a decreased von Frey hair stimulus response threshold. A lower heat threshold was also detected in these mice. Findings also demonstrated that CK1δ mice had a reduced threshold to cortical spreading depression. Taking these measures together may be sufficient to determine that these mice can provide mechanistic and transnationally relevant information for the study of migraine.

Researchers have found that cannabinoid receptors type 1 (CB_1) are abundant in the central nervous system and can be targeted for an analgesic effect. While targeting of these receptors does produced an analgesic effect, it also typically produces the side effects of hypoactivity, motor ataxia, hypothermia, and antinociception. An alternative target—cannabinoid receptors type 2 (CB_2)—were studied to determine their analgesic effectiveness. Studies in animal models mimicking neuropathic pain showed that administration of CB_2 agonists suppressed neuropathic pain without producing tolerance, CB_1-dependent withdrawal, reward, or cardinal signs of CB_1 receptor activation.

Bridging the Gap Between Models and the Clinic

Presenters in the second panel focused on models for sickle cell anemia, chronic overlapping pain conditions, and the effects of adverse childhood experiences and how they may relate to stress and pain.

In the 1980s and 1990s, various mice models for sickle cell anemia were developed that were limited because they expressed both mouse and human sickle cell hemoglobins. However, in 1997 the transgenic Townes and BERK mouse models were developed. These models were different in that they expressed only human alpha and beta globins and no mouse globins.

These models were tested to determine if they could more closely represent human sickle cell anemia. A vasoocclusive crisis was evoked in BERK mice. Results showed higher hyperalgesia in these mice when compared to others. BERK mice were also treated for five days with mast cell inhibitors (cromolyn sodium or Imatinib) which showed a decreased expression of neuropeptides released by mast cells. Studies also showed that Imatinib and genetic mast cell deletion attenuated pain.

Chronic overlapping pain conditions (COPCs) include irritable bowel syndrome (IBS), temporomandibular disorder (TMD), chronic low back pain (cLBP), endometriosis, fibromyalgia, vulvodynea, and other conditions. It is now believed that these conditions may share altered neural, immune, and endocrine mechanisms and also be sensitive to stress.

Most of these chronic pain conditions are more prevalent—or even exclusive—in women. Also, if person has one pain condition, there is a higher likelihood that they could have another. In addition, many of the symptoms seem to be exacerbated by stress.

Rat models for TMD were tested. Results showed that stress in the presence of pain can induce *chronic* visceral sensitivity while stress alone induces *transient* visceral sensitivity in these rats. In addition, the animals that were already in pain when stressed also showed referred back pain.

These studies also demonstrated hypersensitivity to be estrogen dependent. For example, giving females testosterone was shown to block the development of stress-induced sensitivity. In contrast, giving estrogen to males resulted in the development of stress-induced sensitivity that they didn't have before.

The 2012 Adverse Childhood Experiences Study revealed that 27 out of each 1000 children were maltreated and 27 percent of the maltreated children were under 2 years of age. Adverse childhood experiences have been linked to disease, disability, and early death.

An early life stress paradigm was developed in which C57BL/6 mouse pups were separated from their mother as litters for three hours a day from day 1 to 21 and then exposed to stress. The experiments showed that when stressed the female mice developed exacerbation of urinary bladder sensitivity, increased urinary output, and a transient decrease in regulatory hippocampal gene expression, followed by hypothalamic changes.

Applying New Approaches to Pain Research

Presenters in the third panel presented studies to better classify neuron response, understand the role of glial cell activation in pain, and examine the role of neuromodulation as a potential treatment for pain.

While neurons have traditionally been categorized by size or morphology, it is also important to better understand how they can be classified in their response to varied stimuli. Studies were conducted to monitor the activity of TRPV1-lineage neurons with genetically encoded calcium sensors using the GCaMP6 protein. This protein works as an optical probe for imaging calcium transients in neurons. In this mouse model when specific neurons are activated they become brighter and can be seen optically.

An analysis of the neurons activated based on specific stimuli was undertaken. Results showed that some neurons primarily respond to heat stimulation while other neurons respond primarily to both heat and mechanical stimuli. Still other neurons responded almost equally to heat, gentle, and noxious stimulation.

Research is currently being undertaken to analyze the simultaneous in vivo response of approximately 50 neurons. Preliminary results of these neurons showed that they can be grouped. Some were primarily mechanical nocioceptors, others were primarily thermoreceptors, and a yet another group were polymodal nocioceptors.

Studies were also conducted to better understand the involvement of human glial cells in pain. A study was conducted to examine glial activation in 19 humans with chronic low-back pain (cLBP). The translocator protein (TSPO) was used as a marker of glial activation in PET and magnetic resonance (MR) scanning. The study also measured pain levels and blood levels of cytokines.

Findings showed that chronic pain patients demonstrated TSPO elevation in the brain and that human chronic pain is accompanied by glial activation. These findings suggest that glial activation may represent a therapeutic target for chronic pain

Other studies were carried out to test neuromodulation in addressing pain. The study examined invasive motor cortex stimulation in the treatment of chronic pain in human patients with TMD. Direct current was applied in a non-invasive manner to specific targets on the head in an effort to create an analgesic effect. The study showed that after six weeks, nine of the twelve patients experienced a VAS decrease of more than 50 percent, compared with four patients in the sham group.

CONCLUSION

Chronic pain is a significant problem in the United States, resulting in morbidity and mortality, increased 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.

It is clear that the biological, anatomical, physiological, and functional underpinnings of chronic pain must be better understood to improve treatments and care for people with chronic pain. The 11th Annual NIH Pain Consortium Symposium contributed to research efforts by highlighting advances in research models and methods used by pain researchers. This symposium features recent research advances that will serve to guide future directions in pain research.

WORKSHOP SUMMARY

WELCOME AND OPENING REMARKS

John W. Kusiak, PhD, Acting Deputy Director, National Institute on Dental and Craniofacial Research

Dr. Kusiak welcomed all participants to the meeting. He explained that 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 leadership of the consortium involves five NIH directors and 26 NIH Institutes, Offices, and Centers.

Chronic pain is an important public health concern. A 2011 Institute of Medicine (IOM) report stated that nearly 100 million American adults report chronic pain and 25 million report daily pain. An estimated $635 billion are spent in treatment costs and loss of productivity.

Another concern is the increasing rate of opioid abuse. The National Vital Statistics System has reported a significant increase in opioid sales, treatment admissions, and deaths from 1999 to 2010. A 2013 article by Jones et al in *Drug and Alcohol Dependence* stated that 3 out of 4 people who used heroin in the past year took prescription opioids first.

The ongoing Federal Pain Research Strategy is an effort aimed at identifying gaps in basic and clinical research on the symptoms and causes of pain. It aims to fulfill the mission of the Interagency Pain Research Coordinating Committee (IPRCC), which was established to identify critical gaps in basic and clinical research on the symptoms and causes of pain.

Dr. Kusiak reviewed related efforts in the pain field by other expert panels, groups, and Institutes. He also reviewed some current funding opportunities. He walked participants through the agenda for the two-day meeting and introduced Dr. David Clark of Stanford University.

KEYNOTE ADDRESS: CHALLENGES OF TRANSLATIONAL PAIN RESEARCH: WHAT MAKES A GOOD MODEL?

David Clark, MD, PhD, Stanford University

Dr. Clark reviewed the strengths and weaknesses of various animal models. He also made some recommendations to better support studies in the future.

About 25 percent of the population suffers from chronic pain and in some groups, such as veterans, the prevalence could be more than 50 percent. Chronic pain costs the U.S. $600 billion every year in treatment costs and lost productivity.

In addition, chronic pain can be difficult to treat. In drug trials less than 50 percent of participants obtain pain relief of more than 50 percent. Multiple treatments and multimodal treatment is commonly seen in treating chronic pain.

With few exceptions treatments have not typically changed significantly over the past 20 years. Although new formulations do exist, many of the drugs used then and now still involve NSAIDs, acetaminophen, opioids, antidepressants, gabapentin, tramadol, capsaicin, and lidocaine.

Various novel approaches have been studied in trials including CCR_2 antagonists, TRPV-1, FAHH-1, glial inhibitors, and other approaches. Although these approaches seem to work well in animals, none of them have successfully translated to humans thus far.

Also, the cost of pain clinical trials, especially Phase III trials, is generally higher than similar trials for oncology, hematology, dermatology, and other areas. Another challenge is that clinical trial results cannot always be reproduced due to a variety of reasons including study design. The combination of high cost and low reproducibility can be a stumbling block in getting new pain medications to the market.

One of the ways to address this challenge is by making studies more rigorous by using established guidelines such as the Consolidated Standards of Reporting (clinical) Trials (CONSORT) guidelines, Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines, Preclinical Pain Research Consortium for Investigating Safety and Efficacy (PPRECISE) guidelines, and NIH research guidelines.

Genetics can also have an important influence on preclinical models. For example, there is wide genetic variation in the types of available mouse models. Some experiments have shown that a mouse of one strain may respond very differently than another to the same test, such as paw shaking and licking in mice injected with formalin to simulate a neuropathic condition.

Researchers should consider other non-human animal species as candidates for preclinical models. Other animal models may have a physiology and pharmacology that is more similar to that of humans or offer spontaneous demonstration of diseases also seen in humans.

Additional preclinical measures should also be considered. Reflexive or evoked testing is generally measured in animal models but this may not exactly represent the type of pain being experienced by humans.

While measures for reflexive or evoked testing should continue to be used, researchers should also consider developing other measures such as: rodent body language (such as flinching or guarding) as well as postural changes and preference for limb weight bearing. Other measures that could be explored include facial analysis of animals and ultrasonic vocalizations, both of which can be reproduced. Condition based preference, where a rodent prefers one out of two chambers, might also be a useful measure.

Other measures which could be explored are cognitive changes, which are possible to do in animals but take more time. Functional testing such as gait analysis using sophisticated video technology can be used to determine if analgesics are able to reverse gait abnormalities.

Dr. Clark suggested broadly separating models into two categories: models for discovery and models for translation. In models for discovery one should focus on stringently standardizing

experimental conditions and using multiple rigorous, complementary approaches focused on a clear hypothesis (e.g. pharmacological, genetic, biochemical, electrophysiological, optogenetic, etc.).

For translation models researchers should consider the impact of sex, genetics, species, age, disease comorbidities, psychological comorbidities, and pharmacokinetics/pharmacodynamics.

Dr. Clark also suggested considering the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) guidelines as well as the Initiative on Methods, Measurement, and Pain Assessment in Animal Studies (IMMPAAS) guidelines.

IMMPACT guidelines include various outcome domains including:

- Pain (e.g. Patient report and analgesic use)
- Physical function (e.g. Are patients doing more?)
- Emotional function (e.g. Depression and anxiety)
- Patient's impression of change (e.g. Patient global impression of change)
- Symptoms and adverse events
-

IMMPAAS guidelines include various outcome domains including:

- Pain (e.g. Evoked, spontaneous, and operant pain)
- Physical function (e.g. Activity, gait, running)
- Emotional/cognitive function (e.g. Anxiety and memory)
- Side effects, pharmacokinetics, pharmacodynamics, toxicity (Sedation, balance, and organ toxicity)

Researchers should also consider presenting results in accordance to CONSORT or ARRIVE guidelines, based on their study.

PANEL SESSION: LESSONS LEARNED IN TRANSLATIONAL RESEARCH

Yolanda Vallejo-Estrada, PhD, (Moderator), National Institute on Dental and Craniofacial Research

Dr. Yolanda Vallejo-Estrada kicked off the panel by briefly touching on each of the presenter's areas of research. She then introduced each speaker.

DOES MY MOUSE HAVE A HEADACHE? TRANSLATIONAL MODELS OF MIGRAINE

K.C. Brennan, MD, University of Utah

Dr. Brennan reviewed some of the animal models used to study migraine. He explained that migraine goes beyond the typical acute phase that involves head pain.

During the acute phase individuals can experience nausea, vomiting, photophobia, tearing, sweating, pallor, phonophobia, and allodynia. But in about 30 percent of those that suffer from

migraines, the attack can be preceded by an aura. The aura can include visual and somatosensory hallucinations.

In addition, some patients can present symptoms of hunger, thirst, irritability, or other even before the aura (up to 72 hours before an attack). Following the attack patients can experience a "migraine hangover" or the persistence of pain and sensory amplifications.

For individuals that suffer from migraines, three substances have been found to be migraine-including: nitroglycerine, calcitonin gene-related peptide (CGRP), and the pituitary adenylate cyclase activating peptide (PACAP). These substances can also be used as migraine-inducers in some animal models. For example, a 2010 study by Kaiser et al. in *Neuroscience* showed that the use of CGRP in some mice induced an increased aversion to light (photophobia). A 2014 study by Prahdan in *Pain* showed that chronic infusion of nitroglycerin reduced mechanical withdrawal thresholds (allodynia).

For his studies, Dr. Brennan used a casein kinase 1 delta mutation mouse (CK1δ) model. This mutation was found in two human families with familiar migraine with aura. One of the goals of his study was to determine if this mouse model had a migraine-relevant phenotype.

The study used the nitroglycerin infusion model which showed a decreased von Frey hair stimulus response threshold in these mice. A lower heat threshold was also detected in these mice. His team also found that these mice had a reduced threshold to cortical spreading depression (CSD), compared with their litter mates. CSD occurs during the aura and is associated with visual hallucinations.

Taking these measures together may be sufficient to determine that these mice can provide mechanistic and transnationally relevant information for the study of migraine. Dr. Brennan and his team are now examining in vivo whole-cell recordings in these mice to better understand the mechanisms surrounding CSD. They are also using whole-cell recordings to examine mouse behavior during photophobia and other migraine-related events.

BEYOND TRADITIONAL ASSESSMENTS OF PAIN: WHAT CAN ANIMALS TELL US ABOUT ANALGESIC EFFICACY?

Andrea Hohmann, PhD, Indiana University

Dr. Hohmann explained that cannabinoid receptors type 1 (CB_1) are abundant in the central nervous system whereas cannabinoid receptors type 2 (CB_2) are primarily seen in immune tissues and cells. Her research examined the potential analgesic effects associated with CB_2 by using a compound named (R,S)-AM1241, a known CB_2 agonist. More specifically, her studies focused on rats that self-medicated with a nonpsychoactive cannabinoid analgesic to attenuate a neuropathic pain state.

Activation of CB_2 receptors is of particular interest because, unlike activation of CB_1, they can suppress neuropathic pain without the associated central nervous system (CNS) side effects of hypoactivity, motor ataxia, hypothermia, or antinociception. It is also believed that activation of CB_2 mechanisms can suppress pain without producing tolerance or signs of physical dependence.

The experiments involved a spared nerve injury model to simulate neuropathic pain. Three groups of rats were utilized. In the first group, two branches of the sciatic nerve were ligated and cut while the third branch was left intact. In the second group, the nerve branches were exposed but not ligated or cut. In this group the muscle and skin were then sutured closed. In a third group (naïve), the nerves were unmanipulated.

All three groups were surgically implanted with a chronic indwelling jugular catheter to allow for intravenous drug self-administration. The rats pressed one active lever to self-administer AM1241 while another inactive lever administered only the vehicle.

An assessment of mechanical paw withdrawal thresholds in the rats was measured before the surgical procedures as well as before and after the self-administration sessions. Results showed that self-administration of AM1241 induced CB_2-mediated anti-allodynic effects in the injured paw.

The experiment also increased the level of effort the rats had to undertake to self-administer the drug by changing the number of times the lever had to be pressed to self-administer the drug (FR1, FR3, and FR6 reinforcement schedules). Results showed that the neuropathic rats were willing to work harder than shams to obtain AM1241. These and other experiments showed that:

- Neuropathic animals self-medicated with a CB_2 agonist to attenuate pain behavior
- Neuropathic animals worked harder than shams to obtain the CB_2 agonist
- Naïve animals did not reliably self-administer the CB_2 agonist
- Naïve, sham, and neuropathic rats self-administered morphine
- In an extinction test, neuropathic animals perseverated in responding on the lever previously paired with the opioid analgesic, but not the CB_2 agonist

These studies showed that CB_2 agonists can suppress neuropathic pain in preclinical models without producing tolerance, CB_1-dependent withdrawal, reward, or cardinal signs of CB_1 receptor activation. They also showed that opioid analgesics exhibited higher abuse liability in neuropathic pain states compared to CB_2 agonists.

Discussion

A participant said that one of the problems often found in pain trials is the placebo affect. He asked if there are ways to measure pain differences by other means after entering a specific chamber.

Dr. Clark said this would mean going past the simple movement of the animal into the chamber which investigators interpret as being analgesic and asking if there is another measure of pain in that animal. He said they are currently setting up their devices to have floors such that they can measure allodynia in the animal at the same time it enters the chamber. This would yield information on animal movement into the chamber as well as possibly a measurement of another dimension of the pain-like experience in the animal.

An audience member asked if the onset of action for zolendronate was fast, delayed, or if it had to be administered for several days.

Dr. Clark said those experiments were conducted in two different ways. The drug can be administered both after the fracture and during immobilization (or shortly thereafter) with some measured analgesic affect. With complex regional pain syndrome, one can interfere early in the course of the disease because there is an injury or surgery. What is not clear is whether the drug works as well in a chronically affected animal or human. He explained that some people living with pain for years may not respond to the same treatment and this should probably also be studied in the lab.

An audience member asked if Dr. Clark could speak about the differences between inbred and outbred rodent models.

Dr. Clark said that by using outbred animals one could leverage differences among animals to understand the difference in the mechanism of action within a population. One could look at individual animals in the degree of descending inhibition and correlate that with the change in pain measure which would perhaps cast a new light on the mechanism.

An audience member said that in surgery the scalpel is generally only used for one cut and is followed by use of electrocautery. Electrocautery causes changes in the microvasculature of the wound and this seems to be missing from surgery animal models.

Dr. Clark agreed that in incisional models one does not create the tissue disruption typically seen in surgery, such as electrocautery or the dissection of muscle and tendon. Inflammation and tissue damage is also not recreated in this simple model. He said that the measured changes would be more robust and of longer duration if such a model was used.

An audience member asked how we might be able to use the wealth of knowledge available in various inbred strains to come up with groupings of inbred strains which may begin to mimic the heterogeneity seen in a clinical condition

Dr. Clark agreed that not all clinical pain patients are the same. For example, there could be subgroupings of patients within the diagnosis of neuropathic pain with different patterns of hyperalgesia, allodynia, and other quantitative differences in the pain syndrome as well as differences in comorbidities. There might be subgroups in clinical populations that need to be modeled differently in the animals. In other words, the mechanisms that better explain one individual's pain syndrome might be best modeled by a particular genetic background model, environmental conditions under which the animals are kept, stressors that are applied to the animal, and other factors. This may be an example where the clinic has to do a better job of informing the laboratory as opposed to the laboratory simply failing the human.

An audience member said that most of the pain studies use evoked pain by CFA or nerve ligation but in real patients that may not always be the case. She said there are mouse models which either genetically or otherwise mimic different pathobiologies. This is important because different pathobiologies may have different underlying mechanisms and phenotypes of pain. She asked how we could increase the use of such models to provide different answers and more specific insights, which could be more relevant to a particular disease condition.

A panelist said there are phenotypes relevant to different diseases that may have a better "signal-to-noise ratio" in animal model systems. In other words, one could use more than one model. For example, for epilepsy multiple models are used. Another panelist said that animal models are not very good at telling us about the problematic side effects that may be detected with small molecule inhibitors in humans. This is important because drugs can fail for side effects and not just efficacy.

Dr. Clark added that is has been proposed to move from the laboratory to heterogeneous clinical populations by trying specifically to pick up on those types of patients which will best respond to a particular drug and then apply it to a subgroup of patients in the more expensive, long-term term trials such as Phase II or III.

An audience member asked if panelists could comment on PK studies and the translation from animal models to humans. For example, oral analgesics in humans get a first pass through the liver but then start developing metabolites which also may have an analgesic effect. In contrast, in some animal studies most of the drugs are delivered by injection. He also asked what could be done to develop realistic animal models for testing controlled release oral formulations.

Dr. Clark said some drugs fail to move forward because when taken to humans the drug is found to be metabolized differently, or the target is somewhat different in humans than in the animal model. Mouse models with humanized livers have been developed to better predict toxicity for chemotherapeutic agents. These animals have sufficient hepatic tissue from a human so that resulting metabolites represent more faithfully what would be seen in humans. It makes sense to use these animals early on in drug development studies to understand what happens with a candidate molecule in terms of metabolism.

INTRODUCTION TO JUNIOR INVESTIGATOR PRESENTATIONS

Patricia A. Grady, PhD, RN, FAAN, National Institute of Nursing Research

Dr. Grady introduced the junior investigator presentations competing for the Mitchell Max Award. The award was named after neurologist Mitchell Max, MD (1949-2008) who was an authority on the mechanisms of neuropathic pain and the genetic basis for pain.

During his years at the NIH, Dr. Max served as the Chief of the Clinical Pain Research Section and Medical Director of the Pain Research Clinic at the National Institute on Dental and Craniofacial Research. The award honors his lifetime contributions towards pain research.

Dr. Grady reviewed some of the previous awardees and their recent accomplishments. She then proceeded to present the three finalists for the award: Drs. Matt Sapio, Jenny Wilkerson, and Joseph Ditre.

HAPLOINSUFFICIENCY OF THE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) GENE CAUSES REDUCED PAIN SENSITIVITY IN AN ANIMAL MODEL AND HUMANS WITH THE WAGR COPY NUMBER VARIANT

Matt Sapio, PhD, NIH Clinical Center

Dr. Sapio's presentation discussed animal models for humans with WAGR syndrome. WAGR syndrome is a disorder that affects many body systems and is named for its main features: **W**ilms' tumor, **A**nirida, **G**enitourinary anomalies, and Intellectual disability (formerly referred to as Mental **R**etardation). These children have a variable deletion on chromosome 11 which sometimes results in deletions for the brain-derived neurotrophic factor (BDNF).

Studies of BDNF have shown that it is linked to pain. In studies in animal models, injecting a 1 percent carrageenan solution into the hind paw of rodents resulted into the upregulation of genes for BDNF for up to 48 hours.

Dr. Sapio's study involved 12 human WAGR patients: six had the BDNF deletion and the other six did not. Quantitative sensory testing showed that children with the BDNF deletion (BDNF +/-) did not rate painful temperature stimuli as painful, while the other group did. This included temperature stimuli as low as 2 degrees Celsius and as high as 49 degrees Celsius. These results suggests that individuals with the deletion have difficulty in rating noxious stimuli as painful.

Dr. Sapio used rats with a single BDNF gene deletion (BDNF +/-) as the animal model. The rats were exposed to a 100 ms laser pulse (which generated heat) on their heel. Results, in terms of paw withdrawal, showed that at an intensity of 5500 mA the wild-type rats showed a nearly 100 percent response in paw withdrawal, while the BDNF +/- rats showed only a 30 percent response. Similar reports were found using cold stimuli. These results mimic the higher threshold to noxious stimuli seen in humans.

Preliminary data from Dr. Sapio's lab showed that in BDNF +/- rats a number of genes that correspond to peptide precursors decreased (TAC1, CALCA, CARTPT, NPPA, and NPPB). This may be indicative of less excitability, activation, or synthesis of these peptides within primary afferent neurons, which may explain reduced nociception. These findings, in turn, may support the idea of BDNF sequestration as a strategy for pain control.

A DUAL FATTY ACID AMIDE HYDROLASE AND MONOACYLGLYCEROL LIPASE INHIBITOR PRODUCES OPIOID SPARING ANALGESIC EFFECTS IN MICE

Jenny Wilkerson, PhD, Virginia Commonwealth University

Dr. Wilkerson performed studies in mice using a compound named SA-57. This compound that has been found to inhibit both fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Animal studies have shown that the inhibition of such enzymes can lead to analgesic effects. However, at high doses this same compound has shown cannabinoid side effects in the tetrad: locomotor activity, bar test, hypothermia, and tail withdrawal.

Her experiments utilized the chronic constriction injury model, which is a model of neuropathic pain in mice. In this model the sciatic nerve is isolated and ligated loosely with silk sutures. This

results in an inflammatory response and nerve trauma. The comparison group underwent an identical sham surgery except for the ligation.

Allodynia was assessed with calibrated thin monofilaments (von Frey hairs) applied to the hind paw. Results showed that the group with neuropathic pain responded at a much lower stimulus intensity than the sham group. Her team then delivered a dose of SA-57 and compared it to a 1:1 combined dose of morphine *and* SA-57.

Results showed that the combination of SA-57 and morphine resulted in an additive reversal of allodynia in the neuropathic mice. This combination did not show tetrad effects except for antinociceptive tail withdrawal effects. In essence, this combination showed effective anti-allodynia without some of the known cannabimemetic effects.

Her other studies also demonstrated that SA-57 produced diminished heroin seeking behavior in mice. This suggests that the combination strategy may be a promising adjunct therapy to opioids for the treatment of neuropathic pain.

NICOTINE DEPRIVATION INCREASES PAIN SENSITIVITY, NEUROGENIC INFLAMMATION, AND SECONDARY HYPERALGESIA AMONG DAILY TOBACCO SMOKERS

Joseph Ditre, PhD, Syracuse University

Dr. Ditre explained that chronic pain and tobacco smoking are highly prevalent and can be co-occurring conditions. Smokers with co-occurring pain smoke more cigarettes per day and report experiencing more severe nicotine withdrawal and a greater difficulty in quitting.

The study involved 165 daily tobacco smokers from the local community, of which 43 percent were female. The mean number of cigarettes smoked per day per person was 22. Participants were randomized into one of three groups:

- A nicotine deprivation group (abstained from smoking overnight for between 12-24 hours)
- A minimal deprivation group (abstained for two hours)
- A continued smoking group (smoked as usual)

The study used the capsaicin pain model, which approximates key features of neuropathic and inflammatory pain. A capsaicin stimulus was introduced on the forearm of the smoker and the pain intensity and unpleasantness was assessed on a scale of 1 to 10 at 5 minute intervals for up to 30 minutes.

Neurogenic inflammation was measured as the visible area of flare on the forearm. Secondary hyperalgesia was assessed via tactile stimulation at 5 mm points in linear paths radiating from the center of the application site.

Results showed that the nicotine deprivation group expressed higher pain ratings than the two other groups. The continued smoking group expressed the lowest ratings of the three groups. The study also showed that nicotine deprivation increased withdrawal severity, which in turn

was associated with greater pain intensity and unpleasantness ratings. These findings speak about the idea of pain as a potential withdrawal symptom.

In terms of inflammation findings, the study showed that the nicotine deprivation group evidenced a larger area of flare than the continued smoking group, which implicates peripheral mechanism of action. Other results also implicated central mechanisms of action.

In summary, nicotine deprivation seemed to increase spontaneous pain ratings, neurogenic inflammation, and secondary hyperalgesia. In terms of clinical implications, this may suggest that smokers with co-occurring pain may experience a variety of negative pain-related sequelae during the early stages of a quit attempt. These patients may benefit from tailored cessation interventions that account for the antithetical influence of the abstinence-induced amplification of pain.

PANEL SESSION: BRIDGING THE GAP BETWEEN MODELS AND THE CLINIC

Ann O'Mara, PhD, RN, FAAN, National Cancer Institute

Dr. O'Mara opened the panel by describing the background and research of each presenters. She then introduced each speaker.

TRANSLATIONAL ROLE MODELS OF PAIN: THE TRANSGENIC SICKLE MICE

Kalpna Gupta, PhD, University of Minnesota

Dr. Gupta's research focuses on the use of transgenic mice as models for sickle cell anemia. In humans, sickle cell anemia is accompanied by a variety of symptoms, including pain. Due to their shape, sickle cell red blood cells can occlude blood vessels leading to vasoocclusive crises which result in inflammation, ischemia, severe pain, and sometimes organ damage.

Pain can start during infancy and be recurrent throughout life. In addition to chronic pain, individuals can also suffer from acute pain crises. In some cases both chronic and acute pain can occur concurrently in an individual. Relatively high doses of opioids are required to treat the pain, often for long periods of time. In some cases patients can become refractory to treatment.

In the 1980s and 1990s various mice models for sickle cell anemia were developed. They expressed both mouse and human sickle cell hemoglobins. However, in 1997 the transgenic Townes and BERK mouse models were developed. These models were different in that they expressed only human alpha and beta globins and no mouse globins.

The latter models more closely represent human sickle cell anemia including effects such as hemolysis, pain, reticulocytosis, anemia, extensive organ damage, and a foreshortened lifespan. Also, BERK female mice showed more pain-like behavior that males.

A model for acute pain to simulate vasoocclusive crisis was evoked by causing hypoxia in these the BERK mice. The mice were exposed to hypoxia (8 percent oxygen and 92 percent nitrogen) for three hours and then exposed to room air. This approach showed higher hyperalgesia in the BERK mice when compared to other mice.

These mice were then used to test the effect of mast cell inhibitors. Mast cells have shown to have a role in allergic responses and anaphylaxis. They are in lose proximity to vasculature and nerve fibers and can act in an autocrine and paracrine manner. Mast cells can also release cytokines, proteases, prostaglandin, and neuropeptides including CGRP and substance P. Morphine is know to activate mast cells.

BERK mice were treated for five days with either cromolyn sodium or Imatinib (both are mast cell inhibitors). Analysis of mice blood plasma showed decreased expression of neuropeptides released by mast cells such as tryptase, β-hexosaminidase, serum amyloid-protein (SAP), and calcitonin gene-related peptide (CGRP) in mice treated with each of the inhibitors.

Studies also showed that Imatinib and genetic mast cell deletion attenuated hypoxia-evoked pain. This suggests the possibility of targeting mast cells in to improve morphine analgesia and/or stimulate disease modifying effects leading to reduced pain.

PAIN BEGETS PAIN: MODELING CHRONIC OVERLAPPING PAIN CONDITIONS

Richard Traub, PhD, University of Maryland

Dr. Traub's research focuses on utilizing animal models to better understand chronic overlapping pain conditions (COPCs). Examples of these conditions include irritable bowel syndrome (IBS), temporomandibular disorder (TMD), chronic low back pain (cLBP), endometriosis, fibromyalgia, vulvodynea, and other conditions. These conditions comprise pain illnesses that exist in the absence of organic, systemic, or metabolic disease that are likely to explain their symptoms.

A 2015 white paper by the Chronic Pain Research Alliance stated that it was thought at one time that these were individual conditions with distinct peripheral mechanisms at each affected body site. However, it is now believed that these conditions may share altered neural, immune, and endocrine mechanisms.

Most of these chronic pain conditions are more prevalent—or even exclusive—in women. Also, if person has one pain condition, there is a high likelihood that they could have another. In addition, many of the symptoms seem to be exacerbated by stress.

Dr. Traub's research focuses on two of these conditions: IBS and TMD. He utilized an orofacial rat model of TMD. In this model, complete Freund's Adjuvant (CFA) is injected into the masseter muscle or pain is modeled through a chronic constriction injury of the infraorbital nerve. Studies showed that when these rats were exposed to a stressed swim, it resulted in a de novo visceral hypersensitivity as measured by VMR.

Dr. Traub found that if stress was added to rats that were already in pain (i.e. the CFA model) it resulted in chronic visceral hypersensitivity that could last for at six weeks or longer. In comparison, rats that were not in pain when they were stressed showed a hypersensitivity that lasted only from a few days to weeks. In other words, this showed that stress in the presence of pain can induce *chronic* visceral sensitivity while stress alone induces *transient* visceral sensitivity in these rats. In addition, the animals that were already in pain when stressed also showed referred back pain.

These studies also demonstrated the hypersensitivity to be estrogen dependent. For example, giving females testosterone was shown to block the development of the stress-induced sensitivity. In contrast, giving estrogen to males resulted in the development of stress-induced sensitivity that they didn't have before.

As in previous studies, treating these rats with a mast cell stabilizer resulted in blocking the development of hypersensitivity. Other studies showed that the pain was centrally mediated.

His lab is undertaking further research to determine how stress might interact with orofacial and visceral pain by administering an intrathecal 5-HT3R receptor antagonist. Preliminary data show that this approach has resulted in an attenuation of the mechanical hypersensitivity lasting for a few hours.

THE PAINFUL CONSEQUENCES OF STRESS

Julie Christianson, PhD, University of Kansas Medical Center

Dr. Christianson's presentation focused on animal models to better understand the effects of early exposure to stress. The 2012 Adverse Childhood Experiences Study revealed that 27 out of each 1000 children were maltreated and 27 percent of the maltreated children were under 2 years of age.

Adverse childhood experiences have been linked to disease, disability, and early death. A 2008 *JAMA* study by Carbajal et al. showed that newborns in the neonate intensive care unit (NICU) experience an average of 16 procedures a day, of which 10 are considered painful. Nearly 80 percent of these procedures are performed without analgesic intervention. These newborns also experience prolonged periods of maternal separation.

To model these situations, Dr. Christianson used an early life stress paradigm in which C57BL/6 mouse pups were born in-house and separated from their mother as litters for three hours a day from day 1 to 21. These mice experienced Neonatal Maternal Separation (NMS).

In this model, both NMS and naïve mice were exposed to water avoidance stress for one hour. Both groups were then tested for pelvic organ sensitivity, bladder dysfunction, and hypothalamic–pituitary–adrenal (HPA) axis regulation.

Pelvic organ sensitivity was determined by measuring visceromotor reflex (VMR) to the distention of a pelvic organ via air pressure. Female NMS mice showed increased vagina and bladder sensitivity when compared to their naïve counterparts. However, female NMS mice experienced less colorectal sensitivity than their cohort. Male NMS mice showed a decreased threshold for urogenital organ-specific mechanical sensitivity.

Other experiments showed that exposure to water avoidance stress in NMS mice caused:

- Exacerbation of urinary bladder sensitivity in female NMS mice
- Increased urinary output in female NMS mice
- A transient decrease in regulatory hippocampal gene expression, followed by hypothalamic changes
- Mast cell degranulation and associated protein expression in the bladder

Dr. Christianson's studies suggest that exposure to early life stress induces baseline changes within the urogenital organs, and possible priming within the limbic structures, that result in increased susceptibility to stress exposure later in life.

Discussion

An audience member said that nearly all the models presented to evoke stress involved the activation of the sympathetic side of the autonomic nervous system. He asked whether beta blockers or beta-adrenergic antagonists had been examined in these models and whether such approaches may be closer interventions for translation into human models. For example, the catecholamine storms which result from stressors may have profound effect on mast cell degranulation.

A panelist said they are still thinking about that approach. He added that they would like to examine the effects of blocking noradregenic stimulation in the gut.

A participant asked about the role of meditation and yoga as a treatment strategy for pain elevation and pain syndromes.

A panelist said there are some types of allodynia in mice that are completely abolished by exercise. She added that in the wild these mice would running but in the lab they are imposed a sedentary lifestyle. She surmised that perhaps getting pain patients involved in mindfulness activities and exercise might actually decrease the number of drugs used to treat them.

A participant asked, regarding chronic overlapping pain conditions, if one condition induces another or if they have independent origins of peripheral damage.

A panelist said that once an individual gets that first condition then other factors can come into play. Having a pain condition and then adding stress on top of that can result in the generation of other multiple comorbidities that are expressed in these individuals. However, it's not clear whether they are independently converging. Once a person gets one pain conditions it can change brain processes and lead to the generation of multiple conditions in individuals that are susceptible to developing pain conditions.

A PATIENT'S PERSPECTIVE ON PAIN RESEARCH

Christin Veasley, Chronic Pain Research Alliance

Ms. Veasley provided a brief assessment of the public health impact of chronic pain before discussing efforts by the Chronic Pain Research Alliance (CPRA).

To address the impact of chronic pain, the Secretary of Health launched the National Pain Strategy in March 2016. This interagency initiative seeks to reduce the burden and prevalence of pain and to improve the treatment of pain. It will also provide methods and metrics to guide progress towards achieving improved prevention and management of pain in the United States.

From a legislative perspective, The Safe Treatment and Opportunities Pain Act was introduced in the U.S. Senate in March 2016. The Bill aims to direct the NIH to intensify and coordinate fundamental, translational, and clinical research with respect to the understanding of pain, the development and discovery of therapies for chronic pain, and the development of alternatives to opioids for effective pain treatments.

Ms. Veasley explained that the CPRA was created as an advocacy effort to change the lives of those with overlapping pain conditions, such as irritable bowel syndrome, fibromyalgia, chronic migraine, chronic fatigue syndrome, temporomandibular disorders, endometriosis, and other conditions.

It is believed that a common underlying disease mechanisms may impact some or all of these conditions including possibly genetic factors, neuroendocrine and neuroimmune abnormalities, abnormal pain and sensory processing, stress, and other factors.

The CPRA also works to promote a rigorous, standardized, collective, and cost-effective research approach. It hopes to drive the development of safe and effective treatments for chronic overlapping conditions.

The CPRA has been a supporter of large national studies with a specific focus on Chronic Overlapping Pain Conditions (COPCs). These studies include multi-year studies of temporomandibular disorders and the development of overlapping pain conditions including headache, low back pain, irritable bowel syndrome, and widespread body pain.

In order to maximize research on COPCs, the CPRA is working with NIH to develop a case definition, create a common data elements program, and develop a data-sharing repository.

CPRA has also developed a publicly available white paper titled "Impact of Chronic Overlapping Pain Conditions on Public Health and the Urgent Need for Safe and Effective Treatment." The white paper promotes awareness and research of Chronic Overlapping Pain Conditions.

MITCHELL MAX AWARD FOR BEST JUNIOR INVESTIGATOR PRESENTATION

Wilson Compton, MD, MPE, Deputy Director, National Institute on Drug Abuse

Dr. Compton informed participants that the National Institute on Drug Abuse has a substantive portfolio of basic science research—particularly around the opioid system and examining alternatives to opioids. The Institute also has both applied and translational research programs in the area of pain and opioid research. In addition, the Institute is working through a consortium to support educational programs to develop better approaches to the evaluation and treatment of pain.

Dr. Compton presented the Mitchell Max Award to Dr. Joseph Ditre for his work on nicotine deprivation and its increase in pain sensitivity, neurogenic inflammation, and secondary hyperalgesia among daily tobacco smokers.

Dr. Ditre is an assistant professor of psychology at Syracuse University. Most of his work to date has focused on conceptualizing and testing bidirectional associations between the experience of

acute and chronic pain and the self-administration of nicotine an tobacco with an ultimate goal of using these data to inform the development of innovative treatments.

UPDATE FROM THE AMERICAN PAIN SOCIETY

Gerg Terman, MD, PhD, President Elect, American Pain Society

Dr. Terman provided an update on efforts by the American Pain Society (APS). The APS is a multidisciplinary community that brings together a diverse group of scientists, clinicians, and other professionals to increase the knowledge about pain and transform public policy and clinical practice to reduce pain-related suffering.

It is the only professional organization in the U.S. whose primary goal is the promotion of pain science. The organization undertakes efforts in four domains: research, education, treatment, and advocacy and holds various meetings, including its Annual Scientific Meeting. The annual meeting highlights innovative research and educational sessions, among other activities.

The APS offers support to young scientists through its annual Early Career Forum workshops, which shows them how to write a grant, undergo mock study sections, and undertake career development grant discussions.

The society encourages research primarily through small grant programs, including its young investigator travel awards. These awards have helped sixty young investigator trainees to attended this year's scientific meeting.

The society has also partnered with the Rita Allen Foundation to provide two grants for $50,000 per year for a period of up to three years. The Future Leaders in Pain Research grant provides $25,000 for two years. Since its inception, this grant has funded 30 researchers.

The *Journal of Pain*, published by the APS, helps scientists keep abreast of the latest discoveries in the field as well as the latest activities of the society. The society has also revived a pre-event for the annual meeting. During 2015 pre-event held in Palm Springs, California, 165 rapid-fire unpublished data presentations were given on original, unpublished research. Presenters were provided with extensive feedback.

The APS has also been involved in the development of clinical guidelines, along with other organizations. The society has supported the National Pain Strategy developed by the NIH Interagency Pain Research Coordinating Committee.

ENHANCING THE REPRODUCIBILITY AND TRANSPARENCY OF RESEARCH FINDINGS

Shai Silberberg, PhD, National Institute of Neurological Disorders and Stroke

Dr. Silberberg discussed the concept of bias in research findings. A 2011 article published in *Nature Reviews Drug Discovery* by Prince et al. from Bayer HealthCare reported that only one-third of the published studies they reviewed by the academic community could be reproduced in their labs. There are various reasons as to why findings can't always be reproduced:

- The group may not have the know-how or technology to execute complex innovative techniques
- Challenges related to resources (e.g. two labs may run the same experiment but use different cell lines because they are misidentified)
- Confounding variables

Increased transparency in reporting of experimental design, conduct, and analysis could help address the issue of reproducibility. Experimental bias—as well as chance and publication bias—can also be addressed to improve reproducibility. It's important to note that experimental bias can be unintentional and unconscious. This should not be confused with scientific misconduct, which is intentional.

A 2006 *JAMA* study by Hackam and Redelmeir reviewed studies of animal trials that had a high impact (i.e. were cited more than 500 times). The papers reviewed came from seven prominent journals: *Cell, Nature, Science, Nature Medicine, Nature Genetics, Nature Immunology,* and *Nature Biotechnology.* Of the 76 animal trials examined in these studies, less than 20 percent reported whether they blinded or not. Also, less than 20 percent of the trials reported whether or not they randomized to a control group.

Another challenge impacting reproducibility is publication bias. When an experiment supports the hypothesis it generally published, but when it doesn't the experiment is typically filed. A 2010 *PLOS Biology* study by Sena et al. reported that out of 525 unique publications of animal stroke studies, only 2 percent "reported no significant effects on infarct volume." This shows an unusually high level of efficacy.

One should also consider the element of chance. A 2008 study by Scott et al. in *Amyotrophic Lateral Sclerosis* examined the probability of seeing an apparent effect by chance based on the number of animals used per study.

Results showed that in studies using 4 mice per group, 30 percent had chance of obtaining an apparent effect based on chance. In contrast, studies using 10 mice per group had less than a 10 percent chance in obtaining an apparent effect.

Dr. Silberberg explained that the effect of chance, combined with publication bias, can result in published papers reporting a significant effect when none really exists. Dr. Silberberg said his group reviewed the *JAMA* study by Hackam and concluded that more than half of the studies in that paper used 5 animals per group or less.

Dr. Silberberg offered some suggestions to improve reproducibility. One is to increase transparency in reporting when reviewing studies for publication or grants. The *Nature* group was one of the first groups of journals to take a step in this direction. They provided more space for the methods section and ensured that key methodological details are reported. They also encouraged authors to submit raw data.

The NIH has also moved in a similar direction and now requires investigators to discuss the quality of the data used to support their application. The NIH expects applicants to describe the general strengths and weaknesses of prior research being cited by the investigator as being

crucial to support the application. The NIH also expects applicants to describe how they will achieve robust and unbiased results when describing their experimental design and proposed methods. Another parameter to be considered is the authentication of key biological or chemical resources. The NIH expects that key biological or chemical resources will be regularly authenticated to ensure their identity and validity for used in the proposed studies.

Dr. Silberberg also suggested increased education to address unconscious bias and deficient experimental procedures.

NEW TECHNOLOGIES IN TRANSLATIONAL PAIN RESEARCH

Rob Gereau, PhD, Washington University

Dr. Gereau's presentation focused on optogenetics. Over the past 20 years, technological advances in the neurosciences have enabled new findings and discoveries surrounding the biology of pain. These advances include in vivo imaging of circuit activity, techniques for somatic cell reprogramming, technologies to enable the manipulation of identified populations of neurons, and optogenetics.

Dr. Gereau's animal studies aim to better understand Interstitial Cystitis/ Bladder Pain Syndrome. Interstitial Cystitis/ Bladder Pain Syndrome is a severely disabling condition which produces pain on bladder filling. Unfortunately, this condition can be refractory to treatment and many of the strategies used to treat chronic pain can be ineffective.

In his studies, Dr. Gereau utilized a light-sensitive protein found in algae which was then inserted into the DNA of specific mouse neurons. To achieve this, he used a transgenic mouse line that has opsin expression controlled by Cre. The mouse expresses opsins only in nociceptive primary afferent neurons that express TrpV1.

Opsins, or light-sensitive proteins, can be excitatory or inhibitory. When one shines light of a specific wavelength onto an inhibitory opsin, it can suppress the ongoing firing in the neuron—or prevent its firing in response to input.

In his experiments a fiber optic tube, which can turn a light of a specific wavelength on or off, was inserted in the mouse bladder through a catheter. In addition, the mouse bladder was distended using air pressure while the level of contraction of the abdominal muscles was measured via EMG as a response to the distention. The latter measure is considered a surrogate measure for pain.

Results showed that light activation of archaerhodopsin, an inhibitory opsin, suppressed firing in bladder-projecting sensory neurons. This, in turn, suppressed the visceromotor reflex response when the bladder was distended.

One of the concerns regarding the application of this system is that the strategy is dependent on tethering the fiber optic cable to the mouse. To address this, Dr. Gereau's lab in collaboration of other labs developed a wireless implantable LED device. These ultra-miniaturized light sources can be implanted and activated wirelessly in mice. To obtain a perspective on their size, the micro LEDs used measure 50 microns and can fit through the eye of a needle.

The first published reference of the use of these wirelessly activated micro LEDs implanted into a mouse brain appeared in *Science* in 2013. In 2015, an article in *Nature Biotechnology* by Park et al. described a fully implantable device that contained not only the micro LED but an antenna for wireless activation. This device is less than 10 millimeters in length and encapsulated in a soft and flexible material that has a high tolerance to stretch and movement. The device can anchored in a way that does not require a bony anchor point. In other words, the device can be implanted on top of the sciatic nerve, into the epidural space, brain, etc.

Tests showed that these devices were well tolerated. They do not cause any motor impairment on the mouse, produce inflammation, or nerve damage and also produce minimal heat (less than 2 degrees Celsius).

Dr. Gereau presented data for such a device implanted over the mouse's sciatic nerve. In mice expressing TrpV1-ChR2, the activation of the device implanted over the sciatic nerve elicited spontaneous pain behaviors, such as flinching and licking of the paw, when compared to a control group of mice.

A separate experiment implanted a micro LED device into the bladder of mice expressing archaerhodopsin (an inhibitor opsin). The mice were treated with cyclophosphamide to induce cystitis and introduced into a Y-shaped maze. When the mouse entered one side of the maze the investigators turned on an LED while on the other side of the maze the LED was never turned on.

The control animals, which also had induced cystitis but did not express archaerhodopsin, did not show preference to either side of the maze. But the animals with induced cystitis showed a preference for the side of the maze where the LED was turned on. This suggests a preference for a side where the animal feels less pain, which is the side where the LED was illuminated. Preference was measured by the time spent on each side of the maze.

To determine the possibility of translating this technology into humans, a proof-of-concept study was carried out by Valtcheva et al. (*Nature Protocols* 2016, in press). The study took human sensory neurons from organ donors which were transduced with HSV vectors carrying ChR2 or halorhodopsin. Illumination in neurons expressing ChR2 showed light-evoked firing potentials in the neuron, while neurons expressing halorhodopsin when illuminated showed suppression of ongoing action potential firing, or firing induced by a generated potential in the neuron.

Discussion

An audience member asked if researchers are just beginning to investigate the use all of recombinant tools. He asked if the area is still evolving.

Dr. Gereau said he believes the area is indeed still evolving. He said he presented a very limited subset of directed expression. Work using single cell RNA sequencing can help find much more discrete populations of afferent neurons. Deep sequencing technology will tell us what these populations are which may lead to discovering different cell phenotypes.

An audience member asked panelists if they had thought about taking this technology and moving it towards chemogenetics.

A panelist said that chemogenetics uses designer receptors which don't respond to any endogenous ligand but do predictively respond to a ligand that is provided to animals. There are several groups that have been working on this area and which have developed different types of chemogenetic approaches. The advantage of chemogenetics is that it doesn't require a device being implanted. Optogenetics has the ability to have better temporal precision and finer tuning of the dose relative to chemogenetics. Both have advantages and disadvantages and only time will tell which will work better, but both should be pursued.

An audience member asked how long one can use the optogenetics stimulation in the same animal. In other words, how long is the prep viable?

Dr. Gereau replied that the prep is viable as long as the animal lives. There haven't been tests to determine how long the technology lasts, but newer devices have lasted at least four months. The real challenge in terms of long-term viability regarding therapeutic potential is viral expression. In other words, how long of a stable viral expression can one obtain? He said that other researchers are working in this area.

An audience member asked if there was any migration of the device when it was implanted in the epidural space.

Dr. Gereau explained that movement of the device in the epidural space can happen. The team spent a long time working on this issue. It was solved by suturing the device to the epidural space. However, sometimes the suture can come loose and when it does the LED can shift around and one can lose targeting. They are also working on placing the LED over a laminectomy.

An audience member asked if any studies were done to compare archaerhodopsin and halorhodopsin.

Dr. Gereau said they both seem to work as inhibitory opsins, but they haven't directly compared the two to determine which one is more effective.

VISUALIZING TOUCH AND PAIN IN THE PERIPHERY

Alex Chesler, PhD, National Center for Complementary and Integrative Health

Dr. Chesler described some of the research being done as his lab, which is part of NIH's intramural program. His research focuses on studying the somatosensory system using mouse models.

Dr. Chesler explained that neurons come in many shapes and sizes and can be categorized in various ways. One of the most simplistic ways to classify them is to think about them in terms of size: small, medium, and large, with small neurons having a smaller diameter than large ones.

Neurons can also be categorized by the morphology of the nerve ending on the skin. In hairy skin one finds free nerves, circumferential, and lanceolate endings. In glabrous skin, one finds Meissner corpuscles, Pacinian's corpuscles, Ruffini nerve endings, and free nerve endings.

One could also classify neurons by what they respond to. Some neurons primarily respond to temperature stimuli while others may primarily respond to noxious mechanical stimulation, itch, irritants, vibration, muscle control, or even affective gentle touch.

Dr. Chesler's lab has conducted studies to monitor the activity of TRPV1-lineage neurons with genetically encoded calcium sensors using the GCaMP6 protein. This protein works as an optical probe for imaging calcium transients in neurons. In this mouse model, when specific neurons are activated they become brighter. This fluorescent reporter closely follows the electrical activity of a neuron.

Dr. Chesler's team conducted studies that optically imaged the mouse trigeminal ganglia in vivo as various stimulus were applied to the mouse expressing GCaMP6. The mice were exposed to gentle stroking of the cheek's fur in a forward and reverse direction. They were also exposed to noxious mechanical stimuli and temperature variations using a copper probe. The team was able to see the activation of different neurons in real time while the stimuli were introduced.

An analysis of the neurons activated based on specific stimuli was undertaken. Results showed that some neurons primarily respond to heat stimulation while other neurons respond primarily to both heat and mechanical stimuli. Still other neurons responded almost equally to heat, gentle, and noxious stimulation.

While these are the responses of single neurons, the team is currently analyzing the simultaneous response of approximately 50 neurons in vivo. Preliminary results of these neurons showed that they can be grouped. Some were primarily mechanical nocioceptors, while others were primarily thermoreceptors, and a yet another group were polymodal nocioceptors.

These findings will allow researchers to describe neurons not only in terms of their size or morphology, but also identify subtypes of neurons based on their response (i.e. function). It is hoped that this research will contribute to a better understanding of how sensory neuron response properties can change in the context of injury, inflammation, and disease.

IMAGING GLIAL ACTIVATION IN HUMAN PAIN DISORDERS

Marco Loggia, PhD, Massachusetts General Hospital

Dr. Loggia's presentation focused on ways to image glial cells in vivo using Positron Emission Tomography (PET) and examining their role in human pain. He explained that over the past 15 years or so, hundreds of animal studies have implicated the involvement of glial cells in pain.

Studies have shown that when an animal receives a nerve lesion, microglia and astrocytes in the spinal cord and central nervous system (CNS) are able to sense the presence of such a lesion and react to it by undergoing a series of cellular and molecular changes known as "glial activation."

When microglia and astrocytes activate, they undergo a number of phenotypic changes and start to produce cytokines, such as interleukin 6 (IL-6), interleukin 1 beta (IL-1β) and other pro-inflammatory mediators that ultimately sensitize pain pathways inducing a "pain-produces-pain" loop.

Studies have shown that when glial activation is inhibited in animal models using drugs—such as minocycline—the pain is also inhibited. This suggests that glial activation is not just a reaction, but that glial cells may have an active role in the establishment and/or maintenance of pain disorders.

The translocator protein (TSPO) has been used as a marker of glial activation. It is considered a useful marker because it has a very low basal expression in the healthy CNS, but is upregulated by activated microglia and astrocytes. Also, TSPO can be imaged in vivo using PET by using the [^{11}C]PBR28 ligand.

Dr. Loggia and his team conducted a study to examine glial activation in 19 individuals with chronic low-back pain (cLBP). The study used matching pairs with healthy controls matched for sex and age. In addition, all individuals in the study were genotyped for the Ala147Thr polymorphism in the TSPO gene. This was done to ensure that the binding affinity of the ligand was similar in all members in the study, since the ligand can have different binding affinities on different individuals.

The study measured pain levels, blood levels of cytokines (IL-6, IL-1β, and TNF-α), and standardized uptake values, normalized by whole brain. The individuals also underwent PET and magnetic resonance (MR) scanning.

The resulting median images showed a significant activation of the thalamus in cLBP patients when compared with controls. Individual scans also showed significant activation of the thalamus in nearly all patients with cLBP when compared with controls. Chronic low-back patients also showed activation of the brain's paracentral lobule and the postcentral gyrus.

Dr. Loggia explained that other human studies of noxious stimuli to the leg and the lumbar region have shown activation of these two areas. Leg pain was linked to activation of the paracentral lobule while lumbar pain was associated with activation of the postcentral gyrus in such studies. This may suggest that glial activation could be somatotopically organized.

Dr. Loggia's study also showed a negative relationship between [^{11}C]PBR28 signal and both pain and regulatory cytokines. However, there are some studies that show that TSPO may also be a negative regulator of neuroinflammation. He explained that in cell studies where TSPO was overexpressed there was a decrease in inflammation while in studies where TSPO was knocked-down inflammation increased. This may show that TSPO could limit the magnitude of inflammatory responses after their initiation.

Dr. Loggia's studies showed that higher levels of TSPO resulted in decreased cytokine measurements and lower pain levels. In contrast, lower levels of TSPO resulted in higher levels of cytokines and increased pain levels. Further studies are needed to explore and define these relationships, if they indeed exist. In conclusion, his studies showed that:

- Chronic pain patients demonstrate TSPO elevation in the brain
- Human chronic pain is accompanied by glial activation
- Glial activation may represent a therapeutic target for chronic pain

DEVELOPING MODELS FOR NEW TREATMENT TECHNOLOGIES

Alex DaSilva, DDS, Dmed Sc, University of Michigan

Dr. DaSilva is the director of Headache and Orofacial Pain Effort lab at University of Michigan. The lab develops technologies to improve the precision of both pain research and treatment.

He explained that rating pain from 1 to 10 can sometimes be unreliable or subjective. To address this difficulty, his group developed an app to better determine the level and location of pain for patients with TMD.

The phone app shows a three-dimensional head where the patient can locate and mark mild, moderate, or severe pain. The patient can tap on certain areas of the head, such as the cheek, and mark it as either pink (mild), red (moderate), or dark red (severe) pain. The patient can also mark other areas of the head with different pain levels.

For example, the cheek could be marked red (severe pain), while the chin and scalp can be marked pink (mild pain). Dr. DaSilva explained that this model offers a better representation of the location, scope, and severity of pain when compared to a 1 to 10 scale.

The lab is now working on expanding the app to feature the whole body in order to address various pain conditions. This will also help those patients with overlapping pain conditions, such as migraine and fibromyalgia. This approach is useful because if a treatment is provided one can determine, for example, if it is effective only for the migraine or for the whole body.

Dr. DaSilva and his team carried out a study in patients with migraines. More specifically, they wanted to examine μ-opioid activation and allodynia during spontaneous migraine attacks. The study used PET scans to pinpoint the areas of the brain being impacted. During the attack heat was used as a pain stimulus.

Results showed that allodynia was present during the ictal phase of the migraine, which is believe to release of endogenous opioids in the brain. This may explain why the administration of opioids is not always effective to treat migraines, as these receptors are already engaged by endogenous opioids.

A similar study was undertaken with chronic TMD patients. These patients were injected with hypertonic saline to the masserteric area as a pain stimulus. When patients determined that the pain was going beyond moderate pain they could press a button and the injection of saline was automatically reduced thereby reducing the pain stimulus.

This study revealed that chronic TMD patients needed less hypertonic saline to report moderate pain when compared to controls. The study considered patient genetics and found that the catechol-O-methyltransferase (COMT) genotype had the highest sensitivity for pain as well as a higher release of endogenous opioids.

Dr. DaSilva has also carried out studies involving neuromodulation to address pain. The study examined invasive motor cortex stimulation in the treatment of chronic pain in patients with TMD. Direct current was applied in a non-invasive manner to specific targets on the head in an effort to create an analgesic effect.

The study showed that after six weeks, nine of the twelve patients experienced a VAS decrease of more than 50 percent, compared with four patients in the sham group. An interesting result is that while patients reported pain in both sides of the head, stimulation was only applied to one side of the brain. The result was a decrease in pain only in the contralateral side in terms of intensity and area, but no decrease in the ipsilateral side.

Discussion

An audience member asked if there was a relationship between the intensity and duration of pain. He also asked where the signal was seen in the spinal cord.

Dr. Loggia said that, thus far, data do not show a relationship between the two, but warned that the study was very limited. The focus on the spinal cord is on the lower thoracic and upper lumbar spine. More specifically, they are looking at L1, since this is the region receiving innervation from the roots.

An audience member asked whether TSPO—or a pathway within the TSPO signaling pathway—could represent a therapeutic target.

The panelist suggested the possibility of examining the clinical use of TSPO agonists. A review in a *Nature* publication showed that TSPO ligands have shown efficacy across a number of neurological and neuropsychiatric disorders. He advocated using them in humans with pain. He said that another possibility would be using neurosteroids.

An audience member asked if researchers had looked at pathological states with inflammation, and perhaps nerve injury, and examined any differences in the temporal and spatial dynamics. He also asked if they had looked at opioids to tease apart questions related to their inhibitory and excitatory affects that may be involved in activating different isoforms.

A panelist said that it would be helpful to develop a comprehensive dataset at the healthy state to truly understand the changes during inflammation. Pilot studies are showing an increase in spontaneous activity and changes in response profiles, but it's too early to make any final determinations.

An audience member asked Dr. Loggia if he saw any sex differences in TSPO marker of the glial activation.

Dr. Loggia replied that they did not see any differences in their study, but this is an important question that needs to be examined in the context of larger sample size.

CLOSING REMARKS AND ADJOURN

Walter J. Koroshetz, MD, Director, National Institute of Neurological Disorders and Stroke

Dr. Koroshetz reviewed some of the highlights of the two-day meeting. He said that projects focused on various molecules, such as nicotine, BDNF, and others, as well as circuits in the brain and mechanical receptors.

While various projects focused on the new science, he said that people were also important. More specifically, the leading investigators and young investigators presenting their projects.

Dr. Koroshetz said that it's the combination of projects, science, and people that will make a difference. But to get there a community also has to exist. This is why it is important to have meetings such as this one, where investigators can connect and work in teams.

He informed participants that the NIH is currently undergoing a heavy planning stage. Over the summer the Pain Policy Office, along with the IPRCC and numerous other federal partners, will be working to develop a plan for pain research.

Dr. Koroshetz thanked all participants for their attendance and participation. He also thanked Linda Porter and her team for preparing and organizing a successful meeting, the intramural program, and colleagues from other institutes such as NIDA, NCI, and NIDRC.

APPENDIX 1: AGENDA

11th Annual NIH Pain Consortium Symposium on Advances in Pain Research

Innovative Methods and Models

Tuesday, May 31, 2016

8:30 a.m. **Welcome and Opening Remarks**
Martha J. Someman, DDS, PhD, National Institute on Dental and Craniofacial Research

8:50 a.m. **Keynote Address: Challenges of Translational Pain Research: What Makes a Good Model?**
David Clark, MD, PhD, Stanford University

Panel Session: Lessons Learned in Translational Research
Yolanda Vallejo-Estrada, PhD, (Moderator), National Institute on Dental and Craniofacial Research

9:40 a.m. **Does My Mouse Have a Headache? Translational Models of Migraine**
K.C. Brennan, MD, University of Utah

10:00 a.m. **Beyond Traditional Assessments of Pain: What Can Animals Tell Us about Analgesic Efficacy?**
Andrea Hohmann, PhD, Indiana University

10:20 a.m. **Questions and Answers**

10:40 a.m. **Break and Poster Session (*Natcher Atrium*)**

11:10 a.m. **Introduction to Junior Investigator Presentations**
Patricia A. Grady, PhD, RN, FAAN, National Institute of Nursing Research

11:20 a.m. **Haploinsufficiency of the Brain-Derived Neurotrophic Factor (BDNF) Gene Causes Reduced Pain Sensitivity in an Animal Model And Humans With the WAGR Copy Number Variant**
Matt Sapio, PhD, NIH Clinical Center

11:35 a.m. **A Dual Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Inhibitor Produces Opioid Sparing Analgesic Effects in Mice**
Jenny Wilkerson, PhD, Virginia Commonwealth University

11:50 a.m. **Nicotine Deprivation Increases Pain Sensitivity, Neurogenic Inflammation, and Secondary Hyperalgesia Among Daily Tobacco Smokers**

Joseph Ditre, PhD, Syracuse University

12:05 p.m. **Lunch and Poster Session**

Panel Session: Bridging the Gap Between Models and the Clinic
Ann O'Mara, PhD, RN, FAAN, (Moderator), National Cancer Institute

1:25 p.m. **Translational Role Models of Pain: The Transgenic Sickle Mice**
Kalpna Gupta, PhD, University of Minnesota

1:45 p.m. **Pain Begets Pain: Modeling Chronic Overlapping Pain Conditions**
Richard Traub, PhD, University of Maryland

2:05 p.m. **The Painful Consequences of Stress**
Julie Christianson, PhD, University of Kansas Medical Center

2:25 p.m. **Questions and Answers**

2:45 p.m. **Break and Poster Session (*Natcher Atrium*)**

3:20 p.m. **A Patient's Perspective on Pain Research**
Christin Veasley, Chronic Pain Research Alliance

3:45 p.m. **Mitchell Max Award for Best Junior Investigator Presentation**
Wilson Compton, MD, MPE, Deputy Director, National Institute on Drug Abuse

4:00 p.m. **Adjourn**

Wednesday, June 1, 2016

8:30 a.m. **Update From the American Pain Society**
Gerg Terman, MD, PhD, President Elect, American Pain Society

8:50 a.m. **Enhancing the Reproducibility and Transparency of Research Findings**
Shai Silberberg, PhD, National Institute of Neurological Disorders and Stroke

9:20 a.m. **Keynote Address: New Technologies in Translational Pain Research**
Rob Gereau, PhD, Washington University

Panel Session: Applying New Approaches to Pain Research
Dave Thomas, PhD, (Moderator), National Center for Complementary and Integrative Health

10:10 a.m. **Visualizing Touch and Pain in The Periphery**
Alex Chesler, PhD, National Center for Complementary and Integrative Health

10:30 a.m. **Break and Poster Session (*Natcher Atrium*)**

11:00 a.m. **Imaging Glial Activation in Human Pain Disorders**
Marco Loggia, PhD, Massachusetts General Hospital

11:20 a.m. **Developing Models for New Treatment Technologies**
Alex DaSilva, DDS, Dmed Sc, University of Michigan

11:40 a.m. **Questions and Answers**

12:00 p.m. **Closing Remarks and Adjourn**
Walter J. Koroshetz, MD, Director, National Institute of Neurological Disorders and Stroke

12:15 p.m. **Adjourn**

APPENDIX 2: Meeting Participants

PAIN CONSORTIUM EXECUTIVE COMMITTEE

Walter Koroshetz, MD, (Chair), Director, NINDS
Josephine Briggs, MD, Director, NCCIH
Patricia A. Grady, PhD, RN, FAAN, Director, NINR
Martha Somerman, DDS, PhD, Director, NIDCR
Nora Volkow, MD, Director, NIDA

SPEAKERS AND MODERATORS

K.C. Brennan, MD, University of Utah
Alex Chesler, PhD, NCCIH
Julie Christianson, PhD, University of Kansas Medical Center
David Clark, MD, PhD, Stanford University
Wilson Compton, MD, MPE, NIDA
Alex DaSilva, DDS, Dmed Sc, University of Michigan
Joseph Ditre, PhD, Syracuse University
Rob Gereau, PhD, Washington University
Patricia A. Grady, PhD, RN, FAAN, NINR
Kalpna Gupta, PhD, University of Minnesota
Andrea Hohmann, PhD, Indiana University
Walter J. Koroshetz, MD, NINDS
John W. Kusiak, PhD, NIDCR
Marco Loggia, PhD, Massachusetts General Hospital
Ann O'Mara, PhD, RN, FAAN, NCI
Matt Sapio, PhD, NIH Clinical Center
Shai Silberberg, PhD, NINDS
Gerg Terman, MD, PhD, American Pain Society
Dave Thomas, PhD, NCCIH
Richard Traub, PhD, University of Maryland
Yolanda Vallejo-Estrada, PhD, NIDCR
Christin Veasley, Chronic Pain Research Alliance
Jenny Wilkerson, PhD, Virginia Commonwealth University

NIH PAIN CONSORTIUM MEMBERS

NIH Institutes

National Cancer Institute (NCI)
National Eye Institute (NEI)
National Heart, Lung, and Blood Institute (NHLBI)
National Institute on Aging (NIA)
National Institute on Alcohol Abuse and Alcoholism (NIAAA)
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

National Institute of Biomedical Imaging and Bioengineering (NIBIB)
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
National Institute on Deafness and Other Communication Disorders (NIDCD)
National Institute of Dental and Craniofacial Research (NIDCR)
National Institute of Diabetes and Digestive and Kidney Disorders (NIDDK)
National Institute on Drug Abuse (NIDA)
National Institute of General Medical Sciences (NIGMS)
National Institute of Mental Health (NIMH)
National Institute on Minority Health and Health Disparities (NIMHD)
National Institute of Neurological Disorders and Stroke (NINDS)
National Institute of Nursing Research (NINR)

NIH Centers

Fogarty International Center (FIC)
National Center for Advancing Translational Sciences (NCATS)
National Center for Complementary and Alternative Medicine (NCCAM)
NIH Clinical Center (CC)

NIH Office of the Director

Office of Behavioral and Social Sciences Research (OBSSR)
Office of Rare Diseases (ORD)
Office of Disease Prevention (ODP)
Office of Research on Women's Health (ORWH)
Office of Science Policy Analysis (OSPA)
Office of Technology Transfer (OTT)