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Volunteer studies in pain research – Opportunities and challenges to replace animal experiments: The report and recommendations of a Focus on Alternatives workshop

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ABSTRACT

Despite considerable research, effective and safe treatments for human pain disorders remain elusive. Understanding the biology of different human pain conditions and researching effective treatments continue to be dominated by animal models, some of which are of limited value. British and European legislation demands that non-animal approaches should be considered before embarking on research using experimental animals. Recent scientific and technical developments, particularly in human neuroimaging, offer the potential to replace some animal procedures in the study of human pain. A group of pain research experts from academia and industry met with the aim of exploring creatively the tools, strategies and challenges of replacing some animal experiments in pain research with ethically conducted studies of human patients and healthy volunteers, in combination with in vitro methods. This report considers how a range of neuroimaging techniques including functional magnetic resonance imaging, magnetoencephalography and positron emission tomography, singly and combined, can address human pain conditions. In addition, microdialysis in human subjects; genome-wide association research, twin studies and other epidemiological approaches; and in vitro cell and tissue research, are examined for their replacement potential in combination with neuroimaging. Recommendations highlight further opportunities to advance the replacement of animal studies with robust methods of relevance to understanding and treating human pain.

Introduction

Pain is an immensely important biological signal which confers selective advantage by signalling actual or possible injury. In primates, including humans, pain perception involves the interaction of peripheral and cortical structures, coloured by environmental and experiential factors. Some of the ways in which central

nervous mechanisms in the spinal cord and the brain modify incoming nociceptive signals and give rise to different sensations of pain have been described (Dickenson and Suzuki, 2005). Various psychological factors like arousal, attention, attitudes, and cultural and familial learning can all play a role in modifying pain perception and the response to analgesics (Craig, 2002).

Clinically, there is a complex spectrum of pain disorders, both acute and chronic, the impact of which can be devastating. Pain also carries a significant economic burden for the individual and for society as a whole. There are thus significant incentives not only to develop new therapies for the treatment of acute and chronic pain, but also to critically assess current approaches to pain research. This paper explores whether and how some animal experiments that are conducted within this field might be replaced with alternative methods that could shed more light on human pain syndromes.

A broad range of species, from rodents to primates, is currently used in pain research. Procedures may be conducted under anaesthesia or, if conscious animals are used, may result in substantial levels of pain and suffering, as well as death. For ethical reasons such animal procedures are not desirable. Additionally, while providing an understanding of some of the common 'pain pathways' and the role of neurotransmitters and membrane channels, animal models are insufficiently representative of the multidimensional aspects of clinical pain (Coghill et al., 2003).

Our understanding of the nature of human pain, especially the more intransigent syndromes, remains rather basic and dominated by reductionist accounts derived from animal models. Safe and effective analgesia for chronic pain is still elusive (Raffa, 2006). There is thus a pressing need for more detailed knowledge of the clinical picture, including the provision of reliable biomarkers of human pain in order to better understand the plasticity of pain, and to design and monitor more effective pain treatments.

These scientific and ethical concerns about animal-based research, including in the area of pain research (Le Bars et al., 2001), are reflected both in British and European legislation (EEC, 1986), which requires the replacement, reduction and refinement of animal experiments wherever feasible. This places a responsibility on researchers, funders, regulators and policy-makers to implement these concepts in the planning, design, approval and conduct of research. The advent of powerful neuroimaging and complementary neurophysiological methods in ethical studies of healthy and patient volunteers (Reilly et al., 2004) provides tools with significant potential to better understand the central and peripheral pathways implicated in pain perception (Coghill et al., 2003, Borsook and Becerra, 2006 and Klein et al., 2005). This could lead to the replacement of certain animal experiments, and potentially provide more reliable and more cost-effective pathways to drug development and to better treatment regimes.

Focus on Alternatives held an expert workshop to discuss these issues at De Montfort University in 2007. The aim was to review the potential and challenges of using ethically conducted studies of human patients and volunteers to replace animals in certain areas in pain research and in the development of new therapies. Specifically, the remit of the workshop was to consider areas of pain research where human studies might replace the use of animals; discuss how data from human studies compare and contrast with data from animal experiments; look at the barriers to wider acceptance of human studies as robust replacement models in this field; and suggest how progress towards replacing animal experiments might be enhanced.

Participants were asked to approach the issue creatively and to start from the assumption that human-based approaches, where ethical and scientifically feasible, are preferable to experiments on animals of other species. This report reflects the wide-ranging discussions at that meeting and the recommendations that were reached.

Pain – a multifaceted problem

Timely and accurate perception of painful stimuli is essential for survival. Nociceptive signals are generated peripherally and are transmitted centrally for further processing. It has become increasingly clear, over the last twenty years, that many areas of the vertebrate brain are involved in the experience of pain (Dickenson and Suzuki, 2005 and Toga and Mazziotta, 2000). Evidence shows that there is a network of brain regions involved in sensory, emotional, cognitive and motor processing. Combined to varying extents, and dependent upon conditions, these regions interact to generate the unique forms of pain experienced by different individuals (Tracey and Mantyh, 2007). The full range of human pain experience also depends upon biological, psychological and environmental factors; and from one person to the next, variations in traits and past experiences play major roles in how pain is perceived and in the establishment of effective coping strategies.

An improved understanding of the complexity of human pain perception has been, in part, due to the revolution in medical imaging which began with the introduction of X-ray computed tomography around thirty years ago. Powerful neuroimaging devices are now both to clinicians and researchers (Raichle, 2003). For instance, positron emission tomography (PET), magnetoencephalography (MEG), magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) allow researchers to look non-invasively at the brain and other organs within the living human, and also to evaluate their function (Borsook and Becerra, 2006, Raichle, 2003 and Schweinhardt et al., 2006).

The realisation that pain is intimately linked to neuronal plasticity is due largely to the work of Patrick Wall (1985). Wall posited that the nervous activity triggered by an insult can itself alter the ways in which pain is subsequently perceived, with sensitisation occurring either peripherally or centrally (Melzack and Wall, 1965). Such a process explains referred pain, allodynia and phantom limb pain. This basic neuronal circuitry is influenced by psychological factors, previous history of pain and the role of genes, all of which may predispose individuals to experience certain kinds of pain syndrome (MacGregor et al., 2004).

Populations of patients reporting symptoms of pain are heterogeneous and complex. Not only do individual responses to pain vary temporally, but patients also present a wide variety of responses to analgesic and other therapeutic molecules (Dionne et al., 2005). Drug dose-response effects also vary across patients experiencing the same or similar kinds of pain (Suri et al., 1997). It is increasingly obvious that many factors, in addition to the primary cause, are needed for the establishment of chronic pain. Specific genes are also thought to play a role in the susceptibility to different kinds of pain (Couzin, 2006). The heterogeneity of patients experiencing pain is not only a major obstacle to understanding the biology of human pain but also presents a challenge in designing treatments that are tailored to the needs of the individual.

Animals in pain research

Animals have been widely used to model human pain conditions. Worldwide, rodents are the most commonly used species, but rabbits, cats, non-human primates, pigs and dogs are also used experimentally to investigate pain. Animal models have been created in efforts to understand the fundamental biology of pain and its natural history (including the initiation, continuation and subsequent termination of the painful signal). Investigations have ranged from looking at the activities of channels and receptors, through peripheral reception and transmission, to the central processing of stimuli caused by nociceptive insults. Animals are also used to test the efficacy, potential adverse side-effects and pharmacokinetics of candidate therapeutic compounds, and more recently to pinpoint the role of genes

and other predisposing factors in chronic pain (Hatcher and Chessell, 2006 and Wilson et al., 2005). Lastly, studies in animals have been used to provide pointers about the patient sub-groups who may benefit from a candidate drug.

However, by and large animal models offer very limited insights into the complex clinical picture of pain. They tend to simulate only one or two simple aspects of human pain conditions; and there are difficulties in interpretation as behavioural observations are restricted to motor responses (Le Bars et al., 2001). Current animal models tend to focus on the physiological aspects of pain syndromes where similarities with humans are found, including the roles of 5-hydroxytryptamine, gamma-aminobutyric acid and the endogenous opioids. But there are also limitations due to species- and strain-specificities (Shir et al., 2001), such as differences between humans and rodents in the bradykinin receptors (Hawkinson et al., 2007). While non-human primates are closest to humans in terms of brain structures, especially the forebrain and its role in anticipation, there are serious ethical, welfare and economic implications associated with using primates.

A significant number of chronic pain patients do not have a clearly defined nociceptive origin for their pain and these patients are thus not easy to represent in animal models. In many cases animal data lack sufficient translational efficacy for either understanding pain or delivering new treatments, and in animal models the psychological aspects of pain, so important in patients, are very difficult to recognise and assess (Karoly and Ruhlman, 2006). As Wilson and colleagues point out: “The substantial investment in pain research by pharmaceutical companies has failed to deliver novel therapies based on new mechanisms, despite positive animal model data and a better understanding of the pain generation and processing pathways. However, pre-clinical and clinical features are seldom reported fully in detail, making a direct correlation between animal model success and clinical failure difficult to interpret” (Wilson et al., 2005).

The problem of novel compounds unexpectedly failing in clinical trials or after marketing, due to adverse effects or lack of efficacy, does need to be robustly examined (Dieppe et al., 2004 and Li, 2004). Animal models of analgesic efficacy are far from ideal (Lever and Rice, 2007) and different approaches, with human subjects, hold promise (Borsook et al., 2002). Most analgesic compounds that fail in clinical trials do so because of unpredicted adverse effects. However, the potential to replace animals in pharmaceutical toxicology is a separate, complex issue that was outside the scope of our workshop.

In this report, we address current paradigms in pain research and how novel concepts, technologies and data, especially human models of pain that incorporate neuroimaging, could be combined to offer better solutions to understanding and treating human pain.

Human volunteers in pain research

Human volunteers, both healthy and from patient populations, have been used to model various forms of pain (Preston, 2003). Human studies of pain can make use of the ability of the volunteer to report on the detailed experience of pain. This is a distinct advantage as Klein et al. point out in their study of neuropathic pain (Klein et al., 2005). In particular, evaluating the form and quality of human pain as well as its duration and location is more relevant and informative than relying upon motor reflexes in animals. Additionally, laboratory studies with volunteers using neuroimaging, electrophysiological and other investigations (genetic, epidemiological and tissue or cell sampling) are more immediately transferable to patients in terms of species relevance (Klein et al., 2005 and Woolf and Salter, 2000).

Studies of healthy volunteers frequently involve creating a surrogate for the sensory features encountered in pain. These features include burning and shooting pain, allodynia and paraesthesia. All can be induced by a variety of electrical and chemical means, and nervous activity monitored using neuroimaging methods (Borsook and Becerra, 2006). While healthy volunteer studies rely on induced sensory features, research with volunteer patients offers a means to explore the actual pain states of interest (Raichle, 2003, Moisset and Bouhassira, 2007 and Peyron et al., 2000).

Volunteer studies with potential to replace animal experiments

A current limitation to studying pain and its treatment is the inability to sufficiently define patient sub-groups. A more accurate stratification of pain patients would enable volunteer studies to be better focused and more powerful. One way forward is to shift the emphasis away from classifying pain anatomically or in relation to a disease, towards a more physiological approach to defining painful conditions, based on careful studies of human subjects as approved by ethics committees.

Establishing a UK Human Pain Research Network could be an important initiative that would help to spearhead and coordinate research with human subjects. Pain crosses many disease boundaries, and a Network would encourage dialogue and consensus between clinical and other researchers; develop partnerships; facilitate the sharing of a wide range of data (including unpublished and/or negative results); conduct meta-analyses of the predictivity of animal models of pain; and emphasise the replacement of animals. A network has been established in Germany for researching neuropathic pain, and the US National Institutes of Health Pain Consortium similarly promotes collaboration between NIH centres involved in pain research. A UK Network could also act as a focal point for attracting new funding from industry as well as private and government funders, for collaborative research. A Human Pain Research Network would assist in progressing the following range of volunteer studies with potential to replace some animal experiments.

Neuroimaging approaches to pain research

Recent advances in neuroimaging have revolutionised understanding of how the brain processes information. The growth in cognitive neuroscience, including studies of memory, attention, language, motivation, emotion, decision-making and consciousness, owes a great deal to human neuroimaging. Neuroimaging methods used with healthy and patient volunteers have led to a clearer understanding of human brain topography and the plasticity of neuronal circuitry in various healthy and diseased states, not least the central representation of pain perception. Neuroimaging has also been used in conjunction with painful stimulation in volunteers (by laser, heat or cold, electric shock, capsaicin or pressure) to study brain areas activated by such modalities of painful stimuli (Flor and Bushnell, 2005, Becerra et al., 1999 and David et al., 2002). Consequently, there has been an expansion in research articles from imaging centres throughout Europe and North America, there being around 60 such centres at present (Borsook and Becerra, 2006 and Raichle, 2003).

Although functional imaging is a relatively young methodology, it has enabled a new and more nuanced understanding of human pain and offers a number of opportunities to replace the use of animal models in pain research. Borsook and Becerra, reviewing fMRI and investigations of pain, suggest that human neuroimaging has opened new approaches to understanding chronic pain, including: “The ability to evaluate activity and organize active regions into neural circuits that subservise specific pain/analgesia functions (i.e. sensory, emotional, autonomic, endogenous analgesic circuits) [which] is a step forward” (Borsook and Becerra, 2006). The authors go on to suggest that several new fMRI approaches will aid a

better understanding of human pain processing including large-scale systems organisation; techniques that describe circuits and connectivities (Johansen-Berg and Behrens, 2006); and automated parcellation of brain areas including the thalamus.

Other reviews have also agreed that the use of neuroimaging has contributed new insights into how certain regions of the brain are involved in the complex processing of experimental pain in humans (Peyron et al., 2000 and Tracey et al., 2002). For example, neuroimaging of human volunteers can be used to follow brain activity not only during the application of noxious stimuli, but also in anticipation of such stimulation (Fairhurst et al., 2007 and Wise et al., 2007). Functional brain imaging is already being used to compare neural activation patterns related to cutaneous, musculoskeletal, visceral and neuropathic pain.

Neuroimaging has been applied to investigate pharmacological and non-pharmacological interventions in the central processing of pain (Flor and Bushnell, 2005 and Pattinson et al., 2007). Taking the case of opioids as an example, PET has been used to study the changes in regional cerebral blood flow when an opioid, fentanyl, is administered (Casey et al., 2000). Changes in the occupancy of mu-opioid receptors were investigated in healthy volunteers by using carfentanil in a PET study during acute muscle pain (Zubieta et al., 2001). Recently Pattinson and colleagues have applied pharmacological fMRI to investigate opioid effects on the cerebrovascular circulation. It is not known if opioids alter neurovascular coupling, or if their effects are purely neuronal. The mu-opioid receptor agonist remifentanyl was used in volunteers given hypercapnic challenges. Similar methodology was recommended by the authors for evaluating other potentially vasoactive compounds in human volunteers (Pattinson et al., 2007). PET has also been used to investigate spinal cholinergic activation in response to opiate administration, a marker for one site of opiate analgesic action. It is increasingly possible to move into fully characterised volunteer patients to assess opiate activity in painful syndromes. Historically, studies such as these would have used experimental animals.

fMRI has also been used to monitor analgesic effects on both acute and chronic pain in human subjects usually following an applied painful stimulus. Such pharmacological fMRI can also lead to the early assessment of novel drugs and thus reduce reliance upon animal tests and the exposure of patients to molecules with limited or no therapeutic value (Borsook et al., 2002 and Pattinson et al., 2007). Examples include studies of cyclooxygenase (COX) inhibitors (Baliki et al., 2005) and amitriptyline in chronic pain conditions (Morgan et al., 2005) and the effects of drugs on capsaicin-induced hyperalgesia (Borsook et al., 2003). Thus there is certainly potential for using fMRI for objective evaluation of drug efficacy, which could reduce reliance upon animal models for some of the pre-clinical stages of drug development.

Several studies have used fMRI to investigate endogenous modulation of pain (Song et al., 2006 and Borsook and Becerra, 2006) and to map brain regions implicated in the alteration of perceived pain. Such a focus has been used to study the placebo effect and there are signs that neuroimaging will bring some resolution to this area, which is of significance to pain treatment (Cavanna et al., 2007).

Electroencephalographic (EEG) and magnetoencephalographic (MEG) recordings, which can measure brain activity related to painful stimuli, provide direct data on excitatory and inhibitory neuronal processes and thus can augment concomitant fMRI or PET studies (Langley et al., 2000). EEG and MEG have a very good temporal resolution and can capture the central processing of pain in real time (Hillebrand et al., 2005 and Hobson et al., 2006). At the Neuroimaging Research group in Aston University (Birmingham, UK), Furlong and co-workers have, for the last decade, been looking at visceral pain and its associated cortical activations to provide a robust model of pain sensitisation in the human viscera (Hobson et al., 2006 and Hobson et al., 2005). The different strengths of MEG, which provides images of

cortical activity with high spatial (2-3 mm) and temporal (better than 1 ms) resolutions, are combined with MRS which can show the distribution of targeted neurotransmitters and metabolites throughout the brain.

Neuroimaging has also brought greater clarity to our understanding of primary headache, suggesting that such primary headache-related pain is triggered by a central event which activates the trigeminovascular reflex (May, 2006 and Leone et al., 2007). Such studies have led to significant strides forward in understanding the mechanisms of head pain in humans.

At present competitive radioligand binding studies are in their infancy but may have the potential to be used in novel analgesic screening (Song et al., 2005). New small radioligands currently used in animal studies could, with appropriate safeguards, be used in carefully monitored human neuroimaging studies.

At present there are very few available drugs which selectively modulate human pain that could be used as pharmacological tools, to study pain mechanisms and to classify patients into sub-groups to enable more powerful clinical studies. However, there are 'failed' drug candidates and withdrawn drugs for which there are both safety and efficacy data, and such molecules might be very useful in clinical research. Where ethical issues can be resolved, these could be used in carefully selected patient groups to tease apart cellular and biochemical aspects of pain, and companies should be approached to consider making such compounds available.

For example, NK1-receptor antagonists have been evaluated in many pain conditions but are not used therapeutically since they are ineffective despite showing promise in animal studies (Hill, 2000). These compounds, for which full pre-clinical data exist, could be used in careful volunteer neuroimaging studies to identify the role played by pain-related molecules, such as substance P whose transmission function is primarily mediated by NK-1 receptors. Similarly, enkephalins have opiate receptor-binding activity and are found in the thalamus and regions of the spinal cord that have been implicated in pain signalling. Enkephalinase-inhibitors, effective in animal models of pain, were not therapeutically useful but may have also a value as pharmacological tools in human neuroimaging research (Hughes et al., 2007).

Combining various neuroimaging approaches increases their individual resolution and power in both the understanding of human pain and the development of new treatments. As a field neuroimaging in humans is relatively young; however it has already impacted significantly on pain research and has potential to replace a growing number of animal experiments. Under current research paradigms, results from clinical studies are sometimes pursued further in animal models. This paradigm is not ideal and would need to change in order to progressively replace animal experiments. As neuroimaging and related human-based techniques further develop, this becomes increasingly possible.

Complementary human-based approaches

Human neuroimaging is a key technique in the initiative to replace pain research on animals, but cannot address all the areas in which animal experiments are currently conducted. The insights obtained from using neuroimaging techniques can however be extended by combining them with related clinical and other human-based approaches (Kupers and Kehlet, 2006). These include microdialysis, epidemiology, human genomics and human cell and tissue studies. Healthy volunteers and patients with painful conditions have been subjects for a variety of studies involving microdialysis. This technique uses a very fine probe to repeatedly sample extracellular fluid locally from different tissues. Molecules from the tissue diffuse through a semi-permeable membrane into the dialysate solution as it passes through the probe.

The collected fluid is then analysed for the concentration and identity of potentially interesting molecules, and how these fluctuate in real time (Parrot et al., 2004).

Microdialysis has been used to analyse fluid from the skin, muscle, spinal cord and synovium of human subjects, to investigate neurogenic inflammation, critical limb ischaemia, myalgia, inflammation, complex regional pain syndrome and various experimentally induced forms of pain. Despite some technical limitations, microdialysis has provided valuable insight into painful syndromes, especially when combined with other approaches, such as laser Doppler scanning, infusion of test substances, MEG, analysis of biopsy samples and stimulation of nociceptive C-fibres (Hogberg et al., 2006 and Tegeder et al., 2002).

Epidemiology can generate new hypotheses, highlight important research questions, identify at-risk groups for certain conditions (e.g. those with a strong psychological component such as fibromyalgia), and analyse genetic influences on pain sensitivity and the risks of developing painful conditions.

Genome-wide association research, twin studies and long-term developmental studies should provide information relevant to understanding susceptibilities to painful syndromes. Research using the TwinsUK adult registry at St Thomas' s Hospital, London, has already assessed genetic influences on the progression of osteoarthritis (Zhai et al., 2007). Existing high-quality datasets, such as the Avon Longitudinal Study of Parents and Children used to follow inherited effects on a variety of conditions including obesity (Timpson et al., 2007), could be mined for relevant data. National biobank projects, with information on many thousands of people relating the health effects of genes, lifestyle and environment, are likely to provide high-quality data useful to pain researchers (Molnar and Bencsik, 2006).

Fertleman and colleagues have investigated paroxysmal extreme pain disorder, an inherited pain syndrome (Fertleman et al., 2006), by using a genome-wide linkage search followed by a mutational analysis of the candidate gene SCN9A. This identified eight missense mutations in eleven families. They then used *in vitro* functional analysis of three of these mutations to identify the dysfunctional sodium channel linked to the mutation. Both an understanding of this familial pain disorder and clues as to treatment have resulted.

DNA microarray technology applied to human tissues, including blood samples and nerve biopsies, is used for system-wide genomic research and analysis of levels of gene expression. Knowledge of gene regulation networks in different pain conditions, and gene expression changes in anatomical areas that process painful stimuli, is highly desirable and potentially achievable in humans.

These examples from a growing literature combine biochemical and genetic tools at the population level to tease apart the nature of debilitating pain in humans without using animals (Cox et al., 2006 and Couzin, 2006). Human data from genetic epidemiology may be further explored by some researchers in genetically modified animal models, therefore this is an area where better non-animal methods should be sought so as to reduce reliance upon animal studies.

In vitro cell and tissue studies are useful for investigating the molecular and cellular mechanisms of both acute and chronic pain. Using carefully controlled stimuli and relevant end-points, cellular models can provide information on certain aspects of *in vivo* signal transduction between cells or within a nociceptive cellular receptor array.

There is growing scope to use *ex vivo* human primary cells and tissues, either post-mortem or donated by patients undergoing surgery. Relevant human tissues include brachial plexus nerves, brain, neuroma, dorsal root ganglion, spinal nerve roots and spinal cord (Facer et al., 2007, Durrenberger et al.,

2006 and Anand et al., 2006). These are increasingly being used for functional assays in early drug development, in place of efficacy studies in animals; but this work is seriously limited by regulatory and logistical barriers to obtaining reliable supplies of functional human tissues (Thasler et al., 2006 and Sladowski et al., 2005). A network of regional human tissue banks operating within an ethically robust but research-focused framework, is an urgent necessity for this and many other kinds of research, both clinical and fundamental.

Conclusions

Animal models cannot faithfully mimic the complex range of phenomena found clinically in the various manifestations of pain. They provide some pharmacodynamic information during drug development and a partial picture of some of the processes that follow from an experimental painful insult. But there needs to be more understanding of the complexity and heterogeneity of human pain and the role of various genetic, biological and psychological factors in the human perception of and response to pain.

There are strong ethical and legislative obligations to replace animal experiments with alternative methods, and to refine procedures to cause less suffering and reduce animal use. The focus of this review is on replacement approaches. Neuroimaging technologies have extended our knowledge of the complexity and plasticity of the human brain in pain states. Such technologies are being rapidly developed and should contribute to a better understanding of human pain. Imaging resolutions are not, as yet, at the single cell level, but nevertheless neuroimaging has the capacity to replace some current uses of animals in pain research.

An expansion of human-based pain research will aid understanding of pain conditions, improve volunteer studies and advance replacement of animal experiments in particular areas. More detailed sub-typing of human pain conditions will enable better focused studies. Data from neuroimaging research can be augmented by related clinical and other human-based approaches such as microdialysis, epidemiology and *in vitro* research using human cells and tissues. Where these human models of pain are ethical, robust and well characterised they should always be used in preference to animal models.

Recommendations

1. We believe that current paradigms and practices in pain research need reconsideration. A strategy is now required for how existing and novel concepts, and complementary approaches in human-based pain research, especially neuroimaging, could offer better solutions than animal experiments.
2. Researchers and funders should identify areas of pain research, such as acute pain, psychological aspects, and the genetics of pain, where animal experiments can be prioritised for replacement by patient-based and related non-animal approaches.
3. Pharmaceutical companies with failed candidate molecules or withdrawn drugs should consider making these available to researchers where possible. If there are sufficient safety and efficacy data, such molecules could provide vital pharmacological tools to improve careful human studies of pain.
4. Neuroimaging data from human studies can be combined with information from microdialysis research, population data, human genetic studies and *in vitro/ex vivo* research with human cells and tissues. These approaches would contribute to an important multi-disciplinary effort to replace animal experiments.
5. Researchers should prioritise efforts to classify sub-groups of pain patients more accurately and physiologically, so that volunteer studies can become increasingly focused and powerful. This

would also enable better, early clinical studies of the efficacy and tolerability of novel drug candidates.

6. More research is needed, particularly to identify reliable biomarkers of human pain and to understand placebo effects. This knowledge would provide a valuable resource for improved human models of pain.
7. Networks of local human tissue banks for research should be established and adequately funded to facilitate the availability of suitable human tissues and cells.
8. More use should be made of human epidemiology data relevant to pain, for example from twin research, longitudinal child development studies and national biobanks, to identify factors which underlie pain and its perception. These could be assembled into high-quality datasets which would be helpful both to researchers and clinicians.
9. Establishing a UK Human Pain Research Network would encourage dialogue, collaborations and consensus among researchers, and help develop a strategy for human volunteer studies, including with a view to replacing animal experiments. It could also act as a focus for attracting new funding for multi-disciplinary pain research.

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