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REVIEW ARTICLE

Studying human nociceptors: from fundamentals to clinic

Steven J. Middleton,[1,†] Allison M. Barry,[1,†] Maddalena Comini,[1] Yan Li,[2] Pradipta R. Ray,[3] Stephanie Shiers,[3] Andreas C. Themistocleous,[1,4] Megan L. Uhelski,[2] Xun Yang,[1] Patrick M. Dougherty,[4,†] Theodore J. Price[3,†] and David L. Bennett[1,†]

[†]These authors contributed equally to this work.

Chronic pain affects one in five of the general population and is the third most important cause of disability-adjusted life-years globally. Unfortunately, treatment remains inadequate due to poor efficacy and tolerability. There has been a failure in translating promising preclinical drug targets into clinic use. This reflects challenges across the whole drug development pathway, from preclinical models to trial design. Nociceptors remain an attractive therapeutic target: their sensitization makes an important contribution to many chronic pain states, they are located outside the blood–brain barrier, and they are relatively specific. The past decade has seen significant advances in the techniques available to study human nociceptors, including: the use of corneal confocal microscopy and biopsy samples to observe nociceptor morphology, the culture of human nociceptors (either from surgical or post-mortem tissue or using human induced pluripotent stem cell derived nociceptors), the application of high throughput technologies such as transcriptomics, the *in vitro* and *in vivo* electrophysiological characterization through microneurography, and the correlation with pain percepts provided by quantitative sensory testing. Genome editing in human induced pluripotent stem cell-derived nociceptors enables the interrogation of the causal role of genes in the regulation of nociceptor function. Both human and rodent nociceptors are more heterogeneous at a molecular level than previously appreciated, and while we find that there are broad similarities between human and rodent nociceptors there are also important differences involving ion channel function, expression, and cellular excitability. These technological advances have emphasized the maladaptive plastic changes occurring in human nociceptors following injury that contribute to chronic pain. Studying human nociceptors has revealed new therapeutic targets for the suppression of chronic pain and enhanced repair. Cellular models of human nociceptors have enabled the screening of small molecule and gene therapy approaches on nociceptor function, and in some cases have enabled correlation with clinical outcomes. Undoubtedly, challenges remain. Many of these techniques are difficult to implement at scale, current induced pluripotent stem cell differentiation protocols do not generate the full diversity of nociceptor populations, and we still have a relatively poor understanding of inter-individual variation in nociceptors due to factors such as age, sex, or ethnicity. We hope our ability to directly investigate human nociceptors will not only aid our understanding of the fundamental neurobiology underlying acute and chronic pain but also help bridge the translational gap.

1 Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford OX3 9DU, UK
2 Department of Anesthesia and Pain Medicine, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
3 Department of Neuroscience and Center for Advanced Pain Studies, University of Texas at Dallas, Richardson, TX 75080, USA

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4 Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2000, South Africa

Correspondence to: David L. Bennett
Nuffield Department of Clinical Neurosciences
University of Oxford
Oxford, UK
E-mail: david.bennett@ndcn.ox.ac.uk

Correspondence may also be addressed to: Theodore J. Price
University of Texas at Dallas, Department of Neuroscience and Center for Advanced Pain Studies
Richardson, TX, USA
E-mail: theodore.price@utdallas.edu

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Abbreviations: CGRP = calcitonin gene-related peptide; DRG = dorsal root ganglion; IEM = inherited erythromelalgia; IPSC = induced pluripotent stem cell; TG = trigeminal ganglia; TTX-r/s = tetrodotoxin-resistant/sensitive

Introduction

Sherrington[1] was the first to coin the term nociceptor as the neural apparatus responsible for detecting noxious stimuli. Noxious stimuli are those stimuli that can cause tissue injury including extremes of temperature, mechanical, and chemical insults. Many nociceptors are polymodal (responding to combinations of these stimulus types) although there has been recent debate regarding the extent of polymodality in rodent studies[2,3]; this may partly depend on whether nociceptors innervate superficial versus deep targets.[4] A subgroup of nociceptors referred to as mechanically-insensitive, or silent nociceptors, are unresponsive to all modalities in the naïve state and only respond to stimuli in the presence of inflammation, revealing the capacity of nociceptors to become sensitized in disease states.[5]

In healthy systems, the detection of noxious stimuli begins with afferent activation wherein sufficient signal results in a complex pain percept with discriminative and affective components. Peripheral and/or central sensitization of this system—as seen in patients with chronic pain—can be debilitating; in such a situation there is often a poor correlation between the stimulus intensity/degree of tissue injury and pain percept. Chronic pain affects up to 20% of the general population and causes significant reductions in quality of life; unfortunately, treatment remains inadequate.[6]

Nociceptors are an attractive therapeutic target of painful conditions: their sensitization makes an important contribution to many chronic pain states, they are located outside the blood–brain barrier, and they exhibit relative specificity. Even so, several promising preclinical drug targets have failed to translate to clinical use. This reflects challenges spanning the whole drug development process from evolutionary divergence between humans and animal models to the difficulty in assessing pain in analgesic drug trials.

Here, we address the fundamental neurobiology of human nociceptors in both acute and chronic pain states. The molecular profiling, available model systems, and functional assessment of the nociceptive system are discussed drawing on species comparisons, where applicable, and considering therapeutic implications (Fig. 1 and Table 1). Preclinical models show functional age and sex differences in nociceptors[7–11] and although there are age and sex dependent differences in pain perception in humans,[12] these have yet to be directly linked to differences in nociceptors. We look forward to the addition of these datasets, as they will significantly deepen our understanding of human nociceptor function. Our hope is that these emerging techniques and greater availability of cell/tissue samples available to study human nociceptors will help bridge the translational gap seen in pain research.

Human nociceptor morphology and anatomical clinical assessment

The soma of nociceptors reside in dorsal root ganglia (DRG) or trigeminal ganglia (TG), with axons extending terminals into innervation targets such as the epithelium. These terminals are widely distributed in the skin, joints, deep tissues, and cornea as free nerve endings.[13]

Classically, nociceptors can be classified as either C- or A-fibres. C-fibres, comprising the majority of nociceptors, are unmyelinated small diameter neurons with a slower conduction velocity than their A-fibre counterparts. These neurons are commonly subdivided through molecular and electrophysiological criteria, and these distinctions are highlighted throughout this review. Along their axons, C-fibres are closely associated with non-myelinating Schwann cells, forming Remak bundles.[14] Recently, a novel class of cutaneous Schwann cells have been identified in mice.[15] They are closely associated with C-fibre nerve endings in the skin and participate in the transduction of high threshold mechanical stimuli. The presence of this class of Schwann cell has yet to be confirmed in humans.

By contrast, Aδ-fibres are thinly myelinated with a faster conduction velocity.[16] A subset of Aδ-fibres form free nerve endings in the epithelial layer and respond to noxious stimuli. Aβ-nociceptors form a unique group of thickly myelinated high threshold mechanosensors. These neurons have conduction velocities above that seen with Aδ-fibres and are largely insensitive to low-threshold brush stimuli.[17] While sometimes overlooked compared to C- and Aδ-fibres, these nociceptors have been described across species, from mouse to human.[16,17] Unique molecular markers have yet to be shown, and the proportion of Aδ/Aβ nociceptors remains vague because of sampling bias and varying definitions in conduction velocity. In animal models, Aβ nociceptors comprise anywhere from 18% to 65% of nociceptive A-fibres.[16]

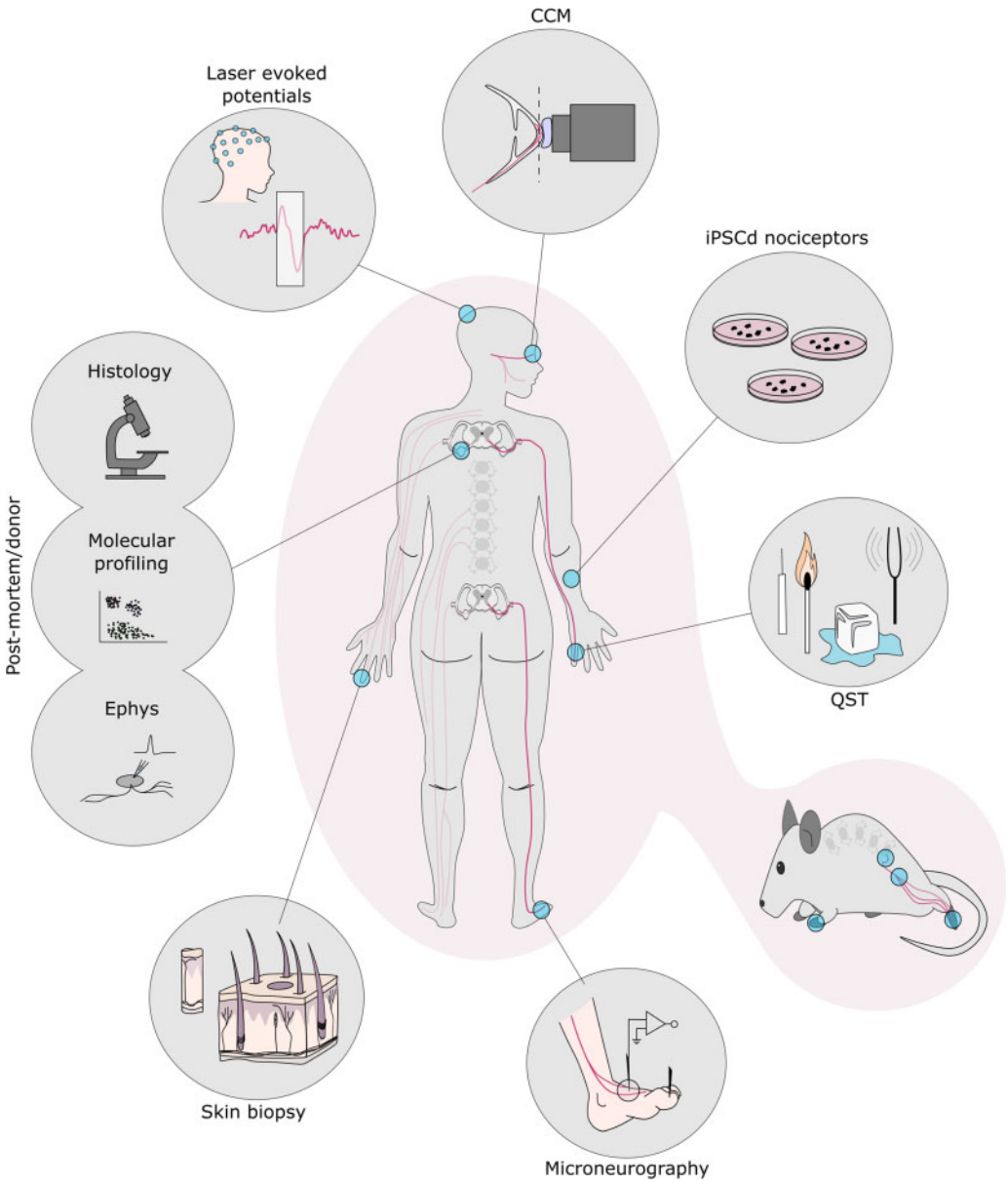

Figure 1 Highlighted methods to study human nociceptor anatomy and physiology. *Clockwise from top*: corneal confocal microscopy (CCM): focal plane (dashed line) lands on the subbasal nerve plexus; IPSC-derived nociceptors; quantitative sensory testing (QST); numerous rodent assays mirror those seen in humans with parallels highlighted throughout this review; microneurography schematic of a peroneal nerve recording; skin biopsy schematic with primary afferents shown in black; post-mortem and donor tissue can be used across applications: histology, molecular profiling and electrophysiology (Ephys); laser evoked potentials, recorded through EEG, measures cortical output in response to heat stimuli.

The morphological and electrophysiological differences between fibre types contribute to the end percept. Generally, A-fibre activation results in a rapid, sharp pain. Correspondingly, nociceptors with C-fibres contribute to enduring, persisting pain sensations.[18] However, it is possible that on-going A-fibre nociceptor activity may also result in burning pain. Electrophysiological recordings of monkey Aδ nociceptors have informed on their branching and structure, suggesting it is possible the long unmyelinated peripheral branches of Aδ nociceptors may blur the temporal separation between Aδ and C-fibre nociceptors.[19] This has yet to be fully resolved, but Nagi and colleagues[17] provide support for A-fibre nociceptor activation, in humans, resulting in fast, sharp pain.

From their innervating target tissues, nociceptors extend central axons to second-order neurons in the trigeminal subnucleus caudalis or the dorsal horn of the spinal cord. In the spinal cord, A-fibres connect with neurons in laminae I, II and V, while C-fibres form synapses in laminae I and II before modulation and/or transmission to the brain.[13,16] In broad anatomical strokes, there are many conserved features between humans and experimental mammals, including general fibre types and projection pathways. Nevertheless, notable differences exist. Compared to mouse and rat for example, human DRGs are larger, contain more satellite glia, and have more connective tissue between neurons.[20,21] There is also a relatively smaller number of unmyelinated axons per human Remak bundle compared to rat.[22]

Cutaneous nociceptors and intraepidermal nerve fibre density

Nociceptors innervating the skin are commonly studied clinically and experimentally. Morphologically, afferents project through the dermis into the epidermis as epidermal nerve fibres. Prior to

Table 1 Technical summary for the study of human nociceptors

Technique	Tissue access[a]	Clinical availability	Advantages	Disadvantages
CCM	*In vivo*	Yes	Non-invasive, rapid, longitudinal tracking, simple analysis, diagnostic relevance	Macrostructure, small-fibre specific
Skin biopsy/IENFD	Biopsy	Yes	Minimally invasive, time course possible, simple analysis, diagnostic relevance, terminal morphology visible	Terminal focus, antibody penetration in the skin is limited, antibody specificity, limited to cutaneous fibres, time course studies require repeated biopsies
Donor IHC/ISH	Donor	No	Multiplexing allows for molecular characterization, *in situ* localization	Tissue availability, antibody specificity, time between death and tissue collection is variable
Deep molecular sequencing	Donor	No	Deep molecular profiling, single cell resolution, data rich	Cell (and nuclei) size provide a technical challenge, cost, computationally complex, variable sequencing depth
IPSC-derived nociceptor cultures	Biopsy/blood sample	No[b]	Minimally-invasive sample, scalable, functional readouts, personalized interrogation of variants, genome editing allows interrogation of causal genes, culture-based system for therapeutic screening	Many different protocols, the full heterogeneity of human DRG lacking, *in vitro* model, technical expertise required, long maturation time
Human DRG cultures	Donor	No	Functional readouts, access to the full diversity of DRG neurons, allows therapeutic assessment in human tissue	*In vitro* model, requires tissue dissociation, technical expertise required, low throughput, not scalable
Human DRG cultures	Surgery	Yes	Functional readouts, access to the full diversity of DRG neurons, allows therapeutic assessment in human tissue, known pain phenotype of patient	*In vitro* model, requires tissue dissociation, technical expertise required, low throughput, not scalable, surgeries are rare
Microneurography	*In vivo*	Yes	*In vivo* functional characterization of cutaneous afferents (sensory and sympathetic), detailed stimulus-response functions possible, can be used in combination with psychophysical tools, pre-post drug treatment recordings possible	Technical expertise required, demanding for experimenter and participant, sampling biases exist, limited to cutaneous fibres, Aδ-fibres difficult to study, informs regional nerve activity only, more invasive than nerve conduction studies

This list does not include clinical/experimental assays targeting sensory percepts [e.g. quantitative sensory testing (QST) or laser evoked potentials]. Techniques available in clinic are specified. CCM = corneal confocal microscopy; IENFD = intraepidermal nerve fibre density; IHC = immunohistochemistry; ISH = *in situ* hybridization; scRNA-seq = single cell RNA sequencing.

[a]Fresh donor tissue access is limited.

[b]IPSCd nociceptors are not routinely studied clinically but participants have benefited from this personalized approach (see 'IPSC-derived nociceptors derived from patients living with chronic pain' section).

penetrating the basement membrane (bordering the dermal and epidermal layers in the skin), these fibre bundles project horizontally and branch extensively. This dense neural network is referred to as the sub-epidermal nerve plexus.

Clinically, the intraepidermal nerve fibre density (IENFD), a measure of individual small fibre terminals per area, can be used to monitor nociceptor density through minimally-invasive skin biopsy[23] and decreased IENFD was developed as a diagnostic measure particularly in small fibre neuropathy.[24,25] Decreased IENFD has been demonstrated in many painful conditions and diseases including diabetic polyneuropathy,[26,27] carpal tunnel syndrome (nerve entrapment),[28] Charcot-Marie-Tooth disease type 1A,[29] chronic ischaemic pain,[30] complex regional pain syndrome,[31] Guillain-Barré syndrome,[32] Fabry disease,[33] and more (for a review see Sommer and Lauria[34]) (Fig. 1). Preclinically, cutaneous nerve fibre densities can also be used to examine fibre changes in mice, providing a translatable parallel.[35]

As well as IENFD quantification, skin biopsies can show morphological changes such as axonal swellings. Axonal swellings are markers of axonal degeneration and contain watery axoplasm, neurofilaments and abnormal mitochondria.[36] An increase in axonal swellings may predict nerve fibre loss.[37] The molecular properties of nociceptors can be interrogated, including expression analyses of regeneration associated genes such as GAP43.[38]

Skin biopsy has also revealed small fibre pathology in conditions which were mainly thought to have central sensitization as a

cause of chronic pain, such as fibromyalgia,[39] and conditions thought to mainly relate to cutaneous pathology, such as the blistering condition dystrophic epidermolysis bullosa.[40]

Corneal nociceptors and corneal confocal microscopy

Corneal nerves originate from the TG and like the skin, form a sub-basal nerve plexus of axons before projecting terminals into the epithelium. The human cornea has an extremely high nerve density and the development of corneal confocal microscopy (CCM) has allowed clinicians and researchers to examine corneal nociceptors in a non-invasive manner. Here, images of nerve fibres within the sub-basal nerve plexus are studied.

Like skin epidermal afferents, nerve density in the plexus is reduced under pathological conditions: the corneal plexus nerve density, visualized though CCM is reduced in patients with neuropathies such as small fibre neuropathy, diabetic neuropathy, and CMT1A (Fig. 1, CCM),[41–45] and has also been reported in fibromyalgia.[46] The ease and non-invasiveness of CCM enable repeated longitudinal measures. These strengths are particularly evident when applied to trials which involve disease-modifying therapies.

Nociceptor subpopulations and gene expression profiles in the human dorsal root ganglion

The simplistic distinction of C- and A-fibre nociceptors on morphological and electrophysiological ground fails to capture the true diversity of primary afferents now demonstrated by molecular profiling. Pain neuroscientists often refer to the peptidergic and non-peptidergic populations of nociceptors. These subpopulations are defined by expression of calcitonin gene-related peptide (CGRP) and/or substance P (SP) for the peptidergic population and binding of isolectin B4 (IB4) and/or P2X3 receptor expression for the non-peptidergic population.[47] In mice, these populations are mostly distinct entities, particularly when distinguished by protein expression. Both *in situ* hybridization and single cell RNA sequencing experiments in mice largely support the presence of these two, distinct subpopulations. Single cell RNA sequencing experiments in mice have further divided these populations into distinct subgroups[48,49] and delineated their developmental lineages.[50] In rats, the segregation of these populations is blurred as there is more overlap between CGRP expression and IB4 binding.[51] There is also overlap in the central projection termination of these afferents in the rat that is not seen in mice.[52]

Efforts to identify the molecular identity of human nociceptors have been hindered by the lack of IB4 binding to human sensory neurons (there are reports of binding in the Rhesus macaque[53]) and difficulties with antibody specificity on human tissues. However, several recent studies have overcome these technical issues, finding differences between murine and human nociceptors.[21,54–56] The most important of these is that CGRP (*CALCA* gene) and P2X3 (*P2RX3* gene) strongly overlap in human DRG (Fig. 2A). There is substantial overlap also in rats,[51,52] but not as extensive as seen in humans. In humans, this likely occurs because a greater proportion of human nociceptors express the *CALCA* gene.[56] In mouse nociceptors, the capsaicin receptor, TRPV1, is nearly exclusively localized to the peptidergic class of nociceptors.[57] In rats, TRPV1 is expressed in both peptidergic and non-peptidergic nociceptors.[51,58–60] However, in human DRG, TRPV1 is found in a substantially larger proportion of neurons suggesting that most human nociceptors express TRPV1.[56] Similar results have been observed for TrkA (*NTRK1* gene), the high affinity nerve growth factor (NGF) receptor,[21] which is exclusively expressed in the peptidergic subset of nociceptors in adult mouse DRG.[47] On the other hand, TRPA1 (an ion channel activated by environmental irritants), which is mostly found in the non-peptidergic nociceptor population in mouse DRG,[61] is found in a smaller subset of human DRG neurons, and this population overlaps with TRPV1 expression.[56]

Larger diameter, Aβ low-threshold mechanoreceptor neurons play an important role in neuropathic pain because they contribute to mechanical allodynia.[62] In mice, these neurons can be labelled with antibodies against heavy chain neurofilament (NF200).[63] In human DRG, antibodies against this protein label all neurons.[21] A potential marker for Aβ low threshold human DRG neurons is *KCNS1*, which encodes the $K_v9.1$ β subunit of voltage-gated K^+ channels (Fig. 2B). This mRNA is selectively expressed in non-CGRP, non-P2X3 mRNA-positive human DRG neurons whereas it is expressed in some peptidergic and non-peptidergic neurons in mice.[56]

From these studies, we conclude that most human nociceptors likely have a peptidergic phenotype (Fig. 2A and B). A subset of human DRG neurons show mixed expression of genes that are found in both the peptidergic and non-peptidergic subsets of nociceptors in mice. Rat nociceptor subtypes may be more closely related to human nociceptors but there are differences, such as the lower proportion of TRPV1-expressing nociceptors in rat versus human.[51,56,58,59,64] Our understanding of populations of human DRG nociceptors would be greatly improved by single cell RNA sequencing datasets. These have been difficult to generate owing to the large size of human DRG neurons, including their relatively large nuclei. However, similar datasets in non-human primates were recently generated. These show broad conservation of sensory neuron populations between primate and mouse; however, there are differences at the individual gene level. Used in combination with human genetic analysis this data provides insight into the cellular origin of human chronic pain with multiple chronic pain conditions appearing to converge on two populations.[65] Similar datasets in rats, which do not currently exist to our knowledge, would enable decisions about appropriate species choice for back-translation studies that will be important for target validation and mechanistic insight. Bulk sequencing datasets have been generated in rats and offer insights into changes in DRG transcriptomes in response to injury.[66,67] Overcoming these technical challenges and gaps in knowledge will advance the field dramatically.

Functional impact of differences between human, murine and rat nociceptor subpopulations

Elegant molecular genetic manipulations in mouse have demonstrated that peptidergic neurons are primarily responsible for thermal pain while the non-peptidergic subset of neurons mediate mechanical pain.[57,68] More complex behavioural analysis in mice complicates this issue somewhat, revealing that while the TRPV1+, peptidergic population is responsible for reflexive heat behaviours, these neurons also mediate 'coping' behaviours in response to a variety of different stimuli.[69] These distinct sets of murine sensory neurons also innervate the dorsal horn differently. The peptidergic subset of mouse nociceptors terminate in lamina I and the outer portion of lamina II. These neurons make synaptic contact with projection neurons indicating that they are capable of driving direct activation of a nociceptive relay to the brain.[70–72] On the other hand, non-peptidergic mouse nociceptors terminate mostly in inner lamina II and do not make direct synaptic connections with projection neurons. Instead, these neurons terminate on interneurons that form a dense network of synaptic connections that regulate the output of projection neurons.[70,72] Synaptic

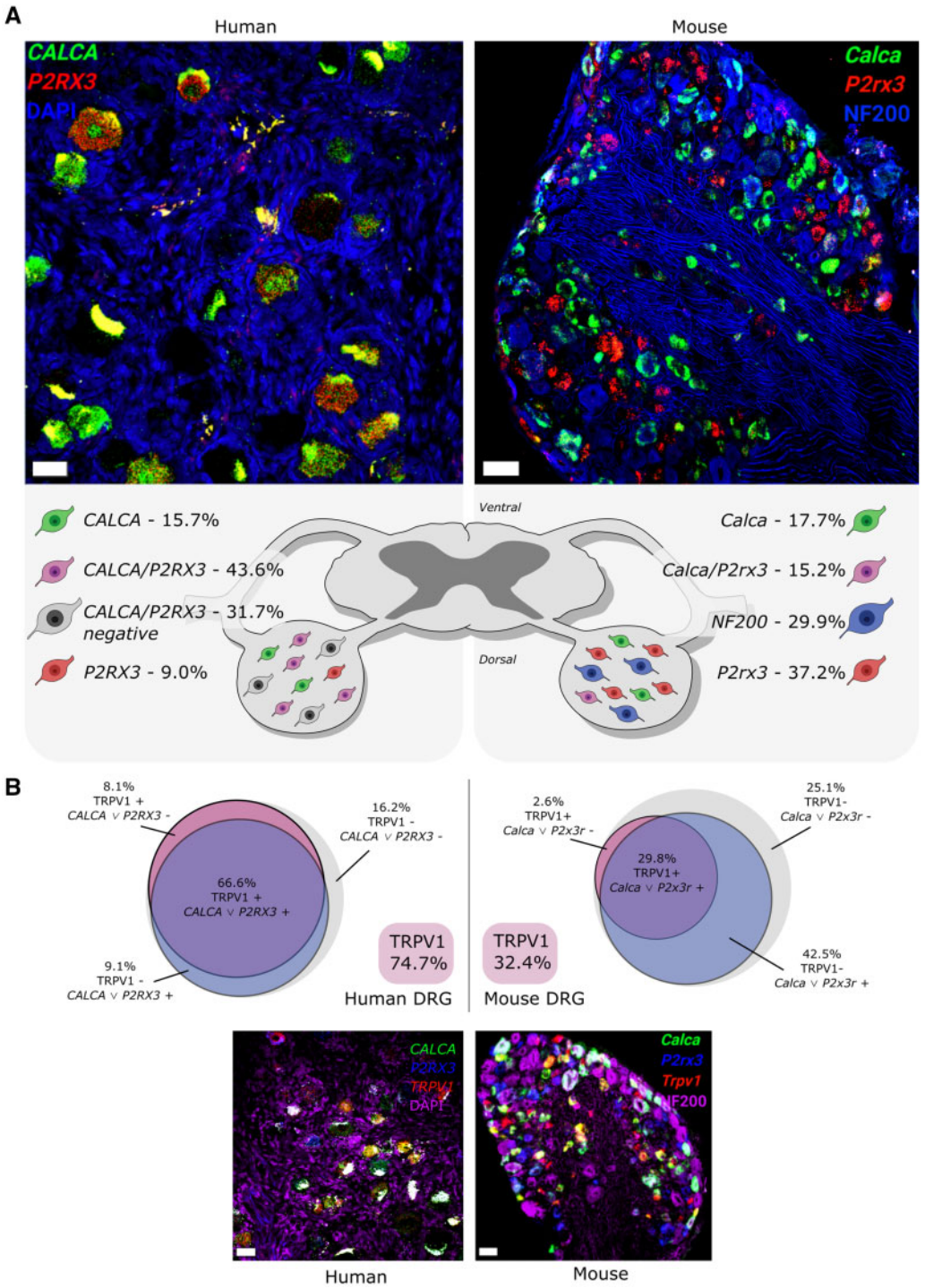

Figure 2 Classic sensory neuron subpopulations are different between mouse and human DRG. (**A**) Mouse and human DRG labelled (at ×20) with RNAscope *in situ* hybridization for *CALCA* (CGRP; green) and *P2RX3* (P2X3R; red) mRNA. Mouse and human DRGs were co-stained for NF200 protein (blue) and DAPI (blue), respectively. Human DRG had a large population of *CALCA*/*P2RX3* co-expressing neurons (43.6%) that was much smaller in mouse (15.2%). This was due to more *CALCA* being found in human DRG than mouse. These data indicate that the peptidergic (CGRP-positive) and non-peptidergic (P2X3R-positive) neuronal subclasses are not separate entities in human DRG and support that the mouse and human nociceptor phenotypes may be divergent. (**B**) Mouse and human DRG (at ×20) labelled with RNAscope *in situ* hybridization for *CALCA* (CGRP; green), *P2RX3* (P2X3R; blue) and *TRPV1* (TRPV1; red) mRNA. Mouse and human DRGs were co-stained for NF200 protein (purple) and DAPI (purple), respectively. *Trpv1* is expressed in a small percentage of neurons (32.4%) in mouse DRG, most of which are *Calca* and/or *P2rx3* positive (29.8%). However, in human DRG, *TRPV1* is expressed in a much larger population (74.7%), the majority of which are positive for *CALCA* and/or *P2RX3*. Data shown are summarized from Shiers *et al.*[56] Scale bar = 50 μm.

arrangements of human nociceptors in the spinal cord have not been delineated, but immunohistochemical studies suggest that CGRP terminals are homogenous throughout lamina II,[73,74] as are TRPV1-positive nerve terminals.[64] Such a difference is consistent with expression of CGRP and TRPV1 in a larger population of human nociceptors.[56,64] Interestingly, both mechanically sensitive and insensitive human nociceptors respond to the TRPV1 agonist capsaicin,[75] consistent with an expansion of TRPV1 expression in subsets of human nociceptors.[56]

In rats, IB_4-saporin has been used to ablate the non-peptidergic population of neurons in adult animals. This approach leads to a different phenotype in rats than would be expected from more recent work in mice. These rats show deficits in both mechanical and thermal pain, both at baseline, and in response to injury.[76,77] This is again consistent with species differences between rats and mice in nociceptor populations that have important impacts on behaviour.

The differences described above have important implications for pharmacology. In mice, the mu and delta subtypes of opioid receptors are distinctly expressed in peptidergic and non-peptidergic subsets of nociceptors, respectively. Consistent with this anatomy, intrathecally applied mu agonists selectively inhibit thermal pain while delta agonists inhibit mechanical pain.[78] A recent study of human nociceptors taken from organ donors suggests that there is substantial overlap of mu and delta opioid receptor functional expression in the human DRG.[54] These mu and delta opioid receptors were both localized to neurons that expressed *TRPV1* mRNA, although not all these neurons were capsaicin responsive. Pharmacological differences between mouse and human nociceptors have also been described for nicotinic acetylcholine receptors.[79] While some studies have found important differences in receptor expression, signalling mechanisms between species appear to be conserved. For instance, opioid receptor activation is linked to inhibition of voltage-gated Ca^{2+} channels in mice and humans[54] and activation of type II metabotropic glutamate receptors (mGluR) inhibits prostaglandin E_2-mediated nociceptor hyperexcitability in both species.[80] Interestingly, while type I mGluRs are prominently expressed in murine nociceptors,[81] mGluR5 (*GRM5* gene) is absent from human DRG.[55]

Another important consideration is nociceptor synaptic connectivity in the dorsal horn. Mouse genetics is now revealing the logic of how distinct subtypes of sensory neurons wire to specific types of dorsal horn neurons.[82] An important next step for this line of work will be understanding the synaptic adhesion or other molecules that determine these synaptic arrangements. A critical piece of this puzzle will be to gain insight into precisely how mouse and human DRG neurons differ in their molecular features so that reasonable hypotheses can be built about wiring diagrams in the human spinal cord. Single cell sequencing technologies, including spatial transcriptomics,[83] will play an important role in making these discoveries and species-to-species translations possible.

Genes with enriched expression in the human dorsal root ganglion

A possible route to effective pain therapeutics is drug discovery centred on genes that are specifically expressed in DRG neurons and mostly not found in other tissues. For instance, the voltage-gated Na^+ (Na_v) channels 1.7, 1.8 and 1.9 are relatively specifically expressed in DRG nociceptors and have been key pain drug targets for decades. Molecules specifically targeting these channels are now in clinical trials. The vast wealth of RNA sequencing data generated across laboratories and fields is uniquely amenable to computational methods that allow for identification of gene expression enrichment in specific tissues. This technique was recently applied to the human DRG to identify ~140 genes that showed enrichment in human DRG compared to other tissues from the Genotype-Tissue Expression (GTEx) consortium transcriptome resource.[55,84] Many of these enriched genes had been identified by previous studies, in particular studies that used subtractive cloning techniques to find DRG-enriched ion channels and G-protein coupled receptors (GPCRs) prior to genome sequencing.[85] They also include transcription factors such as *PRDM12*[86,87] and others that are involved in specifying the nociceptor lineage.

The vast majority of these DRG enriched transcripts are conserved in human and mouse, although with varying degrees of enrichment for many genes.[55,88] Two examples are the *CCKAR* and *IL31RA* genes, which have been implicated in pain and itch, respectively. In humans, *CCKAR* transcripts are more specifically expressed in DRG as compared to mice where a broader expression pattern is seen.[55] *IL31RA* is enriched in both human and mouse DRG but its expression level is far higher in human DRG (Fig. 3). In mouse DRG, the interleukin 31 (IL31) receptor is expressed in a selective set of neurons that also express *Nppb* and are associated with itch.[89] In human DRG, a far greater proportion of nociceptors express the *IL31RA* mRNA[90] suggesting a broader role in pain in humans. IL31 can produce both pain and itch behaviours in mice.[91] A final example is the nicotinic alpha 9 receptor encoded by the *CHRNA9* gene (Fig. 3). This gene is highly enriched in human DRG but not expressed in mouse DRG at all.[55] In human DRG, *CHRNA9* is expressed in a subset of nociceptors.[56] Antagonist studies for this receptor suggest an efficacy against chemotherapy-induced neuropathy in both mice and rats[92] and drug discovery efforts against this receptor are underway for neuropathic pain. It will be interesting to determine the impact of alpha 9 modulation on excitability of human nociceptors given the species expression differences.

Plasticity of human dorsal root ganglion transcriptome in neuropathic pain

Developing therapeutic strategies for neuropathies would be enhanced by a better understanding of molecular changes that occur in the DRG that potentially drive neuropathic pain. To this end, a study was conducted on patients with or without neuropathic pain undergoing vertebrectomy surgery.[93] This surgery provides a unique opportunity because patients have their DRGs removed as part of the surgery. Phenotyping before the surgery allows for pairing of DRG transcriptomes with patient-specific pain information matched to the DRG dermatome. This study led to some striking conclusions about underlying changes in gene expression that may drive neuropathic pain.

The first conclusion was that there are likely important sex differences in neuro-immune interactions in neuropathic pain. Rodent studies indicate that monocyte lineage cells, macrophages, and microglia play a critical role in development of neuropathic pain distinctly in males.[94,95] Human transcriptomic findings broadly support this conclusion.[93] However, in female DRGs associated with neuropathic pain, such striking changes in genes associated with macrophages were not observed. Rather, we find upregulation of a number of GPCRs. One of these, the adenosine type 3 receptor (A3AR), encoded by the *ADORA3* gene, also shows some important species differences. The receptor is not expressed in the mouse DRG,[55] but in rats it is expressed by nociceptors, where its activation inhibits voltage-gated Ca^{2+} channels.[96] In mice, agonists of the A3AR reduce neuropathic pain through an action on non-neuronal cells that increase production and release of interleukin 10 (IL10) that then acts on pain circuitry.[97] Therefore, in female patients with neuropathic pain an inhibitory action on nociceptors coupled with a trophic action on pain-resolving immune cells may have a dramatic analgesic effect.

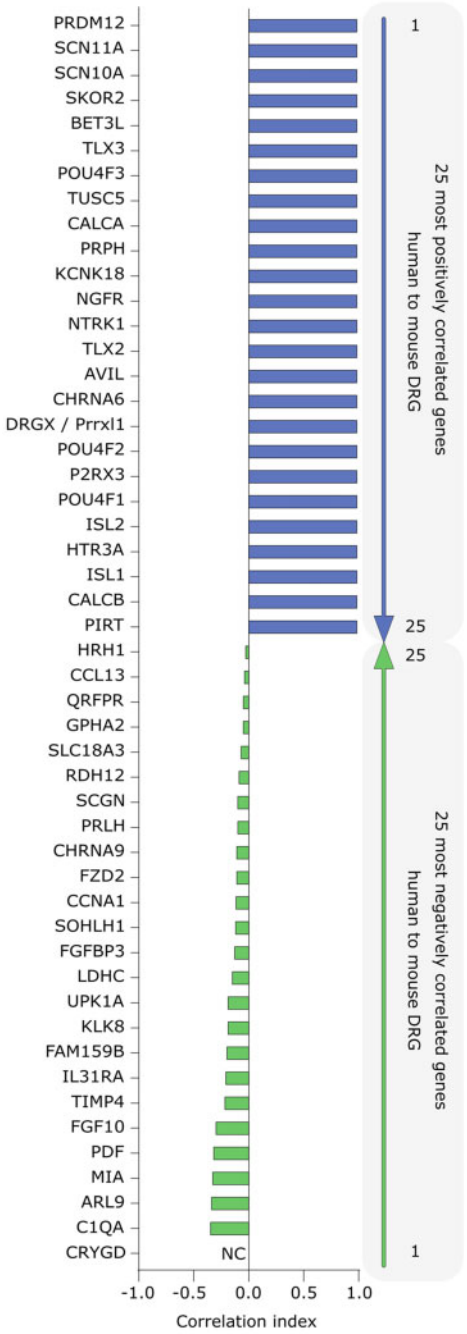

Figure 3 Top 25 positively and negatively correlated DRG-enriched gene expression profiles between pharmacologically relevant human and mouse tissues from RNA sequencing. The figure shows the top 25 most positively and negatively correlated (in blue and green respectively) DRG-enriched gene abundance profiles in 12 tissues across human and mouse gene orthologues. The tissues include the DRG, neural tissue (such as spinal cord and brain subregions), and pharmacologically relevant non-neural tissues (such as skeletal muscle and liver). Only genes with relative abundance >0.1 transcripts per kilobase million (TPM) in human DRGs are shown in either list. The methods are described in detail in Ray *et al.*[55] Among the positively correlated genes, the list is populated with many of the most widely studied genes in the pain and somatosensation fields. Among the anticorrelated genes that are species-specifically enriched in human DRG, most of these genes are relatively unstudied and/or have been validated at the cellular level in independent experiments (e.g. *CHRNA9* and *IL31RA*, discussed in main text). Gene orthologues with different names in human and mice show both names. NC = not calculated since the gene is not expressed in any of the profiled mouse tissues.

Another important finding stemming from these studies is discovery of immune-derived factors that are associated with neuropathic pain in patients. Some of these are well-known from the preclinical literature, but others have not been widely studied. A prime example of the latter is oncostatin M (*OSM* gene).[93,98] OSM is an IL6 family cytokine that acts via the OSM receptor and the GP130 signalling receptor.[99] Its gene expression is increased in DRGs associated with pain and its likely cellular origin is macrophages.[93] Our expression analysis profiling suggests that human nociceptors and satellite glial cells express OSM receptor components (Shiers and Price, unpublished observations). OSM may be an important mediator of neuropathic pain that has not emerged from similar experiments done on rodent DRGs in neuropathic pain models. As the study continues to increase sample sizes from this unique patient cohort, it is expected to have increased power to detect additional potential mediators of neuro-immune interactions driving pain in patients, including potentially sex-specific mediators.

Using human induced pluripotent stem cell-derived nociceptors to understand nociceptor function

As human DRGs are often not readily available, the ability to differentiate human induced pluripotent stem cells (IPSCs) into sensory neurons provides the opportunity to study their function in both naïve and disease states. They can be derived from skin biopsy or a blood sample and are thus less invasive than studying native human DRG neurons (discussed below). They also have the advantage of scalability, investigation of multiple tissue types, and the application of genome engineering. Here, we will briefly outline some of the state-of-art protocols available to generate IPSC-derived sensory neurons and how they have been used to model pain disorders (Table 2). We will also delineate how these *in vitro* models have informed therapeutic design for chronic pain states (Fig. 4). Electrophysiological properties are discussed in a later section.

IPSC differentiation into nociceptors and peripheral sensory neurons

In the past two decades, there have been significant advances in the ability to reprogram rodent and human tissues to generate IPSCs.[123–127] The pioneering work by Chambers *et al.*[128] demonstrated that the synergistic action of Noggin and SB431542, inhibitors of the SMAD and TFG-β signalling, respectively, were sufficient to differentiate both human embryonic stem cells and human IPSCs into peripheral neurons at an efficiency of >80%.[128] This work and subsequent studies led to differentiation protocols that convert stem cells into sensory neuron populations, in particular nociceptors (Table 2).

While the Chambers *et al.*[102] protocol was the first, other differentiation protocols have since followed (Table 2). Two groups simultaneously published their protocols for reprogramming both rodent and human fibroblasts into induced nociceptors (iNoc) and induced peripheral sensory neurons (iSN) using transcription-mediated lineage conversion.[100,101] Both exhibited molecular and physiological properties of mature DRG neurons such as tetrodotoxin (TTX)-sensitive (TTX-s) and resistant (TTX-r) sodium currents and the expression of DRG molecular markers. Wainger *et al.*[100] also demonstrated that human iNoc were able to identify deficits in neurite outgrowth and branching in a patient with familial dysautonomia. However, this protocol has not been as

Table 2 Stem cell differentiation protocols for human sensory neurons/nociceptors and their use in modelling painful pathologies

Original cells	Resulting cells[a]	Exogenous factors	Disease modelling	Reference
Direct induction				
Fibroblasts (mouse/human)	Nociceptive sensory neurons (iNoc)	Ascl1, Myt1l, Ngn1, Isl1, Klf7	Familial dysautonomia	Wainger *et al.*[100]
Fibroblasts	Induced peripheral sensory neurons (iSNs)	Brn3a, Ngn1 or Ngn2	–	Blanchard *et al.*[101]
IPSC protocols				
IPSCs (or ESCs)	IPSC-derived nociceptors	OKSM factors, LDN19318, SB431542, CHIR99021, SU5402, DAPT, Y27632, EGF, FGF, NTFs (Chamber's protocol)	– Migraine	Chambers *et al.*[102] Pettingill *et al.*[103]
IPSCs	IPSC-derived nociceptors	Chamber's protocol + minor modifications	IEM	Meents *et al.*[104]
IPSCs	IPSC-derived nociceptors	Chamber's protocol + minor modifications	Small fibre neuropathy	Namer *et al.*[105]
IPSCs	IPSC-derived nociceptors	Chamber's protocol + minor modifications	CIP	McDermott *et al.*[106]
IPSCs	IPSC-derived peripheral sensory neurons	Chamber's protocol + minor modifications	Varicella-zoster virus infection	Lee *et al.*[107]
IPSCs (from PBMCs)	IPSC-derived peripheral sensory neurons	Chamber's protocol + minor modifications	IEM	Cao *et al.*[108]
IPSCs	IPSC-derived peripheral sensory neurons	Chamber's protocol + minor modifications	Remyelination therapy	Cai *et al.*[109]
IPSCs (from PBMCs)	IPSC-derived peripheral sensory neurons	Chamber's protocol + minor modifications	IEM	Mis *et al.*[110]
IPSCs	IPSC-derived peripheral sensory mechanoreceptors	Chamber's protocol + minor modifications	–	Guimarães *et al.*[111]
IPSCs	IPSC-derived proprioceptive neurons	Chamber's protocol + minor modifications	Afferent ataxia	Dionisi *et al.*[112]
IPSCs	IPSC-derived trigeminal neurons	Chamber's protocol + minor modifications	HSV-1 infection	Zimmer *et al.*[113]
IPSCs	Peripheral mechanoreceptors	OKSM factors, NTFs	–	Schrenk-Siemens *et al.*[114]
IPSCs	NC-iSN mechanosensory neurons	OKSM factors, Y27632, SB431542, Brn3a, Ngn2, NTFs	Piezo2 channelopathy	Nickolls *et al.*[115]
IPSCs	IPSC-NCd sensory neurons	Noggin, LDN193189, SB431542, Y27632, NTFs	–	Umehara *et al.*[116]
ESC/other protocols				
hEPI-NCSC	Peptidergic nociceptive Sensory neurons	SHH, CHIR99021, LDN193189, DAPT, HGF, NTFs	–	Wilson *et al.*[117]
iNPCs	Peripheral sensory neurons (iSNs)	LDN193189, SB431542, CHIR9902, DAPT, SU5402, NTFs	Chemotherapy-induced neuropathy	Vojnits *et al.*[118]
ESCs	Peripheral sensory neurons	LDN193189, SB431542, CHIR9902, DAPT, SU5402, Y27632, NTFs	Peripheral nerve injury	Jones *et al.*[119]
ESCs	Peripheral sensory neurons	SB431542, CHIR9902, BMP2, Y27632, NTFs	–	Alshawaf *et al.*[120]

A substantial number of IPSC protocols are largely based on Chambers *et al.*[102] and have since undergone small modifications; for protocol adaptation refer to each original publication. CIP = congenital insensitivity to pain; ESC = embryonic stem cell; hEPI-NCSC = human epidermal neural crest stem cells; iNPC = induced neural progenitor cells; iSNs = induced sensory neurons; NCd = neural crest-derived; NC-iSN = neural crest derived induced sensory neurons; NTF = neurotrophic factors; PBMCs = peripheral blood mononuclear cells.

[a]Note that 'Resulting cells' is the nomenclature used by each original publication.

widely adopted as the Chambers' one, likely because of a lower efficiency when generating nociceptors.

Alternative approaches have included using human embryonic stem cells to derive neural crest progenitors, which can then be further differentiated into DRG sensory neurons (using media supplemented with a cocktail of neurotrophins).[120,129] Analyses revealed the presence of neuronal subpopulations after 3 weeks of differentiation, suggesting a heterogeneous culture. Distinct subpopulation responses to algogens, temperature, and osmolarity (mechanosensory) changes supports this notion.[120] Recently, it

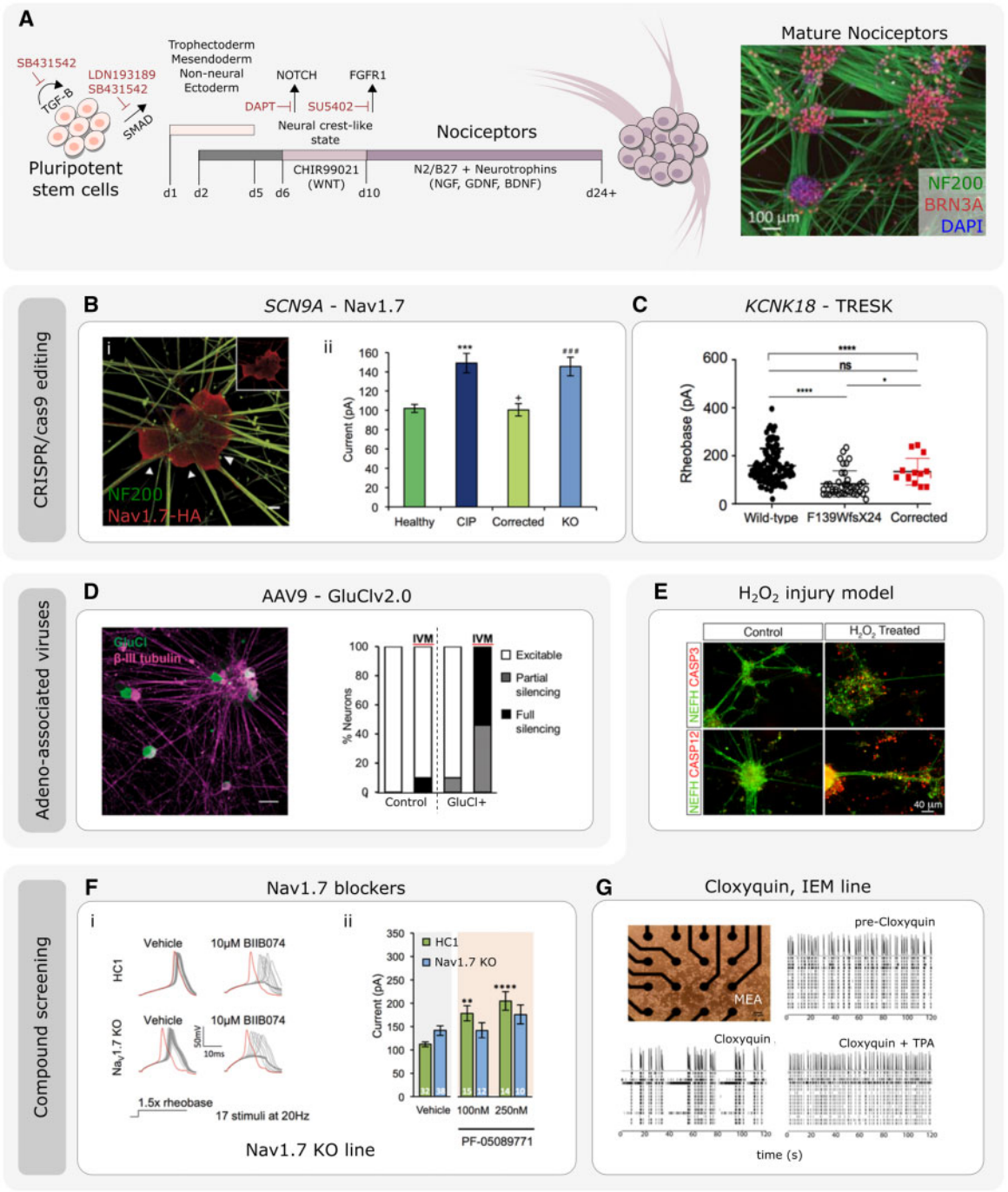

Figure 4 Using IPSC-derived nociceptors to develop therapeutics for pain disorders. (**A**) Illustration of the protocol and timeline of events to generate mature IPSC-derived nociceptors (adapted from Chambers *et al.*[102] and Meentz *et al.*[104]; immunomicrograph adapted from Clark *et al.*[121]). [**B(i)**] The use of CRISPR/cas9 gene editing to generate IPSC-derived nociceptors edited to constituently express HA-epitope tagged $Na_v1.7$. (**ii**) Current-clamp analysis of the current require to elicit and action potential of IPSC-derived nociceptors derived from healthy participants and congenital insensitivity to pain (CIP, due to $Na_v1.7$ loss-of-function) participants. CIP participant IPSC-derived nociceptors were hypoexcitable. The CIP participant hypoexcitability was corrected using CRISPR/Cas9 IPSC editing. CRISPR/Cas9 was used to generate $Na_v1.7$-KO IPSCs that were differentiated into nociceptors that mimicked CIP hypoexcitability (adapted from McDermott *et al.*[106]). (**C**) Current-clamp analysis of the rheobase (minimum current required to elicit and action potential) of IPSC-derived nociceptors from wild-type participants, and participants carrying the F139WfsX24 TRESK loss-of-function mutation. IPSC-derived nociceptors from F139WfsX24 are hyperexcitable compared to wild-type nociceptors. This nociceptor hyperexcitability was rescued when using CRISPR/cas9 to correct the TRESK mutation (adapted from Pettingill *et al.*[103]). (**D**) IPSC-derived nociceptors transduced with the chemogenetic silencing tool GluCl. Scale bar = 50 µm. Patch clamp analysis revealed that following application of the GluCl agonist ivermectin (IVM), GluCl+ nociceptors were rendered either fully silent or partially silent to depolarizing current injections (adapted from Weir *et al.*[122]). (**E**) Example of stem cell derived nociceptors that have been used to model a chemical injury model through the addition of H_2O_2. H_2O_2 treatment leads increased caspase expression and neurite retraction/disassembly (adapted from Jones *et al.*[119]) [**F(i)**] IPSC-derived nociceptors derived from healthy control (HC1) or CRIPSR/cas9 engineered $Na_v1.7$ KO lines. The $Na_v1.7$ blocker BIIB074 promoted action potential failure in both HC1 and $Na_v1.7$ KO lines. (**ii**) Current clamp recordings of HC1 and $Na_v1.7$ KO nociceptors in the presence of vehicle or PF-05089771. Only HC1 but not $Na_v1.7$ KO nociceptors demonstrated changes in excitability in response to the PF-05089771 compound (adapted from McDermott *et al.*[106]). (**G**) Multi-electrode arrays (MEAs) used to assess spontaneous activity of IPSC-derived nociceptors from an IEM participant. Activity at electrodes was reduced after addition of the TRESK activator cloxyquin. Spontaneous activity recovery was lost following co-addition of TPA (TRESK inhibitor) and cloxyquin (adapted from Pettingill *et al.*[103]).

was also proposed that human epidermal neural crest stem cells (hEPI-NCSC) can differentiate into hEPI-NCSC derived nociceptors.[117] Functional assays demonstrated that 30% of these sensory neurons responded to capsaicin.

Across differentiation protocols, derived cells are examined for a variety of sensory neuron markers using transcript expression and functional assays. Detailed RNA sequencing has been performed on IPSC-derived nociceptors from the Chambers' protocol, suggesting a DRG transcript 'signature' when compared to published human RNA sequencing data.[55,106] The ion channel expression profile in mature human IPSC-derived nociceptors (Chambers' protocol) closely resembled that of adult human DRGs. For instance, microarray data suggest 84% of human DRG ion channel genes were expressed, such as those encoding for K_v7.2/7.3, which play a role in controlling neuronal resting membrane potential,[130] and have been recently recognized for their role in the peripheral pain pathway.[131] Additionally, microarray data also showed the expression of *HCN1* and *HCN3* ion channel subunit genes, as well as the presence of acid-sensing ion channels (ASICs).[130] ASICs are highly expressed in nociceptors, with a putative role in inflammatory pain in mice and humans,[132] while modulating HCN ion channels (and in turn nociceptor excitability) might represent a potential avenue for treating chronic pain.[133]

Given the known similarity of mature mouse DRG and TG transcript expression profiles,[134] DRG differentiation protocols are likely informative regarding TG and vice versa. For example, the Chambers' protocol is frequently discussed in the context of DRG, but it has also been used to study migraine-associated channelopathies.[103] An IPSC protocol to derive 'TG'-like sensory neurons has also been developed.[113]

Single differentiation protocols do not recapitulate the full complexity of sensory neuron subpopulations. As we learn more about human DRG expression profiles through transcript analyses, IPSC differentiation protocols may be tailored further. It is advisable that researchers consider the small number of DRG versus TG-specific differences when selecting a differentiation protocol, as well as verify expression patterns to eliminate possible caveats.

While this review is largely focused on nociceptive sensory neurons, it is important to also note that groups have designed novel protocols to generate a variety of human IPSC-derived mechanoreceptors used to study touch biology.[114,115] There is increasing evidence that changes in mechanoreceptors (and their circuitry) post-injury may underlie tactile allodynia.[135,136] Therefore, using IPSC-derived nociceptors and, when appropriate, IPSC-derived mechanoreceptors, may facilitate the successful translation of therapeutics targeted towards chronic pain.

IPSC-derived nociceptors derived from patients living with chronic pain

The use of IPSC nociceptors derived from individuals living with chronic pain has been invaluable in the study of human nociception, novel therapeutics, and even new directions in personalized medicine. For instance, IPSC-derived nociceptors have been generated from participants with inherited erythromelalgia (IEM) due to gain of function mutations in Na_v1.7.[104,108,110] IEM participants experience episodes of severe pain and erythema localized to the extremities and exacerbated by warm temperature. IEM is due to Na_v1.7 gain-of-function mutations increasing nociceptor excitability.[137] IEM IPSC-derived nociceptors have thus enabled valuable insight into the Na_v1.7-dependent mechanisms of IEM in a humanized sensory system. These included aberrant ectopic activity, reduced action potential thresholds, and hyperexcitability to heat stimuli.[104,108,110] Further studies have been able to use these IEM patient IPSC-derived nociceptors to investigate the efficacy of drug interventions. For instance, the development of Na_v1.7 blockers is an attractive avenue to treat IEM, as such, and thus IEM patient IPSC-derived nociceptors are an excellent platform to test the functionality of Na_v1.7-specific blockers. A Na_v1.7-specific blocker was administered to IEM IPSC-derived nociceptors and showed efficacy in reducing their hyperexcitability.[108] This led to a small, randomized, double-blind, placebo-controlled, cross-over study which demonstrated efficacy of this small molecule in reducing heat evoked pain.[108] However, another Na_v1.7 blocker (BIIB074) failed to reach the primary end point in a phase 2 trigeminal neuralgia clinical trial.[138,139] This might reflect the recent data from IPSC-derived nociceptors that lack Na_v1.7, which raised questions over BIIB074's specificity for Na_v1.7 (discussed below).[106]

IPSC-derived nociceptors from participants with IEM have also enabled identification of peripheral mechanisms that underlie interindividual differences and indeed resilience to chronic pain. An example was the study of two IEM participants, both carrying the same Na_v1.7 mutation (S241T) but reporting very different levels of pain. This work identified an additional genetic variant, a K_v7.2 gain-of-function mutation (T730A), which reduced nociceptor excitability and was only present in the participant that experienced the least pain.[110]

Other groups have generated IPSCs from participants with different chronic pain conditions, including painful small fibre neuropathy. This study was based on an individual subject with small fibre neuropathy where genetic sequencing revealed the presence of two mutations (a common Na_v1.9 variant, N1169S, and a rare Na_v1.8 variant, R923H) although it was unclear as to the relative contributions of these mutations to the pain experienced.[105] Regardless, IPSC-derived nociceptors from this subject exhibited increased spontaneous activity compared to controls, and mimicked the increased incidence of spontaneously active C-fibres revealed by microneurography.[105] Lacosamide, an anti-epileptic thought to act by blocking voltage-gated sodium channels, reduced the incidence of spontaneously active IPSC-derived nociceptors and peripheral C-fibres. In this single patient, lacosamide also provided effective pain relief,[105] demonstrating that patient IPSC-derived nociceptor characteristics can guide effective treatment design—a step towards personalized medicine.

IPSC-derived nociceptors have been proven useful also to investigate the sensory system in patients with other sensory dysfunctions. For instance, it has been reported that patients with Parkinson's disease can develop sensory deficits that precede motor symptoms.[140] Taken together, this exemplifies how IPSC-derived nociceptors are a powerful tool that can be used to understand pain mechanisms in classical and non-classical disorders of pain.

CRISPR/Cas9 gene editing in human IPSC-derived nociceptors

CRISPR/Cas9 is a fast and accurate method to edit genomic DNA.[141] This can be applied to human IPSCs to unequivocally link a gene variant to functional outcomes with clinical relevance in the IPSC-derived-nociceptors. It can also be used for experimental manipulations such as tagging endogenous proteins to determine trafficking, and may in itself provide therapeutic opportunities in the future (Fig. 4A).

The first study to use CRISPR/Cas9 editing in human IPSC-derived nociceptors involved IPSCs generated from participants with congenital insensitivity to pain (CIP) due to loss-of-function mutations in *SCN9A* (encoding Na_v1.7).[106] When assessed using patch clamp electrophysiology, IPSC-derived nociceptors from patients with CIP were hypoexcitable to both threshold and suprathreshold electrical stimuli, supporting Na_v1.7's role in nociceptor electrogenesis and excitability.[106,142] CRISPR/Cas9 was

subsequently used to generate a $Na_v1.7$ knock-out (KO) IPSC line, that when differentiated into nociceptors, recapitulated the patient electrophysiological phenotype [Fig. 4B(ii)]. CRISPR/Cas9 gene editing was then used to correct one *SCN9A* allele from one CIP patient. This correction recovered the hypoexcitability phenotype of patient IPSC-derived nociceptors [Fig. 4B(ii)].[106] The same study also generated novel IPSC tools to interrogate human $Na_v1.7$ in a humanized system. A tagged human $Na_v1.7$ IPSC line was generated by introducing a haemagglutinin (HA) epitope at the C-terminus of human $Na_v1.7$ to allow visualization of the subcellular localization of native $Na_v1.7$ in human IPSC-derived nociceptors [Fig. 4B(i)].

Genome editing was also used in IPSCs generated from participants who suffer from migraine in order to understand the causal impact of gene variants in relation to IPSC-derived nociceptor function. Migraine was due to loss-of-function mutations in the *KCNK18* gene, which encodes for the two-pore domain potassium channel TRESK. TRESK mediates a K^+ leak current which stabilizes the resting membrane potential and attenuates depolarizing stimuli. Patient-derived IPSC nociceptors that carried the F139WfsX24 TRESK mutation displayed a loss of functional TRESK currents and neuronal hyperexcitability. This phenotype was reversed using CRISPR/Cas9 to correct the F139WfsX24 mutation (Fig. 4C).[103]

Human IPSC-derived nociceptors as screening tools to test drug and gene therapy efficacy

While CRISPR/Cas9 is a powerful technique for genome editing, many challenges need to be overcome before clinical application, not least the possibility of off target effects. Virus mediated genetic therapies are an alternative that have shown promise clinically.[143] Groups have used adeno-associated viruses (AAVs) to deliver transgenes to human IPSC-derived nociceptors to explore pain therapies in a humanized system. As the availability of human donor nociceptors is limited, IPSC-derived nociceptors can be used, at least in part, to close this translational gap. One approach to treat chronic pain includes silencing of primary afferent activity. Expression of an engineered glutamate-gated chloride channel (GluCl), in the presence of its non-toxic agonist ivermectin, resulted in the silencing of mouse nociceptor activity, inhibition of evoked pain in a mouse model of neuropathic pain, and suppression of post-injury ectopic primary afferent activity.[122] AAV serotype 9 (AAV9) containing GluCl was used to effectively transduce human IPSC-derived sensory neurons (Fig. 4D). Notably, GluCl was able to functionally suppress nociceptor excitability following application of its agonist ivermectin (Fig. 4D).[122] This study demonstrated efficacy of a gene therapeutic in rodent models and a humanized *in vitro* system. Several groups have developed other AAV-transgene mediated silencing strategies to suppress DRG activity; however, to our knowledge their efficacy has yet to be examined in a humanized system.[144,145]

The development of new treatments which show promise in preclinical rodent models often do not successfully translate for human use. This can be due to many factors, including both toxicity and lack of efficacy in human neurons. The use of IPSC-derived nociceptors provides opportunity to interrogate these factors in a humanized system. Numerous efforts have been made to use stem cell technology to screen novel drugs and assess neurotoxicity using human IPSC-derived neurons.[146,147] Some groups have begun to use human IPSC-derived nociceptors (rather than general CNS neurons) as screening tools for drug efficacy and toxicity; for example the use of IPSC-derived nociceptors identified chemotherapeutic and neurotoxic agents that impaired neurite outgrowth.[148,149]

Stem cell-derived nociceptors have also been used to model peripheral nerve injury. For instance, when the reactive oxygen species, hydrogen peroxide (H_2O_2), was added to human embryonic stem cell-derived nociceptors, it resulted in nociceptor cluster disassembly and neurite retraction. This was prevented by co-addition of pan-caspase inhibitors (Fig. 4E).[119] Others have used peripheral blood mononuclear cell-derived, or IPSC-derived sensory neurons, to investigate chemotherapy-induced neuropathy, and offer their high throughput methods as drug-discovery and phenotypic screening platforms.[118,150]

The use of stem cell derived nociceptors has facilitated analysis and investigation into compound toxicity and drug efficacy, but CRISPR/Cas9 targeted KO lines (discussed above) have offered the possibility to test clinically relevant and novel compounds for off target effects in a humanized system. For instance, the $Na_v1.7$ blocker BIIB074 has undergone phase II clinical trials for trigeminal neuralgia.[139] However, this compound was found to be non-selective for human $Na_v1.7$ at clinically relevant concentrations, and following high frequency stimuli BIIB074 altered the excitability of $Na_v1.7$ KO IPSC-derived nociceptors [Fig. 4F(i)].[106] On the other hand, the selective $Na_v1.7$ blocker PF-05089771, which has shown efficacy in preventing spontaneous activity of IEM IPSC-derived nociceptors, and has undergone phase II clinical trials for IEM and painful diabetic neuropathy,[108,151] altered the rheobase only in control but not $Na_v1.7$ KO IPSC-derived nociceptors [Fig. 4F(ii)], suggesting greater human $Na_v1.7$ specificity.[106]

Others have used IEM IPSC-derived nociceptors to examine efficacy of the TRESK activator cloxyquin in IEM and migraine.[103] Using IPSC-derived nociceptors cultured on multi-electrode arrays, the addition of cloxyquin significantly reduced spontaneous activity detected at electrodes (Fig. 4G).[103] This was prevented by the co-addition of tetrapentylammonium bromide (TPA), a TRESK inhibitor, confirming cloxyquin's specificity for TRESK (Fig. 4G).[103] Together, this suggested TRESK activation offers therapeutic potential to human pain disorders where ectopic activity and hyperexcitability are key contributors.

Insights into human nociceptor function through direct electrophysiological recordings

In vitro human dorsal root ganglion recordings

It has become clear that the occurrence of ongoing action potentials, spontaneous activity, arising either in the distal endings of primary afferent fibres and/or ectopically from DRG somata, drives multiple chronic pain states.[93,152–154] Importantly, ectopic spontaneous activity has been identified in multiple models of inflammation and neuropathic pain in rat[155–157] and mouse[158–161] that appear to share key physiological processes. Spontaneous activity was recently also shown to occur in human DRG neurons, and found to be specifically associated with spontaneous ongoing neuropathic pain.[93,162] The shared physiological processes in rat and human DRG neurons with spontaneous activity include a depolarization of resting membrane potential, a hyperpolarization of action potential threshold, and the occurrence of depolarizing spontaneous fluctuations in membrane potential.[162] These changes have also been identified in mouse DRG and TG neurons after axotomy.[160,163] Intriguingly, these membrane events have not been the focus of therapeutic discovery up to now, but given that they are subthreshold, they could have a wide therapeutic safety index. Thus, a key area of study is to define the similarities and differences in the neurophysiological properties of human and rodent DRG.[21] A necessary focus in this regard will be to

understand both the expression and function of the various ion channels critical in the regulation of DRG neuron hyperexcitability and spontaneous activity.[20] It should be noted that spontaneous activity has also been observed *in vitro* from monkey skin-nerve preparations after nerve ligation.[164] To our knowledge, studies of *in vitro* recordings from monkey DRG neurons (with or without the use of experimental pain models) have not been performed.

Recent work on human neurons has shown an important role for voltage-gated sodium (Na_v) and calcium (Ca_v) channels.[165] Human DRG were reported to express a higher proportion of the TTX-s channel $Na_v1.7$ (~50% of total Na_v expression) but lower proportion of the TTX-r channel $Na_v1.8$ (~12% of total Na_v expression) than in mouse (~18% $Na_v1.7$ and ~45% $Na_v1.8$ of total expression, respectively).[166] Similar results were reported when comparing peak TTX-s and TTX-r currents between human and rat DRG.[167] Furthermore, there are species differences in channel properties. Human $Na_v1.8$ demonstrates slower inactivation kinetics and produces larger persistent and ramp currents than other species, resulting in longer lasting action potentials.[168] In addition, differences between rodents and humans in the expression of $Na_v1.7$ and $Na_v1.8$ in the periphery, as well as comparably longer axons, could also contribute to functional differences. The expression of $Na_v1.8$ in mouse (and monkey) appears to be similarly concentrated in distal portions near the terminal end of peripheral nerves[169] and it is possible that the longer sensory axons could accentuate differences. $Na_v1.7$ is expressed in epidermal nerve fibres of the skin of both humans and rats.[170,171]

Changes in expression of both Na_v and Ca_v channels have been described in numerous preclinical models of chronic pain.[137,172] For instance, in the context of a model of painful chemotherapy-induced peripheral neuropathy a comparison of immunohistochemistry images of DRG from vehicle- [Fig. 5A(i–iii)] and paclitaxel-treated rats

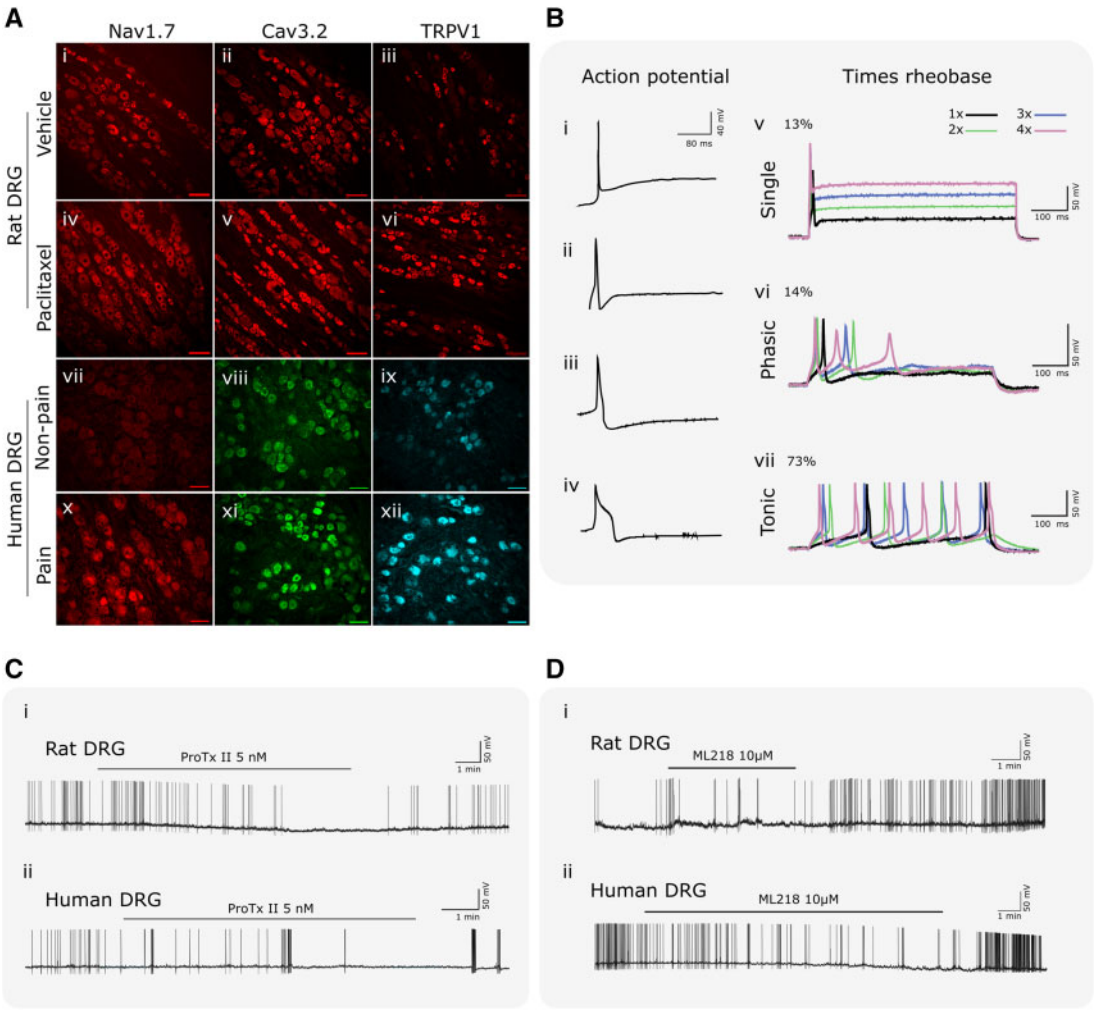

Figure 5 Properties of human versus rat DRG neurons and their response to selective blockers *in vitro*. (**A**) The representative immunohistochemistry illustrates that $Na_v1.7$, $Ca_v3.2$ and TRPV1 are expressed in naïve rat DRG (**i–iii**) and this expression is elevated in rats with neuropathic pain produced using paclitaxel treatment (**iv–vi**).[173–175] All three channels are also expressed at relatively low levels in human DRG where neuropathic pain is absent (**vii–ix**), but also become markedly elevated when neuropathic pain is present (**x–xii**).[173–175] (**B**) The representative analogue traces in **i–iv** show four common types of action potential waveforms observed in whole cell recordings from human DRG neurons. The representative analogue recordings in **v–vii** show the three typical patterns of response shown by human DRG neurons to intracellular currents using graded steps in intensity from rheobase as colour coded at *top right*. (**C**) The representative analogue recordings in show that the $Na_v1.7$ inhibitor ProTxII, given at the time indicated by the solid line over each trace, inhibits spontaneous activity in both rat (**i**) and human (**ii**) neurons. Note the downward (hyperpolarizing) shift in the resting membrane potential in the rat DRG neuron recording during ProTxII administration. (**D**) The representative analogue recordings in show that the $Ca_v3.2$ inhibitor ML218, given at the time indicated by the solid line over each trace, inhibits spontaneous activity in both rat (**i**) and human (**ii**) neurons. Note the downward (hyperpolarizing) shift in the resting membrane potential in the human DRG neuron recording during ML218 administration.

[Fig. 5A(iv-vi)] show that the expression of $Na_v1.7$, $Ca_v3.2$, and TRPV1 is increased with the induction of chemotherapy-induced peripheral neuropathy.[173–175] Similar increases in expression of these ion channels have been shown in other rodent models of neuropathic and inflammatory pain.[176–179] Human DRG neurons show a similar increase in expression of all three channels when that DRG is associated with neuropathic pain due to nerve root compression. The tissue was donated by a patient at MD Anderson Cancer Center who had surgical resection of metastatic oncological disease that involved ligation of bilateral dorsal roots, but only one of which innervated an area of the body with ongoing neuropathic pain. Immunohistochemistry comparing these DRG, one from the side without pain [Fig. 5A(vii–ix)] and one from a side of the body with neuropathic pain [Fig. 5A(x–xii)], reveals that the overall expression of $Na_v1.7$, $Ca_v3.2$, and TRPV1 was markedly increased in the DRG where pain was observed.[173–175] Thus, both rats and humans demonstrate similarity in upregulation of these ion channels in chronic pain states.

The membrane neurophysiological properties of DRG neurons from rats and humans with and without neuropathic pain further the theme that DRG neurons in rodents and humans share many characteristics, but also demonstrate pronounced differences. Examination of data from previous published studies[93,174,175] reveals that rat and human DRG neurons develop spontaneous activity during neuropathic pain and that each species shows the same three logical alterations in neurophysiological properties that would be expected to drive this spontaneous activity.[162] First, both rat and human DRG neurons with spontaneous activity show a similar depolarization of resting membrane potential (rat: –52.01 ± 1.21 mV; human: –50.12 ± 1.91 mV) compared to neurons from the same DRG without spontaneous activity (rat: –59.1 ± 1.6 mV; human: –56.8 ± 1.8 mV) or compared to DRG not associated with neuropathic pain (rat: –58.1 ± 1.3 mV; human –57.9 ± 0.9 mV). Second, the action potential threshold in both rodent and human DRG with spontaneous activity became more hyperpolarized (rat: –24.4 ± 0.9 mV; human: –26.5 ± 1.19 mV) than that in neurons from the same DRG without spontaneous activity (rat: –9.6 ± 1.4 mV; human: –19.6 ± 2.25 mV) or in neurons from DRG not associated with neuropathic pain (rat: –10.2 ± 0.9 mV; human: –18.2 ± 0.9 mV). Third, the cells with spontaneous activity show large, irregular depolarizing spontaneous fluctuations in resting membrane potential that are not present in DRG neurons without spontaneous activity.[93,162] Other changes in membrane properties are also shared between rat and human DRG neurons that develop spontaneous activity and that drive spontaneous pain including action potential amplitude, action potential overshoot, rise time, and magnitude of after-hyperpolarization. Even so, DRG neurons with spontaneous activity in rats and humans show subtle differences in neurophysiological properties. By example, action potential fall time in rat DRG neurons with spontaneous activity (12.24 ± 1.32 ms) was markedly shorter than that in humans (20.9 ± 1.41 ms) and the action potential width at 0 mV in rats (5.4 ± 0.71 ms) was markedly shorter than that in humans (7.72 ± 0.61 ms).

Active electrophysiological properties of human DRG neurons also show both similarities as well as some distinct differences with rat DRG neurons. Action potential waveforms in rat DRG neurons have been categorized into nine distinct types primarily based on action potential duration, the presence or absence of a declining phase shoulder, and after-hyperpolarization duration.[180] Examples in Fig. 5B(i–iv) show four distinct types of action potential waveforms observed in human DRG to date based on the same criteria. Three of these are similar to that reported for rat DRG neurons, but at least one fairly typical waveform is distinct given the very pronounced declining phase shoulder [Fig. 5B(iv)], prolonged action potential duration, and prolonged after-hyperpolarization. This distinctive waveform was observed in at least one other study on human DRG neurons obtained from organ donors.[181] The spike-burst responses of rat and human DRG also show both similarities and distinct differences. Examples of the three patterns of response of human neurons to intracellular current injection are shown in Fig. 5B(v–vii). A tonic firing pattern that grades with increasing current strength is the most common pattern, shown by ~70% of human DRG neurons. The remaining neurons show an even split between either a single spike regardless of current strength, or a brief phasic burst of action potentials. These patterns are also reported in studies of rat neurons, but the reported proportions of neurons showing different types of responses ranged widely. One study in rats reported proportions similar to that observed in human DRG, though no distinction was made between single spike and phasic response types in that study.[162] Two studies in rats reported an apparently equal split between tonic and single spike responses[182,183]; while a third study also in rats reported a proportion of single spike and tonic responses that were the exact opposite of that observed in humans.[184] Combined, these physiological studies suggest that even though the general neurophysiology of DRG neurons in rodents and humans is shared, the underlying molecular biological processes that generate these alterations may be divergent.

A more detailed examination of the neurophysiological characteristics of activation and blockade of $Na_v1.7$ and $Ca_v3.2$ illustrates the discrepancy between species. In Fig. 5C(i and ii) both rat and human DRG neurons with spontaneous action potentials showed a significant reduction in spontaneous activity after bath application of the voltage-gated sodium channel 1.7 ($Na_v1.7$) blocker, ProTxII (5 nM). In neurons with a positive response to ProTxII, action potential properties were analysed before, during, and after washout of ProTxII. In rat DRG neurons, ProTxII hyperpolarizes the resting membrane potential (from –38.5 ± 0.6 to –48.8 ± 1.9 mV) as seen in the representative trace, hyperpolarizes action potential threshold (from –20.1 ± 1.1 to –27.9 ± 1.8 mV), induces a shorter action potential width at 0 mV (from 8.4 ± 1.1 to 5.0 ± 0.3 ms), and also causes a significant decrease in after-hyperpolarization amplitude (from 20.1 ± 1.6 to 15.2 ± 1.4 mV). In contrast, no significant changes in action potential characteristics were observed in human neurons. Thus, while ProTxII decreased neuronal hyperexcitability in both rat and human DRG neurons, contribution of $Na_v1.7$ to different aspects of the action potential in hyperexcitable neurons is not consistent across species.

T-type calcium channels, also called low-voltage-activated calcium channels (LVACCs), are found in primary sensory neurons and activated by minor depolarization of the membrane to initiate action potentials and regulate subthreshold excitability in CNS neurons.[185–187] In Fig. 5D(i and ii), both rat and human DRG neurons with spontaneous action potentials showed a significant reduction in spontaneous activity after bath application of the voltage-gated calcium channel 3.2 ($Ca_v3.2$) blocker, ML218 hydrochloride (10 μM).[174] Like above, action potential properties were analysed in detail for neurons with a positive response to ML218 hydrochloride. The results were the opposite of that when using the $Na_v1.7$ inhibitor. ML218 had no effect on any action potential properties in rat neurons. In contrast, human DRG neurons showed a hyperpolarization of resting membrane potential (from –38.7 ± 0.6 to –43.4 ± 0.9 mV) as seen for the representative example, and decreased after-hyperpolarization amplitude (from 17.7 ± 0.4 to 14.2 ± 0.9 mV). An alternative selective T-type calcium channel blocker, ABT-639, showed preclinical promise in numerous rat[188] and mouse[189] models. However, it failed to show significant analgesia compared to placebo in a phase 2 trial for diabetic peripheral neuropathic pain.[190] This failure was supported by *in vivo* microneurography recordings, with no significant differences in human

nociceptor spontaneous activity following the administration of a single dose versus placebo.[191] This suggests that further work is required to identify why *in vivo* efficacy of T-type calcium channel blockers is limited in neuropathic pain and where their promise lies.

The examples shown here are not isolated. Differences between rat and human have also been shown in the currents evoked by $GABA_A$ and nicotinic acetylcholine receptor agonists.[79,192] This suggests that specific ion channels have evolved to produce different functional outcomes in rats versus humans, and that targeting an ion channel because of its upregulation or modification in a rat model of pain would not necessarily translate effectively in human trials. Future work that may benefit and, in part, overcome this translation hurdle, is combining these *in vitro* human DRG recordings with single cell RNA sequencing of the same cell (Patch-Seq). Patch-Seq allows single cell analysis of both functional and molecular read-outs, which could revolutionize the way we identify promising clinical targets and define nociceptor populations.[193,194]

Electrophysiological characterization of IPSC-derived nociceptors

While *in vitro* human DRG recordings are ideal when investigating compound efficacy, sourcing human nociceptors is not always possible, especially when studying patients who live with chronic pain. The basic electrophysiological properties of IPSC-derived nociceptors and the nociceptor specific ion channels that they express was discussed earlier. While IPSC-derived nociceptors constitute a powerful model, there may be issues with their maturity. Eberhardt *et al.*[195] observed that differentiated IPSC-derived nociceptors showed a discrete presence of $Na_v1.5$ (a TTX-r Na_v that is normally expressed developmentally and then downregulated) in addition to $Na_v1.8$ and $Na_v1.9$. Such TTX-resistant currents showed a more hyperpolarized voltage dependence, in contrast to their counterparts from rodent DRGs, probably due to the different expression of $Na_v1.5$. Voltage-clamp recordings also revealed Na_v-mediated inward currents, followed by sustained outward currents, indicating the ready excitability of IPSC-sensory neurons.[195] Taken together, these data suggest that IPSC-derived nociceptors, at least around 25 days post differentiation, may exhibit an embryonic-like expression pattern of the TTX-r currents, along with specific excitability characteristics. It is therefore important to account for age and maturity of IPSC-derived nociceptors when used in electrophysiological studies. For this reason, many studies have used IPSC-derived nociceptors at 50–70 days post-differentiation, when their maturity and gene expression changes have plateaued.[106,122,130]

Acknowledging the caveat of maturity, IPSC-derived nociceptors can successfully recapitulate nociceptor action potentials and thus allow the direct investigation of neuronal excitability. IPSC-derived nociceptors can generate single and tonic action potentials in response to current injections.[104–106,108,110,122] This has also been shown for direct fibroblast to nociceptor conversion methods and embryonic stem cell-derived nociceptors.[100,119] This is similar to responses observed in human DRG neurons. However, the identification of all four distinct types of action potential waveforms seen in human DRG neurons (above) are lacking in IPSC-derived nociceptors. This is likely due to the limitation that protocols do not give rise to the full range of DRG neuronal subpopulations.

Importantly, IPSC-derived nociceptors, in particular those derived from patients living with chronic pain, exhibit increased incidences of spontaneous activity (as discussed above) which has been valuable for understanding disease mechanisms. In addition, they can also be used in screening pharmacology on an individualized basis, and may potentially be extended into medium throughput screens, given their scalability.[103,105,110]

In vivo human nociceptor afferent recordings

Microneurography is the only technique in which direct (*in vivo*) recording of nociceptor axonal electrical activity in humans is possible. The use of microneurography in healthy participants and patients living with chronic pain can identify novel insights into the peripheral basis of nociception and chronic pain.

Microneurography can provide exquisite detail of C-fibre nociceptor activity *in vivo*, and most of our knowledge of axon conduction properties of human nociceptors is based on the study of cutaneous afferents, as they are readily accessible. However, visceral pain is a large clinical problem and there exists a large body of rodent literature demonstrating differences in somatic versus visceral nociceptors. Therefore, the development of new techniques to study visceral afferents innervating resected human bowel tissue should ultimately aid understanding of human visceral afferents.[196]

While C-fibre nociceptors are studied using microneurography, Aδ nociceptors remain difficult to study.[197] Only recently were a class of human nociceptors with Aβ conduction velocities identified.[17] Hence, we have a limited understanding of human A-fibre nociceptor physiology and their role in chronic pain states. As such, the role of A-fibres and A-fibre nociceptors are often studied in rodents. While anaesthetized microneurography and teased-fibre recordings in rats and monkeys has been achieved and offers comparison to human work, it is not widely practiced.[19,198–202] In the mouse, the *ex vivo* skin-nerve preparation is often used to obtain a detailed characterization of mouse primary afferents at the single fibre level,[203,204] offering primary afferent characterization following genetic or pharmacological manipulation, alongside comparison with human nociceptors.

C-fibre nociceptor subpopulations identified using microneurography

Human cutaneous C-fibre nociceptors are divided into two major classes on functional grounds, C-mechanosensitive and C-mechanoinsensitive afferents, according to their response (or lack of response) to mechanical, electrical, thermal, and chemical stimuli (Table 3).[205–212]

Most C-mechanosensitive afferents are polymodal (Table 3) and it is thought that they are responsible for signalling mechanically induced pain in uninjured skin. While they are activated by mechanical stimuli below the intensity for evoking painful sensations,[213,214] a correlation exists between the increasing force of mechanical stimuli into the noxious range and the perception of mechanical pain.[215] These findings suggest that coding of mechanically evoked pain by C-mechanosensitive afferents is intensity dependent.[197]

Most C-mechanosensitive afferents are termed C-mechano-heat afferents (Table 3)[5] and are thought to encode heat pain. Similar to mechanical stimuli, activation temperatures are below the noxious range,[207,212,216,217] and suprathreshold encoding appears important in the signalling of heat pain.[212,217,218] However, the relationship between firing rate and the sensation of pain is complex, with both temporal and spatial summation playing a role.

A smaller proportion of C-mechanosensitive afferents, termed C-mechano-heat-cold afferents (Table 3), are activated by heat and prolonged stimulation to cold (~20°C) stimuli.[219] It is thought they may mediate the burning sensation felt at extreme hot or cold temperatures.[197] Their maximum firing frequency to cold

Table 3 Characterization of human C-fibre nociceptors using microneurography

Nociceptor afferents and subpopulations	Response characteristics	Neurophysiological properties	Receptive fields
C-mechanosensitive ('polymodal')	Mean mechanical response ~30 mN (range 3.4–750 mN). Majority respond in 10–300 mN range Weak and transient response to algogens[a] Respond to cowhage and histamine	ADS: Low latency changes during low-frequency stimulation e.g. <2% during 5 min 0.25-Hz stimulation High (>10%) latency changes during 2-Hz stimulation CV ~1 m/s 10 mA activation threshold[b]	100 mm^2 (10–363 mm^2) Uniform shape, some irregular; size varies depending on body site
C-mechano-heat	Mean temperature response ~40°C; >45°C linear response between stimulus temperature and firing frequency		
C-mechano-heat-cold	Also respond to cold <20°C		
C-high threshold mechanoreceptors	Only respond to mechanical stimuli; no thermal response		
C-mechanoinsensitive	Unresponsive or very strong mechanical stimulus >750 mN Thermal response at >48°C Strong and sustained response to algogens (unresponsive to endothelin 1)	ADS: High latency changes during low-frequency stimulation e.g. >5% during 0.25-Hz stimulation High (>10%) latency changes during 2-Hz stimulation CV ~ 0.8 m/s >30 mA activation threshold[b]	500 mm^2, discontinuous patches, irregular in shape, heterogeneous physiological response properties across receptive field
C-mechanoinsensitive-heat-insensitive ('silent')	Unresponsive to mechanical/thermal stimulus; can be sensitized after algogen (mustard oil/capsaicin) application		
C-mechanoinsensitive-histamine-positive ('pruriceptors', C-itch afferents)	Very sensitive to histamine, no response to cowhage		

From human *in vivo* microneurography studies C-fibre nociceptive afferents comprise two classes. This table summarizes key differences between C-mechanosensitive nociceptors and C-mechanoinsensitive afferent responses to mechanical, thermal, and chemical stimuli. C-mechanosensitive nociceptive afferents are also called type 1A fibres and C-mechanoinsensitive type 1B fibres. ADS = activity-dependent slowing; CV = conduction velocity.
[a]Algogens include mustard oil, capsaicin, bradykinin, acetylcholine, serotonin, prostaglandin E2, and endothelin 1.
[b]In response to monopolar skin surface stimulation.

stimuli is lower than for mechanical and heat stimuli.[217] This suggests a unique mechanism for the encoding of noxious cold temperatures.

C-mechanoinsensitive afferents are activated by intense mechanical stimulation and the majority respond to heat (Table 3).[212] A subset of C-mechanoinsensitive afferents are 'silent' nociceptors and only respond after chemical sensitization.[5,209,220] However, it is important to recognize that thermal assessment of (unsensitized) silent afferents might be limited by sensory ending depth and the necessity to keep skin temperature <50°C. The observation that capsaicin injections activate most 'silent' afferents,[75] suggests they might indeed be capable of detecting heat stimuli. Whether or not silent afferents are heat sensitive in humans remains an open question. In monkeys silent afferents make up ~30% of cutaneous C-fibres,[201] in rats they make up ~15–26% of cutaneous C-fibres,[221] and in mice they make up ~10% of cutaneous C-fibres.[222] Particularly in mice, this is lower than that observed in humans (~24%)[5] and an important translational point to consider. Recently, a population of murine silent nociceptors were identified and shown to innervate deeper structures, such as muscle, viscera and joints, but not skin.[223] Another subpopulation of nociceptive C-mechanoinsensitive afferents are termed pruriceptors, as they signal itch and are sensitive to histamine.[224–226] Taken together, C-mechanoinsensitive afferent discharges are related to pain evoked by tonic pressure[227] and to chemically induced sensations.[75,225,228] It is likely that C-mechanoinsensitive afferents signal ongoing pain after tissue injury, where they become sensitized after the release of inflammatory mediators. While both C-mechanosensitive and C-mechanoinsensitive afferents are activated by a variety of algogens (pain) and pruritogens (itch), stronger and more sustained responses are elicited from C-mechanoinsensitive afferents.[75,224–226,228–231]

C-mechanosensitive and C-mechanoinsensitive afferents exhibit distinctive changes during low frequency electrical stimulation (Table 3), and thus this can be used to distinguish the fibres in the absence of peripheral natural stimulation of the skin.[209,212,232–234] Using repetitive, low frequency electrical stimulation, C-fibres exhibit the phenomenon termed activity dependent slowing, where C-fibre conduction velocity slowing occurs (C-fibre latency increases). C-mechanoinsensitive afferents exhibit greater activity dependent slowing (Table 3), which is maintained under pathological conditions.[202,235–238]

C-fibre nociceptor subpopulations play different roles in nociceptive physiology in both health and disease. Microneurography provides valuable *in vivo* information that can link findings from *in vitro* studies of human nociceptors. For example, human *in vivo* findings that the majority of C-fibre nociceptors respond to capsaicin[75] parallels the recent molecular profiling that revealed a broad (~75%) TRPV1 distribution within human DRG[56] (Fig. 2). As novel molecular and *in vitro* electrophysiological profiling of nociceptor subpopulations becomes available, other C-fibre nociceptor subpopulations may be identified and characterized using microneurography.

The clinical relevance of microneurography

Microneurography has identified fundamental mechanisms that govern human nociception. It has also facilitated the direct investigation of nociceptor afferent changes in chronic neuropathic pain states. For instance, C-mechanoinsensitive afferents are sensitized and display increased spontaneous activity under pathological conditions, whereas C-mechanosensitive afferents do not display such marked changes.[202,235,236,238–240] An increase in spontaneous activity and mechanical sensitization of C-mechanoinsensitive afferents was related to neuropathic pain in patients with painful polyneuropathy.[235] A similar pattern was seen in patients with small fibre neuropathy, fibromyalgia, and IEM,[238,239] where there is no evidence of tissue injury or inflammation. The presence of aberrant activity seen *in vivo* mirrors that seen *in vitro* when studying human DRG neurons or IPSC-derived nociceptors from patients living with chronic pain. The findings that pharmacological suppression of aberrant activity within nociceptor afferents leads to lower reported pain supports the link between nociceptor afferent activity and chronic pain.[105,153] In this regard, microneurography can be used to assess aberrant C-fibre activity in clinical studies using compounds that target peripheral hyperexcitability, especially when guided by efficacy data from *in vitro* human nociceptor models.

The use of microneurography in the study of participants with rare high impact genetic mutations, such as IEM and CIP,[106,239] is clinically relevant. These studies have yielded insights for those disorders, and can also be more broadly applied to common causes of neuropathic pain, such as painful diabetic neuropathy.[241] This has been an area of limited study and a number of unanswered questions remain. For instance, what are the pathophysiological mechanisms of human afferent sensitization, how does abnormal afferent activity relate to the intensity and presence of chronic neuropathic pain/sensory profile, and what roles do A-fibres play in chronic neuropathic pain?

Conclusions and future directions

We have reviewed the increasingly diverse means to study human nociceptors with a focus on chronic pain pathophysiology. While we acknowledge that other methods such a laser evoked potentials and quantitative sensory testing can be used to engage the nociceptive system, these techniques do not only measure nociception and nociceptor activity; they often rely on stimulus saliency, and the engagement of the entire nociceptive pathway (for reviews, see Valeriani *et al.*[242] and Treede[243]). It is clear that there are important differences between human, mouse, and rat nociceptors, both in terms of molecular profile and functional properties. There is more that can be learned about the molecular profiles of nociceptors across species and new technologies enable this important characterization. Rodent models have a critical place in pain research. Rodents still provide the unique opportunity to assess the integrative action of the nociceptive system. Similarly, new imaging methodologies in freely behaving rodents and circuit level approaches provide deep mechanistic insight into nociception and pain. These should be seen as important complementary approaches, but we have to better understand how these models relate to clinical translation in quantitative ways. Assays of human nociceptors (both *in vitro* and *in vivo*) provide a critical stepping stone in the translational pathway. These studies can confirm and identify novel pathophysiological mechanisms, as well as target engagement and potential biomarkers in experimental medicine studies. Publicly accessible databases are likely to be a potent research resources for understanding with high fidelity gene and protein expression in human DRG and the distinct nociceptor populations. Ultimately, these need to be expanded to relate such features to genotype and demographic variables, functional properties, and clinical phenotype using transcriptome and proteome wide association studies. We are still in the infancy of understanding sex, ethnicity, lifestyle, and age-related changes in human nociceptor function. Finally, we need to develop better techniques to functionally interrogate the 'hard to reach' human nociceptors such as joint and visceral afferents, given that dysfunction in such neurons is responsible for such a great deal of human morbidity.

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Competing interests

D.L.B. has acted as a consultant in the last 2 years for Amgen, Bristows, CODA therapeutic, Lilly, Mundipharma, Orion, Regeneron, and Theranexus on behalf of Oxford University Innovation. He has an MRC Industrial Partnership grant with AstraZeneca funded by the BBSRC. T.J.P. has acted as a consultant in the last 2 years for Grunenthal and Synerkine Pharma and holds a research grant from Merck and Hoba Therapeutics. T.J.P. is cofounder of 4E Therapeutics, PARMedics and Doloromics. P.R.R. is cofounder of Doloromics.

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