Preclinical Assessment of Candidate Analgesic Drugs: Recent Advances and Future Challenges


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ABSTRACT

In analgesic drug development, preclinical procedures are widely used to assess drug effects on pain-related behaviors. These procedures share two principal components: 1) a manipulation intended to produce a pain-like state in the experimental subject and 2) measurement of behaviors presumably indicative of that pain state. Drugs can then be evaluated for their ability to attenuate pain-related behaviors. In the simplest procedures, the pain state is produced by delivery of an acute noxious stimulus (e.g., a warm thermal stimulus), and the primary dependent measures focus on withdrawal responses or other nocifensive behaviors that increase in rate, frequency, or intensity in response to the noxious stimulus. This approach has been refined in two ways. First, new methods have been developed to induce more clinically relevant pain states. In particular, pain requiring clinical intervention is often associated with inflammation or neuropathy, and novel procedures have emerged to model these conditions and their ability to produce hypersensitive pain states, such as allodynia and hyperalgesia. Second, studies are incorporating a broader array of pain-related behaviors as dependent measures. For example, pain not only stimulates nocifensive behaviors but also suppresses many adaptive behaviors, such as feeding or locomotion. Measures of pain-suppressed behaviors can provide new insights into the behavioral consequences of pain and the effects of candidate analgesics. In addition, functional magnetic resonance imaging has emerged as a noninvasive tool for investigating changes in neural activity associated with pain and analgesia. Integration of these complementary approaches may improve the predictive validity of analgesic drug development.

Pain is a pervasive public health problem, and analgesic drugs play a central role in its treatment. Historically, the most widely used analgesics have included μ-opioid agonists such as morphine, anti-inflammatory steroids such as cortisone, and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin. Although these drugs are useful across a wide range of conditions, they are not uniformly effective, and undesirable side effects often limit their use. Consequently, one long-standing focus of drug discovery has been the search for novel analgesics.

Meaningful research on pain and analgesia depends on the development of validated procedures for identifying the presence of pain and quantifying its magnitude. Pain has been defined by the International Association for the Study of Pain (1979) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Analgesia is a selective reduction in pain without altering sensitivity in other sensory modalities. Thus, pain and analgesia are essentially subjective experiences, and their existence in humans is typ-
ically assessed using verbal reports. However, verbal reports are obviously not suitable for measuring pain and analgesia in animals, and this presents a challenge to preclinical research. How does one tell if an animal is in pain, and how does one tell if a candidate analgesic is effective in reducing that pain?

Assays of Acute Nociception

All procedures used in analgesic drug development share two principal components: 1) a manipulation intended to produce a pain-like state in the experimental subject and 2) measurement of behaviors presumably indicative of that pain state. For nearly a century, preclinical researchers interested in such issues as the genetics, neurobiology, and pharmacology of pain and analgesia have focused largely on withdrawal responses or other nocifensive behaviors that increase in rate, frequency, or intensity following the presentation of an acute noxious stimulus (Barbour and Maurer, 1920; Dykstra, 1985; Bennett, 2001; Le Bars et al., 2001; Mogil et al., 2001). The radiant-heat tail-flick test exemplifies this approach (D’Amour and Smith, 1940). In this procedure, which was based on methods originally used in humans, a rat’s tail is placed beneath a radiant heat source (i.e., a light bulb), the heat source is activated, and the primary dependent variable is the latency to tail withdrawal. Rapid tail withdrawal is considered evidence of “nociception” (i.e., the ability to detect a noxious stimulus), and nociception is thought to be functionally related to pain. Attenuation of the tail-withdrawal response by drugs is considered preliminary evidence of “antinociception,” which is thought to be related to analgesia.

The early evolution of this type of procedure was quickly influenced by the importance of two key independent variables: stimulus intensity and stimulus modality. As stimulus intensity increases (i.e., as temperature increases in assays of thermal nociception), the rate, frequency, or intensity of the evoked response also typically increases (Fig. 1a). In addition, the evoked response also becomes more resistant to attenuation by drugs. The value of this ability to modulate both evoked behaviors and drug effects by manipulating stimulus intensity rapidly became apparent, and preclinical studies of pain and analgesia often incorporate measures of

![Fig. 1. Shown are assays of acute thermal nociception (a and b) (E. Bilsky, G. Stevenson, J. Lowery and H. Martinez, unpublished data), inflammatory thermal hyperalgesia (c and d), and neuropathic thermal hyperalgesia (e and f) (T. W. Vanderah, unpublished data). a, effect of stimulus temperature on tail-withdrawal latencies in a warm-water tail-withdrawal procedure in male ICR mice. As temperature increased, tail-withdrawal occurred more rapidly, and latencies decreased. b, effects of the μ-opioid receptor agonist morphine and the NSAID ibuprofen (s.c., 30 min pretreatment) on tail-withdrawal latencies from water heated to 55°C. Data are expressed as % maximum possible effect (%MPE, with %MPE = [(test latency – baseline latency)/cutoff – baseline latency] × 100. Morphine produced dose-dependent antinociception, whereas ibuprofen was ineffective. c, time course of thermal hypersensitivity elicited by administration of 2% carrageenan into the hind paw of male Sprague-Dawley rats. Under baseline (BL) conditions before carrageenan injection, rats withdrew their paws from the thermal stimulus in approximately 20 s. Three hours after carrageenan administration, there was a significant decrease in thermal paw-withdrawal latencies, and this hypersensitivity was sustained for at least 7 h after carrageenan. d, effects of morphine and ibuprofen (subcutaneously, 60 min pretreatment) on carrageenan-induced thermal hyperalgesia. Data are expressed as %MPE, with %MPE = [(test latency – carrageenan baseline latency)/precarrageenan control latency – carrageenan baseline latency] × 100. Both morphine and ibuprofen were fully effective. e, time course of thermal hypersensitivity elicited by a neuropathic pain manipulation (spinal nerve ligation at the level of L5/L6) in male Sprague-Dawley rats. Under baseline conditions before nerve injury, rats withdrew their paws from the thermal stimulus in approximately 20 s. Hypersensitivity was apparent 1 day after nerve injury and was sustained for at least 7 days. f, effects of morphine and ibuprofen (subcutaneously, 60 min pretreatment) on nerve-injury-induced thermal hyperalgesia. Data are expressed as %MPE, with %MPE = [(test latency – neuropathic baseline latency)/(preneuropathic control latency – neuropathic baseline latency)] × 100. Morphine was fully effective, whereas ibuprofen was only weakly active.

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behaviors evoked by a range of noxious stimulus intensities (Dykstra, 1985; Bennett, 2001; Mogil et al., 2001). Moreover, by testing a range of stimulus intensities, the threshold intensity for evoking the target behavior can be determined, and this threshold can then serve as a dependent measure for evaluating drug effects (Negus et al., 1993).

Stimulus modality has also been extensively manipulated in assays of nociception. In addition to thermal stimuli, other commonly used modalities include electrical, mechanical, and chemical noxious stimuli (Dykstra, 1985; Bennett, 2001; Le Bars et al., 2001; Mogil et al., 2001). Electrical stimuli directly activate primary afferents, including nociceptors, whereas mechanical noxious stimuli physically deform tissue (e.g., by pinching or applying pressure via probes). As with thermal tests, the dependent measure in tests using electrical or mechanical stimuli is usually the latency to a withdrawal response or the threshold stimulus intensity for evoking a withdrawal response. Commonly used chemical noxious stimuli include intraperitoneal injections of dilute acetic acid or intraplantar injections of formalin. When chemical noxious stimuli are used, withdrawal from the stimulus is rarely possible, and the primary dependent variables are typically behaviors such as writhing or paw-flinching. The incidence of these behaviors can then be counted during a set observation period. It should also be noted that chemical noxious stimuli often produce initial features of acute pain followed by subsequent effects involving inflammatory processes. This issue will be discussed further in the next section.

There are both advantages and disadvantages to the use of acute nociception assays in analgesic drug development. Advantages include their technical simplicity and their utility for pharmacologic characterization of some classes of known analgesics, such as morphine-like opioid agonists (Coop, 2005). The most significant disadvantage of these procedures for analgesic drug development has been their imperfect predictive validity with nonopioids. For example, drugs may decrease nocifensive behaviors by impairing the subject’s ability to respond (a false positive effect) rather than by decreasing sensitivity to the noxious stimulus. To control for potential motor effects, one common strategy has been to compare a drug’s potency for producing antinociception versus motor impairment (e.g., disruption of locomotor activity or rotorod performance) (Seguin et al., 1995). However, it is not always clear what measures of motor behavior are optimal, or what potency differences are sufficient to justify continued drug development. False negative effects are also possible. For example, assays of acute nociception are generally sensitive to μ-opioid agonists but insensitive to clinically useful analgesics such as steroids and NSAIDs (Fig. 1b). The insensitivity of acute pain models to NSAIDs is apparent even when relatively low-intensity noxious stimuli are used (e.g., Seguin et al., 1995). This potential for false positive and false negative effects has stimulated two types of advances in the development of procedures for analgesic drug development: a) development of new methods to induce more clinically relevant pain states and b) incorporation of additional dependent measures to provide evidence of pain.

Toward More Clinically Relevant Pain States

Models of Inflammatory Pain. Pain that requires clinical intervention is often associated with inflammation, and one active area of research has been the development of procedures that model inflammatory pain (Bennett, 2001; Luo, 2004). Inflammation can result from tissue damage (e.g., a surgical incision or burn), exposure to chemical stimuli (e.g., the chemical constituents of a bee sting), or autoimmune processes (e.g., some forms of arthritis). In each case, stimulation of the immune system results in the release of inflammatory mediators, such as bradykinin and prostaglandins. These mediators in turn produce numerous effects, including sustained activation and sensitization of both primary nociceptors and higher order neurons involved in the transmission of nociceptive input (Marchand et al., 2005). This hypersensitivity of nociceptive pathways contributes to the behavioral phenomena of allodynia (pain-like responses to normally innocuous stimuli) and/or hyperalgesia (enhanced pain-like responses to normally noxious stimuli), and the goal of drug treatment is to normalize pain sensitivity.

Most models of inflammatory pain include the injection of inflammatory mediators themselves or of chemical substances that provoke an immune response. For example, one model of acute inflammatory pain involves the subcutaneous administration of formalin into the hind paw of rats (Dubuisson and Dennis, 1977). As noted above, formalin injection elicits an acute nociceptive response characterized by intense paw flinching/licking that lasts for approximately 5 min. In addition, after a quiescent period of 10 to 15 min, a second phase of paw flinching ensues for approximately 60 to 90 min. This second phase is associated with plasma extravasation and is thought to involve the release of inflammatory mediators. Drugs can then be evaluated for their ability to suppress this second phase of paw flinching. Importantly, flinching during the second phase of the formalin response is decreased not only by morphine-like opioids but also by steroid and NSAID analgesics that have established clinical efficacy (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987; Damas and Liegeois, 1999; Taylor et al., 2000). Carrageenan (a family of sulfated polysaccharides extracted from red seaweeds) is another substance commonly used in models of inflammatory pain (Vinegar et al., 1976). Injection of carrageenan into the paw does not produce the robust flinching response observed with formalin; however, it elicits substantial paw swelling and both thermal and mechanical allodynia and hyperalgesia in the affected paw for up to 7 h (Fig. 1c). Injection of carrageenan or other compounds (e.g., iodoacetate, Freund’s complete adjuvant) into the knee or ankle joint produces even more protracted allodynia/hyperalgesia, lasting for several days to weeks, and these joint injections of inflammatory compounds are used to model more chronic inflammatory conditions such as osteoarthritis. As with the second-phase formalin response, inflammation-associated allodynia/hyperalgesia can be attenuated, and nociceptive sensitivity can be normalized by morphine-like opioids as well as by steroid and NSAID analgesics (Fig. 1d) (Jett et al., 1999; da Silva Filho et al., 2004; Whiteside et al., 2005).

As with assays of acute nociception, drug effects in these models of inflammatory pain may be influenced by the specific characteristics of the procedure. For example, NSAIDs are most effective in procedures that produce large amounts of edema and in which thermal or mechanical sensitivity are measured using low to medium intensity stimuli. Moreover, drugs may be more effective in models of acute inflammation than in models of chronic inflammation, because chronic inflammation may recruit sustained activity of both C-fibers
and Aδ fibers (Smolen et al., 2005). Accordingly, a comprehensive assessment of any given test drug will compare the effects of that drug with effects of known analgesics across a range of procedures.

Overall, the introduction of procedures for producing inflammatory pain states has contributed to a reduction in false negative effects (e.g., with steroid and NSAID analgesics). However, these procedures generally remain dependent on the same behavioral measures as described above for assays of acute nociception. Thus, the second-phase formalin response is characterized by an increase in flinching behaviors. Likewise, thermal and mechanical hypersensitivity are typically assessed by measuring withdrawal responses from thermal and mechanical stimuli. As a result, these procedures remain vulnerable to false positive effects associated with drug-induced motor impairment. It is also well recognized that conventional approaches to the measurement of withdrawal responses may introduce experimenter bias in the precise methods used to deliver provocative stimuli and define withdrawal responses (Chesler et al., 2002). To reduce experimenter bias, a different approach has been developed that provides an automated measure of weight bearing in adjuvant arthritic rats (T. W. Vanderah, unpublished observations) (Fernihough et al., 2004; Pomonis et al., 2005). In this procedure, the subject is placed into a container that holds the rat in a "rearing" position, so that all weight is distributed to the rear paws. The floor of the container consists of two scales, one for each paw, and weight bearing on each paw can be assessed by comparing the distribution of weights on the two scales. Under control conditions, weight is distributed evenly across both paws. Injection of inflammatory agents into one knee promotes a shift in weight bearing to the uninjured leg. More importantly, compounds that relieve pain and inflammation clinically also produce a dose- and time-dependent normalization of weight bearing. Although this approach reduces experimenter bias, it remains sensitive to motor disruption and false positive effects, because drug-induced motor impairment might compromise the subject’s ability to sustain a shift in weight distribution.

Models of Neuropathic Pain. Acute or inflammatory pain may be unpleasant, but these types of pain result from normal functioning of an intact nervous system and are adaptive insofar as they promote avoidance of noxious stimuli and protection of inflamed tissue. However, pain requiring clinical intervention may also result from trauma or disease that damages the nervous system and chronically disrupts normal pathways of pain processing. Examples include pain associated with traumatic injury (e.g., phantom limb pain after amputation), chemically induced nerve damage (e.g., neuropathy caused by cancer chemotherapies), and diseases affecting the nervous system (e.g., diabetic neuropathy). Pain associated with damage to the nervous system is referred to as "neuropathic" pain, and it is characterized by abnormal pain sensations that may include spontaneous pain (i.e., pain in the absence of apparent stimulation) as well as thermal and/or mechanical allodynia and hyperalgesia (Martin and Eisenach, 2001; Backonja and Stacey, 2004; Irving, 2005). Current approaches to the treatment of neuropathic pain include use of morphine-like opioids and anticonvulsant medications such as gabapentin. However these, medications are often only partially effective, and neuropathic pain is usually not responsive to steroid or NSAID analgesics. Accordingly, the development and validation of neuropathic pain models has been another active area of research (Bennett, 2001).

All neuropathic pain models begin with a manipulation intended to produce nerve damage. The most widely used strategies for nerve injury target the sciatic nerve, because this nerve is readily accessible and because it innervates the hind limbs, which can be readily probed with provocative stimuli. Neuropathic pain models that target the sciatic nerve differ in the location of injury (proximal, medial, or distal sciatic nerve) and type of injury (compression, crush, ligation, or transection). For example, commonly used models involve spinal nerve ligation of the L5 and/or L6 spinal nerve(s) (Fig. 1e), partial sciatic nerve ligation, spared nerve injury, and chronic constriction injury of the sciatic nerve (Bennett and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992; Decosterd and Woolf, 2000). Experimental nerve injuries to model clinical neuropathies can also be achieved by treatment with various chemical agents. For example, cancer chemotherapies, such as vincristine and paclitaxel, produce neuropathic pain in humans, and these compounds also produce neuropathy-associated allodynia and hyperalgesia in rodents (Higuer and Luo, 2004). Diabetic neuropathies can be modeled by the cytotoxic destruction of pancreatic β-cells through the administration of streptozotocin (Fox et al., 1999; Calcutt, 2004). The poor health of streptozotocin-treated animals complicates behavioral analysis; however, refinements of the model include insulin replacement to maintain animal health while preserving the development of hypersensitivity (Fox et al., 1999; Calcutt, 2004). Naturally occurring neuropathies may also provide a basis for model development. For example, under normal laboratory conditions, the Goto-Kakizaki rat strain develops signs of type 2 diabetes, including neuropathies and altered sensory function (Ueta et al., 2005).

Once a neuropathic manipulation has been implemented, pain sensitivity is then typically evaluated using the same approaches described above, which again rely primarily on withdrawal responses from provocative thermal or mechanical stimuli. Tactile (mechanical) sensitivity is the most commonly evaluated endpoint in models of neuropathic pain, and it is typically quantified using von Frey monofilaments applied to the plantar footpad. Other forms of mechanical sensitivity also develop, such as hypersensitivity to blunt or pinprick pressure. Increases in sensitivity to warm stimuli may develop; however, sensitivity to cold stimuli develops more robustly in these models. Importantly though, the profile of hypersensitivity across different stimulus modalities varies across the models. For example, heat hypersensitivity is most apparent in the chronic constriction injury model and less apparent or absent in the spared nerve injury and chemotherapy models. Moreover, hypersensitivity to different stimulus modalities can wax and wane over time, and this temporal variability in baseline levels of thermal or mechanical sensitivity can influence drug effects (Jasmin et al., 1998; Suzuki et al., 1999). Evaluation of compounds long after injury might be expected to model the clinical condition more accurately, because clinical pharmacologic interventions are often initiated weeks, months, or even years after the initial injury. However, a systematic test of this hypothesis has not been conducted.

Ultimately, the predictive validity of neuropathic pain pro-
proved to be high, stable through time and across individuals,
and readily measured during short observation periods.

In order to achieve a closer alignment between preclinical and clinical measures of pain has been to include
measures of pain-related behavioral suppression. Although
most current models of acute, inflammatory, and neuropathic
pain rely on conventional measures of pain-stimulated behav-
iors, it has long been appreciated that pain is also often
associated with suppression of normally adaptive behaviors.
For example, several pain inventories used in human clinical
medicine assess suppression of daily activities (e.g., walking,
performance of household chores, and social interactions)
(Ostelo and de Vet, 2005). The efficacy of interventions is
then measured in part by a restoration of these pain-sup-
posed behaviors. Measures of behavioral suppression play
an even more important role in the diagnosis of pain in veterinary medicine. For example, cardinal signs of chronic
pain in laboratory animals include reduced feeding and
weight loss, reduced locomotor activity, and a decrease in
behavior (National Research Council, 1986). The use of
these signs in the clinical diagnosis of pain and analgesic
efficacy suggests that measures of pain-suppressed behavior
may also be useful in the preclinical evaluation of candidate
analgesics.

Studies of pain-suppressed behavior can focus on any of a
range of normally occurring behaviors, including spontane-
ous locomotor activity, feeding, or social interactions (Fleck-
nell, 1994; Karas, 2002). However, for the purposes of pre-
clinical drug evaluation, control rates of the target behavior
should be high, stable through time and across individuals,
and readily measured during short observation periods.
These goals are best achieved by training animals in a be-
behavioral task. Once training is complete, an acute, inflam-
matory, or neuropathic pain manipulation can be introduced
and evaluated for its ability to suppress the target behavior,
and drugs can be evaluated for their ability to restore pain-
suppressed behaviors. In an early example of this approach,
Rodriguez and Pardo (1968, 1974) trained dogs to locomote
on a treadmill task. Intra-articular injections of dilute for-
malin into one hind limb produced inflammation of the knee
joint and suppressed usage of the affected leg during the
task. Hind limb function could be restored by both morphine-
like opioids and NSAIDs, and the potencies of analgesic
drugs in this procedure were similar to their clinical poten-
cies. In addition, the procedure was reasonably specific for
analgesics, because a range of nonanalgesic drugs failed to
reverse formalin-induced functional impairment. A more re-
cent study directly compared drug effects on pain-stimulated
and pain-suppressed behaviors in mice (Fig. 2) (Stevenson et
al., 2006). Intraperitoneal injection of dilute acetic acid pro-
duced a concentration-dependent increase in abdominal
stretching (a pain-stimulated behavior) as well as suppres-
sion of consumption of a preferred liquid food (a pain-sup-
pressed behavior). Morphine reduced acid-stimulated ab-
dominal stretching and increased acid-suppressed feeding, a
profile of effects consistent with the conclusion that mor-
phine decreased sensitivity to the noxious stimulus. In con-
trast, the nonanalgesic neuroleptic haloperidol attenuated
acid-induced abdominal stretching but failed to increase ac-
id-suppressed feeding, a profile of effects consistent with
motor impairment. Stable baselines of operant responding
have also been used effectively to study pain-suppressed
behavior and the effects of analgesic drugs (Martin et al.,
2004).

Taken together, these studies suggest at least four advan-
tages to inclusion of assays of pain-suppressed behaviors in
drug development testing batteries. First, drugs that produce
motor impairment are less likely to produce false positive
effects in assays of pain-suppressed behavior than in assays
of pain-stimulated behaviors. Of course, assays of pain-sup-
pressed behavior will be vulnerable to their own confounds
(e.g., nonspecific stimulation of the target behavior); how-
ever, these vulnerabilities are different from and complemen-
tary to those that beleaguer assays of pain-stimulated behav-
iors. As a result, a given drug would be less likely to produce
false positive effects in assays of both pain-stimulated and
pain-suppressed behaviors than in one or the other type of
assay alone. Second, pain-suppressed behaviors can be mea-
sured remotely using automated equipment (e.g., locomotor
activity boxes, operant response chambers). This greatly re-
duces investigator-related sources of variability inherent in
some assays of pain-stimulated behavior. Third, assays of
pain-suppressed behavior may incorporate behavioral meas-
ures that are routinely used for diagnostic purposes in vet-
inary and human clinical medicine, and this in turn may
facilitate translation of preclinical results to clinical settings.
Lastly, it is of interest to note that measures of pain-sup-
pressed behaviors may be especially useful in exploring af-
fective consequences of chronic pain. As noted above, pain
has been defined as “an unpleasant sensory and emotional
experience...” (International Association for the Study of
Pain, 1979), and chronic pain is often associated with signs of
clinical depression (Bair et al., 2003). As such, pain can be
conceptualized as a negative affective state, and there is an
increasing interest in developing experimental tools that will
permit investigations of this affective component of pain
(Price, 2002). Various approaches to assessment of negative
affect have been developed, and one general approach has
been to assess the degree to which highly motivated behav-
iors are suppressed (e.g., “depression” as indicated by de-
It would be straightforward to adapt these procedures to studies of pain-suppressed behavior. Overall, studies of pain-suppressed behaviors could provide a useful complement to more conventional assays of pain-stimulated behaviors while also providing a means for research on affective dimensions of pain.

**Functional Magnetic Resonance Imaging in Studies of Pain and Analgesia.** Functional magnetic resonance imaging (fMRI) has emerged as a second approach toward achieving a closer alignment between preclinical and clinical measures of pain and analgesia (Borsook et al., 2002; Borsook and Becerra, 2003). fMRI can be used to measure “blood oxygen level-dependent” changes in blood flow across capillary beds (as measured by changes in paramagnetic signal due to oxygen extraction in tissue with a consequence of changes in relative concentrations of oxyhemoglobin and deoxyhemoglobin). Changes in the blood oxygen level-dependent signal are considered to reflect changes in neural activation. As a result, activation across the whole brain associated with noxious stimulation or delivery of analgesic drugs may be assessed noninvasively. Patterns of fMRI activation not only serve as potential physiological correlates of pain or analgesia but also describe neural circuits that may underlie these sensations and their associated patterns of behavior.

The use of fMRI to study pain and analgesia has been pioneered in studies with human subjects (Davis, 2000; Tracey, 2001). For example, studies using acute thermal stimuli in humans have identified a consistent set of supraspinal structures that is activated by noxious stimuli and associated with the sensation of pain. This set includes structures thought to be involved in the sensory-discriminative aspect of pain (lateral thalamus, primary and secondary somatosensory cortex, and insular cortex), affective and attentional aspects of pain (anterior cingulate cortex), motor areas potentially involved in preparing nocifensive responses (striatum, cerebellum, and supplementary motor area), and areas involved in descending modulation of spinal or primary afferent activity (periaqueductal gray). Moreover, fMRI has been used in analgesic drug development to characterize the
effects of analgesics on pain-induced changes in brain activation (Tracey, 2001; Borsook et al., 2002). For example, the morphine-like opioid agonist remifentanil attenuated thermal pain-associated fMRI signals in humans, especially in contralateral insular cortex (Tracey, 2001; Wise et al., 2002).

fMRI is also being used with increasing frequency to study pain and analgesia in animals. In one recent example (Hess et al., 2006), thermal heat stimuli promoted increases in brain activation in such areas as sensory-motor cortex, cingulate cortex, and periaqueductal gray in rats—all homologous to areas also activated by noxious heat in humans. Moreover, treatment of one hind paw with the inflammatory agent zymosan produced swelling, thermal hyperalgesia as measured using standard paw-withdrawal behavioral tests, and enhanced fMRI activation in response to thermal stimuli. fMRI studies in animals are also being used to examine effects of analgesics. For example, treatment of the paw with formalin produced bilateral increases in cerebral blood flow in somatosensory cortex, cingulate, and the periaqueductal gray area in rats, and pretreatment with morphine attenuated these formalin-induced fMRI effects (Shah et al., 2005). Morine also blocked pain-associated fMRI responses to electrical stimulation and injection of capsaicin (Tuor et al., 2000; Malisz and Docherty, 2001).

A significant advantage of fMRI procedures is that very similar experimental protocols can be conducted in both humans and research animals, which should facilitate the translation of experimental findings. As such, fMRI permits assessment of pain-related effects in brain areas thought to be important in processing both sensory and affective components of the pain experience across species, allowing “circuit function” to be the language of translation. As a consequence, fMRI procedures may be extremely useful for predicting effects of drugs on both sensory and affective components of pain.

Counterbalancing the potential promise of fMRI procedures are at least two significant obstacles. First, the infrastructure in equipment, personnel, and technical expertise required to conduct fMRI experiments is considerable and far beyond what is required for any other preclinical procedures used in analgesic drug development. The expanding popularity of fMRI for a whole host of basic science applications is beyond what is required for any other preclinical procedures used in analgesic drug development. The expanding popularity of fMRI for a whole host of basic science applications is steadily reducing this barrier, but for now that barrier remains appreciable. A second disadvantage is that body movements, and in particular head movements, must be virtually eliminated during fMRI data acquisition, and such an elimination of movement may be difficult to achieve during presentation of noxious stimuli. To date, the elimination of head movement has been accomplished in preclinical rodent studies primarily by anesthetizing the subject; however, the introduction of anesthesia creates obvious confounds for research on pain and analgesia. More recently, imaging in awake subjects (nonhuman primates and rodents) has been accomplished by acclimating the subject to magnetic resonance imaging-compatible restrainers that permit minimal head movement (Andersen et al., 2002; King et al., 2005).

Conclusions

Preclinical assays of pain and analgesia are critical for analgesic drug development. These procedures all include two basic components: 1) a method for generating a pain-like state in the experimental subject and 2) measurement of pain-related behaviors. In the simplest assays of pain and analgesia, the pain-like state is produced by delivery of an acute noxious stimulus, and the target behavior is a withdrawal response or other nocifensive behavior. This basic approach has been refined by a development of new approaches to generate inflammatory and neuropathic pain states that more closely mimic clinical pain states and by incorporation of new dependent measures, including pain-suppressed behaviors and fMRI measures of cerebral blood flow and brain activation. The continued evolution of these procedures will provide new tools for use in analgesic drug development. Iterative preclinical and clinical studies will help identify procedures or sets of procedures that provide optimal predictive validity for treatment of clinical pain states.

References


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