Neuron
Perspective

Composite Pain Biomarker Signatures for Objective Assessment and Effective Treatment

Irene Tracey,[1,*] Clifford J. Woolf,[2,*] and Nick A. Andrews[2]
[1]Nuffield Department of Clinical Neurosciences, University of Oxford, West Wing, John Radcliffe Hospital, Oxford OX3 9DU, UK
[2]Kirby Neurobiology Center, Boston Children's Hospital and Department of Neurobiology, Harvard Medical School, Boston, 02115 MA, USA
*Correspondence: irene.tracey@ndcn.ox.ac.uk (I.T.), clifford.woolf@childrens.harvard.edu (C.J.W.)
https://doi.org/10.1016/j.neuron.2019.02.019

Pain is a subjective sensory experience that can, mostly, be reported but cannot be directly measured or quantified. Nevertheless, a suite of biomarkers related to mechanisms, neural activity, and susceptibility offer the possibility—especially when used in combination—to produce objective pain-related indicators with the specificity and sensitivity required for diagnosis and for evaluation of risk of developing pain and of analgesic efficacy. Such composite biomarkers will also provide improved understanding of pain pathophysiology.

What Is Pain?

Pain is our body's alarm system, warning us of danger in the environment, injury, or the presence of disease. It is necessarily and intrinsically unpleasant (hurts), which enables protection by driving immediate attention, action, and adaptive learning. To be effective, protective pain must be so overpowering that we cannot ignore it. Consequently, it is intimately linked to negative emotions. Physiologically, it is triggered by the activation of high-threshold primary sensory neurons (the noxious stimulus detecting nociceptors) and by specific transduction machinery in their peripheral terminals (Woolf and Ma, 2007). This is the nociceptive pain evoked by pin prick, touching something too hot, or any other potentially tissue-damaging chemical, thermal, or mechanical stimulus. Lack of the capacity to experience nociceptive pain is catastrophic: tips of fingers and toes are damaged, lips and tongues are mutilated, and life expectancy is reduced, as witnessed in individuals with congenital insensitivity to pain due to rare recessive gene mutations resulting in either loss of nociceptors or their functional disruption (Bennett and Woods, 2014).

Post injury or infection, adaptive processes are engaged in both the peripheral and central nervous systems (PNS and CNS, respectively) that manifest as an amplification of noxious inputs resulting in an exaggeration and prolongation of pain (hyperalgesia) due to peripheral and central sensitization (Hucho and Levine, 2007). Additionally, there is the generation of hypersensitivity such that normally innocuous inputs now produce pain—the phenomenon of allodynia: a warm shower becomes painful after sunburn or a skin cut is tender to touch; this helps drive guarded protection of the injured area until it heals (Latremoliere and Woolf, 2009). Both nociceptive and acute inflammatory pain and the neurobiological mechanisms responsible are then key to survival. Treatments that block nociceptive and inflammatory pain need, therefore, to be used with caution. Elimination of the protective elements of acute pain are only required temporarily and in a highly controlled fashion, as for surgery, obstetrics, or dentistry. Control of inflammatory pain needs to be a tight balance between reducing suffering and enabling healing, such as postoperatively or after trauma. There is a particular challenge though for pain reduction in arthritis patients using either non-steroidal anti-inflammatory drugs or a promising new treatment with anti-nerve growth factor antibodies, where pain needs to be managed such that the underlying joint disease is not worsened by overuse of pain-free but still damaged joints (Denk et al., 2017).

Chronic pathological pain is entirely different from acute nociceptive pain. Chronic pain is defined clinically as pain that persists beyond normal tissue healing time—normally 3–4 months. This includes chronic inflammatory pain, such as rheumatoid arthritis, neuropathic pain arising from injury to or disease of the nervous system, such as painful diabetic neuropathy or post-herpetic neuralgia, and dysfunctional or idiopathic pain, such as fibromyalgia or irritable bowel syndrome (https://www.iasp-pain.org/terminology). Chronic pathological pain rarely may also be the consequence of gain-of-function mutations in voltage-gated sodium ion channels expressed in nociceptors (Bennett and Woods, 2014; Dib-Hajj et al., 2015). Pathological pain includes conditions in which the pain is not signaling the presence of an ongoing noxious stimulus (i.e., it is not nociceptive) and ones that are not enabling healing (i.e., not acute inflammatory pain). The pain here is not protective and is instead pathological. Persistent pathological pains are major sources of disability globally with 1 in 5 adults fulfilling this definition and a consequential high socioeconomic burden (~$600 billion per annum in the USA) (Institute of Medicine (IOM), 2011). The current opioid crisis in the USA is a stark indicator of the suffering and urgent need for better non-addictive analgesics (https://www.nih.gov/research-training/medical-research-initiatives/heal-initiative).

Such pathological or clinical pain has a complex biology and pathophysiology with multiple diverse pathways affected. These include abnormal peripheral drivers such as ectopic activity in injured axons, alterations in transmission, and processing in the spinal cord and higher brain centers due to sensitization, amplification, and disinhibition, through to modifications in perception. Exactly what is responsible for the transition of acute to chronic pain and why some individuals are more susceptible than others are areas of active research, mitigating the development of persistent pathological pain by targeting the responsible mechanisms being the goal. Figures 1A and 1B provide a simplified overview of acute and chronic pain pathways and some mechanisms.

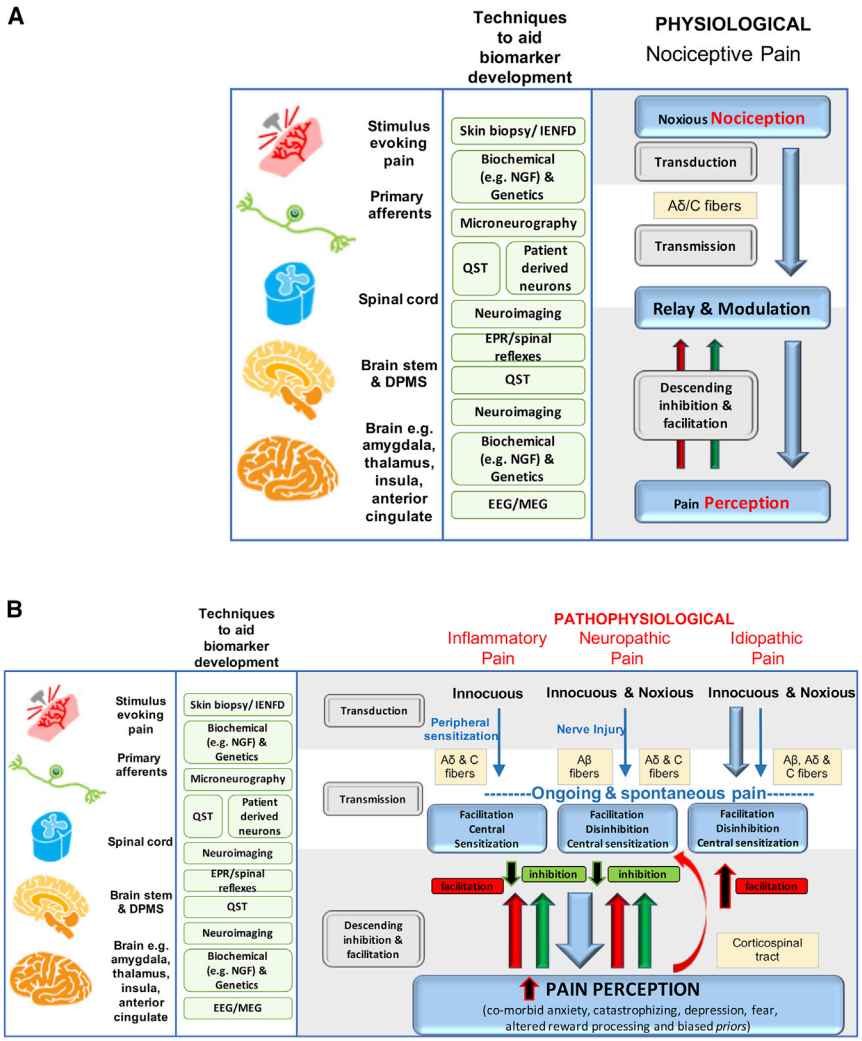

Figure 1. Mechanisms of Acute and Chronic Pain

(A and B) The normal physiological (A) to an acute noxious stimulus and pathophysiological (B) processes is illustrated with the involvement of Aδ- and C-fiber nociceptors transducing the input from the periphery to the superficial laminae of the dorsal horn of the spinal cord where they can be modulated. From the spinal cord, signals are relayed to the brainstem and to the brain where pain emerges as a perception. Here the sensory and emotional context and learning is applied to interpret and aid future avoidance of the stimulus. The major classes of chronic pain and the processes that are believed to lead to chronic pain in susceptible individuals are depicted in red. During inflammation, while the stimulus (e.g., activated immune cells or a skin incision) is present, there exists a status of peripheral sensitization (PS) characterized by erythema and tenderness to innocuous stimuli, typically heat. PS goes away once the peripheral pathology resolves. Stimuli activating nociceptors that are noxious, repeated, and sustained (e.g., following nerve injury) induce the process of central sensitization (CS) in the dorsal horn of the spinal cord. Initially CS is protective and enables the organism to avoid further injury due to a heightened awareness of its surroundings, but at some point, CS becomes pathological. CS produces pain in non-inflamed tissue by co-opting novel inputs (e.g., Aβ fibers); thus, mechanical pain is typical of CS and heat pain is more typical of peripheral sensitization. Recently, it has been shown that a subset of corticospinal neurons (CSNs) known to originate in the primary and secondary somatosensory cortex and to directly innervate the spinal dorsal horn via CST axons can directly modulate normal and pathological tactile sensory processing in the spinal cord. Facilitation and descending inhibition are processes that occur due to different regions of the brain and brainstem inhibiting or activating (or even disinhibiting) nociceptive inputs to the spinal cord. The effect can be seen on both mechanical and heat sensations in different forms of chronic pain, and an imbalance in this system (less inhibition, more facilitation) is a key mechanism, as are changes in the brain's neurochemistry, structure, and functional activity. Shown in green are the current methodologies that are used to define biomarkers at the particular levels of nociceptive and pain processing that apply to acute and chronic pain

Chronic Pain as a Symptom or Disease

The recognition that most chronic pain is maladaptive and mechanistically quite different from acute protective pain has been a major conceptual breakthrough within the pain field. Nevertheless, it is hard to manage chronic pain effectively because the processes in the nervous system driving the pain are not easily identified and targeted for treatment. Pain in these circumstances is not simply a symptom of some distinct disease pathology but rather the expression of a pathologically functioning nervous system. Of course, there are some conditions, such as osteoarthritis, in which there is a primary peripheral pathological driver and replacement of the joint can ameliorate the chronic pain. However, even here the response to such interventions is not universally beneficial, as the pain might become centralized in some patients (De Oliveira Silva et al., 2018; Soni et al., 2018). Furthermore, the most effective treatment of pain (especially its prevention) needs to be not simply symptom suppressing (switching off the sensation of pain) but rather targeted at the underlying pathophysiological mechanisms within the nervous system that drive the chronicity and are, in consequence, disease modifying. For example, within the PNS, a local anesthetic that temporarily silences ectopic activity in an injured nerve is symptom suppressing while one that reduces expression of those ion channels that drive this pacemaker-like activity would be disease modifying. Within the CNS, a short-lived inhibition of activity in nociceptive projection neurons in the spinal cord would be symptom suppressing while one that prevented long-lasting changes in synaptic input strength would be disease modifying. The International Association for the Study of Pain has recently classified chronic pain for the International Classification of Diseases (ICD-11) as a disease or symptom with either chronic primary pain, such as fibromyalgia for which there is no known trigger, or chronic secondary pain, such as chronic neuropathic pain that develops after a lesion to the nervous

system (Treede et al., 2019). This classification is welcome as it recognizes chronic pain as a disease in its own right.

So, although we use a single word "pain," multiple distinct mechanisms contribute to the generation and maintenance of this complex, multidimensional, and subjective experience. The ACTTION-American Pain Society Pain Taxonomy (AAPT) provides an evidence-based and multi-dimensional (or composite) approach to classifying chronic pain that attempts to address the need for mechanism-based assessment and treatment (Fillingim et al., 2014). We similarly argue for an approach where pain is defined by the primary pathophysiological drivers responsible (Vardeh et al., 2016), as this will help better identify targets for successful intervention. However, to do this well, we need biomarkers of mechanisms.

Composite Biomarker Signatures for Pain

Similar to other complex neurological diseases like Alzheimer's, it is highly unlikely that one biomarker will be able to capture "pain" in its entirety. Exploiting advanced analytical tools like neural networks, artificial intelligence, or machine-learning algorithms to combine multiple objective biomarkers into "composite pain biomarker signatures" is more likely to be successful for understanding pain and developing new treatments (Baskin et al., 2016). We need to abandon the view that pain categorized as mild, moderate, and severe determines therapeutic choice and move to a model in which we recognize that the pain patient's experience is the consequence of multiple diverse neurobiological processes, each of which offer an opportunity to be managed differently. We recognize that this is not trivial to achieve. We must develop therapies (drug, surgical, physical, and psychological) that selectively target these mechanisms and objective ways to assess the presence of the mechanisms and the efficacy of any modulation of them. We need to follow oncology where there has been a shift from general cytotoxic agents to treatments aimed specifically at the unique features of the cancer in an individual patient.

In this Perspective, we ask the question: are objective biomarkers of pain and the mechanisms that drive it possible? However, we first need to ask why relying solely on subjective ratings is inadequate for pain assessment and treatment.

The Challenge of Measuring Pain

The International Association for the Study of Pain currently defines pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." This definition does not capture the fact that pain may be both protective and pathological and also misses key features of clinical pain conditions; pain may arise either in the absence of any stimulus or in response to a stimulus that would normally only evoke an innocuous sensation. Finally, it doesn't readily serve non-verbal individuals as it requires the experience to be described.

At its simplest, pain comprises two core dimensions of intensity (magnitude) and unpleasantness (effect). When it comes to clinical pain, there are many subjective variants in how the sensation is described; stabbing, burning, throbbing, pulsing, grinding, and shock-like are a few examples. Although an enormous amount of effort has been devoted to examining these pain descriptors, looking, for example, to see whether the subjective sense of burning specifically reflects neuropathic pain, the results are, to date, generally not clear cut. Much of how we describe pain is learned, conditioned by our experiences or shaped by our language and society or clinicians (Bourke, 2014). While report of the presence of pain is critical, as in duration, periodicity, location, and intensity, descriptors are unlikely to share a simple one-to-one mapping with any underpinning mechanism.

Nonetheless, the standard way to assess the presence and magnitude of pain is to ask a person to describe it using rating scales and symptom-based questionnaires. For rating scales, the challenge is how variable is an individual's assessment of their pain—if you rate your pain at 7 out of 10 today, how accurately can you gauge whether your pain tomorrow is identical or different? Is your worst pain, moreover, the same as someone with a different genetic, cultural, or environmental upbringing? Pain experiences are also malleable to various factors, such as mood, context, and cognition, making the consequential pain report both complex and variable even to the same nociceptive input. Neuroimaging (discussed below) has shown that these factors influence the pain experience and consequent self-report via different mechanisms to the ones driving the underlying pathology. So, while these factors are a key part of the multidimensional pain experience, their contribution needs to be distentangled and mechanistically understood, particularly if we want to know what our therapy is targeting "underneath" the pain experience that is a mixed represention of all these inputs and influences.

Figure 2A illustrates the common tools we have to measure pain. Figure 2B illustrates how we measure pain in children and adults ranging from the awake and cognitively able to the deeply sedated or anesthetized and cognitively impaired. Figure 2C illustrates how pain is measured in animals.

Current Attempts to Measure Pain: Ratings

For an awake, conscious, and cognitively able individual, the most common way to measure pain is by rating intensity using a visual analog (VAS), numeric rating (NRS), or verbal rating (VRS) scale, but the sensitivity and robustness and reliability of these measures is low, even with training (Smith et al., 2016). Subjective ratings alert us to the patient's report of the presence of pain, but it can be challenging to verify whether the report is genuine or not. Capturing pain qualities on a range of single-item descriptors is also difficult; therefore, multidimensional outcome measures are used to try to reveal the mix of sensory and affective pain components, but they are empirically based and reveal little about the specific nature or degree of the pain. Helpful disease-specific scales have also been developed (e.g., DN4 and PainDetect for neuropathic pain) and their specificity, sensitivity, and reliability are being validated (Bouhassira et al., 2005; Freynhagen et al., 2006; Haanpää et al., 2011).

The subjective assessment of pain—whether acute or chronic—can capture if pain is present, some degree of its severity and duration, and, probably most reliably, where anatomically it feels to be occurring. Additional features of the pain experience can also be captured, such as if it is spontaneous or evoked, intermittent or continuous, deep or superficial. However, the presence of pain as a common endpoint tells us very

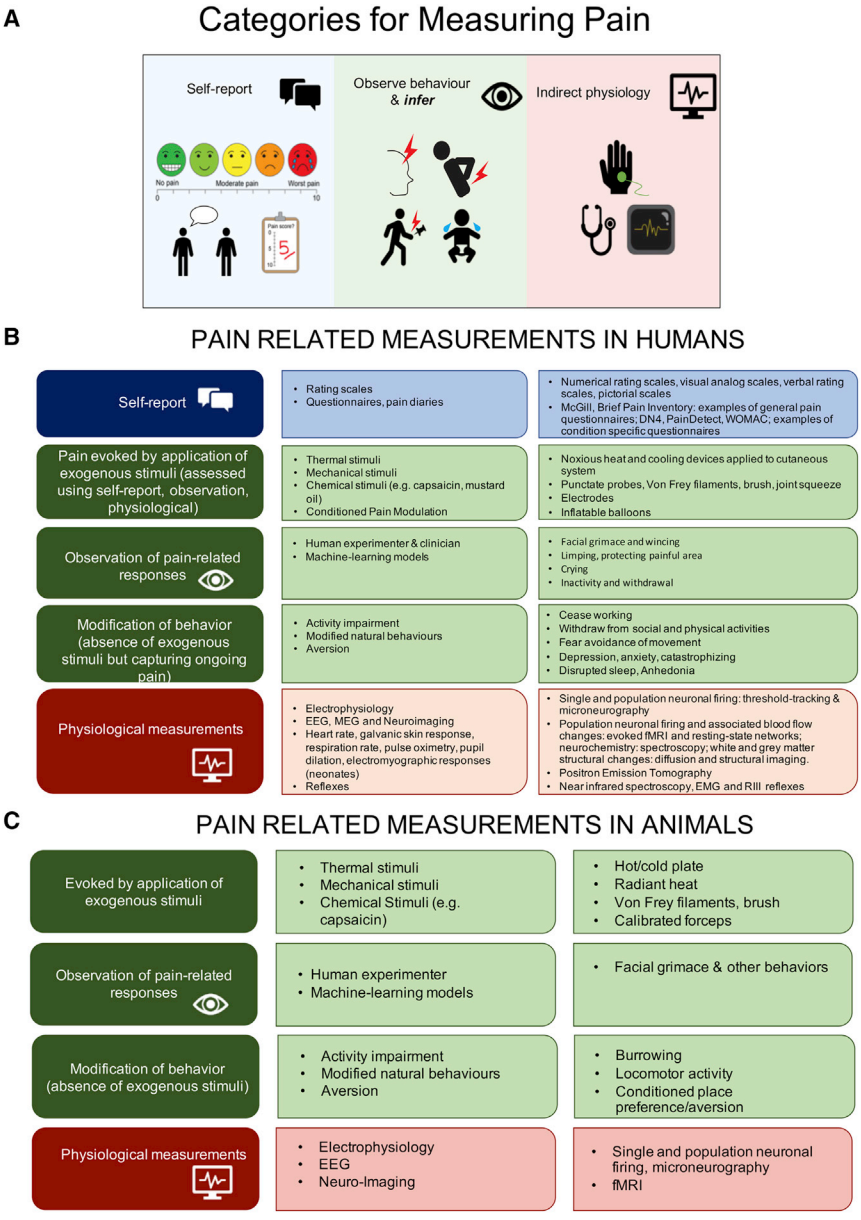

Figure 2. Current Pain Measurement Methods

(A) Categories for measuring pain.

(B) How we currently "measure pain" in humans. This falls into the following broad categories: (1) self-reports using rating scales/descriptors/questionnaires to exogenous stimuli or any ongoing and spontaneous pain; (2) observed measures of pain-like behavior; (3) indirect measures of physiology/autonomic changes. (2) are currently subjective and may suffer from cultural and social biases/influences, as well as a lack of sensitivity and specificity, but artificial intelligence/machine-learning methods may remove the subjectivity and identify more sensitive components and (3) are indirect assessments and make significant assumptions when relating these physiological measures to the underlying subjective state.

(C) How we currently "measure pain" in animals. Preclinical pain-related categories are shown with increasing complexity from top to bottom. The vast majority of testing still involves the use of exogenously applied thermal and mechanical stimuli (typically to the plantar surface of the hindpaws of rodents) and reflexive output measures. Other measures of behavior based on choice (avoidance of an activity or avoidance of an aversive condition) and complex measures of physiological function are being increasingly introduced. Finally, we anticipate that human observation on animal behavior will be supplanted by computer-based machine-learning approaches to identify specific pain-related behaviors, such as facial grimace.

little or arguably nothing about the presence of the multiple distinct pathologies that can drive pain. One other important issue with ratings is that high variance leads to reduced assay sensitivity, i.e., the ability of a randomized control trial to detect an active treatment. First identified by Harris and colleagues (Harris et al., 2005) in a trial studying the effect of milnacipran on fibromyalgia pain ratings, the effect of high pre-treatment pain variability was found to significantly predict a treatment response specifically in the placebo arm. Farrar and colleagues (Farrar et al., 2014) also found the same phenomenon when they analyzed clinical trials involving diabetic neuropathy (n = 1,226) and peripheral herpetic neuralgia (n = 1,514) patients. Both groups suggested that this problem be mitigated by excluding patients that showed the highest baseline pain rating variability (the top 20%–25%) from the trial. It was made clear that exclusions must be performed prior to randomization into the treatments to avoid violating internal validity. However, overall, even with these recommendations on how to manage the variability of pain ratings, we need to recognize that there is something problematic and too insensitive with how we're currently measuring pain and relief. As patient self-report remains the gold standard, development of more sensitive rating scales and even "composite" scales combining rating with physical functioning shows promise in this regard (Dworkin et al., 2012; Patel et al., 2018; Smith et al., 2016). However, it's time we argue for an additional strategy.

What If You Cannot Rate Pain: Indirect Measures to Assess Pain

Obviously animals cannot self-report pain, but neither can a baby, a demented elderly person, or an anesthetised or comatose patient. Crying, grimacing, and guarding are taken to reflect pain in non-verbal awake individuals and in animals, but the extent to which they reflect the *intensity* of the pain is not clear and specificity is low—babies cry also because they are hungry or uncomfortable. A baby's inability to verbally report pain poses an obvious barrier to pain management, and assessing pain in the elderly demented is equally problematic (Corbett et al., 2012). Responses to noxious stimulation can

Table 1. Biomarker Definitions with Present and Future Examples for Pain

Type of Biomarker	Definition	Pain Examples (top rows, present; bottom rows, future)
Diagnostic	To detect or confirm the presence of a disease or condition.	QST, EEG, intra-epidermal nerve fiber density
		Microneurography, neuroimaging, genetics
Monitoring	To assess status of a disease or condition or effect of a medical product by any biomarker that is measured serially.	QST, compound levels in plasma, CSF
		Neuroimaging, EEG, intra-epidermal nerve fiber density
Pharmacodynamic/ Response	To show that a biological response occurs in an individual exposed to a medical product.	QST, neuroimaging, EEG, changes in cytokines
		Specific mechanistic/biochemical pain drivers, intra-epidermal nerve fiber density
Predictive	To identify individuals with more likely than individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product.	Genetics
		Neuroimaging, EEG, intra-epidermal nerve fiber density
Prognostic	To identify likelihood of a clinical event, disease recurrence, or progression in patients with disease of interest.	Genetics
		Neuroimaging, EEG, intra-epidermal nerve fiber density
Safety	Measured before or after an exposure to a medical product to indicate likelihood, presence, or extent of toxicity.	Treatment related, e.g., sedation, tolerance, constipation, respiratory depression
		Neuroimaging, EEG
Susceptibility/Risk	Potential for developing a disease or medical condition	Genetics
		Neuroimaging, EEG

Adapted from "BEST (Biomarkers, Endpoints, and other Tools) Resource," a publication produced by the joint FDA-NIH Biomarker Working Group, December, 2016 (FDA-NIH Biomarker Working Group, 2016).

One early approach by neuroimagers has been to deconstruct the complex signal of the pain experience into constituent elements, whether as activity within a network or a particular set of brain regions for subsequent selective diagnostic monitoring or targeting (Ploghaus et al., 1999). Chronic pain is often accompanied by co-morbidities such as depression, anxiety, catastrophizing, fatigue, sleep disturbance, and poor cognition, and these all contribute to the pain experience. The relationship, therefore, between a patient's self-report of pain and their concurrent regional brain activity is complex, and studies mostly using acute pain stimuli in healthy volunteers have shown how these factors profoundly alter the neural processing of nociceptive inputs—almost acting as central neural amplifiers or attenuators of the experience (Berna et al., 2010, 2018; Eippert et al., 2009a; Ploghaus et al., 2001; Sprenger et al., 2012; Tracey et al., 2002; Vachon-Presseau et al., 2016a; Wiech and Tracey, 2009). Related, even the analgesic effect measured by self-report of a defined concentration of an intravenous dose of the fast-acting mu-opioid remifentanil can be enhanced by positive expectation and obliterated by negative expectation (Bingel et al., 2011). Such experiments reveal that subjective pain reports are highly malleable and that the processing of nociceptive inputs is modulated by factors like expectation, anxiety, and mood. We now know that as pain becomes chronic, these influences alongside neural expression of ongoing, spontaneous, or evoked pain in chronic pain patients become more important and complex (Harper et al., 2017; Hashmi et al., 2013; Vachon-Presseau et al., 2016b). If we can identify the presence of such modulating influences, this would greatly help assessment of the value of self-report, for example, in analgesic efficacy trials.

Neuroimaging Tools in Pain Research

An array of neuroimaging tools are used in the study of acute and chronic pain: functional magnetic resonance imaging (fMRI) and associated advanced magnetic resonance methods such as spectroscopy, quantitative cerebral blood flow, diffusion imaging for white matter tract delineation, structural MRI, and voxel-based morphometric analysis; positron emission tomography (PET); EEG; and magnetoencephalography (MEG). A growing body of work using EEG/MEG to characterize nociceptive and pain signals exists but is not discussed further here (see Ploner and May, 2017). Most functional brain imaging studies use blood-oxygen-level-dependent (BOLD) imaging and require a phasic stimulus to elicit a measurable signal. Measuring the brain's functional response to an applied noxious stimulus or provoking a clinical symptom, like brush-evoked allodynia or a painful joint squeeze, while producing a painful experience also recruits regions of the brain contributing to the entire multidimensional experience, including attention, fear, and anxiety. While the whole network is a reflection of the multidimensional experience, giving a drug or providing an intervention that produces changes only in a component of the experience may not target those elements related to nociceptive processing or pathogenic mechanisms. For example, midazolam, a drug targeting anxiety, attenuates brain activity during pain, but this should not be interpreted therefore as "analgesic." A novel paradigm design that identified brain regions related to pain anticipation/anxiety rather than receipt of nociceptive afferents (Ploghaus et al., 1999) revealed it was the anticipatory/anxiety-related and not nociceptive brain regions that were preferentially modulated by the drug (Wise et al., 2007). Other research has further dissected the particular roles brain regions subserve in the multidimensional pain experience (Woo

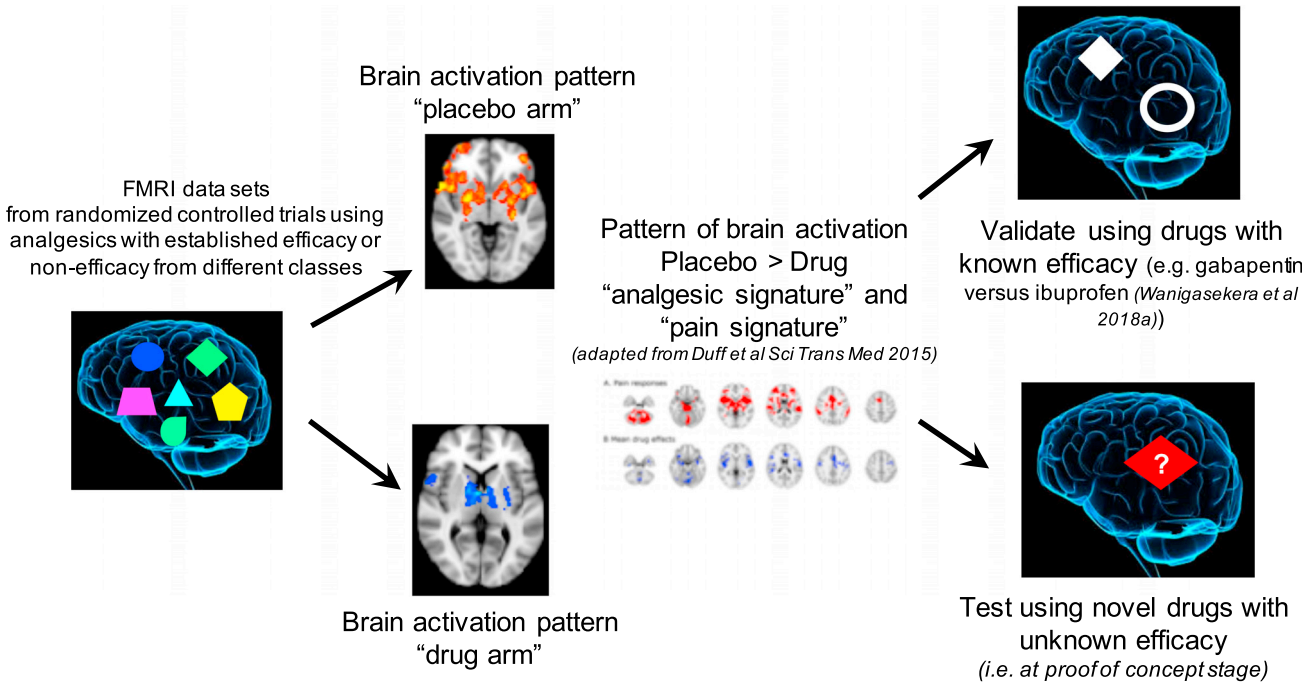

Figure 5. Machine Learning for Analgesic Drug Development
Illustration of the Pain-Analgesia signature being updated, validated, and potentially used for novel analgesic drug development.

(n = 24) from a double-blind, randomized, placebo-controlled, three-way crossover study in a healthy volunteer model of central sensitization (not used in the database and signature generation), the reduction in the brain response to painful stimuli by gabapentin could be correctly distinguished from placebo in 79% of individuals (p = 0.003) using the signature. In contrast, responses following ibuprofen (non-efficacious in this model and so a negative control) could not be distinguished from placebo (45%, p = 0.72). In the assessment for analgesic efficacy, the classifier correctly identified the gabapentin arm in 17 of 24 subjects (p = 0.03), whereas for ibuprofen discrimination was below chance (p = 0.92). Specific analgesic compounds may have distinct signatures; a classifier trained on an independent study of gabapentin reliably identified gabapentin (p = 0.03) while classifiers trained on studies of the opioid remifentanil failed to identify gabapentin (p = 0.5) but successfully identified the effects of remifentanil (Wanigasekera et al., 2018a). Such studies highlight the potential for this approach to aid analgesic drug development in an unbiased way. Figure 5 illustrates the approach.

Cautionary Note about Machine-Learning Approaches in Pain

These approaches, while promising, rely on the self-report to train the classifier (e.g., pain or no pain), and additional ways to classify training datasets without self-report would be of interest. If a patient complains of pain and a neuroimaging biomarker confirms its presence, then this needs to be regarded seriously even if a cause for the pain cannot be defined (e.g., idiopathic pain). Furthermore, emotional pain is as relevant to the patient and should not be treated as "second class" pain despite being challenging to understand and diagnose. ML methods that generate emotional pain signatures/biomarkers, provided they have specificity and sensitivity, would be helpful diagnostically in this regard (Krishnan et al., 2016; Woo et al., 2014). However, the brain decoding of thoughts, feelings, and perceptions, while offering unprecedented opportunities scientifically (Kragel et al., 2018), might have serious societal and personal/legal consequences, and caution is advised to avoid misuse of such data. For example, what to do if there is no neuroimaging pain biomarker/signature but the patient complains of pain—should the subjective self-report usurp the imaging data or vice versa? These precise issues have been discussed by a recent taskforce (Davis et al., 2017).

Neuroimaging-Based Biomarkers of Pathogenic Mechanisms

Neuroimaging can generate a suite of potential biomarkers of mechanisms driving pain as verified in preclinical models. Structural, functional, and neurochemical changes, particularly when combined, all have potential as composite pain biomarker signatures.

Anatomical Changes

Changes in gray matter volume (voxel-based morphometric increases and decreases) and abnormalities in white matter and brain connectivity are observed in individuals with different chronic pain conditions (Apkarian et al., 2004; Tatu et al., 2017; Wartolowska et al., 2012), with some predictive of the conversion from acute to chronic back pain (Vachon-Presseau et al., 2016b). However, these anatomical changes reverse upon resolution of the pain condition and so might relate to co-morbid features that also resolve upon cessation of pain (Gwilym et al., 2010;