


Special Section on The Opioid Crisis

The Opioid Crisis and the Future of Addiction and Pain Therapeutics^S

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ABSTRACT

Opioid misuse and addiction are a public health crisis resulting in debilitation, deaths, and significant social and economic impact. Curbing this crisis requires collaboration among academic, government, and industrial partners toward the development of effective nonaddictive pain medications, interventions for opioid overdose, and addiction treatments. A 2-day meeting, *The Opioid Crisis and the Future of Addiction and Pain Therapeutics: Opportunities, Tools, and Technologies Symposium*, was held at the National Institutes of Health (NIH) to address these concerns and to chart a collaborative path forward. The meeting was supported by the NIH Helping to End Addiction Long-TermSM (HEAL) Initiative, an aggressive, trans-agency effort to speed scientific solutions to stem the national opioid crisis. The event was unique in bringing together two research disciplines, addiction and pain, in order to create a forum for crosscommunication and collaboration. The output from the symposium will be considered by the HEAL Initiative; this article summarizes the scientific presentations and key takeaways. Improved understanding of the etiology of acute and chronic pain will enable the

discovery of novel targets and regulatable pain circuits for safe and effective therapeutics, as well as relevant biomarkers to ensure adequate testing in clinical trials. Applications of improved technologies including reagents, assays, model systems, and validated probe compounds will likely increase the delivery of testable hypotheses and therapeutics to enable better health outcomes for patients. The symposium goals were achieved by increasing interdisciplinary collaboration to accelerate solutions for this pressing public health challenge and provide a framework for focused efforts within the research community.

SIGNIFICANCE STATEMENT

This article summarizes key messages and discussions resulting from a 2-day symposium focused on challenges and opportunities in developing addiction- and pain-related medications. Speakers and attendees came from 40 states in the United States and 15 countries, bringing perspectives from academia, industry, government, and healthcare by researchers, clinicians, regulatory experts, and patient advocates.


Introduction

The opioid misuse and addiction public health crisis in the United States is rapidly evolving. In 2016, the opioid crisis was responsible for 42,000 overdose fatalities in the United States

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ABBREVIATIONS: CSDS, chronic social defeat stress; DRG, dorsal root ganglion; GPCR, G-protein-coupled receptor; LHA, lateral hypothalamic area; MD, molecular dynamics; MRI, magnetic resonance imaging; NAC, *N*-acetylcysteine; Na_v, voltage-gated sodium channel; NK1R, neurokinin 1 receptor; OUD, opioid use disorder; RGS, regulator of G-protein signaling; TRP, transient receptor potential.

develop anti-addiction medications, and to better understand the transition from acute to chronic pain (Volkow and Collins, 2017).

To help address these needs, the National Institutes of Health National Center for Advancing Translational Sciences, in collaboration with the National Institutes of Health National Institute on Drug Abuse and National Institute of Neurologic Disorders and Stroke, sponsored the Opioid Crisis and the Future of Addiction and Pain Therapeutics: Opportunities, Tools, and Technologies Symposium on February 7 and 8, 2019, in Bethesda, MD (agenda included as Supplemental Material). The meeting was supported by the National Institutes of Health Helping to End Addiction Long-TermSM Initiative, an aggressive, trans-agency effort to speed scientific solutions to stem the national opioid crisis. The symposium convened scientific leaders from academia, industry, healthcare, and government to highlight challenges and opportunities in the precompetitive, preclinical stage of development for addiction- and pain-related medications, and to provide a framework for more focused efforts within the research community. The speakers and attendees came from 40 states in the United States and 15 countries, and contributed their perspectives as researchers, clinicians, regulatory experts, and patient advocates.

The overarching purpose of the symposium was to join two relatively independent research disciplines, addiction and pain, in a forum to promote communication and collaboration among these fields. The 2-day agenda encompassed a wide range of lectures and discussions, including 62 poster presentations. Keynote lectures provided a high-level overview of novel targets and pathways in pain and addiction. The first session of lectures highlighted current and next-generation targets as well as lessons learned from clinical successes and failures. Lectures of the second session focused on biomarkers that can enable clinical trials. Speakers of the third session described assays to improve predictive therapeutic efficacy and misuse/addiction liability. Lectures of the fourth session focused on new discovery technologies and methodologies to screen for or rationally design therapeutics targeting pain and addiction. The symposium videocast was archived and the videos can be accessed from the first day (<https://ncats.nih.gov/pain-addiction-symposium/day1>) and second day (<https://ncats.nih.gov/pain-addiction-symposium/day2>).

High-Level Overview of Novel Targets and Pathways in Pain and Addiction

A Multipronged Approach to Capturing Novel Pain Targets. Dr. Clifford Woolf of the Boston Children's Hospital and Harvard Medical School (Boston, MA) emphasized that there has been a failure thus far to identify novel targets that could lead to effective pain treatments without involvement of the μ -opioid receptor. Currently, only a limited number of targets exist for pain management and most of these have been known for decades. Historically, analgesic drugs were discovered first, and their targets were identified much later. In addition, current pain treatments unfortunately have limited efficacy. For example, meta-analyses on pharmacological treatments for knee osteoarthritis and use of opioids for noncancer pain has found very limited or no efficacy for both (Busse et al., 2018; Gregori et al., 2018).

Today, most analgesics act by reducing nociceptor activation, decreasing inflammation, modulating excitability, increasing inhibition, or blocking synaptic transmission. To move forward, the field should recognize the complexity of pain; there are several quite distinct types driven by different mechanisms that require different therapeutic interventions, and the field should learn from past mistakes (what went wrong and why in analgesic drug development). Dr. Woolf emphasized that there is a need for multiple, independent, and unbiased approaches to target identification for pain therapeutics that include omics profiling using transcriptomic and proteomic analysis of relevant cells and circuits in healthy and diseased conditions, as well as combining patient phenotypic and genotypic approaches (Cobos et al., 2018; Khera et al., 2018). There is also a need for targeting disease modification, not just symptom control, by identifying those at risk and preventing diseases from manifesting. Given the genetic complexities of pain and high heritable contribution, large-scale studies of polygenic risk factors for chronic pain could aid in the identification and development of new targets, as they have done successfully for coronary artery disease, atrial fibrillation, type 2 diabetes, inflammatory bowel disease, and breast cancer (Khera et al., 2018).

Preclinical models that accurately capture the disease phenotype present in patients could lead to more effective treatments. There is a need for better surrogate measures of pain in animal models, such as nonreflexive models and surrogates of spontaneous pain. One methodological example that should be widely adopted is to look at disease phenotypes in neurons from human patient-derived induced pluripotent stem cells to model disease, conduct CRISPR genome-wide screens to identify targets, and run drug screens to find compounds that act selectively on diseased human neurons. A proof-of-principle study used nociceptors differentiated from a single patient's induced pluripotent stem cells to predict pain relief for an experimental treatment (Namer et al., 2019), which suggests this method could also eventually lead to a precision-medicine approach.

Transcriptional and Epigenetic Mechanisms of Drug Addiction: Guide for Drug Discovery. Dr. Eric Nestler of the Icahn School of Medicine at Mount Sinai (New York, NY) proposed a novel way to discover targets for medication development. While drugs with misuse potential initially act at the synapse, addiction requires repeated drug exposure and can, therefore, be viewed as a form of drug-induced neural plasticity mediated in part by altered gene expression. Virtually all past medication development efforts have focused on neurotransmitter receptors and transporters. This approach has left a large gap in our knowledge of postreceptor mechanisms, namely, the tens of thousands of intracellular signaling proteins that control all aspects of brain function including the abnormalities that underlie an addicted state (Walker and Nestler, 2018). An unbiased method for drug discovery with a focus on both transcriptional and epigenetic mechanisms that includes the key biochemical pathways affected most prominently by drugs with misuse potential was proposed.

Addiction risk is roughly half genetic, with hundreds of genes each contributing a small fraction of the risk. The other half of addiction risk is environmental, which reinforces the importance of epigenetic mechanisms. RNA sequencing can identify long-lasting changes in gene expression in specific

brain regions during the life course of drug self-administration in animals, including relapse, and machine learning approaches can be used to rate the degree of addiction behavior, potentially creating an addiction index. These tools can be combined to identify genes with long-lasting changes in gene expression that are associated with individual self-administration behavior (Walker et al., 2018). Going forward, Dr. Nestler proposed that research should define chromatin scars as primed and desensitized genes, which act with drug-related transcription factors through combinatorial analysis of multiple genome-wide chromatin assays. By using an unbiased approach, genes, proteins, and biochemical pathways crucial for the addiction process can guide drug discovery to previously unexplored targets (Egervari et al., 2017).

Opioid Addiction: The Gain in the Brain Is in the Pain. Dr. George Koob of the National Institute on Alcohol Abuse and Alcoholism discussed the role of negative affect in addiction. Negative emotional states cause physical and emotional pain that may be a driving force in addiction. Drug addiction represents a dysregulation of incentive salience and executive function systems, but also a dysregulation of reward-stress function mediated by the extended amygdala neurocircuitry. Individuals with addictions experience a hypersensitive negative emotional state during acute and protracted withdrawal, also known as hyperkatifeia. Hyperkatifeia is defined as the increased intensity of negative emotional/motivational signs and symptoms observed during withdrawal (Shurman et al., 2010). In opioid users, hypersensitivity to pain can persist for years after the last dose of heroin.

There is significant overlap in the engagement of brain circuits mediating negative emotional states (hyperkatifeia) and pain, which may explain the role of alcohol and opioids in deaths of despair. Dr. Koob mentioned that there seems to be a relationship between loss of reward and gain of stress. The stress system contributes to addiction. For example, an antagonist of a stress-related neurochemical, such as the corticotrophin releasing factor-1 receptor, blocks development of heroin escalation and withdrawal-induced hyperalgesia in rats (Park et al., 2015). κ -Opioid receptor antagonists also block the development of heroin escalation in an animal model (Schlosburg et al., 2013). The extended amygdala may be the interface between addiction and stress. Targeting the negative affect stage of withdrawal may offer novel targets for addiction treatment.

Next-Generation and Current Targets with Lessons Learned from Clinical Successes and Failures

Targeting the Primary Afferent Nociceptor for Analgesia: Insights from Natural Products. Dr. David Julius of the University of California, San Francisco (San Francisco, CA), focused on ion channel targets that are activated by natural products. Target-based approaches to drug discovery can still be useful with natural products (such as capsaicin, menthol, isothiocyanates, and thiosulfonates) known to elicit irritation or pain by activating nociceptors. These molecules have enabled the identification of ion channels [transient receptor potential (TRP) channels] that represent promising targets for novel analgesics (Julius, 2013; Mickle et al., 2016). Primary afferent sensory nerve fibers that project into the brain contain varying amounts of different kinds of TRP channels (TRPV1, TRPM8, and TRPA1) that sense diverse

kinds of pain such as heat, cold, and chemical. TRP channels work as polymodal signal integrators. Understanding how these channels work and respond to pain allows further understanding of how these molecules operate as molecular signaling machines. Work is ongoing to identify antagonist molecules that will modulate TRP channels and alleviate pain in inflammation and cancer-induced pain (Carnevale and Rohacs, 2016; Moran and Szallasi, 2018; Muller et al., 2019). For example, using the crystal structures of the TRP channels, researchers can visualize how an agent in drug development, such as A-967079, fits into the channel pocket. Additional work has identified the electrophilic site where agonists modify the channel to induce gating. Understanding how this allosteric signaling nexus can transduce the information from agonist binding to channel gating is a key element in understanding how to develop antagonists that block the channel under specific physiologic conditions.

Venomous toxins from various animals are also considered natural products. Recently, Hm1a/b spider toxins, excitatory agents for a subgroup of somatosensory neurons *in vivo*, revealed a role for voltage-gated sodium channel (Na_v) 1.1 in pain. Nociception results from Hm1a/b enhancing Na_v 1.1 currents by inhibiting channel activation. This led to the recognition of a role for Na_v 1.1 in peripheral pain mechanisms. Activation of pain fibers that contain Na_v 1.1 contribute to mechanical hypersensitivity. Using natural products to identify important mechanisms of pain transduction has led to several potential new targets for pain signaling; also, products including cannabinoids that modulate cannabinoid receptors, such as cannabinoid receptors 1 and 2, and the opioid receptor agonist mitragynine (from the plant kratom), are receiving attention for their potential to treat pain (Vučković et al., 2018).

Challenges and Opportunities for the Development of Na_v 1.7 Inhibitors. Dr. Bryan Moyer of Amgen presented a review of drug discovery efforts to identify inhibitors of Na_v 1.7, a voltage-gated sodium channel that is being aggressively pursued as a target for novel pain therapeutics based on learnings from human genetics. Human loss-of-function mutations in the gene encoding Na_v 1.7 (SCN9A) result in the inability to sense pain (Cox et al., 2006). Conversely, human gain-of-function mutations result in extreme pain disorders (Yang et al., 2004; Fertleman et al., 2006; Faber et al., 2012). Na_v 1.7 plays a critical role in initiating action potentials in dorsal root ganglion (DRG) nociceptor neurons. Compounds engaging different binding pockets and blocking various channel gating states are being pursued, including sulfonamides, pore blockers (tetrodotoxin and saxitoxin), peptides (from spider venoms), and local anesthetics (e.g., lidocaine).

The main challenges for Na_v 1.7 drug discovery are isoform selectivity, the need to block action potential firing in nociceptors (which generally requires drug concentrations above the IC_{50} value for Na_v 1.7), and biodistribution of large molecule therapeutics (including peptides and biologics) to axons behind the blood-nerve barrier. Examples were provided for drug discovery efforts utilizing peptides, saxitoxin, and sulfonamides. Venom screens and structure-activity relationship analyses of tarantula peptides identified potent and selective Na_v 1.7 inhibitors that were 1000 \times selective over Na_v 1.5 and Na_v 1.4 and that blocked action potential firing in both DRG neurons as well as C-fibers. Engineering of the natural product saxitoxin identified derivatives with

1000× selectivity over the other human Na_v isoforms. Sulfonamide antagonists have been optimized with 100–1000× selectivity over other human Na_v isoforms. These compounds block action potential firing in human DRG neurons and block mouse pain behavior in multiple translatable models, demonstrating target access and engagement. Ongoing work to prosecute potent and selective Na_v1.7 inhibitors in clinical trials will ultimately test the hypothesis that acute pharmacological blockade of Na_v1.7 can decrease human pain.

Translational Assays: Supporting a Small Molecule Na_v1.7 Inhibitor Drug Discovery Program. Patients with SCN9A mutations (encoding Na_v1.7) cannot feel pain but have tactile and pressure sensitivity and loss of olfaction and do not respond to histamine-induced itching. Based on this validation, several pharmaceutical companies have pursued this target as described by Dr. Moyer at Amgen. Dr. Houghton from Merck (West Point, PA) presented a very informative account of the effort in this program to develop translatable assays that could be used preclinically and clinically to demonstrate target modulation and efficacy to ensure clinical success. The current approach was compared with the historic approach used for MK-0759, a nonselective sodium channel inhibitor that failed to demonstrate efficacy in the clinic. The compound MK-0759 was effective in a rat spinal nerve ligation model of neuropathic pain and a rat complete Freund's adjuvant model of inflammatory pain. The spinal nerve ligation model was used to calculate the human dose using the minimum effective concentration. However, in the human clinical trial the compound demonstrated no efficacy at exposures that worked in the rodent spinal nerve ligation model, ending development of this compound. The compound was well tolerated.

Dr. Houghton outlined that translational assays should be used, where possible, to measure target engagement, target modulation, physiologic responses, efficacy, and safety of the compound in a dose-dependent manner. Dr. Houghton emphasized that it is important to consider these criteria during the drug discovery phase, and as more of the questions regarding these criteria are answered during the discovery process, the greater likelihood there is for success during the clinical trial phase. Dr. Houghton also pointed out that it is essential to consider the anticipated human dose early in the process to ensure that the molecule can be dosed in humans with good safety margins.

Using these principles, translatable preclinical models were developed for Merck's novel selective Na_v1.7 inhibitors. Assays were back-translated from the clinic to primate models; for example, primate assays of acute nociception to a noxious thermal stimulus (Vardigan et al., 2018) and microneurography were established. Furthermore, an isoamyl acetate-induced biomarker assay was developed to monitor olfactory bulb activity with functional magnetic resonance imaging (MRI) (Zhao et al., 2016). However, when trying to translate this assay to the clinic in an experimental medicine study it failed since the size of the olfactory bulb in man was too small, resulting in the assay having insufficient sensitivity. Zhao et al. (2016) demonstrated that Na_v1.7 inhibitors slowed and blocked C-fiber activity in the primate microneurography experiments and inhibited responses to acute noxious thermal responses. Both of these assays can be used in phase 1 trials to demonstrate target modulation and efficacy.

Complexity of Biased Agonism and the Implications for Opioid Analgesics. Dr. Laura Bohn of The Scripps Research Institute (Jupiter, FL) discussed biased agonism and the complexities of opioid analgesics. Functional selectivity (ligand-directed signaling and biased agonism) of G-protein-coupled receptor (GPCR) activation refers to the binding of a compound to a receptor directly affecting receptor conformation. The receptor conformation will influence interactions with a complement of intracellular signaling proteins such as G-proteins, cAMP, and β -arrestins, to turn on specific pathways of cellular and physiologic responses. The intracellular protein complement differs between cell types as well as in tissues, which may have a differential impact on how analgesia and side effects are mediated. In this context, Dr. Bohn pointed out that the receptors are considered as microcircuit integrators rather than on/off switches, and cell-based assays may be indicators of toggles of receptor states. This means that ligands can also be designed to prefer one path over another and can be tested in vivo. Genetic models can be used to identify key players involved in drug actions. Hence, the contexts of in vitro and in vivo assays are critical components in identifying compounds with safe and effective analgesic characteristics.

β -Arrestins play diverse roles in regulating receptors. Genetic knockout of β -arrestin2 led to a decrease in morphine-induced respiratory effects in mice. Studies were presented of a μ -opioid agonist series (Schmid et al., 2017; Kennedy et al., 2018) with favorable bias factors for guanosine 5'-O-[γ -thio]triphosphate and cAMP over β -arrestin2 showing that in vivo widening of the therapeutic window may be possible. These compounds were shown to provide antinociception with limited respiratory suppression. However, it was emphasized that introducing bias may not prevent the induction of side effects and that very potent agonists, although biased, may still induce enough signaling at other pathways to produce adverse events. The information from these assays can help in the development of cellular models that can predict dosing and safety profiling.

Mathematical modeling may be required to analyze the differential effects of biased agonists to a reference agonist in biologic systems. In this context, Dr. Bohn also presented several examples of measuring bias factors for [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin, morphine, and buprenorphine, and showed they may be assay dependent (Stahl et al., 2015). In addition, Dr. Bohn presented interesting data on the effect of triazole 1.1 on κ -opioid receptors that maintain efficacy in the chloroquine phosphate mouse itch model without eliciting sedation, decreasing dopamine in mice, or showing signs of dysphoria in rats (Brust et al., 2016). Dr. Bohn suggested that measurement of biased agonism in opioid receptors to specifically minimize β -arrestin signaling would be of significant value in improving the therapeutic index and minimizing certain side effects generally induced by μ -opioid receptor agonists.

Intracellular Targets for the Treatment of Chronic Pain. Dr. Venetia Zachariou of the Icahn School of Medicine focused on intracellular targets for pain therapy. Dr. Zachariou's research takes three main directions, including 1) optimizing the actions of opioids used for the treatment of severe pain conditions, 2) understanding the mechanisms modulating the onset of action and efficacy of antidepressant medications used for the treatment of pain, and 3) uncovering new targets

for the treatment of neuropathic pain, including epigenetic modifiers and G-protein modulators.

Dr. Zachariou discussed regulator of G-protein signaling (RGS) proteins that are of interest to optimize the action of opioid analgesics. RGS proteins are multifunctional regulators of G-coupled protein receptor signaling; they vary in structure and cellular distribution, are differentially expressed throughout the brain, and also differ in preference for receptor subtypes. Several RGS proteins, including RGS9-2 and RGS4, modulate opioid actions in an agonist-dependent manner (Han et al., 2010; Psifogeorgou et al., 2011; Gaspari et al., 2017). Recently, Dr. Zachariou's laboratory discovered that downregulation of the RGS protein RGSz1 promotes μ -opioid receptor signal transduction in the periaqueductal gray, enhancing the analgesic effect of clinically used opioids while reducing their rewarding efficacy. Notably, inhibition of RGSz1 delayed the development of morphine tolerance without affecting physical dependence (Gaspari et al., 2018). RGS proteins may also be targeted to accelerate the onset of antidepressant drug actions in models of neuropathic pain. Specifically, Dr. Zachariou's laboratory found a prominent role of striatal-enriched RGS9-2 on the onset of action and anti-allodynic efficacy of desipramine and other monoamine-targeting antidepressants (Mitsi et al., 2015). RGS9-2 controls the nuclear shuttling of histone deacetylase-5, which represses a number of genes necessary for antidepressant-mediated plasticity. Dr. Zachariou also presented data from RNA sequencing studies (Descalzi et al., 2017) on gene expression adaptations in models of peripheral neuropathy, which helped identify novel intracellular targets for the treatment of pain (histone deacetylase-5, histone deacetylase-6, MEF2c, and RGS4).

Intersection between Pain and Addiction: Implications for κ -Opioid Receptors. The role of κ -opioid receptors in chronic pain was addressed by Dr. Catherine Cahill of the University of California, Los Angeles (Los Angeles, CA). Patients with co-occurring psychiatric illnesses and chronic pain often have heightened pain sensitivity, increased pain-related disability, and increased risk of opioid misuse. κ -Opioid receptor antagonists may be an effective therapeutic strategy to alleviate the affective dimension of chronic pain. In humans, κ -opioid receptor agonists promote depression, anxiety, discomfort, agitation, and dysphoria. κ -Opioid receptor agonists enhanced place aversion in neuropathic pain models, but only in male animals; κ -opioid receptor mRNA and receptor activation of 5'-O-[γ -thio]triphosphate were increased in the nucleus accumbens of the male, but not female, pain group (nerve injury method).

Opioids stimulate release of dopamine in the striatum, which contributes to both the motivational and rewarding effects of opioids. New evidence suggests that opioid-evoked release of dopamine is also important in the modulation of pain transmission. In chronic pain states, opioids fail to evoke dopamine release, suggesting that ongoing pain causes dysregulation of mesolimbic dopaminergic circuitry, which may contribute to chronic pain. Administration of κ -opioid receptor antagonists can restore this dopamine release. κ -Opioid receptor antagonists do not alter sensory pain thresholds. A series of preclinical experiments demonstrated that the κ -opioid receptor contributes to the aversive components of pain, and this is driven by κ -opioid receptor-driven reduction in dopamine tone where the effect is blunted by deletion of the

κ -opioid receptor from dopamine neurons. Future studies will investigate whether κ -opioid receptor antagonists can reduce pain intensity or pain-related disability in patients with chronic pain, and thus reduce the risk for opioid misuse.

Dr. Cahill mentioned that future work will determine whether sex differences in pain processing are hormonal or genetic. Preliminary data suggest that it is genetic. The take home message is that negative effects need to be treated differently in males and females.

Developing New Opioid Addiction Therapeutics Based on Habenular Modulation. Continuing with the discussions on the theme of new evidence in the opioid and receptor targets, Dr. Paul Kenny of the Icahn School of Medicine discussed the contributions of the habenular region in addiction. The habenula is a key hub that connects fore-brain and midbrain actuation centers, and may be responsible for aversion responses to drugs that can be misused. This brain region has a high concentration of nicotinic and opioid receptors (Gardon et al., 2014). The highest concentrations of μ -opioid receptors in the brain are in the medial habenula (Gardon et al., 2014). There are several preclinical studies supporting a possible role of this brain region in opioid addiction. For example, in animals chronically exposed to heroin, there is decreased activation of the medial habenula (Martin et al., 1997).

Gpr151 is an orphan GPCR expressed almost exclusively in the habenula, particularly in cells that also express μ -opioid receptors (Broms et al., 2015; Wagner et al., 2016). Gpr151 may inhibit activity of the habenula/interpeduncular nucleus circuit and regulate the actions of opioids on this circuit. Gpr151 knockout mice are resistant to addiction-relevant actions of opioids. High-throughput screening identified novel small molecule modulators of GPR151. A compound has been developed and testing is underway to investigate its effects on the reinforcing and analgesic effects of opioids.

Blockade of orexin-1 receptors decreases consumption of nicotine (and other drugs that can be misused) in rodents and primates (Kenny, 2011). A molecule suitable for drug discovery was developed (AZD4041) to mimic this effect. AZD4041 also decreases oxycodone consumption and drug-seeking behavior in rats.

Orexin is known to be coreleased with the opioid neuropeptide dynorphin, which acts at κ -opioid receptors. κ -Opioid receptors play an important role in regulating aversive behavioral states. Considering that the habenula is also thought to regulate aversive behavioral states, this raises the possibility that dynorphin acting at κ -opioid receptors could modulate drug intake by acting on habenula neurons to control aversion. However, Dr. Kenny indicated that there is limited information on the role of κ -opioid receptors in the habenula circuit in the context of drug addiction, but it seems likely considering their roles in aversion.

Biomarkers to Enable Clinical Trials

Opioids and the Brain: Lessons from Brain Imaging. Dr. David Borsook of the Boston Children's Hospital emphasized that functional imaging can be used as a continuum in drug development from preclinical to clinical as a language of translation. While human and rat brains are quite different, older circuits of pain can be used (Becerra et al., 2013). For example, in a migraine rat model, neuroimaging showed activation in very similar brain regions between rats and

humans. Imaging could also help to improve preclinical models by looking at basic circuits in key animal models to see if there are parallels to the human condition. Furthermore, functional imaging can be used across all phases (I–III) of drug development.

In translational imaging, Dr. Borsook highlighted an example of the use of functional MRI and pharmacologic MRI in spontaneous and evoked pain in animals in preclinical studies to define the pain phenotype in humans. This would be of great value in brain circuit targeting of drugs in development, in addition to being a useful tool for establishing dosing and pharmacokinetic/pharmacodynamic data interrogation (Upadhyay et al., 2012; Borsook et al., 2013).

Imaging in phase I clinical trials can enhance information obtained in early phase trials by looking at dosing, functional measures (reward/aversion), effect size, potential side effects, and pharmacokinetic/pharmacodynamic correlates. For example, a finding of increased activity in the habenula led to research looking for functional connectivity and circuits. Imaging in phase II clinical trials may provide a number of advantages including evaluation of sex differences related to drug (Gear et al., 2013) or disease state, responder versus nonresponder, and early subclinical readouts of brain regions implicated in long-term changes. Smaller cohorts may also be used to evaluate novel analgesics in N-of-1 trials. For example, patients that have activation in certain regions or pathways may be more responsive to specific agents that act on that pathway. New approaches to measure ongoing pain and analgesic efficacy are needed. While brain changes in response to acute painful stimuli have been reported (Wager et al., 2013), clinical trials may not be long enough to observe adaptive brain changes. Few trials extend beyond 6–8 weeks; many drugs, if tested longer, may show diminished efficacy or increased side effects, opioids being a good example. Notwithstanding costs and perhaps exposure, for analgesics, a minimum of 3 months would seem reasonable. Imaging may facilitate new measurement approaches by observing ongoing pain and the efficacy of analgesics over longer time periods.

Digital Biomarkers for Chronic Pain: A Needed New Horizon. Briefly, digital therapeutic options were described by Dr. Robert Conley from Eli Lilly & Company. Digitally enabled research and care may enable progress in developing effective chronic pain therapy. By accessing consented data derived from smartphones, connected sensors, wearables, and voice input there is the potential to measure and understand a patient's health. Ultimately, the goal is to develop validated biomarkers from these kinds of data. Pain-related neuroscience areas where digital health could be relevant include migraine prevention, acute migraine treatment, osteoarthritis pain, and chronic low back pain. For example, in a chronic low back pain study, step counting was used to measure movement after treatment. Treated patients increased their movement 5-fold after the treatment, but the patients were unaware that they had such dramatically increased daily movements (Tomkins-Lane et al., 2019). Digital health is a complex ecosystem made of many parts, but can form an interconnected system of care that improves outcomes and value for patients and payors.

There are a variety of devices that can be used outside of the doctor's office to monitor health. The data from multiple sources (patient, provider, and payor) can be combined to uncover new insights. In the field of diabetes, a method to

predict brittle patients with diabetes (patients that cannot regulate their insulin levels very well) was developed using data analysis. This ability provides a way to improve diagnosis and better monitor patients (Fagherazzi and Ravaud, 2018). In the pain field, this kind of data analysis has predicted a way to detect patients that have become oversensitized to pain treatments and potentially less acutely responsive to therapy. This is being tested in studies of patients with migraine and cluster headache disorder as well as chronic low back pain.

Machine learning algorithms delivered via devices can be used in certain situations to provide treatment. In the regulatory landscape, platform issues (iOS vs. Android; compatibility) and data objectivity (detection and reliability) are the next important considerations. While there are very few validated digital biomarkers, there is great potential to improve treatment of individual patients (identification, therapeutic engagement, and measured response to therapy), passively collect data (daily behaviors and determining norms), and determine response to therapy (Lee et al., 2019).

Genomic Biomarker Development in Opiate Addiction. Dr. Pierre-Eric Lutz of the Centre National de la Recherche Scientifique discussed the genomic, transcriptomic, and epigenomic biomarkers in addiction and opioid use disorder (OUD). The Psychiatric Genomics Consortium has an ongoing study to perform genome-wide association studies in over 100,000 addicted patients to identify genetic risk scores in order to develop clinically relevant biomarkers. To overcome classic limitations in psychiatric genomics (high interpatient phenotypic variability and small effects of individual common variants), the findings from genome-wide association studies are also being increasingly correlated with quantitative addiction-related endophenotypes. For example, during both methadone maintenance and surgical anesthesia, opiate dosage associates with single nucleotide polymorphisms located 300 kilobases upstream of the μ -opioid receptor, a primary mediator of opiate effects in the brain (Smith et al., 2017). Randomized controlled trials will be necessary to document the predictive validity of these genetic biomarkers for OUD, and to enable their clinical use. Dr. Lutz also emphasized the development and use of transcriptomic and epigenomic measures that can be obtained in peripheral samples as a strategy to identify biomarkers of OUD (Belzeaux et al., 2018).

Behavioral epigenetics can be used to understand the interplay between life experience and brain function (Lutz et al., 2017a). Dr. Lutz discussed the severe effects of early life adversity (such as child abuse) on gene expression, histone modifications, and DNA methylation, and how these affect the lifetime risk for substance misuse and OUD (Lutz et al., 2017b, 2018). There is an ongoing project at supervised consumption sites (also referred to as overdose prevention sites) in France to study potential biomarkers for OUD. The study will take a multidisciplinary approach to study social and psychiatric risk factors, life trajectories, and opiate consumption profiles, in combination with peripheral samples (blood, hair, and saliva) to investigate opiate metabolism, gene expression, and epigenetic mechanisms. Compared with previous studies on patients stabilized under maintenance therapy, the goal of the project is the genome-wide identification of peripheral biomarkers in patients that are in the active phase of opiate addiction.

Assays to Improve Predictive Therapeutic Efficacy and Misuse/Addiction Liability

What's Wrong with Animal Models of Pain? Earlier sessions clearly demonstrated the lack of translatability of preclinical *in vitro* and *in vivo* data to the clinic in both pain and addiction. Dr. Jeffrey Mogil of McGill University (Montreal, Canada) addressed this issue and pointed out that there is a failure in translation from preclinical models to humans in a variety of fields, not just pain research. Basic scientists have traditionally ignored epidemiologic reality (particularly in pain). There are three facets to consider in preclinical models of pain; subjects, assays, and outcome measures. When choosing animal models, there are often mistakes made in the choice of sex, genetics, and age or duration of injury. As of 2015, 79% of all preclinical studies published in the journal *Pain* used only male rats and mice (Mogil, 2016). The choices matter greatly, because biologic mechanisms underlying pain can differ qualitatively in these different subject populations. For example, many studies suggest that microglia play an important role in pain signaling in the spinal cord. However, this does not hold true in females (Sorge et al., 2015). As another example, calcitonin gene-related peptide mediates thermal pain in some mouse strains, but not others (Mogil et al., 2005).

Environmental laboratory factors also play a large role in animal behavior and increase variability in both inbred and outbred mouse strains. There is a common belief that inbred mice have less variance, and are thus preferred by investigators studying specific research outcomes. However, a meta-analysis of the biomedical literature by Tuttle et al. (2018) of studies where inbred and outbred strains were tested contemporaneously found no difference in variance between inbred and outbred mice in any trait class. Outbred mouse strains are more resilient to environmental factors because of their genetic diversity and should be used in most behavioral experiments unless the hypothesis requires the use of a specific mouse strain.

Emerging evidence suggests that typical pain studies, even of chronic pain, may be too short in duration to detect the relevant underlying pathophysiology. There are almost no preclinical studies in pain lasting longer than 3 months. The spinal nerve injury model is a particularly long-duration chronic pain model. Using this model, Mogil et al. (2005) found that *Terc* null mutant ($-/-$) mouse, with shortened telomeres, are more sensitive to pain, and when given spared nerve injury have even further shortened life spans. One proposed mechanism was cellular senescence in the spinal cord observed starting at 4 months postinjury, but not in younger mice.

During the discussion, Dr. Mogil stated that the mouse grimace scale (Langford et al., 2010) is useful for the measurement of postoperative pain in animals, but is only reliably detectable for around 24 hours after the injury. Other useful preclinical assays may include wheel running and conditioned place preference. The field needs a measure of spontaneous chronic pain that is user-friendly.

During the discussion, Dr. Mogil agreed that by waiting 6 months postinjury, the mice would be considered middle aged. This would also correspond to the clinical literature suggesting that chronic pain peaks in middle age. On the other hand, the biologic and physiologic events involved in pain happen at the same speed in rodents and humans, despite the differing life spans.

Iterating between Neurobiology and Clinical Trials to Identify Relevant Behavioral Phenotypes for Clinical Translation and Target Discovery. To create better animal models of addiction, we need to identify translatable symptoms of addiction. Most research on addiction has focused on either the pre- or postsynapse, according to Dr. Peter Kalivas of the Medical University of South Carolina (Charleston, SC). Another way of thinking about the synapse is the tetrapartite synapse consisting of the pre- and postsynapse, the presynaptic astroglial process, and the extracellular matrix (Mulholland et al., 2016). A glutamate imbalance may contribute to the pathogenesis of addiction. Methods were developed to study the structure of astroglia with confocal microscopy to allow for faster data analysis. During withdrawal from heroin, glial contact with synapses retracts; during relapse subpopulations of glia make contact again, and this change disappears a few hours later.

Another active area of preclinical and clinical research suggests that *N*-acetylcysteine (NAC) normalizes extracellular glutamate by restoring the activity of the glutamate transporter. A clinical trial with NAC showed a decrease in post-traumatic stress disorder symptoms and drug craving (Back et al., 2016). NAC was also able to alleviate intrusive thoughts (cravings) and reduce rumination in depression. NAC administration does not appear to be effective in active drug users, but it may be useful in facilitating and maintaining abstinence. Accordingly, it was suggested that it might be useful for relapse prevention in combination with suboxone or methadone by reducing intrusive thoughts (cravings). Hence, the role of astroglia at the synapses, glutamate homeostasis, and the emerging evidence of the role of NAC in addiction should be studied in both preclinical and clinical settings.

During the discussion, it was mentioned that glutamate imbalance in the brain and the involvement of tetrapartite synapatic interaction with microglia is an active area of research in addiction. The role of NAC in restoring glutamate homeostasis and reversing addiction is also under investigation.

Sleep-Related Endpoints in Preclinical Studies of Pain and Opioid Withdrawal. Anecdotal reports from clinicians indicate that pain and opioid withdrawal disrupt sleep. However, there is very little information in the scientific literature on the effects of pain and opioid withdrawal on sleep. Dr. Bill Carlezon, of Harvard Medical School (McLean Hospital, Belmont, MA), discussed the possibility that sleep disturbances and the stress related to sleep alterations may serve as drivers for opioid addiction, and act to promote drug-seeking behaviors. If true, then reducing sleep disruption in patients with addiction issues may aid in mitigating the opioid crisis.

Vigilance states—wakefulness, slow-wave sleep, and paradoxical sleep (also known as rapid eye movement sleep)—can be monitored in humans and rodents (Baker et al., 2018). Wireless telemetry devices implanted in mice can provide continuous data, including electromyography and electroencephalogram measurements, which are supplemented by simultaneous video recording. Data are analyzed with digital algorithms. Chronic social defeat stress (CSDS) was used to study the effects of stress on vigilance states (Wells et al., 2017). CSDS increased the time spent in paradoxical sleep, but this returned to baseline after the stressor was removed. However, CSDS increases the number of paradoxical sleep

bouts—the number of times the animals go in and out of paradoxical sleep, which does not recover upon termination of the stress. Dr. Carlezon also described a preclinical pain model (acid-stimulated stretching) in which a painful stimulus dramatically increases the latency to slow-wave sleep. Similarly, precipitated opiate withdrawal increases the latency to slow-wave sleep.

Dr. Carlezon also described the designer receptor exclusively activated by the designer drugs–based chemogenomic system that can regulate the activity of cells expressing D1 receptors in the nucleus accumbens. Chemogenomic inhibition of D1 receptor–expressing cells in the nucleus accumbens can mimic CSDS effects on paradoxical sleep, suggesting a mechanism by which specific sleep stages could be controlled for research or therapeutics.

The next step will be to address major empirical gaps in the understanding of the relationships between pain/addiction and sleep quality, and whether there are sex differences in these relationships, and to perform proof-of-concept studies with the National Institute on Drug Abuse's 10 most wanted list, including orexin-1/2 and κ -opioid receptor antagonists. Following proof-of-concept studies, these agents will also be tested in other pain models (such as spinal ligation to model chronic pain) and drug withdrawal models (spontaneous opioid withdrawal). Use of sleep measures may offer opportunities to better align neuroscience and psychiatry, since data points passively collected on cell phones and wearable devices in human subjects are becoming more easily accessible, enabling cross-species hypothesis testing. There may be a biologic link between sleep deprivation and opioid withdrawal symptoms—driven by increases in brain orexin levels—that should be investigated in the context of opioid addiction.

Scaling Up: Zebrafish Assays of Pain and Addiction.

Dr. Randy Peterson of the University of Utah (Salt Lake City, UT) presented a discussion on addiction behaviors in zebrafish for preclinical discovery. Key brain regions in mammals and teleosts are highly conserved and zebrafish exhibit a rich repertoire of behaviors such as sleep, learning, aggression, and addiction-like behaviors similar to those observed in mammals. The genes, cells, and circuits that enable these behaviors are very similar to those involved in human central nervous system disorders.

Because 1000 zebrafish can be housed in a standard 96-well plate, zebrafish can be used in *in vivo* high-throughput screening experiments. Zebrafish screens have been used successfully to discover new therapeutic molecules for a variety of diseases. However, there are limits on genetic and physiologic conservation and differences in drug metabolism, and 20% of compounds discovered in zebrafish have failed to produce similar results in rodents.

Rudimentary high-throughput assays of pain have been developed. A zebrafish hot plate assay, similar to the assay in rodents, demonstrated that morphine blocks and naloxone restores the pain response in zebrafish. Motion tracking software can be used to analyze behavioral responses. Light-dependent ligands (e.g., optovin) for sensory neurons can be used to transiently activate channels with light. Optovin binds to the TRPA1 channel but does not gate the channel. A proof-of-concept trial demonstrated that light could activate the channel and zebrafish demonstrated pain response behavior.

Opioid receptors and associated peptides have orthologs in zebrafish, suggesting that zebrafish could potentially serve as

preclinical models of opioid use disorder. A self-administration arena was designed for zebrafish. The arena contains two platforms, one active platform that releases opioids (hydrocodone) when the fish swim on it and another inactive platform. The arena is monitored with cameras to record behavior. A pilot study trained adult zebrafish ($N = 15$) for 50 minutes/day for 5 days. Animals quickly learned to spend time on the active platform during training. Conditioned zebrafish were tested in the progressive ratio test, which requires animals to perform an increasing number of operant behaviors to trigger a drug dose. Results showed that zebrafish would increase the operant behaviors to maintain the same opioid dose. Zebrafish exhibit attributes of human opioid addiction including willingness to engage in risky behaviors to seek a dose, exhibiting signs of withdrawal, and prevention of drug-seeking behaviors with naloxone. This zebrafish model is currently being used to screen compounds that block opioid-seeking behaviors. Finasteride, a medication that alters neurosteroid metabolism, reduced opioid seeking in zebrafish. Current work is systematically testing these neurosteroids to determine which ones might alter opioid-seeking behavior in rats and warrant continued mechanistic studies.

New Technologies and Methodologies to Screen or Rationally Design Therapeutics Targeting Pain and Addiction-Related Proteins and Pathways

Molecular Simulation for the Design of Finely Tuned Drugs. Dr. Ron Dror of Stanford University (Stanford, CA) highlighted that crystal structures provide a static snapshot, which can be misleading when trying to understand the behavior of highly dynamic receptors. Molecular dynamics (MD) simulation can function as a computational microscope, providing a way to probe conformational flexibility and dynamics (Hollingsworth and Dror, 2018). This type of information is critical to understanding how proteins function and how drugs interact with their targets.

Allosteric modulators, which bind in noncanonical binding sites, are highly promising as drugs for GPCR targets. MD simulations have captured the process by which modulators spontaneously associate with GPCRs, revealing exactly how these modulators bind (Dror et al., 2013). These MD results were supported experimentally by radioligand binding measurements with a series of receptors containing point mutations intended to increase or decrease modulator affinity. Additionally, MD simulations have identified mechanisms that contribute to positive and negative allosteric modulation of classic ligand binding. Together, this information enabled the successful rational design of an allosteric modulator with desired properties.

Recent structural data have suggested that related receptors often have very similar allosteric binding pockets. For example, the allosteric modulator BQZ12 binds to the M1 muscarinic acetylcholine receptor, but hardly binds to M2, M3, M4, or M5. Such selectivity for M1 is highly desirable for treating cognitive disorders such as schizophrenia and Alzheimer's disease. Mutagenesis studies showed that BQZ12 binds in M1's extracellular vestibule. However, the extracellular vestibules of M1, M2, M3, and M4 are very similar. MD simulations revealed a spontaneous opening of a cryptic pocket in the M1 receptor that cannot be seen in the crystal structures. This cryptic pocket, which does not open in M2,

M3, or M4, allows BQZ12 and certain other ligands to bind very selectively to M1.

Certain ligands that bind GPCRs selectively stimulate certain signaling pathways without stimulating other signaling pathways controlled by the same GPCRs. G-protein–biased ligands are promising at the μ -opioid receptor since they could deliver pain relief with fewer side effects associated with existing opioids, such as respiratory depression. MD simulations have demonstrated how GPCRs trigger G-protein signaling (Dror et al., 2015) and arrestin signaling (Latorraca et al., 2018). Work is ongoing to determine whether mechanistic studies of biased signaling at the atomic level will enable the rational design of drugs with fewer side effects, and preliminary results are promising. In a recent study, for example, simulations identified ligand–receptor interactions associated with arrestin-biased signaling at the D2 dopamine receptor and enabled the design of new ligands with desired biased signaling profiles (McCorvy et al., 2018).

Endosomal Platforms for the Signaling Train to Pain. GPCRs mediate pain and neurogenic inflammation. GPCRs are dynamic signaling proteins that change conformation and subcellular localization when activated. Dr. Nigel Bunnett of Columbia University (New York, NY) explained that upon activation, many GPCRs translocate from the plasma receptor to endosomes. Endosomes play a role in signal transduction, but through different mechanisms than those that occur at the plasma membrane. Biophysical approaches were used to study the link between endosomal trafficking and signaling in subcellular compartments of the substance P neurokinin 1 receptor (NK1R) (Jensen et al., 2017). Bioluminescence resonance energy transfer–based biosensors were used to examine the proximity between NK1R and proteins resident to the plasma membrane and endosomes. Fluorescence resonance energy transfer–based biosensors were used to examine compartmentalized signaling. Results suggested that substance P activation of NK1R resulted in endocytosis. Inhibitors of clathrin and dynamin prevented endocytosis. Endocytosis of NK1R mediates activation of nuclear extracellular signal–regulated kinase. Dynamin and clathrin inhibitors prevented sustained substance P–induced excitation of neurons in spinal cord slices *in vitro* and attenuated nociception *in vivo*.

Since the NK1R in endosomes generates signals that control pain transmission, the NK1R in endosomes—rather than at the plasma membrane—may be a key target for the treatment of pain. Several approaches can be used to target the NK1R in endosomes. One approach is to develop small molecule antagonists that preferentially interact with receptors in acidified endosomes. Another approach is to encapsulate small molecule antagonists into nanoparticles that disassemble in the acidified endosomal environment. A third approach is to conjugate antagonists to transmembrane lipids that deliver drugs to endosomes. *In vivo* experiments demonstrated that these approaches amplify and prolong the antinociceptive actions of NK1R antagonists. Targeting endosomal GPCRs is a new frontier in drug delivery and a novel therapeutic strategy with broad implications. Previous failures with GPCR antagonists may reflect the inability to target internalized receptors during chronic disease.

Optogenetic Assays of Sensory Neuron Function to Accelerate Discovery of Pain Therapeutics. Dr. Kit Werley of Q-State Biosciences, Inc. (Cambridge, MA), explained

how the Optopatch enables all-optical electrophysiology (Hochbaum et al., 2014) with a pair of engineered proteins: action potentials are stimulated with blue light using the light-gated ion channel CheRiff, and they are recorded with far-red light using the voltage-sensitive fluorescent protein, QuasAr. Pairing Optopatch and the custom ultrawidefield Firefly microscope (Werley et al., 2017b), the platform can simultaneously record from ~100 cells with 2.7- μ m spatial resolution and 1-millisecond temporal resolution, maintaining the richness of manual patch-clamp recording with 10,000 \times higher throughput. With environmental controls, a pipetting robot, and fully automated 96-well plate scanning, the platform can record from 1000 wells per day with 300 neurons per well to enable phenotypic drug screening or therapeutic characterization. The Optopatch platform is compatible with diverse types of primary (Werley et al., 2017a; Nguyen et al., 2019) and human-induced pluripotent stem cell–derived neurons (Williams et al., 2019).

The Optopatch system aims to use cell-based models to bridge the gap in drug discovery between target-based high-throughput screens and *in vivo* models. Dorsal root ganglion sensory neurons are used to develop a pain-in-a-dish model for osteoarthritis pain. DRG neurons are treated with a sensitizing pain reagent composition, a cocktail of inflammatory molecules found at elevated levels in the joints of arthritic patients; receptors for each sensitizing pain reagent composition ingredient are expressed in DRG neurons and the ingredients induce pain when injected in animals. The DRG neurons become hyperexcitable when treated with sensitizing pain reagent composition—their firing rate is increased by more than 3-fold and action potentials are elicited in response to a gentler stimulus. The hyperexcitability phenotype is partially reversed by several analgesic drugs, and tool compounds with diverse targets have sensible effects on neuronal electrophysiology. Future work will identify DRG neuronal subtypes and the pharmacological responses of these subtypes, and run a phenotypic screen on a diverse, 14,000-compound library.

Resolving Brain Reward Circuits for Addiction. Dopamine release from ventral tegmental area neurons is universally required for motivated behaviors. The lateral hypothalamus interfaces with the ventral tegmental area and indirectly controls the activity of dopamine neurons (Nieh et al., 2016). The lateral hypothalamic area (LHA) is known for control of feeding and reward behavior (Olds and Milner, 1954). Dr. Garret Stuber of the University of Washington (Seattle, WA) explained how optogenetic targeting of neural circuit elements has recently been used to activate or inactivate specific cell types in the brain in preclinical models to study LHA circuit function. Photostimulation of GABAergic neurons in the LHA increases feeding behavior, enhances motivation for food, and is inherently reinforcing (Jennings et al., 2015). Genetic ablation of LHA GABAergic neurons results in decreased reward-seeking behavior. Mini-epifluorescence microscopes were used to record cellular activity while an animal was performing reward-seeking behaviors. Subpopulations of neurons were found that responded to nose pokes or consumption, but rarely to both. Optogenetic inhibition of lateral hypothalamic/lateral habenula glutamatergic fibers produced a place preference, whereas optogenetic stimulation of the same fibers had the opposite effect (Stamatakis et al., 2016).

Dr. Stuber explained that using genetic profiling in subpopulations of cells allows the study of specific cell types. Early work has demonstrated the ability to identify certain genes that are associated with a specific cell type. Going forward, the plan is to leverage single-cell transcriptional profiling data to target more specific cell types in order to study their role in addiction-associated behaviors.

Modern Approaches for Dissecting Neuromodulatory Circuits in Behavior. GPCRs make up the largest gene family in the mammalian brain, but are poorly understood in the brain. Dr. Michael Bruchas of the University of Washington presented several new methods (both biologic and hardware related) to control and measure neural circuit activity in freely moving animals. To study the activation of opioid receptor circuits and signaling in awake animals, optogenetically sensitive GPCRs were developed. For example, Siuda et al. (2015) developed optically sensitive μ -opioid-like receptor systems for further study. These receptors allow spatiotemporal control of opioid signaling in vitro and in vivo, and match the canonical signaling profile of native opioid receptors. Additionally, ultrathin layered devices with light sources, detectors, and sensors capable of being inserted into precise locations of the deep brain were developed (Kim et al., 2013; Shin et al., 2017).

This wireless optogenetic device can also be combined with a microfluidic delivery system that allows remote drug delivery in awake freely moving animals (Qazi et al., 2019). The optogenetic approaches have been combined with pharmacology, as well as multianimal and multidrug on-demand control. One experiment demonstrated this, expressing Chr2 in vGAT+ neurons of the bed nucleus of the stria terminalis and placing the wireless optofluidic stimulation device in the lateral hypothalamus. Inhibition of GABA receptors (gabazine) in a conditioned place preference assay was able to block the ontogenetically evoked behavior. Wireless photometry is also in development for use with this system as well (Lu et al., 2018). The device will allow monitoring of fluorescent activity dynamics for proteins like GCaMPs and jRGECOs in deep-brain structures in freely moving animals. These devices are undergoing further refinement to develop better sensors and minimally invasive hardware to bridge molecular- and system-level questions.

Opioids and the Brain Connectome: at the Crossroads of Mechanistic and Biomarker Research. Dr. Brigitte Kieffer of McGill University explained how the three opioid receptors (μ , κ , and δ) contribute to all facets of addiction and play distinct roles in hedonic homeostasis and emotional control (Darcq and Kieffer, 2018). The μ -opioid receptor is involved in drug reward, the κ -opioid receptor contributes to the dysphoric state of drug misuse and addiction, and the δ receptor can improve mood. The nociceptin opioid receptor, a member of the opioid receptor gene family, also regulates mood.

The μ -opioid receptors are the target for both clinical pain treatment and opioid misuse. The μ -opioid receptor facilitates drug-seeking behavior in reward circuits. A well-established mechanism is that the μ -opioid receptor in GABAergic interneurons of the ventral tegmental area disinhibits dopamine release, which in turn contributes to opioid reward. Importantly, there are also μ -opioid receptors densely expressed in several other brain regions. Receptors in the striatum have been shown to be critical for motivation. μ -Opioid receptors also play a role in aversion-related

behaviors and are expressed in areas of the brain associated with these behaviors such as the bed nucleus of the stria terminalis, amygdala, and medial habenula. Recent data suggest that in the medial habenula, μ -opioid receptors reduce aversive behaviors. A mouse model of protracted opioid abstinence was developed, which lead to depression-like behaviors and social withdrawal. Dr. Kieffer's group showed that μ -opioid receptor knockout in the dorsal raphe nucleus before opioid exposure resulted in normal physical withdrawal, but these mice did not develop social withdrawal, indicating that overstimulation of the receptor in this brain region contributes to social interaction deficits of protracted opioid abstinence.

Noninvasive and translation neuroimaging was performed with functional MRI (resting state and fiber tracking) in mice. Targeted deletion of the μ -opioid receptor gene (*Oprm1*) induced widespread remodeling of the brain functional connectome in mice (Mechling et al., 2016). The greatest amount of change occurred in brain areas associated with pain or aversion networks. This study demonstrated that gene-to-connectome mapping is feasible in the mouse. The μ -opioid receptor gene A118G is polymorphic for both mice and humans, and in humans it is associated with social hedonic capacity in drug misuse. Ongoing work in human brain tissue is analyzing receptor density for μ -opioid receptors. Work is also ongoing to identify a connectome associated with μ -opioid receptors by using a variety of techniques including imaging after acute doses of opioids and μ -opioid receptor knockout mice to determine opioid drug effects in the brain.

Key Takeaways

Targets. Key questions were addressed during the symposium to highlight the challenges in developing new pain and addiction therapeutics, including: why have so many clinical trials failed to offer therapeutics that provide symptom relief equal to or greater than opiates without safety concerns; is a selective-ligand single-target strategy preferable to therapeutics that interact with multiple targets; and how do we do better at translating animal experimental data to human studies? Answers to these questions, and other key questions, lie in deeper knowledge of the underlying circuitry of pain and addiction and the possible unique localization and regulation of protein targets within these circuits. Genomics, proteomics, epigenetics, lipidomics, single-cell RNA sequencing, and related methods will continue to help identify these circuits, biochemical pathways, and biomarkers that are important for identifying disease risk factors and potential targets for treating pain and addiction. An unbiased method for drug discovery with a focus on both transcriptional and epigenetic mechanisms that includes the key biochemical pathways in pain and addiction was proposed. Natural products may offer the pharmacological complexity required to provide robust efficacy, although there is risk in not fully understanding the targets engaged. Since opioids remain the most robust therapeutics to treat a broad number of pain conditions, ways to better harness the efficacy and reduce the safety concerns were presented. For example, κ -opioid receptor antagonists might meet these criteria. Exploring single-cell mRNA transcriptional profiling to differentiate opioid gene expression in distinct lateral hypothalamus cell types may allow selective targeting of these specific cell types. Biased opioid ligands may harness specific signaling pathways that weigh the beneficial

over the deleterious pathways. Studying the connection between sleep disruption and opioid withdrawal might provide opportunities to better understand withdrawal symptoms and suggest solutions in circadian cycle control or improved sleep patterns.

Preclinical Models. Little innovation has occurred in the development of surrogate models of pain and addiction in recent times. Most pain models are based on reflexive responses since they are relatively easy to measure with current technology. However, these assays offer poor translation to clinical success (Mogil, 2009; Yeziarski and Hansson, 2018), and thus may not report pain within the complete physiologic context. Nonreflexive models and surrogates of spontaneous pain are being explored to potentially align better to the pain phenotype. Longitudinal chronic pain studies in animal models should extend beyond 3 months, which might improve markers associated with chronic pain. Outbred mice should be considered for assays as well as the trend to include both sexes in studies. Preclinical studies frequently show clear differences between males and females regarding pain processing, and further research on the underlying processes that contribute to sex differences of pain is needed. Zebrafish provide an interesting preclinical model for opioid-seeking behavior and are amenable to high-throughput screening. Could these easy-to-manage models be more broadly helpful in studies of pain and addiction?

Drug Discovery. The themes for the drug discovery focus of the symposium revealed challenges in prosecuting promising targets and opportunities with new technology. One of the targets best linked genetically to pain is the sodium channel $Na_v1.7$. This is a difficult ion channel in which to create selective drugs since there are a number of closely related family members. Progress has been made, but after years of effort no selective drug has reached the market. Similar challenges have been seen with the TRP ion channel family since these, like many ion channel targets, are expressed well beyond pain pathways and frequently provide undesirable nonselective effects beyond pain. The lead molecule discovery process has typically started with target expression followed by high-throughput screening of large diverse compound libraries. Secondary screening and lead development require chemists, biologists, statisticians, and project support staff, quickly making this an expensive, time-consuming iterative and labor-intensive process. Technologies currently being applied to improve the efficiency and success of hit, lead, and candidate discovery include target X-ray crystallography, cryo-electron microscopy, and MD simulations. Seeking targets in specific addiction pathways such as the habenula/interpeduncular nucleus circuit, which regulates aversion to drugs, may provide new therapeutic opportunities. GPCR proteins represent roughly 35% of marketed drugs and remain attractive since many in this super family of proteins have yet to be screened for tool compounds or linked to specific diseases. Endosomal-localized GPCRs continue to provide rich signaling inside the cell environment and are being exploited for longer-lasting efficacy for new therapeutics targeting this compartment.

Biomarkers. There is considerable evidence that biomarkers significantly increase the probability of success in therapeutic development from phase I to approval (Thomas et al., 2016). Thus, validated biomarkers remain a key component in any successful drug discovery program. Imaging

biomarkers might improve translation in preclinical research, enhance information obtained in phase 1 clinical trials, and improve accuracy in phase II clinical trials. Imaging biomarkers may also facilitate new objective pain readouts rather than the currently used subjective 10-point scale of pain. Digital biomarkers look very promising for migraine prevention, acute migraine treatment, osteoarthritis pain, and chronic low back pain. Genetic biomarkers for chronic pain and opiate use disorder are needed; however, high interpatient variability has been confounding. Epigenetic biomarkers could also provide understanding of the interface between experience and brain function. Target engagement measures and biomarker discovery and validation are areas of active research and key to improving the rate of successful research and development efforts. Improved biomarkers may help address some of the problematic issues currently facing clinical trials in pain (Häuser et al., 2011; McKeown et al., 2015).

Conclusions

Given the rapidly growing occurrence of opioid use disorder and associated overdose deaths, as well as the lengthy time required for new targets to reach patients in need, there is a renewed urgency to develop better treatments for pain and addiction. There was strong agreement among all of the symposium participants that to develop new therapeutics for pain and addiction, a better understanding of the circuits, pathways, genetic changes, transcriptional and epigenetic mechanisms, and key disease-relevant targets within these circuits is needed. There must also be evidence that any new targets are druggable. Druggable targets would: 1) be expressed exclusively or enriched in pain and addiction pathways to help avoid unwanted side effects; 2) be genetically linked to pain and/or addiction; 3) have a medicinal chemistry history of being successfully modulated by small drug-like molecules; and 4) have translatable measures of target engagement and biomarkers. There is also an opportunity to further explore established target family classes, such as ion channels and GPCRs, to discover novel pharmacological regulation. Examples include: 1) leveraging allosteric binding pockets to gain selectivity and uniquely modulate natural protein function; 2) designing biased ligands to encourage disease modification/symptom control pathways over deleterious unhealthy pathway control; 3) leveraging unique protein-protein interactomes such as GPCR hetro- and homodimers/multimers and chaperone proteins to gain central nervous system region/circuit-specific regulation; 4) targeting intracellular signaling to gain new therapeutic results; and 5) expressing genetically modified targets in specific brain areas to introduce novel circuit control mechanisms in order to alleviate disease with a better safety profile. Constructs such as optogenetically or magnetically controlled proteins and designer receptors exclusively activated by designer drugs are emerging examples of truly novel approaches to improved therapeutics. Additionally, innovative methods and technologies to rapidly create validated tool compounds to de-risk targets and improved forward and reverse translatable preclinical and clinical assays for pain and addiction will be needed for new therapeutics in this space. Progress in developing novel treatments and therapeutics will require

researchers to think, act, and execute impactful science differently.

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