Preclinical Assessment of Pain: Improving Models in Discovery Research

Tamara King and Frank Porreca

Abstract To date, animal models have not sufficiently "filtered" targets for new analgesics, increasing the failure rate and cost of drug development. Preclinical assessment of "pain" has historically relied on measures of evoked behavioral responses to sensory stimuli in animals. Such measures can often be observed in decerebrated animals and therefore may not sufficiently capture affective and motivational aspects of pain, potentially diminishing translation from preclinical studies to the clinical setting. Further, evidence indicates that there are important mechanistic differences between evoked behavioral responses of hypersensitivity and ongoing pain, limiting evaluation of mechanisms that could mediate aspects of clinically relevant pain. The mechanisms underlying ongoing pain in preclinical models are currently being explored and may serve to inform decisions towards the transition from drug discovery to drug development for a given target.

Keywords Ongoing pain · Affective · Motivational behaviors · Translation

Contents

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Pain in humans is a multidimensional experience with cognitive, motivational, and sensory components (Melzack and Casey 1968). Nociceptive pain, typically resulting from traumatic injury (e.g., bone fracture), serves protective functions including escape/avoidance of the pain-generating stimulus and promotes recuperative and protective behaviors to facilitate healing (Costigan et al. 2009). Injuries can sometimes lead to chronic pain that reflects maladaptive plasticity of the nervous system. Unlike nociceptive pain, chronic pain does not offer survival advantages and is often associated with pain that occurs in the absence of external stimuli (ongoing or spontaneous pain), from normally innocuous stimuli (allodynia) and with enhanced and longer lasting pain due to normally painful stimuli (hyperalgesia). Examples of chronic pain states include chronic non-malignant inflammatory (e.g., low back pain) and neuropathic (e.g., post-herpetic neuralgia) pain conditions as well as multimodal conditions such as cancer pain. Additionally, dysfunctional pain can occur in the absence of apparent tissue injury (e.g., fibromyalgia) (Clauw 2009; Goldenberg 2009).

Current treatments for pain continue to rely largely on non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, therapies that have been in existence across millennia. These drugs remain the gold standard for pain management, but are associated with a large array of adverse side effects that can compromise the patient's quality of life. This limits the therapeutic goal of providing complete pain relief by limiting dosing to effect, thus impairing the beneficial outcome of pain management. Advances in pain therapeutics are needed and depend on understanding of the neurobiology associated with specific pain conditions. Animal models with apparent relevance to clinical pain conditions have been developed and have informed the basics of our understanding of mechanisms associated with pain syndromes. While our knowledge of biological mechanisms of pain has been immensely aided by the use of animal models, there is increasing concern that these models are not sufficiently "predictive" to gain insight into mechanisms relevant to the human experience of pain (Vierck et al. 2008; Mogil 2009; Mao 2012; Percie du Sert and Rice 2014). Thus, the disproportionate lack of availability of new treatment strategies relative to our gains in understanding the neurobiology underlying pain have been fairly or unfairly linked to a failure of animal models to capture essential features of clinical pain. It should be noted that the issue of translation of mechanism to novel therapy is complex and the impediments associated with preclinical models represent only one of many hurdles. While it is essential to recognize the limitations of animal models in providing insights into the multidimensional human experience of pain, it is also important to recall their contribution to the tremendous advances in the understanding of sensory

neurobiology including the molecular underpinnings of transduction, transmission, and modulation in response to stimuli that typically elicit sensations of pain.

Most preclinical studies of pain have emphasized output measures that rely on responses to evoked stimuli (Vierck et al. 2008; Mogil 2009). While such stimuli engage the nociceptive pathway and likely accurately reflect the mechanisms associated with acute nociceptive pain, these reflexive responses are unlikely to capture components of pain that are most relevant to clinical chronic pain syndromes. Reflexive behaviors can often be observed in decerebrated animals (Woolf 1984) and do not require learning (Vierck et al. 2008), an essential feature of physiological pain. For this reason, investigators involved in preclinical pain research have developed a number of novel strategies aimed at capturing features of pain that might have increased translational relevance. Such approaches are generally intended to measure features of pain without the need for an evoked reflexive withdrawal response. In this review, we highlight some of the recent advances in how "pain" is measured in the preclinical setting.

1 Preclinical Studies: Animal Models

Animal models are not intended to mimic the human pain experience. Many models have been developed that allow measurement of neurochemical and neurophysiological mechanisms of nociception and of behavioral responses that likely have relevance to aspects of pain in humans. Models allow for evaluation of output measures before (baseline readings) or at multiple time points following an injury for testing of mechanistic hypotheses that form the basis of novel chemistry and drug discovery. Mogil has suggested that a preclinical pain model is comprised of three basic components: the subjects, the assay, and the outcome measure (Mogil 2009). Each component requires careful consideration in order to optimize potential translational value to the proposed human pain syndrome being modeled.

2 Preclinical Studies: Subjects

The predominant subjects used in preclinical studies within basic science focusing on mechanisms underlying pain, as well as for drug development and testing of proof of concept for improved analgesics are rodents, especially rats and mice. Subjects are chosen to elicit reliable and reproducible responses to represent the pain state of interest and for this reason, most experiments are performed in only a limited number of rodent strains in laboratories around the world. However, it is known that many strains do not respond well to injuries presumed to be eliciting pain syndromes (e.g., nerve injury models of neuropathic pain) (Mogil et al. 1997, 1998, 1999a, b; Yoon et al. 1999). This is especially notable given observations that injuries in humans can lead to chronic pain, but in most cases they do not.

Thus, in patients, the incidence of chronic pain resulting from amputation or coronary artery bypass surgery is estimated to be approximately 30–50 % while Caesarean section or inguinal hernia repair produces an incidence of chronic pain of 10 % (Kehlet et al. 2006). Thus animal subjects that do not develop chronic pain in the setting of injury are likely to more accurately reflect the human condition and important mechanistic insights may be gained by comparisons across strains within a species. Ethical and practical considerations may limit widespread evaluation of animal strains that are "resistant" to the development of chronic pain. Nevertheless, strains demonstrating variable outcomes in response to injury may provide important information regarding mechanisms that are necessary, but not sufficient, for driving pain, chronification of pain as well as for pain relief (Mogil et al. 1997, 1998, 1999a, b; Yoon et al. 1999; De Felice et al. 2011).

3 Preclinical Studies: Pain Assays

Historically, the first pain assays used were observations of behavioral responses to acute administration of a noxious stimulus to the hindpaw, tail, or abdomen. The measured behaviors are typically reflexive withdrawal from the noxious stimulus, or other simple behaviors (e.g., writhing, flinching, licking) that can be easily observed and scored. These assays relate to acute noxious experience in humans, for example touching a hot stove, and have served to elucidate many of the basic neurobiological mechanisms that underlie transduction, transmission, and modulation of acute pain states (Basbaum et al. 2009; Woolf 2011; von Hehn et al. 2012). However, their relevance to persistent or chronic pain states typically found in the clinical setting is limited.

Animal assays of persistent or chronic pain often involve the induction of inflammation and/or nerve injury. These assays are artificial by design, and meant to reveal mechanisms driving pain in the experimental setting. These assays have been successful in dissociating biological mechanisms likely to be associated with inflammation or nerve injury (Schaible et al. 2011; Xu and Yaksh 2011; von Hehn et al. 2012) and provide insight into time-dependent mechanisms of some clinical syndromes. Persistent pain can be elicited by localized administration of noxious inflammatory agents (e.g., capsaicin, formalin, mustard oil), resulting in immediate behavioral responses such as flinching and licking and associated with neurochemical and neurophysiological changes within the spinal cord and the brain. Other agents (e.g., carrageenan, CFA) have diminished immediate effects, but produce longer lasting responses in peripheral tissues and in the central nervous system (CNS) that are characterized behaviorally as hypersensitivity to evoked stimuli (e.g., thermal and tactile stimuli). Such hypersensitivity likely reflects mechanisms of peripheral and central sensitization that can be explored to reveal plasticity and adaptive responses to noxious stimuli (Woolf 2007, 2011). Multiple assays of nerve injury have also been developed with differential patterns of hypersensitivity to evoked stimuli possibly reflecting different driving mechanisms

(Dowdall et al. 2005; Campbell and Meyer 2006; Mogil 2009). The differences between these assays may provide insight into specific injury-induced changes that may reflect clinical observations of differences in pain phenotype. This possibility is supported by differences in pain phenotype characterized clinically by the German Neuropathic Pain Network suggesting the need for individualized treatment strategies in individual neuropathic pain patients (Baron 2006; Backonja and Woolf 2010; von Hehn et al. 2012).

The assays described above are mostly designed to capture mechanisms driving persistent inflammatory pain. In addition, assays have been developed that are designed to mimic, at least in part, disease processes that can produce pain including diabetes, bone cancer or chemotherapy-induced neuropathic pain. Additionally, assays have now been characterized to gain insight into disease-specific mechanisms associated with migraine, bone fracture, osteoarthritis, low back pain, spinal cord injury, pancreatitis, oral cancer, bowel pain, and others (Kesslak and Keirstead 2003; Vera-Portocarrero et al. 2003; Rosenzweig and McDonald 2004; Freeman et al. 2008; Bove et al. 2009; Meng et al. 2011; Okun et al. 2012; Farrell et al. 2014; Mantyh 2014a, b). These assays allow the examination of time-dependent mechanisms associated with the progression of the disease as well as the discovery of disease modifying treatments that may influence pain. For example, Denosumab, a human IgG2 monoclonal antibody with affinity and specificity for human RANKL, has been used to treat cancer-induced remodeling of the bone and was shown in clinical trials to have a strong consequent effect on pain (Honore et al. 2000; Luger et al. 2001; Lipton and Balakumaran 2012; Cleeland et al. 2013). An example of a potentially disease modifying mechanism is anti-NGF antibodies that have shown clinical efficacy for back pain and osteoarthritis (Seidel et al. 2013). Preclinical studies demonstrated that anti-NGF antiserum blocks thermal and tactile hypersensitivity following nerve injury (Ugolini et al. 2007; Wild et al. 2007), fracture pain, and cancer-induced bone pain (Halvorson et al. 2005; Sevcik et al. 2005; Koewler et al. 2007). Another important example is the discovery and development of anti-CGRP antibodies that have been clinically validated for migraine prophylaxis (Peroutka 2014). This work was built on preclinical observations of the cardinal role of CGRP in migraine (Meng et al. 2011; De Felice et al. 2013) and clinical observations that CGRP receptor antagonists were effective in migraine (Olesen et al. 2004; Doods et al. 2007).

4 Preclinical Studies: Pain Outcome Measures

4.1 Reflexive Withdrawal

The most commonly used behavioral measures are easily scored and rely on recording reflexive withdrawal of a limb, usually a hindpaw or the tail in rats and mice (e.g., tail-flick test), in response to exposure to a noxious (e.g., heat, high intensity mechanical) or non-noxious (e.g. tactile) stimulation in an inflammatory

or injured condition. These behaviors correspond to responses to acute nociceptive stimuli in humans. These assays allow for relatively rapid analysis of time-course of drug effect as well as determination of dose response curves for test compounds (i.e., PK/PD relationships). Other measures rely on spinal-bulbospinal reflexes in response to a noxious stimulus (e.g., hot plate test, formalin, acetic acid), such as licking, flinching, biting or scratching, abdominal stretching, or ultrasonic vocalization as measures of pain. However, many of these responses persist in decerebrated animals (Woolf 1984; Xu et al. 1992), indicating that they do not require cortical processing of the nociceptive stimuli, a critical aspect of the pain experience. Therefore, the relevance to understanding mechanisms promoting ongoing pain in humans is questionable (Costigan et al. 2009; Mogil 2009). Of note, it should be emphasized that pharmacological mechanisms that modulate nociception at the spinal level have shown a very strong correlation to efficacy in humans (e.g., opioids, α2 adrenergic agonists, N-type calcium channel blockers, local anesthetics) and that spinal delivery of drugs is an important, and in some cases necessary strategy for management of pain in many patients (Mercadante et al. 2012; Pope and Deer 2013).

Reflexive measures remain important for drug discovery and for initial proof of concept. Their predictive value for translation across species for spinal modulation of acute pain is unquestionable. However, these measures may not sufficiently capture dimensions of pain that are important in humans including affective or cognitive components (Melzack and Casey 1968; Fields 1999). This deficiency has led to increased efforts to develop novel measures based on existing assays in an effort to improve the drug discovery process and to enhance translation of mechanism identified in preclinical studies to clinical settings.

5 Measures of Use and Function

Decreases in use or function of an injured body part likely reflect the presence of either ongoing pain or of tenderness to evoked stimuli. For example, outcome measures in preclinical assays of osteoarthritis (OA) usually rely on evaluation of relative weight bearing of the rat hind limbs following injection of chemicals (e.g. monoiodoacetate, MIA) or surgical damage to the knee (Schott et al. 1994). Whether weight bearing differences reflect evoked hypersensitivity or the presence of ongoing pain is not completely clear. Okun and colleagues (Okun et al. 2012) demonstrated that systemic administration of NSAIDs could reverse weight bearing in a preclinical assay of advanced OA pain but failed to block ongoing pain (see below). Guarding has been used as an outcome measure in assays of post-operative, inflammatory and cancer pain (Schwei et al. 1999; Djouhri et al. 2006; Xu and Brennan 2009, 2010). Notably, guarding behavior has been linked to increased spontaneous activity of nociceptive fibers and dorsal horn neurons in rats with hindpaw incision of skin and deep tissue, linking the behavior with spontaneous neural activity associated with nociception (Xu and Brennan 2009, 2010).

Gait patterns have also been employed in assays of cancer-induced bone pain and in nerve injury-induced neuropathic pain (Schwei et al. 1999; Vrinten and Hamers 2003). These measures provide information on time-course of pain related mechanisms and potential recovery to pre-injury states. However, whether these measures accurately reflect the presence of ongoing pain or hypersensitivity resulting from ambulation is not clear. Finally, these behaviors may also reflect learned avoidance of activities that evoke pain in the hypersensitive injured area.

5.1 Depression of Voluntary Behaviors

Measures of pain-induced suppression of voluntary behaviors as indicators of a more global impact of pain on the animal have been recently introduced and are in the process of being characterized. One approach has been to assess behavioral outcome measures that are ethologically relevant to a social and prey species. These include species-specific behaviors, such as burrowing behaviors in the laboratory rat, proposed to be a measure of pain-induced suppression of an ethologically relevant behavior (Andrews et al. 2012; Huang et al. 2013; Lau et al. 2013; Rutten et al. 2013a, b). Several studies have demonstrated injury-induced decreases in these behaviors that can be reversed by treatments used in the clinical setting. For example, gabapentin reverses nerve-injury (tibial nerve transection) induced reductions in burrowing, and ibuprofen, naproxen, gabapentin, and morphine, reversed inflammation (CFA)-induced reductions in burrowing (Andrews et al. 2012; Rutten et al. 2013a, b). A suggested advantage of burrowing over evoked reflex testing is that drug-induced sedation or motor impairment would further dampen the behavior, rather than produce an increase as would be expected with analgesics, reducing the possibility of a false positive in these instances. Initial characterization of burrowing behavior revealed sensitivity to a wide array of conditions and the behavior was reported to be altered by "anything that affected the well-being of the animal" (Deacon et al. 2001; Guenther et al. 2001; Deacon 2006, 2009). Reductions in burrowing have been reported due to prion disease and Scrapie disease as well as in response to lipopolysaccharide, which induces nausea (Deacon et al. 2001; Guenther et al. 2001; Deacon 2006, 2009). Therefore, further characterization of this behavior is required to demonstrate that alterations in burrowing behavior are specific to pain and not the consequence of other factors such as anxiety, stress, or illness.

Similar confounds are associated with suppression of exploration, particularly of open areas such as the center of an open field. Recent studies have demonstrated increased thigmotaxis, or increased time spent in the peripheral zone close to the walls, in preclinical assays of herpes zoster and nerve injury-induced pain (Huang et al. 2013). Notably, however, this behavior was altered by anxiolytic drugs, such as diazepam, (Huang et al. 2013), raising concerns of specificity of mechanism of action to pain (Ablin and Buskila 2013; Borsook et al. 2013; Finan and Smith 2013; Goesling et al. 2013).

Other studies have reported pain-induced depression of voluntary wheel running behavior (Stevenson et al. 2011; Cobos et al. 2012). Cobos and colleagues recently demonstrated a notable decrease in voluntary wheel running in rats following injection of CFA into the hindpaw (Cobos et al. 2012). An important factor is that the diminished wheel running was observed only following bilateral injection of CFA, likely reflecting the ability of animals that are quadrupeds to compensate by running on uninjured legs. Cobos and colleagues characterized the effects of therapeutically available agents commonly used to treat inflammation-associated pain in patients in the wheel running measure. Opioids were the most effective in blocking the CFA-induced diminished wheel running, followed by corticosteroids and then NSAIDs, wherein diclofenac was more effective than ibuprofen or celecoxib (Cobos et al. 2012). These observations are consistent with the relative efficacy of these drugs in patients (Cobos et al. 2012). An important observation in this study is that the effective dose of ibuprofen that produced recovery of wheel running behavior was approximately 20-fold lower than the dose required to reverse evoked tactile hypersensitivity (Cobos et al. 2012). This is consistent with the 20-fold higher plasma concentrations observed at doses required to reverse tactile hypersensitivity in preclinical assays compared to therapeutic plasma concentrations in humans (Cobos et al. 2012). The higher sensitivity of voluntary wheel running to drug effects compared to reflexive withdrawal measures, such as tactile hypersensitivity, has been suggested to more accurately predict efficacious drug doses in humans. It was acknowledged by the authors that the reduction in wheel running could be due to ongoing pain, injury-induced touch sensitivity, or to avoidance of activity that produced pain (e.g., evoked pain) (Cobos et al. 2012). However, this situation was suggested to be similar to humans where a painful condition may induce a loss of motivation and avoidance of activities that may evoke pain in the injured area or aggravate pain already there (Cobos et al. 2012) reflecting the "everyday pain experience". As this measure required inflammation of both hindpaws to produce a measurable decrease in wheel running that allowed analysis of drug effects or creation of dose response curves, its use may be limited in other types of pain (e.g., nerve injury, trigeminal pain).

6 Affective Pain Measures

Pain in humans is assessed on the basis of its "intensity" by self-report using a variety of rating scales (e.g., VAS, numerical rating scale, etc.). Very few studies assess sensory thresholds in pain patients as a primary endpoint, though such changes are well documented (Rolke et al. 2006; Maier et al. 2010; Pfau et al. 2014). Pain is fundamentally aversive and it is this feature that is the main complaint of patients (Fields 1999; Vierck et al. 2008). Our relative inability to study mechanisms mediating affective, or unpleasant, dimensions of pain in the preclinical setting is likely to have been an important barrier to the discovery of

new medications. Until recently, assays that focused predominately on the unpleasant/affective component of chronic pain were lacking. Collective preclinical success in measuring affective components of pain will likely provide critical complementary information to studies emphasizing sensory neurobiology.

6.1 Measuring the Affective/Motivational Aspect of Pain

A proposed measure of the affective component of pain is the facial grimace scale developed by Mogil and colleagues (Langford et al. 2010; Sotocinal et al. 2011; Matsumiya et al. 2012). Facial expressions were characterized by comparing videos of mice before or after administration of acetic acid known to induce abdominal constriction (Langford et al. 2010). The changes to facial features including orbital tightening, nose bulge, cheek bulge, ear position, and whisker change were coded on a 3-point scale as a measure of pain (Langford et al. 2010). This scale was applied across a variety of preclinical assays ranging from transient pain lasting seconds to pain models lasting minutes to hours to chronic pain models associated with pain lasting days, weeks, months, or longer, with testing occurring 1, 7, or 14 days following injury. While nociceptive stimuli of moderate duration, lasing 10 min to 12 h produced changes within the facial grimace scale (Langford et al. 2010), assays of longer duration such as nerve injury-induced pain did not produce changes in facial grimace (Langford et al. 2010). Lesions of the insular cortex were found to block pain-induced facial grimace but lesions of the amygdala and the anterior cingulate cortex, areas implicated in affective and motivational components of pain by imaging studies (Rainville et al. 1997; Rainville 2002) as well as preclinical studies (Johansen et al. 2001; Nandigama and Borszcz 2003; Johansen and Fields 2004; LaGraize et al. 2004, 2006; Harte et al. 2011; Qu et al. 2011) failed to do so (Langford et al. 2010).

Vocalization after-discharge in response to noxious tail shock has been characterized as a measure of pain affect by Borszcz and colleagues (Borszcz 1993, 1995; Nandigama and Borszcz 2003; Harte et al. 2011; Spuz and Borszcz 2012). Electrical shock-induced avoidance was found to correlate with induction of vocalization after-discharge, but not with shock-induced spinal reflexes (Borszcz 1993) suggesting correspondence with the motivational aspects of the painful shock. Administration of opioids suppressed vocalization after-discharge at doses significantly lower than those required to block vocalization during shock or the spinal motor reflex; however, this was also true with the anxiolytic diazepam (Borszcz et al. 1994). Lesions of the rostral anterior cingulate cortex (rACC) and medial thalamus were found to block shock-induced vocalization after-discharge linking this measure to affective components of pain (Harte et al. 2011). Other key brain sites that play a role in this measure include the amygdala (Nandigama and Borszcz 2003; Spuz and Borszcz 2012), periaqueductal gray (PAG) and ventral medulla (Borszcz 1995), hypothalamus (Borszcz 2006), ventral tegmental area (VTA) (Kender et al. 2008), and parafascicular nucleus (Harte et al. 2005).

Four recently developed measures attempt to capture pain affect in assays of chronic pain. These measures exploit the motivational behaviors elicited by ongoing pain following tissue injury. The place escape avoidance paradigm (PEAP) measures the motivation to escape and avoid unpleasant painful stimulation applied by the experimenter by withdrawing, or moving away from the stimulus, and is based on the assumption that if an organism escapes and/or avoids a noxious stimulus, then the stimulus is aversive to the organism (LaBuda and Fuchs 2001; LaGraize et al. 2004; Fuchs and McNabb 2012; Uhelski et al. 2012). Conditioned place aversion (CPA) to a chamber paired with normally non-noxious tactile stimulation following nerve injury or inflammation injury demonstrates the negative affective component of repeated tactile hypersensitivity (allodynia) (Hummel et al. 2008). Notably, this tactile stimulation-induced CPA was reversed by doses of morphine that did not produce analgesia (Hummel et al. 2008). The self-administration paradigm measures the reinforcing effects of drugs that induce pain relief, and demonstrates that animals in pain will work to acquire pain relief (Martin et al. 2006, 2007). Similarly, conditioned place preference (CPP) measures negative reinforcement associated with removal of the aversive component of pain and corresponding reward from pain relief (Navratilova et al. 2012). These measures have been characterized within several pain assays using various drugs and manipulations known to be clinically effective in modulating the aversiveness of pain in humans (LaBuda and Fuchs 2001; Martin et al. 2006; Hummel et al. 2008; King et al. 2009; De Felice et al. 2013). Moreover, these measures allow dissociation of the affective/motivational and sensory components of pain.

King et al. demonstrated that pairing a context with an effective and rapidly acting pain relieving treatment can produce single-trial CPP (King et al. 2009, 2011; Qu et al. 2011). Pain has a strong emotional component exemplified by its unpleasantness. The unpleasantness of pain serves as the "teaching signal" that leads to avoidance of stimuli that can potentially produce damage to tissues (Johansen et al. 2001; King et al. 2009). Chronic pain can be envisioned as an aversive state that provides strong motivation to seek relief. Moreover, pain relief is rewarding, as indicated by human imaging demonstrating that offset of an acute pain stimulus produces a positive BOLD signal in the nucleus accumbens, an area associated with reward-aversion processing in humans (Becerra and Borsook 2008). Reward achieved from removal of an aversive stimulus produces "negative reinforcement" and is applicable to alleviation of an aversive state induced by chronic pain. Pairing pain relief with a distinct context increases time spent in that context (King et al. 2009). Importantly, conditioned place preference (CPP) to a context that is paired with pain relief is only observed in rats with injury, demonstrating the "unmasking" of ongoing or spontaneous experimental inflammatory or neuropathic pains (King et al. 2009, 2011; Qu et al. 2011).

The concept that pain relief may induce conditioned place preference (CPP) was proposed by Sufka (1994). In this initial study, CPP was observed in rats with hindpaw injection of complete Freund's adjuvant (CFA) following multiple learning (e.g., conditioning) trials with systemic MK-801 but not with indomethacin. Additionally, systemic morphine produced CPP in both CFA and saline-treated rats

suggesting that the effect might not be specific for pain and could result from the inherently rewarding properties of the drug rather than the reward associated with pain relief (Sufka 1994). Reasons for these variable results are not clear but could be related to uncertain kinetics associated with systemic delivery where possible changes in pain state occur at times at which associations with the context are not easily made. Validation of single trial CPP as a measure for detecting ongoing, or non-evoked, pain, has been performed with rats across preclinical assays of experimental nerve injury (i.e., spinal nerve ligation or spared nerve injury) (King et al. 2009), inflammation-induced pain (Okun et al. 2011); incision pain (Navratilova et al. 2012), and in a preclinical assay of osteoarthritis pain (Liu et al. 2011; Okun et al. 2012). In all cases, the route of administration of the treatments were carefully chosen to avoid confounding influences of pharmacokinetics or direct stimulation of the reward pathways.

One important aspect of developing measures with potential clinical translation abilities is validation through comparison with clinical findings and reports. Consistent with observations in the self-administration measure, clonidine, but not adenosine, delivered spinally produced CPP selectively in rats with nerve injury and not in sham operated controls (Martin et al. 2006; King et al. 2009). Such observations support specificity for pain-induced motivational behaviors revealing the presence of ongoing pain and allowing for the study of underlying mechanisms. Notably, spinal administration of ω-conotoxin was also effective in producing CPP selectively in animals with nerve injury. These preclinical observations are consistent with human observations. In a small clinical trial, spinal clonidine was effective against ongoing neuropathic pain (Eisenach et al. 2000; Wermeling and Berger 2006), whereas adenosine blocked secondary hyperalgesia, but did not block ongoing pain (Eisenach et al. 2003). Additionally, ω-conotoxin is marketed as ziconotide (Prialt), as an effective pain reliever in humans. The findings that spinal clonidine and ω-conotoxin produce CPP preclinically suggest that this measure could facilitate translation of new therapeutics.

Axotomy of the sciatic nerve has been useful as an assay for electrophysiological evaluation of injured nerves but has been difficult to study behaviorally as it produces denervation of the hindpaw (Devor 1991, 2009). As a consequence of denervation, it has been difficult to conclusively demonstrate whether ongoing pain is actually present since: (a) ectopically discharging axons in a neuroma are not identified nociceptors, (b) evoked behavioral hypersensitivity following axotomy is difficult or impossible to measure due to denervation, and (c) axotomy-induced autotomy or self-mutilation might be due to loss of sensation in the limb rather than pain (Rodin and Kruger 1984; Devor 1991). However, CPP can be demonstrated selectively in animals with either partial or complete hind paw denervation following either spinal clonidine or RVM lidocaine confirming an aversive state likely reflecting spontaneous neuropathic pain (Qu et al. 2011). These data also suggest that spontaneous pain arises from injured nerve fibers, consistent with findings in humans (Qu et al. 2011). Additionally, these observations in animals with complete denervation of the hind paw also provide an important control, eliminating concerns for pain resulting from tactile stimulation potentially arising

from ambulation within the testing apparatus (Qu et al. 2011). These data cannot address, however, whether additional contributions to spontaneous pain, or evoked hypersensitivity, may result from uninjured, but abnormal adjacent fibers in partial nerve injury assays.

Using the CPP measure, a new preclinical assessment of advanced OA pain was recently reported. Advanced OA pain in patients is associated with a constant dull/aching pain punctuated by short episodes of often unpredictable intense pain (Hawker et al. 2008). Notably, patients with advanced OA pain are resistant to NSAIDs and must undergo joint replacement therapy (Hawker et al. 2008). Within an established and well-characterized preclinical measure of osteoarthritis in which monosodium iodoacetate (MIA) is injected into the intra-articular space of the knee joint, Okun and colleagues demonstrated that commonly used doses of MIA that produce weight asymmetry and hindpaw tactile hypersensitivity failed to elicit ongoing pain (Okun et al. 2012). A higher dose of MIA was required to elicit persistent ongoing pain that is characteristic of advanced OA pain (Liu et al. 2011; Okun et al. 2012). Notably, NSAIDs at a dose sufficient to block MIA-induced weight asymmetry failed to block ongoing pain, observations consistent with patients with advanced OA pain (Okun et al. 2012). Further, the MIA-induced ongoing pain was not blocked by either a TRPV1 or TRPA1 receptor antagonist (Okun et al. 2012), two molecular targets with compounds in clinical development for pain management, including for potential use for OA pain (Honore et al. 2009; Puttfarcken et al. 2010).

An important feature of the PEAP, CPA, self-administration, and CPP approaches is that they are based on the aversive aspect of the injured state. The unpleasantness of pain is perhaps the most important facet of the pain experience. An important aspect of validating these measures as reflecting the affective/motivational aspect of pain is assessing the role of the rACC. Early studies demonstrated that the affective and sensory components of pain are distinguishable, and rely on differential processing within cortical areas. Fields and colleagues initially demonstrated that lesions of the rostral, but not caudal, anterior cingulate cortex blocked CPA to a distinctive context paired with hindpaw formalin, but failed to block the formalin-induced licking and flinching of the formalin treated hindpaw (Johansen et al. 2001). Similar observations have been made in the PEAP and CPP measures of spontaneous/ongoing pain (LaGraize et al. 2004; Qu et al. 2011).

Using the PEAP assay, Fuchs and colleagues demonstrated that bilateral lesions of the ACC did not alter mechanical hypersensitivity induced by tight ligation of the L5 spinal nerve, but significantly attenuated the shift from the dark side of the chamber to the light side of the chamber in response to mechanical stimulation of the injured hindpaw (LaGraize et al. 2004). Further, morphine microinjection into the ACC caused an attenuation of place escape/avoidance behavior with no alteration in mechanical hypersensitivity (LaGraize et al. 2006). A similar dissociation in the affective and somatosensory aspects of nerve injury-induced pain was observed in the CPP measure of SNL-induced spontaneous pain. Lesions of the rACC failed to alter SNL-induced hypersensitivity, but blocked the

SNL-induced spontaneous pain (Qu et al. 2011). Notably, the rACC lesions did not alter CPP to a positive reinforcing agent such as cocaine (Qu et al. 2011).

The importance of limbic system structures in pain processing is supported by human brain imaging studies that have found a positive relationship between the self-reported unpleasantness of experimental pain with ACC activation (Rainville et al. 1997, 1999, 2002; Hofbauer et al. 2001; Rainville 2002). Indeed, the altered perception of unpleasantness in the absence of a change in stimulus intensity correlated with activity in the ACC, but not the somatosensory cortex (Rainville et al. 1997; Rainville 2002). Moreover, brain imaging studies studying basal (non-evoked) activity in patients with chronic neuropathic pain indicate increased activity in the insula and ACC without significant changes in the somatosensory cortices (S1 and S2) (Moisset and Bouhassira 2007), whereas brush evoked allodynia is predominately associated with changes in the lateral thalamus and S1, S2 somatosensory cortices rather than the ACC and insula (Peyron et al. 1998, 2002, 2013; Ducreux et al. 2006; Witting et al. 2006). In addition, patients with cingulotomies report diminished pain related unpleasantness, but discrimination of stimulus intensity or localization of the noxious stimulus was unaltered (Foltz and White 1962; Ballantine et al. 1967; Hurt and Ballantine 1974). The consistency between the preclinical observations of selective blockade of motivational aspects of pain without alteration of behavioral signs of evoked hypersensitivity with these clinical observations strengthen the argument that these assays are capturing affective components of injury-induced pain.

7 Enhancing Discovery Through Improved Animal Models

An animal model reflects variables of subjects, assays, and outcome measures, each of which contributes to the ultimate validity of conclusions about the clinical pain syndrome of interest. It is important that models undergo rigorous validation (e.g., lesion of pain pathways, selective response to clinically effective drugs) for specificity to pain rather than other related conditions (e.g., anxiety, depression). Lack of clarity that the measure is reflective of pain makes interpretation of mechanisms, circuits, and drug effects difficult. Reverse translation may be achieved through characterization of drug effects reflecting clinical experience; one example is the observation that corticosteroids were more effective in restoring CFA-suppressed wheel running compared to NSAIDs demonstrating corresponding efficacy across drug families (Cobos et al. 2012). There should also be evidence for corresponding efficacy across pain syndromes. For example, current treatment guidelines for inflammatory pain states recommend NSAIDs, acetaminophen, or local steroids while those for treatment of neuropathic pain recommend different classes of agents such as serotonin-norepinephrine reuptake inhibitors or pregabalin (Attal et al. 2006; Dworkin et al. 2007; Sarzi-Puttini et al. 2012; Whittle et al. 2012; Ablin and Buskila 2013). Another way to increase confidence in the predictive value of the animal model is through correspondence of clinical

observation of differential drug effects on evoked hypersensitivity and ongoing or spontaneous pain, as was observed in response to adenosine (Eisenach et al. 2003; Martin et al. 2007; King et al. 2009). Finally, the dose range of drug effects should correspond to clinically effective doses, as observed in ibuprofen-induced restoration of wheel running (Cobos et al. 2012), blockade of the PEAP response to mechanical stimulation following incision injury (LaBuda and Fuchs 2001), and blockade of CPA to tactile stimulation following nerve injury or carrageenan by non-analgesic doses of morphine (Hummel et al. 2008). In conclusion, it is clear that mechanistic differences exist between evoked behavioral responses of hypersensitivity and ongoing pain. The mechanisms underlying ongoing pain in preclinical models are currently being explored and may help in the process of filtering targets informing decisions to engage in the transition from drug discovery to drug development for a given target.

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