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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 non-invasive 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 mu opioid agonists such as morphine, anti-inflammatory steroids such as cortisone, and non-steroidal 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 as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain, 1979). 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 typically 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 (Figure 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 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 non-opioids. 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 vs. 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 mu opioid agonists but insensitive to clinically useful analgesics such as steroids and NSAIDS (Figure 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 minutes. In addition, following a quiescent period of 10-15 min, a second phase of paw flinching ensues for approximately 60-90 minutes. 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 sulphated 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 hr (Figure 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 (Figure 1d) (Jett et al., 1999; da Silva Filho et al., 2004; Whiteside et al., 2005).

As with assays of acute nociception, drug effect 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 A∂ 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. Similarly, 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 (Fernihough et al., 2004; Pomonis et al., 2005, Vanderah, unpublished observations). 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) (Figure 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 (Higuera 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, but 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,

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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 procedures has been difficult to

gauge, in part because of the variable clinical efficacy of existing drugs. These procedures are

generally sensitive to morphine-like opioids and gabapentin-like anticonvulsants, and they are

insensitive to NSAIDS (Figure 1f). In view of such findings, one recent literature review

reported successful prediction rates of 88% (chronic constriction injury), 68% (spinal nerve

ligation), 61% (partial nerve ligation) and 70% (streptozotocin) for different neuropathic pain

models (Kontinen and Meert, 2003). However, these apparently high levels of predictive

validity may be misleading, in part because failed clinical trials often go unreported. Limitations

to the predictive validity of neuropathic pain models result from at least two factors. First, these

models typically rely on withdrawal responses as the dependent measure, and as a result, these

procedures remain vulnerable to false positive effects produced by drugs that produce motor

impairment. Second, key clinical features of neuropathic pain often include spontaneous

burning, shooting, and lancinating pain, but these types of pain are not adequately measured or

quantified in preclinical behavioral procedures (Backonja and Stacey, 2004; Blackburn-Munro,

2004; Mogil and Crager, 2004).

Beyond Nocifensive Behaviors

  Incorporating Measures of Pain-Suppressed Behavior: One step toward achieving 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 behaviors, it has long

been appreciated that pain is also often associated with a 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, social

interactions; Ostelo and de Vet, 2005). The efficacy of interventions is then measured in part by

a restoration of these pain-suppressed 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 mating behavior (National Research Council, 1996). The

utility of these signs in the clinical diagnosis of pain and analgesic efficacy suggest 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 spontaneous locomotor activity, feeding or social interactions (Flecknell,

1994; Karas, 2002). However, for the purposes of preclinical 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 behavioral task. Once training is complete, an acute, inflammatory 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 (Rodriguez and Pardo, 1968; Rodriguez and Pardo, 1974)

trained dogs to locomote on a treadmill task. Intraarticular injections of dilute formalin 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

potencies. In addition, the procedure was reasonably specific for analgesics, because a range of

non-analgesic drugs failed to reverse formalin-induced functional impairment. A more recent

study directly compared drug effects on pain-stimulated and pain-suppressed behaviors in mice

(Figure 2; Stevenson et al., 2006). Intraperitoneal injection of dilute acetic acid produced a

concentration-dependent increase in abdominal stretching (a pain-stimulated behavior) as well as

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a suppression of consumption of a preferred liquid food (a pain-suppressed behavior). Morphine

reduced acid-stimulated abdominal stretching and increased acid-suppressed feeding—a profile

of effects consistent with the conclusion that morphine decreased sensitivity to the noxious

stimulus. Conversely, the non-analgesic neuroleptic haloperidol attenuated acid-induced

abdominal stretching but failed to increase acid-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 advantages 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-suppressed behavior will

be vulnerable to their own confounds (e.g. non-specific stimulation of the target behavior);

however, these vulnerabilities are different from and complementary to those that beleaguer

assays of pain-stimulated behaviors. 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 measured remotely

using automated equipment (e.g. locomotor activity boxes, operant response chambers). This

greatly reduces investigator-related sources of variability inherent in some assays of pain-

stimulated behavior. Third, assays of pain-suppressed behavior may incorporate behavioral

measures that are routinely used for diagnostic purposes in veterinary 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-suppressed behaviors may be especially

useful in exploring affective 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 behaviors are suppressed (e.g. "depression" as indicated by decreases in positively reinforced responding; Holmes, 2003). 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.

fMRI 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" (BOLD) changes in blood flow across capillary beds (as measured by changes in paramagenetic signal due to oxygen extraction in tissue with a consequence of changes in relative concentrations of oxyhemoglobin and deoxyhemoglobin). Changes in the BOLD 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 non-invasively. Patterns of fMRI activation not only serve as potential physiological correlates of pain or analgesia, but also

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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, insular cortex), affective and attentional aspects of pain (anterior cingulate cortex), motor areas potentially involved in preparing nocifensive responses (striatum, cerebellum, 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 periaqueductal gray in rats, and

pretreatment with morphine attenuated these formalin-induced fMRI effects (Shah et al., 2005).

Morphine also blocked pain-associated fMRI responses to electrical stimulation and injection of

capsaicin (Tuor et al., 2000; Malisza 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.

Consequently, 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 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 (non-

human primates and rodents) has been accomplished by acclimating the subject to MRI-

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compatible restrainers that permit minimal head movement (King et al., 2005; Kaufman et al., 2006).

Conclusions. Preclinical assays of pain and analgesia are critical for analgesic drug development. These procedures all include two basic components: (a) a method for generating a pain-like state in the experimental subject, and (b) 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 (b) 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.

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FOOTNOTES

This commentary is based on a symposium presented at the 2005 Experimental Biology meeting and sponsored by the ASPET Division of Behavioral Pharmacology.

FIGURE LEGENDS

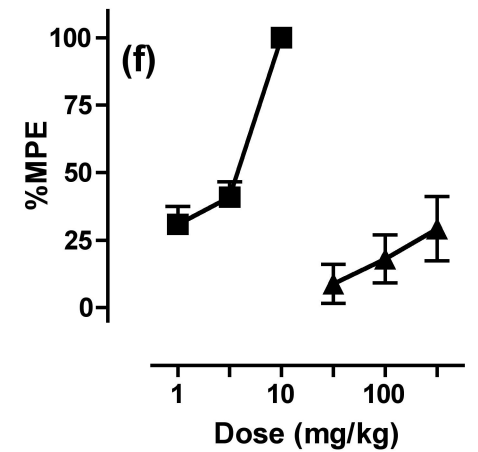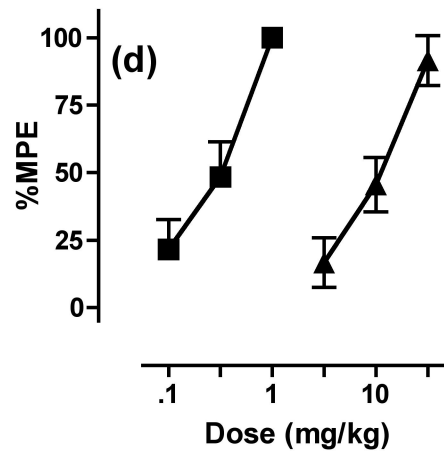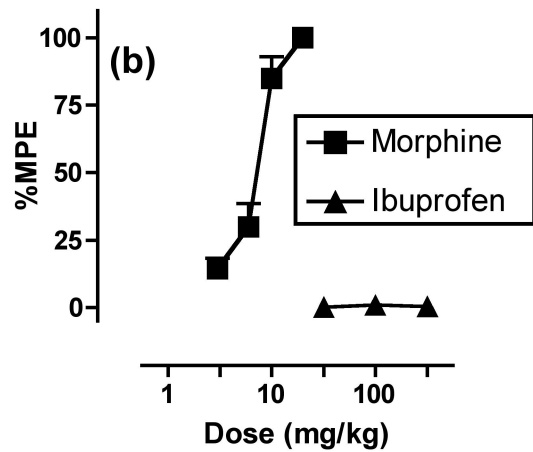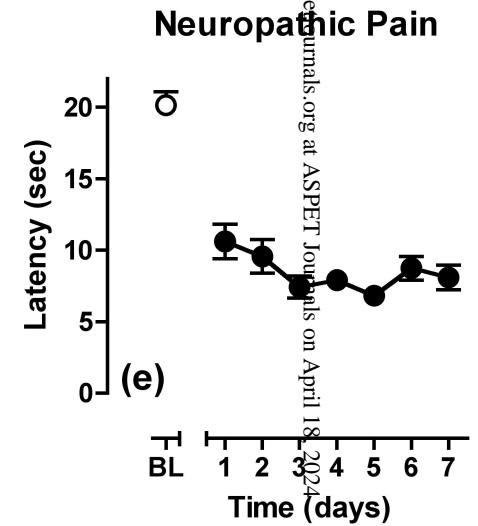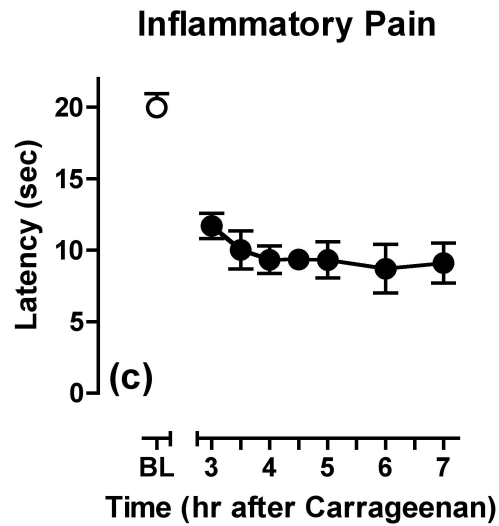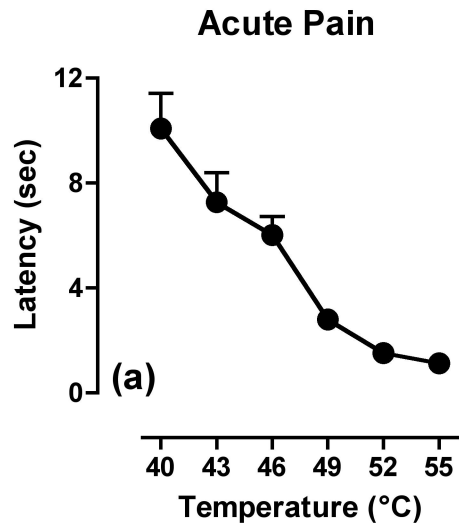**Figure 1. Assays of acute thermal nociception (a,b), inflammatory thermal hyperalgesia (c,d), and neuropathic thermal hyperalgesia (e,f).** (a) Effect of stimulus temperature on tail-withdrawal latencies in a warm-water tail-withdrawal procedure in male ICR mice. As temperature increased, tail-withdrawal occurred more rapidly, and latencies decreased. (b) Effects of the mu opioid receptor agonist morphine and the non-steroidal anti-inflammatory drug (NSAID) ibuprofen (SC, 30 min pretreatment) on tail withdrawal latencies from water heated to 55°C. Data are expressed as %Maximum Possible Effect, with %MPE=[(Test Latency-Baseline Latency)/(Cutoff-Baseline Latency)] x 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 sec. Three hours after carrageenan administration, there was a significant decrease in thermal paw-withdrawal latencies, and this hypersensitivity was sustained for at least 7 hr after carrageenan. (d) Effects of morphine and ibuprofen (SC, 60 min pretreatment) on carrageenan-induced thermal hyperalgesia. Data are expressed as %MPE, with %MPE=[(Test Latency-Carrageenan Baseline Latency)/(Pre-Carrageenan Control Latency-Carrageenan Baseline Latency)] x 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 (BL) conditions before nerve injury, rats withdrew their paws from the thermal stimulus in approximately 20 sec. Hypersensitivity was apparent one day after nerve injury and was sustained for at least 7 days. (f) Effects of morphine and ibuprofen (SC, 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)] x 100. Morphine was fully effective, whereas ibuprofen was only weakly active. (a,b

unpublished data by E. Bilsky, G. Stevenson, J. Lowery and H. Martinez; c,d,e,f unpublished
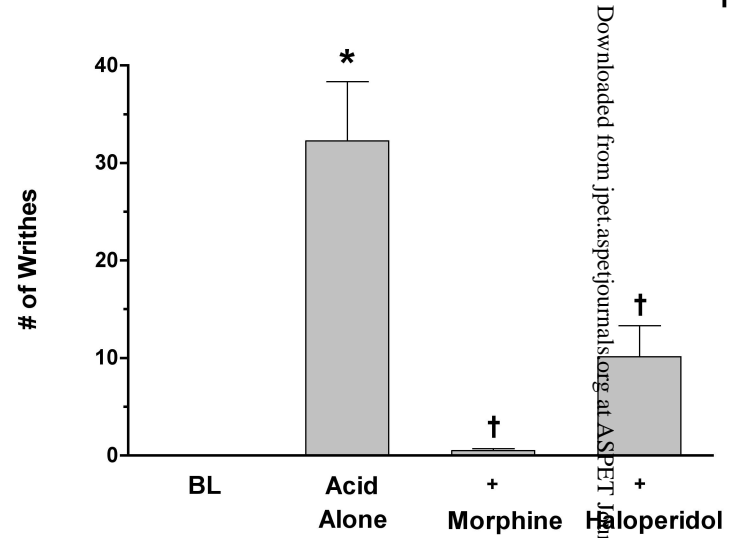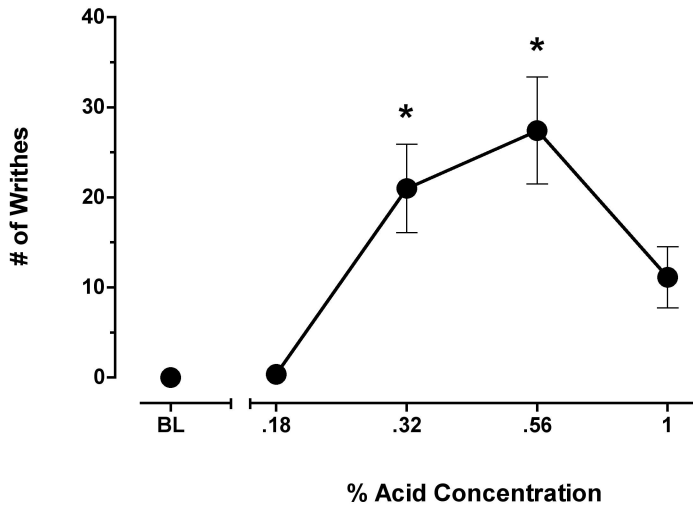
data by T. Vanderah)

Figure 2. Comparison of drug effects in pain-stimulated and pain-suppressed behaviors in

mice. Abscissae (left panels): % Acetic acid solution injected IP in mice. Points above "BL"

show baseline in the absence of an IP injection.   Abscissae (right panels): Treatment condition—

baseline (BL), 0.56% acetic acid alone (Acid Alone), 0.56% acetic acid + 1.0 mg/kg morphine (+

morphine), 0.56% acetic acid + 1.0 mg/kg haloperidol (+ haloperidol).   Ordinates (top panels).

Writhes counted during observation period. Ordinates (bottom panels): Consumption of liquid

food solution (g consumed per g body weight). All points/bars show mean ± SEM of 6-12 mice.

* Indicates significantly different from "BL," $p < 0.05$. † Indicates significantly different from

"Acid Alone," $p < 0.05$. (Adapted from Stevenson et al., 2006)

Figure 1

Pain-Stimulated Behavior

Pain-Suppressed Behavior