The Opioid Crisis and the Future of Addiction and Pain Therapeutics


National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, Maryland (N.P.C., G.S.S., S.G.J., M.D.H.); Orvos Communications, LLC. (H.E.G.); National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland (A.P.T.); Q-State Biosciences, Cambridge, Massachusetts (O.B.M.); VP Discovery Research, Karuna Therapeutics, Boston Massachusetts (C.C.F.); and National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland (K.R.)
Running Title: The Future of Addiction and Pain Therapeutics

Corresponding Author:

Kurt Rasmussen, Ph.D.
Director, Division of Therapeutics and Medical Consequences
National Institute on Drug Abuse
6001 Executive Blvd, Rm 4133, MSC 9551 Bethesda MD 20892 U.S.A.
+1 301.827.3502 (office)
+1 301.526.5227 (mobile)
+1 301.443.2599 (fax)

kurt.rasmussen@nih.gov

Number of Text Pages: 41
Number of Tables: 0
Number of Figures: 0
Number of References: 89
Number of words in the Abstract: 250
Number of words in the Introduction: 465
Number of words in the Discussion: 1,227

List of nonstandard abbreviations:

BACPAC, back-pain research consortium
BNST, bed nucleus of the stria terminalis
BRAIN, Brain Research through Advancing Innovative Neurotechnologies

BRET, bioluminescence resonance energy transfer

cAMP, cyclic adenosine monophosphate

CRF, corticotrophin releasing factor

CRISPR, clustered regularly interspaced short palindromic repeats

DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin

DRG, dorsal root ganglion

EPPIC-net, early-phase pain investigation clinical network

fMRI, functional magnetic resonance imaging

FRET, fluorescence resonance energy transfer

GABA, gamma-aminobutyric acid

GPCR, G-protein-coupled receptor

GTP-gamma-S, guanosine 5'-O-[gamma-thio]triphosphate

GWAS, genome-wide association studies

HDAC, histone deacetylase

iPSCs, induced pluripotent stem cells

LHA, Lateral Hypothalamic Area

MD, molecular dynamics

NAc, nucleus accumbens

NAC, N-acetylcysteine

Nav1.7, a voltage-gated sodium channel and target for novel pain therapeutics

NCATS, National Center for Advancing Translational Sciences

NIDA, National Institute on Drug Abuse
NINDS, National Institute of Neurological Disorders and Stroke

NK1R, substance P neurokinin 1 receptor

NOP, Nociceptin Opioid Receptor

OUD, opioid use disorder

phMRI, pharmacologic magnetic resonance imaging

PK/PD, pharmacokinetic/pharmacodynamic

PSPP, preclinical screening platform for pain

PTSD, post-traumatic stress disorder

RGS, regulator of G protein signaling

RNA-seq, RNA sequencing

SAR, structure-activity relationship

SCN9A, gene encoding Nav1.7

SNP, single nucleotide polymorphism

SPARC, Sensitizing PAin Reagent Composition

TRP, transient receptor potential

VTA, Ventral Tegmental Area
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 towards the development of effective non-addictive pain medications, interventions for opioid overdose, and addiction treatments. A two-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 to address these concerns and to chart a collaborative path forward. The meeting was supported by the NIH HEAL (Helping to End Addiction Long-term℠) 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, to create a forum for cross-communication 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 two-day symposium focused on challenges and opportunities in developing addiction- and pain-related medications. Speakers and attendees came from 40 US 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 (Volkow and Koroshetz, 2019). Moreover, more than 25 million adults in the United States are affected by chronic pain (Collins et al., 2018). With a lack of effective and safe non-opioid options for pain management, for some, there is no relief in sight. These numbers do not capture the full extent of the damage of the opioid crisis, which reaches across every domain of family and community life—from lost productivity and economic opportunity, to intergenerational and childhood trauma, to extreme strain on community resources, including first responders, emergency rooms, hospitals, and treatment centers. Moving forward, there is a major need to develop therapies for pain without addiction liability, to 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’s National Center for Advancing Translational Sciences (NCATS), in collaboration with NIH’s National Institute on Drug Abuse (NIDA) and National Institute of Neurological Disorders and Stroke (NINDS), sponsored *The Opioid Crisis and the Future of Addiction and Pain Therapeutics: Opportunities, Tools, and Technologies Symposium* on February 7-8, 2019, in Bethesda, Maryland (agenda included as supplementary material). The meeting was supported by the NIH HEAL (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 pre-competitive, 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 US 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 two-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 Boston Children’s Hospital and Harvard Medical School emphasized that there has been a failure so 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 non-cancer 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 includes ’omic profiling using transcriptomic and proteomic analysis of relevant cell 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 who is at risk and preventing this 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 non-reflexive 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 (iPSCs) 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 iPSCs 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 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 post-receptor 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-seq 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 corticotrophin releasing factor (CRF)-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 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 thiosulfimates 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., 2018). 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 key to 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 Nav1.1 in pain. Nociception results from Hm1a/b enhancing Nav1.1 currents by inhibiting channel activation. This led to the recognition of a role for Nav1.1 in peripheral pain mechanisms. Activation of pain fibers that contain Nav1.1 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, natural products including cannabinoids that modulate cannabinoid receptors such as CB1 and CB2, and the opioid receptor agonist mitragynine (from the plant kratom), are receiving attention for their potential to treat pain (Vuckovic et al., 2018).

**Challenges and Opportunities for the Development of Nav1.7 Inhibitors.** Dr. Bryan Moyer of Amgen presented a review of drug discovery efforts to identify inhibitors of Nav1.7, 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 Nav1.7 (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). Nav1.7 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 Nav1.7 drug discovery are isoform selectivity, the need to block action potential firing in nociceptors (which generally requires drug concentrations above the Nav1.7 IC\textsubscript{50} value) 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 (SAR) analyses of tarantula peptides identified potent and selective Nav1.7 inhibitors that were 1000x selective over Nav1.5 and Nav1.4 and that blocked action potential firing in both DRG neurons as well as C-fibers. Engineering of the natural product saxitoxin identified derivatives with 1,000x selectivity over the other human Nav isoforms. Sulfonamide antagonists have been optimized with 100-1000x selectivity over other human Nav 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 Nav1.7 inhibitors in clinical trials will ultimately test the hypothesis that acute pharmacological blockade of Nav1.7 can decrease human pain.
Translational Assays: Supporting a Small Molecule Nav1.7 Inhibitor Drug

Discovery Program. Patients with SCN9A mutations (encoding Nav1.7) can’t feel pain, but have tactile and pressure sensitivity, a loss of olfaction, and do not respond to histamine-induced itching. Based on this validation, several pharma companies have pursued this target as described by Dr. Moyer at Amgen. Dr. Houghton from Merck presented a very informative account of their effort on this program to develop translatable assays that could be used preclinically and clinically to demonstra target modulation and efficacy to ensure clinical success. The current approach was compared to the historic approach used for MK-0759, a non-selective sodium channel inhibitor, that failed to demonstrate efficacy in the clinic. The compound MK-0759 was effective in the rat spinal nerve ligation (SNL) model of neuropathic pain and the 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 SNL 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, physiological responses, efficacy, and safety of the compound in a dose dependent manner. She emphasized that it is important to consider each of these during the drug discovery phase, and the more of these answered during the discovery process, the greater likelihood 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 Nav1.7 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. Further, an isoamyl acetate-induced biomarker assay was developed to monitor olfactory bulb activity with fMRI (Zhao et al., 2016). However, when they tried to translate this assay to the clinic in an experimental medicine study, it failed, as the size of the olfactory bulb in man was too small, resulting in the assay having insufficient sensitivity. They demonstrated that Nav1.7 inhibitors slow and block 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 to demonstrate target modulation and efficacy.

**Complexity of Biased Agonism and the Implications for Opioid Analgesics.** Dr. Laura Bohn of The Scripps Research Institute discussed biased agonism and the complexities of opioid analgesics. Functional selectivity (ligand-directed signaling, biased agonism) of GPCR activation refers to the binding of a compound to a receptor and 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 physiological responses. The intracellular protein complement differs between cell types and as well as in tissues which may have differential impact on how analgesia and side effects are mediated. In this context, she 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 tested \textit{in vivo}. Genetic models can be used to identify key players involved in drug actions. Hence the context of \textit{in vitro} and \textit{in vivo} assays are critical components of identifying compounds with safe and effective analgesic characteristics.

\(\beta\)- Arrestins play diverse roles in regulating receptors. Genetic knockout of \(\beta\)-arrestin2 led to a decrease in morphine-induced respiratory effects in mice. Studies were presented of a \(\mu\)-opioid agonist series (Schmid et al., 2017; Kennedy et al., 2018) with favorable bias factors for GTP\(_{\gamma}\)S and cAMP over \(\beta\)-arrestin2 showing that \textit{in vivo} widening of the therapeutic window may be possible. These compounds were shown to provide antinociception with limited respiratory suppression. It was emphasized, however, 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 biological systems. In this context, Dr. Bohn also presented several examples of measuring bias-factors for DAMGO, morphine, and buprenorphine, and showed it may be assay dependent (Stahl et al., 2015). In addition, she presented interesting data on the effect of Triazole 1.1 on \(\kappa\)-opioid receptors that maintains efficacy in chloroquine phosphate mouse-itch model without eliciting sedation, decreases in dopamine in mice nor 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 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. Her 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.

She discussed 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, they are differentially expressed throughout the brain, and they 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, her 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.
pain. Specifically, her laboratory found a prominent role of the striatal enriched RGS9-2 on the onset of action and antiallodynic efficacy of desipramine and other monoamine-targeting antidepressants (Mitsi et al., 2015). RGS9-2 controls the nuclear shuttling of the histone deacetylase HDAC5, which is repressing a number of genes necessary for antidepressant-mediated plasticity. She 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 (HDAC5, HDAC6, MEF2c, RGS4).

Intersection Between Pain and Addiction - Implications for Kappa Opioid Receptors. The role of κ-opioid receptors in chronic pain was addressed by Dr. Catherine Cahill of the University of California. 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 GTPγS is increased in the nucleus accumbens (NAc) 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 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 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 suggests that it is genetic. The take home message is that negative affect needs 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 forebrain 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 a decreased activation of the medial habenula (Martin et al., 1997).

Gpr151 is an orphan G-protein coupled receptor that is expressed almost exclusively in the habenula, particularly in cells that also express the µ-opioid receptors (Broms et al., 2015; Wagner et al., 2016). Gpr151 may inhibit activity of habenula-interpeduncular nucleus circuit, and regulates 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 co-released 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 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 the human and rat brain are quite different, older circuits of pain can be utilized (Becerra et al., 2013). For example, in a migraine rat model, neuroimaging showed activation in very similar brain regions between the rat and human. 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, he highlighted an example of the use of fMRI and phMRI 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 PK/PD 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 PK/PD 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 non-responder 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 this kind 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, payor) can be combined to uncover new insights. In the field of diabetes, a method to predict ‘brittle diabetics,’ patients that can’t regulate their insulin levels very well, was developed using data analysis. This ability provides a way to improve diagnosis and better monitor patients (Fagherazzi et al., 2018). In the pain field, this kind of data analysis has predicted a way to detect patients that have become over-sensitized 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 versus 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 for individual patients (identification, therapeutic engagement, measured response to therapy), passively collect data (daily behaviors, determine 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 (GWAS) in over 100,000 addicted patients to identify genetic risk scores to
develop clinically relevant biomarkers. To overcome classical limitations in psychiatric genetics (high inter-patient phenotypic variability, small effects of individual common variants), GWAS findings are also being increasingly correlated with quantitative addiction-related endophenotypes. For example, during both methadone maintenance and surgical anesthesia, opiate dosage associates with SNPs located 300kp 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. He 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). He discussed the severe effects of early-life adversity, such as child abuse, on gene expression, histone modifications, and DNA methylation, and how it affects the lifetime risk for substance misuse and OUD (Lutz et al., 2017b; Lutz et al., 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, 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 addressed this issue and pointed out that there is a failure of translation from preclinical models to humans in a variety of fields, not just pain research. Basic scientists have traditionally ignored epidemiological reality (particularly in pain). There are three facets to consider for 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 biological 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 (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 and colleagues found that the Terc null mutant (-/-) mouse, with shortened telomeres, are more sensitive to pain and, when given spared nerve injury, have even further shortened lifespans. One proposed mechanism was cellular senescence in the spinal cord observed starting at 4 months post-injury, 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 post-operative pain in animals, but 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 six months post-injury, 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
biological and physiological events involved in pain happen at the same speed in rodents and humans, despite the differing life span.

**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 post-synapse, according to Dr. Peter Kalivas of the Medical University of South Carolina. Another way of thinking about the synapse is the ‘tetrapartite synapse’ consisting of the pre- and post-synapse, the pre-synaptic 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 retract; 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 glutamate transporter. A clinical trial with NAC showed a decrease in PTSD 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 to facilitate and maintain abstinence. Accordingly, it was suggested that it might be useful for relapse prevention in combination with suboxone or methadone by reducing intrusive thoughts (craving).
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 tetrapartide 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), 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 REM sleep)—can be monitored in humans and rodents (Baker et al., 2018). Wireless telemetry devices implanted in mice can provide continuous data, including EMG and EEG 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 Designer Drugs (DREADD)-based chemogenomic system that can regulate the activity of cells that express 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, whether there are sex differences in these relationships, and to perform proof-of-concept studies with NIDA’s ’10 Most Wanted’ list including orexin-1/2 antagonists 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 other 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 biological 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 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 1,000 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, 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 demonstrate pain response behavior.
Opioid receptors and associated peptides have orthologues 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 per 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 continue the 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 highlighted that crystal structures provide a static snapshot that 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 non-canonical binding sites, are highly promising as drugs for G-protein-coupled receptor (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 classical ligand binding. Together, this information enabled the successful rational design of an allosteric modulator with desired properties.

Recent structural data suggests 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 as 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 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 (BRET)-based biosensors were used to examine the proximity between NK1R and proteins resident to the plasma
membrane and endosomes. Fluorescence resonance energy transfer (FRET)-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 (ERK). Dynamin and clathrin inhibitors prevented sustained SP-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 GCPR 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. 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-µm spatial resolution and 1-ms temporal resolution, maintaining the richness of manual patch clamp recording with 10,000x higher throughput. With environmental controls, a pipetting robot, and fully-automated 96-well plate scanning, the platform can record from 1000 wells/day with 300 neurons/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 (hiPSC) derived neurons (Williams et al., 2019).

The Optopatch system aims to use cell-based models to bridge the gap in drug discovery in between target-based high-throughput screens and in vivo models. Dorsal root ganglion (DRG) 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 (SPARC), a cocktail of inflammatory molecules found at elevated levels in the joints of arthritic patients; receptors for each SPARC ingredient are expressed in DRG neurons and the ingredients induce pain when injected in animals. The DRG neurons become hyperexcitable when treated with SPARC – 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 (VTA) neurons is universally required for motivated behaviors. The lateral hypothalamus interfaces with the VTA 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 explained how optogenetic targeting of neural circuit elements has recently been used to activate or inactive 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 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 biological and hardware 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. have developed optically-sensitive µ-opioid-like receptor systems for further study (Siuda et al., 2015). 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, 2019 et al., Nature Bioengineering, in Press). The optogenetic approaches have been combined with pharmacology, as well as multi-animal and multi-drug on-demand control. One experiment demonstrated this, expressing ChR2 in vGAT+ neurons of the bed nucleus of the stria terminalis (BNST) 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 systems 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 (NOP), 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 VTA 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 BNST, 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 interactions deficits of protracted opioid abstinence.

Non-invasive and translation neuroimaging was performed with fMRI (resting state and fiber tracking) in mice. Targeted deletion of the μ-opioid receptor gene (Oprm1) induced widespread remodeling of 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 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; how do we do better at translating animal experimental data to human studies? Answers to these and other key questions lies in deeper knowledge of 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 RNAseq, 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 though there is risk in not fully understanding the targets engaged. As 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 as they are relatively easy to measure with current technology. Yet these assays offer poor translation to clinical success (Mogil, 2009; Yezierski and Hansson, 2018) and thus may not report pain within the complete physiological context. Non-
reflexive 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 the 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 NaV1.7. This is a difficult ion channel to create selective drugs for as 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 as these, like many ion channel targets, are expressed well beyond pain pathways and frequently provide undesirable non-selective 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 which quickly becomes 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 molecular dynamic simulations. Seeking
targets in specific addiction pathways, such as the habenula-IPN circuit which regulates
aversion to drugs, may provide new therapeutic opportunities. GPCR proteins represent
roughly 35% of marketed drugs and remain attractive as 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 is 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 to 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 but high inter-patient 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
R & D efforts. Improved biomarkers may help address some of the problematic issues currently facing clinical trials in pain (Hauser et al., 2011; McKeown et al., 2015).

**Conclusion**

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: a) be expressed exclusively or enriched in pain and addiction pathways to help avoid unwanted side effects; b) be genetically linked to pain and/or addiction; c) have a medicinal chemistry history of being successfully modulated by small drug-like molecules; and, d) 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: leveraging allosteric binding pockets to gain selectivity and uniquely modulate natural protein function; designing biased ligands to encourage disease modification/symptom control pathways over deleterious unhealthy pathway control; leveraging unique protein-protein interactomes such as GPCR hetero- and homo-dimers/multimers and chaperone proteins to gain CNS
region/circuit specific regulation; targeting intracellular signaling to gain new therapeutic results; and, expressing genetically modified targets in specific brain areas to introduce novel circuit control mechanisms to alleviate disease with a better safety profile. Constructs such as optogenetically or magnetically controlled proteins and DREADDs 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.

Acknowledgments

The authors wish to thank Judith M. Orvos, ELS of Orvos Communications, LLC for assistance in capturing information from the symposium. The authors wish to thank Lillian J. Coussens for her assistance with editing the manuscript.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Coussens, Sittampalam, Jonson, Hall, Gorby, Tamiz, McManus, Felder and Rasmussen.


McCorvy JD, Butler KV, Kelly B, Rechsteiner K, Karpiak J, Betz RM, Kormos BL, 
Shoichet BK, Dror RO, Jin J and Roth BL (2018) Structure-inspired design of 

McKeown A, Gewandter JS, McDermott MP, Pawlowski JR, Poli JJ, Rothstein D, Farrar 
JT, Gilron I, Katz NP, Lin AH, Rappaport BA, Rowbotham MC, Turk DC, Dworkin 
RH and Smith SM (2015) Reporting of sample size calculations in analgesic 

Mechling AE, Arefin T, Lee HL, Bienert T, Reisert M, Ben Hamida S, Darcq E, Ehrlich A, 
BL and Harsan LA (2016) Deletion of the mu opioid receptor gene in mice 
reshapes the reward-aversion connectome. Proc Nati Acad Sci U S A 
113:11603-11608.

Mickle AD, Shepherd AJ and Mohapatra DP (2016) Nociceptive TRP Channels: 
Sensory Detectors and Transducers in Multiple Pain Pathologies. 
Pharmaceuticals (Basel) 9.

Mitsi V, Terzi D, Purushothaman I, Manouras L, Gaspari S, Neve RL, Stratinaki M, Feng 
determine the onset of action and efficacy of antidepressants in neuropathic pain 

10:283-294.


Footnotes

This work was supported [in part] by the Intramural Research Program of the NIH, National Institutes of Health National Center for Advancing Translational Sciences (NCATS) Division of Pre-Clinical Innovation [Grants 1ZIATR000346, 1ZIJTR000340]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.