Comprehensive review

The necessity of animal models in pain research

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A B S T R A C T

There exists currently a fair degree of introspection in the pain research community about the value of animal research. This review represents a defense of animal research in pain. We discuss the inherent advantage of animal models over human research as well as the crucial complementary roles animal studies play vis-à-vis human imaging and genetic studies. Finally, we discuss recent developments in animal models of pain that should improve the relevance and translatability of findings using laboratory animals. We believe that pain research using animal models is a continuing necessity–to understand fundamental mechanisms, identify new analgesic targets, and inform, guide and follow up human studies–if novel analgesics are to be developed for the treatment of chronic pain.

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1. Introduction

Basic scientific understanding of pain processing and modulation has greatly increased over the past few decades. However, our knowledge of the intricate molecular, cellular, and systems organization of nociception remains substantially incomplete. Furthermore, the management of pain in both acute and especially chronic settings remains far from optimal. Although the pharmaceutical industry has made substantial investments in analgesic drug development, a paucity of analgesics acting at novel molecular targets have been approved. Also lacking are new, more effective surgical targets and behavioral strategies for pain control, despite the clear need to improve upon the relatively modest efficacy of current treatments for many chronic pain conditions. This unfortunate state of affairs – whether accurate or simply “looking at the glass half empty” – has engendered considerable cynicism in the value of the animal models of pain that are currently at the core of the research and drug development enterprise. Simultaneously, new options have presented themselves in pain research using humans as subjects. The number of pain-related neuroimaging studies on human volunteers and patients has exploded, and there is increased interest in complementary human experimental techniques including quantitative sensory testing, microdialysis, epidermiology, physiology (e.g., nerve conduction studies), ex vivo studies of human cells and tissues, and both DNA- and RNA-based genetic studies. The perceived failure of animal studies for analgesic drug development and the increasing interest in human-based techniques has led some to call for the replacement of animal pain experiments with human volunteer studies [34]. We believe this would be a grievous mistake. The following represents a case for the continued reliance on, and necessity of, animal models in pain research.

It is instructive to begin with a consideration of the current state of pain research. Of all primary research papers published in the journal Pain, from its inception in 1975 until 2007 [46], approximately two-thirds were human-based, studying either patients with pain (47.8%) or healthy human volunteers (17.2%) as experimental subjects. The remaining third of published papers were based on studies in laboratory animals. In almost two-thirds of those, the subjects were awake and behaving, with the overwhelming majority testing rats and mice [40]. Thus, humans, not animals, have always represented the lion’s share of pain research. Most of these studies, however, are primarily aimed at characterizing pain states; only a small percentage of human studies directly test the anatomical, biochemical or physiological mechanisms of pain [46].

Two quantitative trends observed in this analysis [46] are of interest here. First, the percentage of studies published in Pain with awake animals as subjects has increased steadily over 30+ years, from less than 10% to more than 30%. Closer analysis reveals that studies including a behavioral pharmacology component underlie most of this increase. Correspondingly, the percentage of human studies has decreased, significantly in the case of human patient studies. However, the percentage of imaging studies has increased, such that they represented almost 10% of published studies in Pain by 2007.
2. The value of animal models in understanding pain

Over a decade ago Pat Wall lambasted neuroscientists for their attempts to understand pain as a result of activity within dedicated line systems because of his view of pain as far too complex for such simplicity [61]. Bud Craig and colleagues responded by explaining that approaching the mammalian nervous system as being functionally and anatomically well-organized has allowed for notable progress in understanding both the brain and pain [10]. Wall and Craig were both correct. Pain is psychologically complex and, as well as being a proper subject matter for basic scientists, will become fully comprehensible only when subjected to psychological and sociological interrogation. If you want to know what pain feels like then you must interrogate a conscious person and not a nerve fiber. But if you want to know how a degenerate sciatic nerve creates an afferent barrage – often experienced as pain in a conscious person – then it is a better idea to interrogate the sciatic nerve and not the patient. This Cartesian divide may be unsatisfactory for many reasons but it continues to serve us well and, critically, neuroimaging, genotyping, tissue databanks and epidemiological statistics do not challenge that divide. Conscious persons can no more be found in colorful brain images, DNA, tissue fragments or population statistics than they can be found in animal models.

Technically, animal models offer extremely fine characterization of neurochemistry and anatomy, and the excellent temporal and spatial resolution and direct recording of electrophysiology. Animal models also have obvious advantages over human subjects with respect to standardization of genetic and environmental backgounds, safety, and economy. Although DNA can be as easily obtained from humans as from animals, mRNA from pain-relevant tissues can usually only be obtained from animals except in unusual circumstances. Most importantly, animal studies allow controlled investigation of chronic pain conditions that are simply impossible to perform in humans. A feature of several animal models of peripheral neuropathic pain, for example, is partial denervation. Loose ligation of the whole peripheral nerve [2], tight ligation of a part of a large peripheral nerve [57], or a tight ligation of an entire spinal segmental nerve [28] are all common techniques to create a mixture of intact and injured fibers. Human models of neuropathic pain can, obviously, not be created in this way. Is capsaicin-induced sensitization in human “normals” really a better model of neuropathic pain? The subject (human vs. rodent) is more appropriate, of course, but the assay (capsaicin vs. actual nerve damage) is a mere proxy, only as appropriate as the assumptions that underlie it. One could use actual patients as subjects instead of human normals, but this introduces difficult to control secondary factors that greatly complicate interpretation of experimental data.

One way in which humans and animals are identical with respect to pain research is that pain is a subjective phenomenon in both species. We might never be able to know what pain feels like in a rat, but we will never know what pain feels like in you either. In both humans and animals we infer pain based on behavior. Humans, however, have the unique behavior of speech, and speech provides relatively rapid and direct access to subjective experience via introspective ratings and descriptors. The ability of human subjects to reflect on their subjective experience is of considerable importance to pain research. In human sub-jects, the subjective experience can at least be associated with objective measures such as those obtained from neuroimaging, and it is obvious that any objective correlate of the pain experience will more closely model the human condition when studied in hu-mans. A further, and relatively new, advantage of human subjects over rodents is that they can be persuaded to stay motionless in scanning devices without training or anesthesia; human subjects can generally follow complex instructions quickly and readily.

3. Do animal models predict analgesic efficacy in humans?

The advantages of animal models in the exploration of basic physiological mechanisms of pain is only one of two major reasons animals have been employed in pain research; the other is the prediction of analgesic efficacy leading to clinical drug development. One can explore the relevance of animal models towards this second aim empirically. Despite the impressive achievements of human imaging studies, this approach has not yet discovered specific brain regions not previously implicated in pain based on animal experiments, although the organization of such brain areas into networks can be derived from human neuroimaging in a manner not previously possible using electrophysiology. Neither has a molecule or pain-related phenomenon (e.g., counter-irritation, stress-induced pain modulation) ever been found in humans that did not have a rodent counterpart.

Nonetheless, there are celebrated examples of failed “transla-tion,” where efficacy in animal models predicted efficacy in human clinical trials, but no efficacy was found. The most definitive example of this failure to translate is with neurokinin-1 (NK1) antagonists (i.e., MK-869), which failed despite demonstrations of adequate exposure, penetration, and occupancy [21]. There are other less-openly reported failures as well, including glycine-site antagonists [63] and sodium channel blockers [62] for neuropathic pain, and neuronal gap-junction blockers for migraine [19]. In 2010 alone, analgesic drugs failing to show efficacy in phase 2 or phase 3 clinical trials include ralfinamide (a mixed Na,1.7 blocker and NMDA antagonist) and indantadol (a mixed monoamine oxidase inhibitor and NMDA antagonist). This problem of translation, however, is not specific to the pain field. In fact, from 1991 to 2000 there was a 17% success rate from “first-in-man” (i.e., clinical trial phase 1) to drug registration for pain-related pharmaceuticals compared to 11% for all drug categories [29], although it should be noted that many of the successful pain drugs are simply new formulations or congeners of old compounds. More worrisome is the fact that as of 2000 the primary reason for attrition was lack of efficacy, responsible for over 25% of clinical failures [29]. In other words, these are drugs that worked in animal models, with no intractable pharmacokinetic or toxicity issues, but were subsequently demonstrated as no more effective than placebo in pain patients.

Are animal models to blame? It’s difficult to know for sure. First, virtually all clinically effective compounds have been “back-trans-lated” to show efficacy, at appropriate exposure levels, in existing animal models [30,65]. Second, at least one compound represents an obviously successful “forward” translation, the snail conopep-tide, ziconotide (Prialt®) (see [54]). The drug is a synthetic form of the Conus magus peptide, ω-MVIIA. The peptide was found to be neuroactive after intracranial injection, and later found to bind with high-affinity to N-type voltage sensitive calcium channels, which themselves are upregulated in the dorsal horn after inflammation or nerve damage. Intrathecal ziconotide was then found to produce strong analgesic effects in a broad array of animal models, and after three positive randomized control trials is now approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMEA) for the treatment of severe chronic pain. Ziconotide is no panacea, requiring intrathecal injection and featuring serious adverse effects, but it does represent a clear example of successful “rational” analgesic drug development. It should also be noted, however, that although animal models successfully predicted the efficacy of ziconotide, they failed to predict its side effects.
Ultimately, enthusiasm among stakeholders in analgesic drug development regarding the value of animal models in drug development at any point in time seems very much to depend on a running "tally" of successes and failures. In terms of recent developments, at the time of writing it appears that TRPV1 antagonists have failed (due to side effects), but tanezumab (a humanized monoclonal nerve growth factor antibody) has just successfully passed a phase 3 knee osteoarthritis pain study.

4. The value of animal models in complementing human neuroimaging

One might question whether animal models are still needed to study pain given the advancement of human neuroimaging. We argue that they are, given limitations imposed by human experimentation and technical limitations of neuroimaging to reveal the function and structure at the cellular level.

There are several technical limitations of current human neuroimaging technologies that impact specificity and sensitivity of the findings. A review of some of these issues has been previously discussed [11,12] and so only three main factors will be mentioned here. First and most critical are those limitations that preclude studies of individual neuronal events that provide the basic information code about pain. For example, it is not always possible to distinguish the precise location of where pain functions reside compared to other related functions. Electrophysiological studies in humans have revealed that neurons that encode pain, and neurons that encode various cognitive/attention or emotional salient information, are located within the same area of the cingulate cortex [13,14,22]; this co-localization cannot be resolved with human neuroimaging. The specificity and sensitivity of electrophysiology provide a unique window into the workings of the elemental components that form the basis of the nociceptive system. Although there are a few laboratories that have had unique opportunities to record from neurons in the human brain during neurosurgical procedures, these studies are rare and data are only obtainable from patients being treated for a serious intractable condition. Thus, our knowledge of the normal physiological properties of single neurons must be derived from animal studies. The second main limitation of neuroimaging with functional magnetic resonance imaging (fMRI) that is the blood oxygen level-dependent (BOLD) signal can reach a ceiling, and so it may not be able to distinguish very high levels of neuronal activity. A third limitation of human neuroimaging is that the technology is not yet able to interrogate very small areas of the central nervous system – such as specific lamina of the dorsal horn, the dorsal root ganglia, or the peripheral nervous system – with any precision. This limitation is unlikely to be resolved because most neuroimaging techniques are based on indirect proxies of neuronal activity, or integrate electrical signals across large sampling regions.

Clearly, an essential role of animal models of pain is to guide, inform and complement imaging and other types of human-based studies. First, animal studies are important in focusing human studies to relevant questions. The ability to interrogate the entire human brain in a functional or structural imaging study can provide a wealth of information. However, this can also lead to "information overload" and statistical multiple comparison problems because whole-brain analysis typically involves over one million data points (i.e., there can be >1 million imaging voxels in a given brain). A hypothesis-driven approach to test reasonable predictions is often used to avoid this problem. The vast knowledge accumulated from animal studies of subcortical nociceptive pathways and spinal and thalamic neuronal responses to noxious stimuli provides the fundamental basis from which we can interpret neuroimaging findings [4,11,66]. However, we know very little about cortical nociceptive neurons and their anatomical connections. Thus, we propose that animal studies now need to focus on the function and organization of cortical nociceptive neurons and pathways. Indeed, although much animal research continues to be focused on the dorsal root ganglion, spinal cord, and brain stem nuclei, increasingly rodent studies of cortical pain processing are being successfully implemented (e.g., [24,25,33,67]). In tandem with this effort human studies should then ask reasonable questions based on strong fundamental knowledge of neurons and pathways implicated in pain based on the animal models.

Second, animal studies can help develop appropriate neuroimaging experimental designs, tasks, stimuli and controls. Although there is a segregation of nociceptive and non-nociceptive neurons at the spinal level, and some clustering at brainstem and thalamic areas, the few electrophysiological studies that have recorded from cortical neurons (e.g., in S1, S2, insula, cingulate) indicate that these brain regions likely contain more non-nociceptive neurons than nociceptive neurons (e.g., [27]). A good experimental design requires strong knowledge about such neuronal response properties, the stimuli that can influence their activity and even the basal ("resting state") activity of neurons contained within each imaging data voxel. These crucial factors are not clearly understood at the cortical level and so impede interpretation of imaging data derived from a sampling space (i.e., voxel) in the order of several square millimeters that contain, at a minimum, thousands of neurons (see [11]). Therefore, a greater understanding of these fundamental issues must be derived from animal studies to inform neuroimaging design to yield specific and relevant information about the human pain system.

Third, animal studies can inform the interpretation of neuroimaging findings in light of the spatial and temporal limitations of imaging technologies. For example, data in each functional MRI voxel reflect information derived from hemodynamic changes over several seconds presumably due to activity in thousands of neurons. Similarly, structural MRI measures of brain density/volume and cortical thickness derive from voxels containing an unknown mix of neurons, axons, glia and other elements. Thus, interpretation of the meaning of functional and structural imaging data requires an understanding of the physiological and anatomical properties of the elements within the voxel (e.g., excitatory and inhibitory neurons, synaptic activity, dendritic spines, glia) (see [11,12]). The potential sources of neuronal influences on the elements within that voxel are also needed for a complete picture of the mechanisms underlying MRI data. A detailed understanding of neuronal circuitry is also needed to fully understand white matter structural imaging data obtained from diffusion tensor imaging and functional connectivity findings obtained from task-free "resting state" functional MRI studies.

Decades ago, pioneers in the pain field such as Pat Wall, Ron Melzack and Ken Casey all recognized that the pain experience included an emotional/motivational/affect component and could be shaped by attention and cognitive factors [37–39]. There is also electrophysiological evidence for attentional and multisensory effects on brainstem and cortical nociceptive responses (e.g., [17,23]). Clearly, more animal studies of this nature are needed to understand the location, preponderance and capacity of neurons to modulate their response to noxious stimuli and thus to properly design experiments that take these cognitive effects into consideration.

5. The value of animal models in complementing human genetics

Another new technique that some would suggest be used to replace animal studies is investigation into the human genomics of pain. Indeed, DNA can be obtained from patients or normals via
blood or buccal swab, and both linkage and association methodologies are in common use in humans. Linkage mapping in mice has proven its translational value, as pain-relevant genes identified in mice [45,47] (at a fraction of the cost of human genetics studies) have been shown to play analogous roles in humans [47, W. Maixner, unpublished data]. Linkage mapping works in humans as well, as virtually all known monogenic disorders of pain have their responsible gene currently identified [see [32]]. However, all such disorders are extraordinarily rare, and genes affecting common disorders can only be identified in humans by association study or large-scale sequencing projects. As has happened in other fields, candidate gene-based association studies of pain have thus far largely failed to replicate [32,41]. Hugely expensive genome-wide association studies (GWASs) have not yet been performed on pain-related traits, and even their utility has also been questioned [20,36]. Eventually, costs will decrease to the point where pain will be the subject of both GWASs and sequencing efforts. But what then? The great advantage of the genetic approach is that novel trait-related molecules can be identified via their genomic position alone, without any prior evidence for their involvement. When such genes are eventually identified by GWAS or sequencing, how will we ever know how they function as mediators of pain without follow-up studies in animals (e.g., transgenic knockout mice)? What otherwise would be the point of their identification?

6. New developments in animal models of pain

Whatever one’s thoughts are about the value of classical animal models of pain, it is obvious that major developments have occurred in recent years (see [40]). There are essentially six main criticisms of the dominant paradigms in animal pain testing. The first is that too much emphasis has been placed on reflexive withdrawal from mechanical or thermal (hot and cold) stimuli as a dependent measure [60]. The measurement of reflexive withdrawals is thought to be suboptimal both in terms of its poor match with human symptoms and also the considerable experimenter bias involved [5,8]. There is some irony here, because at the same time the trend in human testing has been to move away from rating scales and questionnaires to quantitative sensory testing paradigms that use very similar psychophysical approaches [52]. Recently, a number of conditioning paradigms have been developed, based either on classical conditioning (i.e., place aversion to environments paired with pain or place preference to environments paired with pain relief), training rodents to escape from painful stimuli (i.e., operant conditioning), or using motivational conflicts between pain and other drives (e.g., thirst) (see [40]). The use of such paradigms often leads to different or even opposite conclusions compared to reflex models; for example, low doses of morphine attenuated rats’ tendency to escape from a heated plate (44–50 °C) but increased reflexive guarding responses [59].

The second criticism is that all the emphasis has been placed on measuring pain itself, with almost none placed on important states that accompany pain, either as sequela or comorbidities. Chronic pain patients experience disability, anxiety, depression, cognitive dysfunction, sleep loss, loss of libido, social withdrawal, and any number of other symptoms that could be modelled in animals experiencing chronic inflammatory or neuropathic states. Thus far the results are decidedly equivocal, but there is definitely some evidence at least that similar changes may occur in rodents (see [40]). There have been tremendous recent advances in the ability to automatically track not only animal position and locomotion but gross behavior (e.g., grooming, drinking, eating, social approach) [53], and any number of studies are ongoing that aim to provide a full “ethogram” of the 24-h-a-day behavior of rats and mice with chronic pain.

The third criticism is that the existing models are too artificial, with inflammatory mediators such as formalin, carrageenan and Freund’s adjuvant standing in for arthritis, and surgical nerve damage standing in for painful diabetic neuropathy and post-herpetic neuralgia. Great strides are being made here too, for example with the development of post-operative (incision) pain [6], cancer pain [56] and chemotheraphy-induced neuropathic pain [49,58] that have far more obvious face validity to human (patho)physiological conditions featuring pain.

A fourth criticism is that whereas the primary symptoms of chronic pain in humans are spontaneous pain (either continuous or paroxysmal), numbness and dysesthesia [1,55], basic scientists overwhelmingly measure thermal (in 48% of studies) and mechanical (in 42% of studies) hypersensitivity [43]. For both neuropathic and non-neuropathic pain, mechanical and especially thermal hypersensitivity are symptoms of limited prevalence, importance and correlation with global ratings of pain severity [1,55]. There are exceptions to this generalization, of course; disorders like post-herpetic neuralgia and vestibulodynia feature mechanical hypersensitivity almost exclusively. As reviewed previously, any number of dependent measures of spontaneous pain in rodents have been proposed, but most are problematic in terms of their specificity, frequency, practicality, and susceptibility to confound [43]. A recent study specifically evaluated the utility of a number of these measures in the context of nerve injury in mice. We found that hypolocomotion and decreased rearing, licking, licking, flinching and shaking are simply not present in nerve-damaged mice at levels exceeding sham-operated or unoperated controls, and that although changes in dynamic weight bearing (i.e., gait abnormalities) were often present, the severity of the gait changes could not be used as a measure of any pain-related phenomenon [44]. In contrast, in certain pain states mice present with reliably and accurately scored facial expressions of pain [33], and these of course are spontaneously emitted behaviors. Although previous investigations were negative [26,64], a new study suggests that with improvements in design and habituation, chronic pain states are accompanied by increased ultrasonic vocalizations in mice [31].

Finally, there exists an obvious mismatch between the epidemiological realities of chronic pain prevalence in the human population and the usual choices of animal model subjects. That is, although chronic pain sufferers are overwhelmingly female [3], the prevalence of chronic pain is higher in the middle-aged and elderly than in young adults [18], and humans feature considerable genetic variability [48], pain research is overwhelmingly performed on young adult, male rats and mice of a single strain [40,42]. This choice is based on convenience and inertia, and on (empirically false [42]) expectations of higher variability in populations other than young male animals. There are any number of examples of conclusions from animal studies later found to be utterly dependent on the choice of subject sex or strain (e.g., [7,45,47]). As researchers become more sensitized to the robust influence of such factors on the results of animal pain experiments, the utility and predictiveness of findings obtained should improve.

Finally, it has been argued [51] that design issues and reporting standards in animal experiments are greatly inferior to those currently prevailing in human clinical trials. Specifically, details regarding blinding, randomization, and data dropouts are rarely reported in animal studies of pain, likely leading to high experimental bias. Of course, only a minority of rodent pain studies are specifically designed to assess analgesic efficacy, but especially in those more methodological detail would only enhance transparency and efforts to replicate. Many journals now have online methods (or supplementary methods) sections with no practical word limits.

Overall, then, we contend that despite any perceived limitations of current animal models of pain, we have at the present time the
ability to greatly improve their implementation, and the near future will feature animal pain research of ever greater relevance to clinical pain in humans.

7. Ethical objections to pain research

We understand that many people object to animal research, and especially animal pain research, on principle [50]. A detailed discussion of such objections belongs elsewhere [9,15,16]. Our aim here is to provide a scientific rationale for the continuation of animal research as an important mechanism in advancing our understanding of pain. Those that object to animal research on principle will, understandably, be unmoved by the scientific advances animal research provides.

8. Conclusions

The need for greater understanding of the fundamental physiology underlying pain will persist at least as long as treatment of chronic pain patients remains suboptimal. As a scientific matter, we see no scenario in the short-to-medium term whereby true advances are possible without the participation of animal models. That being said, we believe that the combination of human imagination (and other human) studies along with ever-more-relevant animal studies will lead to far more effective and tractable science than has been the case thus far. Thus, the time is ripe for true translational (“bedside-to-bench-to-bedside”) pain research.

Conflict of interest statement

None of the authors have a conflict of interest.

References


