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Somatosensation a la mode: plasticity and polymodality in sensory neurons

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Our understanding of how peripheral damage-sensing neurons (nociceptors) respond to noxious stimuli is fundamental to the development of effective analgesics. To date, numerous studies have presented diverging hypotheses on how nociceptors encode modality-specific stimuli, including labelled-line, intensity dependence or pattern theory. In this short review, we appraise data from electrophysiological, behavioural, imaging and molecular expression studies from the last 60 years, in order to obtain a coherent view of modality-specific sensing in peripheral sensory neurons. We propose a mechanistic explanation for the broad range of values obtained for the incidence of polymodal nociceptors that reconciles apparently contradictory data.

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Introduction

A fundamental characteristic of the somatosensory system is the ability to detect and distinguish innocuous and noxious stimuli in order to initiate behavioural responses to evade or minimise harm. Theories of sensation have invoked modality-specific labelled lines, intensity coding or patterned input to explain the link between sensory neuron activity and sensation, and for more than 50 years, countless studies have investigated the mechanisms by which damage-sensing neurons (nociceptors) are activated, with the aim of developing effective analgesics [1]. An intriguing observation from these studies, is that a significant proportion of nociceptors are sensitive to multiple types of noxious stimulation (such as noxious heat and noxious mechanical stimulation), thus making them polymodal. However, this observation is difficult to reconcile with the modality-specific sensations associated with pain, and the evidence for modality-specific pain pathways obtained from behavioural studies after the ablation of subsets of sensory neurons. Indeed, experiments performed by Magnus Blix and Alfred Goldscheider, conducted almost 150 years ago, provided evidence for modality-specific spots on human skin (see Ref. [2] for review). The debate about polymodality has important practical consequences for drug development: if all nociceptors are polymodal, then all pain may be considered a single pathology; a view at odds with much experimental data. Here, we summarise our understanding of modality sensing in sensory neurons, considering electrophysiological, behavioural, imaging and molecular expression data. Taken together, these studies show that plasticity in somatosensation, particularly in response to inflammatory mediators, can reconcile the apparently disparate data in the literature to give a coherent view of modality sensing in peripheral somatosensory neurons.

Electrophysiological analysis of sensory neuron modality

Electrophysiological studies of nociceptor function have been performed on a variety of animal species. This work began in earnest in the late 1960s, with the work by Burgess and Perl (1967) and later Bessou and Perl (1969). These seminal papers characterised a subset of peripheral sensory neurons that were exclusively activated by noxious stimuli, demonstrating the existence of specialised nociceptors [3,4]. A key observation from the work of Bessou and Perl in 1969 was that some unmyelinated neurons responded to more than one noxious modality (e.g. heat, mechanical and chemical), giving rise to the term 'polymodal nociceptor' [4]. To date, the majority of sensory physiology studies use the term polymodal to define a neuron that is responsive to both mechanical and thermal stimulation, mainly driven by the fact that chemical sensitivity is infrequently tested. Here, the term polymodal will refer to sensory neurons that respond to more than one noxious modality (e.g. mechanical and heat). Using this more testable definition, numerous studies have characterised the modality responses of nociceptors from different species, yielding variable results. While the reported incidence of C-fibre polymodality is high in some studies (67–100%) [5–10], it is much lower in others (11–56%) [4,11–14]. Beyond the variability in reported levels of polymodality, there are also significant inconsistencies in the response profiles of polymodal nociceptors. For example, several studies investigating the polymodality of C-fibres found that none of the fibres that responded to noxious heat and noxious mechanical stimulation responded additionally to

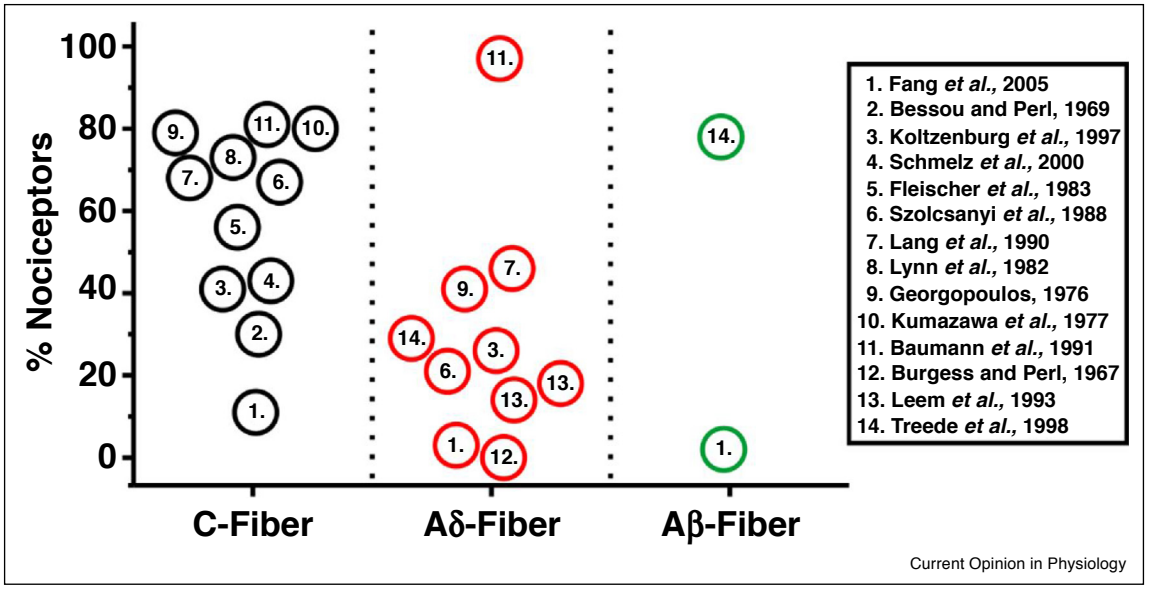
noxious cold [5,14,15]. In contrast, a study by Baumann *et al.* in 1991 showed that 78% of C-fibre nociceptors responded to noxious mechanical, heat and cold stimuli [10].

How can such substantial differences between studies be reconciled? While factors such as species, site of stimulation, as well as the mode and strength of a given stimulus, are all likely causes of variation between studies, the degree of variation may also reflect the plasticity of the nociceptive system. It is well documented that nerve injury and/or inflammation induces functional changes in nociceptors [see Ref. [16] for review]. Of note, Simone and Kajandar (1997) showed that while ~20% of Aδ-nociceptors are excited at 2°C, this proportion increases to almost 100% at the tissue damaging temperature of –18°C [17]. Similar variations in modality sensitivity have also been reported for heat and mechanical stimuli following the actions of neurotrophic factors [18–21], nerve injury and/or inflammation [22], and between *in vivo* and *in vitro* experimental preparations [23]. In addition, a significant population (up to a quarter) of nociceptive afferents are unresponsive to noxious stimulation under basal conditions in both rodents [21,23,24] and humans [25]. These afferents become awakened following injury and/or inflammation, and typically exhibit sensitivity to noxious mechanical stimulation, with some also responding to noxious heat stimulation. Understanding the physiology of silent nociceptors is fundamental to our understanding of the nociceptive system, as they offer clear insight into the plasticity of the system. Relatively little is known about silent nociceptors, perhaps caused by their variable incidence, which, as outlined above, is likely to be affected by local inflammatory mediators.

To date, the majority of polymodal classification studies have been restricted to C-fibres. However, a significant proportion of Aδ and Aβ fibres are also activated by noxious stimulation, and are therefore considered nociceptors [26]. Unfortunately, far fewer modality studies have been performed on A nociceptors compared to C-fibres, and even fewer on the largely overlooked Aβ population. One of the first studies to investigate the noxious sensitivity of myelinated fibres was performed by Burgess and Perl in 1967. From the 513 fibres recorded, 74, mainly Aδ fibres, responded exclusively to noxious mechanical stimulation [3]. Intriguingly, none of these fibres responded to noxious heat, noxious cold, acid application or bradykinin injection. Subsequent electrophysiological assessments from rats, cats and monkeys have reported the incidence of polymodality to vary between 3–97% in Aδ fibres [3,5,6,8,10–12,27,28]. Within the Aβ nociceptor population, one study on rats showed that only 3% were polymodal, with the vast majority (86%) responding to noxious mechanical stimulation [11]. In contrast, a study in monkeys showed that 78% of Aβ nociceptors were responsive to both noxious mechanical and heat stimulation [28]. The observed incidence of polymodal nociceptors between, C, Aδ and Aβ fibres is summarised in Figure 1.

From the large number of electrophysiology studies undertaken, it is clear that polymodal neurons exist; however, their relative incidence within the nociceptor

Figure 1



Reported incidence of polymodality from nociceptors. Summary of the reported incidence of mechano-heat polymodal nociceptors as a proportion of total C-fibre, Aδ-fibre and Aβ-fibre nociceptors, obtained from electrophysiological studies.

population, as well as their response profiles, remains to be fully understood, and due to the reasons outlined above, these issues are unlikely to be resolved through electrophysiological studies alone. Therefore, alternative approaches that interrogate neuronal physiology need to be considered.

Behavioural studies identify modality-specific subsets of neurons

Our ability to distinguish between discrete environmental stimuli suggests that there are specific receptors within sensory neurons that are adapted for their detection. Numerous molecular transducers (e.g. Trpv1, Trpa1, Trpm8, Piezo 2) have been identified that respond to a range of noxious and innocuous stimuli. Knockout studies on mice have shown that individual thermal or mechanical transducers are largely dispensable for pain perception, demonstrating redundancy in nociceptive mechanisms. In contrast, ablation of specific neuronal populations has provided strong evidence for labelled modality specific lines of sensation [29–33,34••,35]. Ablation of $Na_v1.8$ expressing neurons (about 85% of the peripherin-positive C-fibre associated neurons) was found to result in a complete loss of noxious mechanosensation with no effect on acute noxious heat responses [29], likely due to a surviving subset of sensory neurons expressing one of the three molecular transducers necessary for heat transduction [36•]. Additional studies by Cavanaugh *et al.* investigated the behavioural effect of ablating non-overlapping subsets of sensory neurons, marked by either Trpv1, or Mrgprd [30]. The authors found that the ablation of the Mrgprd population of neurons led to significant deficits in noxious mechanical sensation, without affecting noxious heat sensitivity [30]. In contrast, ablation of the Trpv1-expressing population had no effect on noxious mechanical sensitivity, but sensitivity to noxious heat was completely absent. Additional studies investigating the role of the Trpm8-expressing population of sensory neurons, a population that is distinct from Mrgprd and Trvp1, have also been undertaken. Ablation of the Trpm8-expressing population causes a significant loss of noxious cold sensitivity (at temperatures >0°C), while having no effect on noxious mechanical or noxious heat sensitivity [32,33]. A summary of behavioural data obtained following the ablation of specific neuronal populations is shown in Table 1.

Table 1

The effect of ablating specific sub-populations of peripheral sensory neurons on nocifensive behaviours in mice. The targeted neuronal population is shown along with the method of ablation (capsaicin treatment – experimenter controlled; diphtheria toxin fragment A (DTA) – genetically controlled; diphtheria toxin receptor activation (DTR) – experimenter controlled). Changes in nocifensive phenotype to noxious heat, mechanical (Mech.) and cold are shown (deficit: red; no change/normal: green; not studied: grey)

Neuronal population	Ablation type	Nocifensive behaviour			Reference
		Heat	Mech.	Cold	
Trpv1	Capsaicin				Cavanaugh *et al.*, 2009
Trpv1	DTA				Mishra *et al.*, 2011
Trpv1	DTR				Pogorzala *et al.*, 2013
Mrgprd	DTR				Pogorzala *et al.*, 2013
Mrgprd	DTR				Cavanaugh *et al.*, 2009
Trpm8	DTR				Pogorzala *et al.*, 2013
Trpm8	DTR				Knowlton *et al.*, 2013
Trpa1	DTR				Yarmolinsky *et at.*, 2016
CGRP	DTR				McCoy *et al.*, 2013
$Na_V1.8$	DTA				Abrahamsen *et al.*, 2008

Deficit; Normal; Not studied.

The observation that distinct populations of neurons are responsible for transducing modality-specific behaviours is compelling, offering strong support for the specificity theory. However, how can discrete modalities be carried by neurons that respond to multiple modalities? A possible answer to this conundrum is that the pattern/combinatorial behaviour of incoming afferent activity allows for modality discrimination at the level of the spinal cord and beyond. However, the ablation of peripheral Trpv1-expressing afferents causes a near complete loss of noxious heat-induced activity from both superficial and deep dorsal horn neurons, without affecting the coded response to noxious mechanical stimulation, or cooling [37]. This suggests that while Trpv1-expressing afferents are essential for detecting noxious heat, Trpv1-negative afferents alone are not sufficient to activate dorsal horn neurons following noxious heat stimulation. This result is also consistent with data from combined electrophysiological and immunohistochemical studies showing that Trpv1 expression is restricted to DRG neurons that are responsive to noxious heat, but not noxious mechanical stimulation [22,38]. These data strongly suggest that, under basal conditions, Trpv1-expressing neurons represent a modality-specific population responsible for noxious heat sensing, however, given the broad expression of Trpv1 among C-fibre nociceptors [39,40,41••], it is difficult to reconcile these data with the high incidences of polymodality reported for C-fibres.

Beyond ablation studies, there is also evidence of analgesics targeting specific subsets of sensory neurons to affect distinct modalities. In 2009, Scherrer *et al.* showed that μ-opioid and δ-opioid receptors are expressed by different subsets of sensory primary afferents in mice, and moreover, the use of specific μ- (DAMGO) or δ- (SNC80) agonists specifically reduced acute heat or mechanical pain, respectively [42]. More recently, it has been shown that local administration of DAMGO significantly attenuates post-operative mechanical and heat pain, supporting the hypothesis that injury induces changes in the modality sensitivities of sensory neurons [43].

Resolving anomalies: the role of *in vivo* imaging

Recent studies have exploited genetically encoded calcium indicators (e.g. GCaMP) to monitor neuronal

activity *in vivo* in sensory neurons [34••,44–51,52•]. An obvious limitation of these studies is that, by definition, monitoring changes in intracellular Ca^{2+} provides an indirect means to monitoring neuronal electrical activity (i.e. action potential generation); however, numerous studies have championed the fidelity of this technique, showing that low frequency electrical stimulation (equivalent to a single action potential) reliably coincides with a reproducible change in GCaMP fluorescence [46•,48,49•]. A major advantage of *in vivo* imaging is that any associated damage caused by culturing neurons is avoided. Importantly, a number of studies have used *in vivo* GCaMP imaging to investigate the modality specificity of DRG neurons in mice. One of these studies used single photon confocal microscopy to study modality responses of individual DRG neurons to mechanical, heat and cold stimulation, before and after PGE2-induced inflammation. This study also reported on the number of polymodal neurons observed within each preparation, which was observed to be ~15% of the mechanically sensitive population [46•]. Importantly, following the injection of the pro-inflammatory mediator PGE2, there was a substantial increase in the number of neurons responding to noxious heat stimulus, as well as those that were deemed polymodal, supporting the hypothesis than endogenous inflammatory mediators can regulate the modality responses of sensory afferents. Beyond heat sensitivity, a more recent study has shown that noxious cold sensing can be attributed to two discrete neuronal populations based on whether the stimulus is likely to be damaging [52•].

In vivo imaging studies using two-photon microscopy have been performed to investigate the incidence of polymodality within DRG neurons. Wang *et al.* observed that in response to plantar stimulation, the vast majority (~70%) of pinch sensing neurons did not respond to noxious heat stimulation [49•]. Despite these low levels of observed polymodality, the authors of this study conclude that >50% of sensory neurons are polymodal. In the study by Emery *et al.*, (2016), neurons that responded to brush stimulation were excluded in order to reduce the artefactual inclusion of low-threshold mechanically sensitive neurons. However, in the study by Wang *et al.*, (2018), the authors deemed neurons that responded to both brush and pinch stimuli as polymodal, despite the fact that some of these neurons did not respond to thermal stimulation. Thus, while the data are similar between these studies, the interpretation differs.

Beyond the DRG, *in vivo* imaging studies have also been performed on trigeminal ganglia. Although restricted to temperature sensing, Yarmolinksy *et al.* recently showed that heat and cold sensing neurons form non-overlapping populations, consistent with specific peripheral pathways for thermal discrimination [34••]. Moreover, they observed that injury, caused by the application of a 55°C stimulus for 15 s, profoundly altered neuronal responses to discrete thermal stimuli, highlighting the dynamic plasticity of the peripheral nociceptive system [34••]. Similarly, imaging studies from spinal cord neurons have shown that there is an almost complete absence of heat-induced neuronal activity following diphtheria-mediated ablation of Trpv1-expressing DRG neurons [50•], consistent with electrophysiological recordings from the dorsal horn following capsaicin-mediated afferent ablation [37].

Conclusions

Data from electrophysiological, behavioural, molecular and imaging studies all support the existence of modality-specific nociceptive neurons, yet the prevailing view within the pain field is that the vast majority of nociceptors are polymodal. As discussed above, polymodality within peripheral sensory neurons is a commonly observed phenomenon; however, its incidence is clearly dependent upon environmental context. If a specific stimulus is intense enough to cause tissue damage, or if such damage is caused by experimental preparation, the ensuing inflammatory response is likely to increase the number of responsive sensory neurons, as well as regulate their modality sensitivity, increasing the overall incidence of polymodality. These effects reconcile the variability in the reported incidence of polymodality between different studies, and more importantly, highlight the remarkable plasticity of peripheral sensory neurons in the detection of noxious stimuli in acute and chronic pain states.

Conflict of interest statement

Nothing declared.

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