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# Recent advances toward understanding the mysteries of the acute to chronic pain transition

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Chronic pain affects up to a third of the population. Ongoing epidemiology studies suggest that the impact of chronic pain on the population is accelerating (Nahin *et al.*, 2019). While advances have been made in understanding how chronic pain develops, there are still many important mysteries about how acute pain transforms to a chronic state. In this review, I summarize recent developments in the field with a focus on several areas of emerging research that are likely to have an important impact on the field. These include mechanisms of cellular plasticity that drive chronic pain, evidence of pervasive sex differential mechanisms in chronic pain and the profound impact that next generation sequencing technologies are having on this area of research.

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## Defining the transition from acute to chronic pain

Chronic pain is defined as pain that persists for at least 3–6 months [2]. This definition is clinically useful but it does not give any insight into mechanism. It also does not point out when the transition from acute to chronic pain occurs. A reading of the existing *clinical* literature on the topic suggests that the transition point is presumed to occur around the 3–6 month point; however, we will argue in this review that the *preclinical* literature suggests that the transition point is actually far earlier than the three month time point [3]. Given what we now know about the profound cellular plasticity that is brought about by injury that drives sensitization of the nociceptive system in the peripheral (PNS) and central nervous systems (CNS), it is likely that the transition likely occurs at time points near injury and may even occur concurrently with injury.

Why would such a system exist? Responding to injury is fundamentally important for all organisms. Nociceptors, neurons that are tuned to respond to injurious or potentially injurious stimuli, appeared early in nervous system evolution [4]. These neurons are remarkably plastic. They demonstrate the ability to increase their excitability upon sustained activation in every species where studies have been done to examine this form of intrinsic plasticity [4]. This creates a powerful teaching signal that enhances survival [5] while allowing the organism to recover from insult. An important question is whether this plasticity resolves or if its default mode is persistence. If it is indeed the latter, then injury that can cause sensitization of nociceptors may be doomed to cause a transition to a chronic pain state in most cases.

We know, however, that this is not the case. People have remarkably similar injuries everyday wherein only a minority will continue to have pain after the injury resolves [6]. How, then, can we reconcile our clinical observations with what we now understand about the physiology of nociceptor plasticity? The answer likely lies in a second type of plasticity that is driven by pain resolution mechanisms [7]. The pioneering work of Serhan revolutionized the way that we think about inflammation from a physiological process that runs its course to one that will persist far beyond its usefulness unless it is actively resolved by a novel class of lipid mediators called resolvins [8]. This idea has now permeated areas of neurobiology. We now also know, for instance, that the forgetting of aversive memories does not happen through the gradual decay of plasticity mechanisms involved in the original learning, but is instead driven by extinction mechanisms that require new learning [9]. With these paradigms in mind, it may be useful to think of a transition from acute to chronic pain as a default mode that can be overridden by pain resolution mechanisms. This idea is now emerging from numerous studies in the field. Here we will argue that this notion has the potential to revolutionize pain medicine, but that discovery in this area will likely be hard fought, largely because we lack understanding of the basics of nociceptive plasticity in females (of all species) [10].

## Modeling the acute to chronic pain transition

Defining preclinical models as ‘chronic’ is challenging because researchers rarely study experimental animals that have had pain for more than one month, much less three or six. The argument is often made that this is justified based on proportions of life-span to disease

duration, but this defies the physics of time. Having said that, there is still a strong argument to be made for studying plasticity mechanisms relatively early after injury because these mechanisms are likely key drivers of the transition to chronic pain.

### Inflammation

Two similar models have been extensively studied in the past few years in terms of understanding how inflammation might lead to the transition to chronic pain: hyperalgesic priming [11–13] and latent sensitization [14]. In the hyperalgesic priming model an animal receives a ‘priming’ stimulus, that is usually an inflammagen (e.g. carrageenan) or a defined inflammatory stimulus (e.g. interleukin 6, IL6) and allowed to recover from the initial nociceptive hypersensitivity (usually thermal and mechanical hyperalgesia). The animal is then challenged with an otherwise subthreshold dose of a different mediator that can now induce a long-lasting pain state. This system has been applied to the hindpaw and also to the cranial dura, creating a new model for migraine headache-like pain [15,16]. In the latent sensitization paradigm the animal is similarly primed but the animal is subsequently treated with an antagonist or inverse agonist of a G-protein-coupled receptor (GPCR) that then reveals a hypersensitive state that was not previously apparent [17–21].

Both of these models suggest forms of plasticity in the PNS and CNS that are persistent after the original injury, indicating the presence of a transition to a new homeostatic point for the organism. In the case of hyperalgesic priming it is clear that even though nociceptors may not be actively generating signals, they can easily become activated by stimuli which would ordinarily not have an effect. Interfering with specific translation regulation signaling pathways at the time of injury seems to be the most effective way to impede the development of priming [22–27]. However, several recent studies from Levine’s group have demonstrated multiple forms of priming that have very distinct molecular mechanisms [28–30]. If priming occurs in patient populations it may be critical to understand what mechanism the insult engages to reduce the probability of development of chronic pain. In the case of latent sensitization, CNS-driven, GPCR-mediated mechanisms actively suppress signals that can cause nociceptive hypersensitivity to very rapidly re-emerge if these GPCRs are blocked [17–21]. This suppressive mechanism may be one important way that pain hypersensitivity resolves after severe inflammation or surgery. While a first attempt to demonstrate latent sensitization in people was unsuccessful [31], a subsequent trial showed a statistically significant unmasking of latent sensitization upon administration of high-dose naloxone [32,33].

### Nerve injury and neuropathic pain

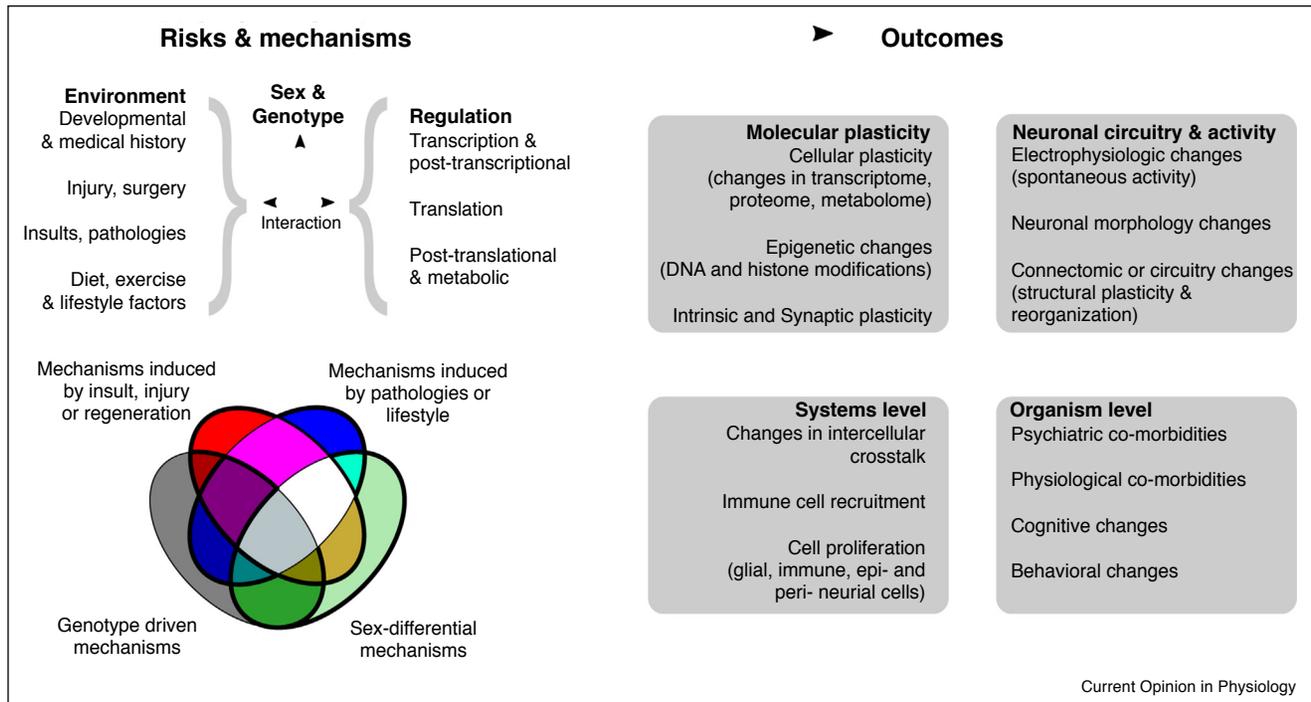
The most widely accepted models of chronic pain fall into the category of neuropathic pain and usually involve traumatic injury to a nerve (e.g. spared nerve injury, SNI), drug-induced neuropathy (e.g. chemotherapy induced painful neuropathy, CIPN) or metabolic disease (diabetic neuropathy). Some of the earliest evidence for an acute to chronic transition came from studies where it was shown that descending modulatory circuits from the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) are crucial for promoting pain in the first few weeks after injury [34]. More recent studies have demonstrated that rodents which are protected from the development of neuropathic pain after injury show engagement of descending inhibition at the time of injury to suppress later neuropathic pain [35]. This finding provides good evidence for the idea that the acute to chronic pain transition can occur very early after pain begins and that protection from the transition is due to endogenous pain resolution mechanisms. Strikingly, some human imaging studies provide evidence that people who are protected from developing chronic pain may do so through enhanced descending inhibition [36].

The acute to chronic pain transition in neuropathic pain is not entirely driven by central mechanisms, and is a complex interaction of central and peripheral processes with inherited and environmental risks (Figure 1). Recent human studies have demonstrated that blocking peripheral nerves in patients with neuropathic pain leads to almost immediate pain resolution that returns only after the nerve block wears off [37,38]. This, surprisingly, also occurs in patients who have neuropathic pain driven by stroke [39], suggesting that even CNS injuries may ultimately cause nociceptors to become sensitized and fire action potentials ectopically [40]. These studies point out an important point about CNS plasticity in neuropathic pain (which is often referred to as central sensitization [41]): these mechanisms amplify signals via synaptic gain and may not be capable of generating signals on their own. Therefore, they require an afferent signal to amplify and that afferent signal is likely provided by ectopic or evoked activity in peripheral nerves. Importantly, neuropathic pain in animals and humans is accompanied by the generation of ectopic activity in peripheral nerves [42,43].

### Mechanisms of acute to chronic pain transition involve cellular plasticity

The phenotypic changes occurring in nociceptors after injury driving the transition to a chronic pain state require changes in the molecular profile of these cells [44]. Many recent RNA-seq experiments have somewhat surprisingly shown that wholesale transcriptional changes do not seem to occur in DRG neurons and that those that are reliably found after nerve injury are mostly involved in the regeneration response [45,46]. Instead, translation regulation seems to be the key mechanism that nociceptors use to alter their phenotype

Figure 1



Chronic pain is typically the outcome of interaction between complex inherited polygenic risk, sex, environmental factors, developmental and medical history and triggers like insult or injury. Long term outcomes of chronic pain include changes in molecular configuration and profile, persistent immune signaling, changes in electrophysiology and the connectome, and resulting organism level changes in behavior.

toward profound hyperexcitability [26<sup>\*</sup>,44,46<sup>\*</sup>,47]. Two pathways that have recently emerged as critical for translation of new proteins that play a key role in the development of chronic pain are the MNK–eIF4E signaling axis [26<sup>\*</sup>,46<sup>\*</sup>] and poly-A binding protein (PABP) [48]. Genetic manipulation of MNK–eIF4E signaling renders mice less susceptible to injury-induced nociceptive hypersensitivity and almost completely abolishes hyperalgesic priming [26<sup>\*</sup>]. Tomivosertib (formerly known as eFT508) is a clinical candidate drug for cancer that specifically targets MNK [49,50]. This drug reverses mechanical hypersensitivity, ongoing pain and nociceptor ectopic activity in mouse CIPN models (in males and females) suggesting that this drug may be effective for treating chronic neuropathic pain in people [46<sup>\*</sup>]. MNK–eIF4E signaling regulates translation at the 5' end of mRNAs while PABP controls translation at the 3' end. Recent evidence suggests that 3' end processing of mRNAs is critical for localization of mRNAs in neurons, as well as neuronal plasticity. Blocking PABP function with a novel RNA-mimetic drug reduces development of chronic pain in response to inflammatory mediators and incision in mice [48]. These studies point to two, mechanistically distinct intervention points for preventing the acute to chronic pain transition.

Ion channels have long been considered prime targets for the treatment of chronic pain. One of the most important in this class is Nav1.7, where the rationale for targeting

this protein is clear based on the human genetics [51], although complicated somewhat by recent evidence of anatomical changes brought about by Nav1.7 loss of function mutation [52]. The studies that built this rationale largely identified mutations that led to profound gain or loss of function phenotypes. Interestingly, other, rare mutations have been discovered that lead to enhanced susceptibility of developing a chronic pain state in the presence of other variables, like diabetes and diabetic neuropathic pain [53]. This study establishes a paradigm wherein the interaction of genetics with environment may be a critical factor in determining whose pain will transform to chronic pain states. As DNA sequencing continues to advance into the clinical space, new discoveries along these lines will unquestionably be made. An excellent example of this is the case of two related people with gain of function mutations in Nav1.7 wherein one had a mild pain phenotype and the other severe pain. Whole exome sequencing revealed that a point mutation in Kv7.2 causing a gain of function decreased excitability in induced pluripotent stem cells (iPSCs)-derived sensory neurons from the person with less pain [54<sup>\*\*</sup>]. This gene–gene interaction illustrates the way that modern genetic sequencing technologies can be combined with the utility of iPSC-derived sensory neurons to make fundamental discoveries about pain phenotypes found in people. This study also highlights the key role that voltage gated

potassium channels (VGKCs) play in the development of chronic pain. These channels show decreased expression after nerve injury, which results in enhanced excitability [55]. Many of these channels are downregulated at the transcriptional level via an epigenetic mechanism involving G9a [56].

Central circuits play a key role in regulating the transition from acute to chronic pain. Plasticity mechanisms in the spinal dorsal horn involving long-term potentiation (LTP) and/or loss of GABAergic efficacy have long been recognized as key factors. The past several years have seen rich gains in our understanding of dorsal horn circuitry but much of this work is still focusing on acute pain, or ablation of circuits before injury [57]. It will be interesting to see if different circuits contribute to acute versus chronic pain. An example where this has already been shown is the descending dopaminergic circuit from the A11 nucleus [58]. This circuit inhibits acute and neuropathic pain through a D2-like-mediated mechanism [59]. In hyperalgesic priming models, once the primed state is established, the circuit plays a key role in maintaining priming via D1 and D5 dopamine receptors. Interestingly, this circuit relies on D5 receptors in male mice and D1 receptors in female mice [60]. This brings us to one of the strongest recent themes in pain research: sex differences.

### **Mechanisms of acute to chronic pain transition are sex differential**

Sex differences in basic pain mechanisms is one of the most rapidly expanding areas of discovery in the field (Figure 2) [61]. Most of this experimentation has highlighted sex differences in how pain becomes chronic. The most widely studied of these differences is the role of glia and immune cells in chronic neuropathic pain. It is now clear that there are major differences in the role of astrocytes and microglia wherein certain signaling pathways in these cells play a strong role in promoting pain in male but not female rodents [61–66]. A very recent paper from the Salter lab suggests that this difference boils down to sex differences downstream of the P2X4 receptor including p38 MAPK and IRF5-mediated transcription [67]. Many studies have suggested that this sex difference is driven by sex hormones [65]. However, injury to neonatal mice that drives important changes in adult pain behavior is also sex differential with microglial mechanisms playing a key role in male mice [66]. An outstanding question to be addressed is whether microglia play a role in chronic pain in females but simply use different mediators. In support of this conclusion, most of the studies cited above have observed robust microglial ‘activation’ in females in chronic pain models [65,68]. A similar literature is emerging in our understanding of macrophage (these cells share a common developmental lineage with microglia) contributions to chronic pain in rodents [69]. It may be the case that some mechanisms are

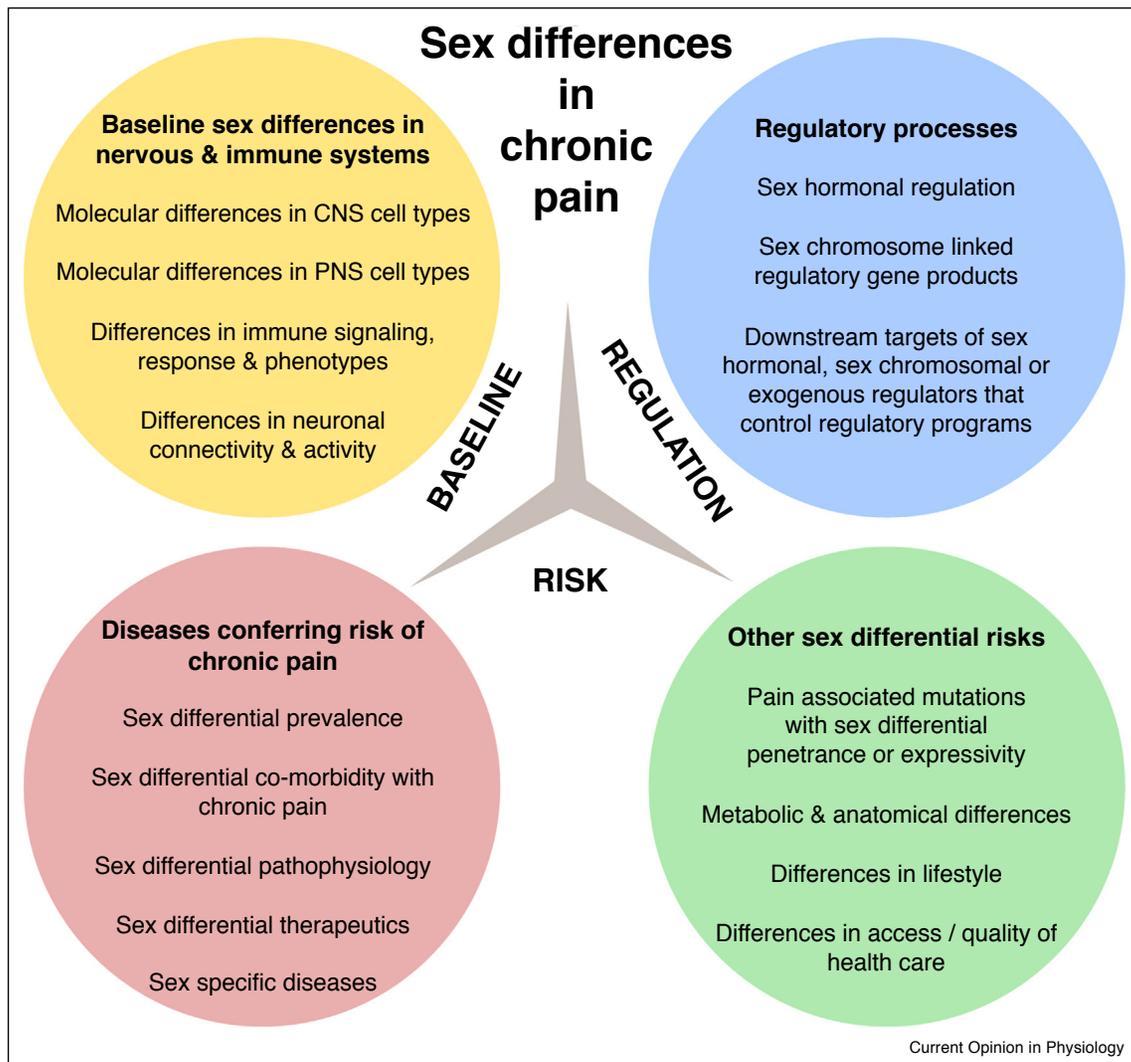
sex-specific while others, like redox signaling, can be engaged in males and females [70].

Are there immune cells that promote transition to chronic pain specifically in females? Some studies suggest that T-cells may serve such a function [65], but much less is known about signaling mechanisms through which this occurs. This is also controversial because while some studies have shown that T-cells promote chronic pain, others have shown that T-cells can play a critical role in pain resolution [71,72]. Finally, recent work from the Mogil lab demonstrates that T-cells regulate opioid analgesia in male and female rodents but that this effect is stronger in females [73]. The emerging picture from all of these studies is that T-cells are key mediators of pain plasticity in females but we are just scratching the surface in terms of our understanding of how these cells interact with the nervous system to promote or inhibit chronic pain.

While a great deal of recent work has focused on immune cells and sex differences, many differences in the nervous system have also been discovered. An example in the PNS is that activity-dependent slowing of C-fiber action potentials is more pronounced in female rats than males. This effect is substantially decreased with inflammation, contributing to greater hyperalgesia in females compared to males [74]. Baseline gene expression in human tibial nerves also show sex differential gene expression in several genes known to be neuronally expressed, raising the possibility of differential axonal mRNA transport between sexes [75]. In the CNS, sex differences in neuronal plasticity mechanisms are emerging. We recently showed that neuropathic pain causes shortening of axon initial segments in prefrontal cortex neurons of male but not female mice [76]. This structural change drives cognitive dysfunction that is far more pronounced in male than female mice. Synaptic plasticity mechanisms in pain plasticity also show sex dimorphisms. Although controversial, atypical PKCs (aPKC), in particular PKM $\zeta$ , have been described as mediators of maintenance of LTP at many CNS synapses [77]. Several previous studies, all conducted exclusively in male mice and rats, suggested that spinal aPKCs are required for the transition from acute to chronic pain [78,79]. More recent studies have demonstrated that this spinal effect is specific for male mice. Removal of the gene encoding PKM $\zeta$  or applying aPKC blockers to the spinal cord inhibit or reverse, respectively, chronic pain in male but not female mice [80]. Spinal inhibition of aPKC also reverses a stress-mediated pain hypersensitivity caused by placing mice in an environment where they previously received a tonic pain stimulus. Strikingly, this effect only occurs in male mice and can be replicated in male, but not female, human volunteers in an experimental setting [81].

A major gap in knowledge is the extent to which these sex differences will be represented in human populations.

Figure 2



Sex differences in chronic pain can be envisioned as being built upon the building blocks of baseline differences in the nervous and immune systems, with sex differences becoming more pronounced as a result of sex differential risks (including risk of diseases that share co-morbidity with chronic pain), and sex differences in molecular regulation.

The latter study [81] suggests the possibility that many of these differences will translate to humans. If this turns out to be the case, sex differences in chronic pain mechanisms will have a profound impact on therapeutic development, clinical trial design and clinical care.

### The RNA sequencing (RNA-seq) revolution

RNA-seq is now widely accessible to most laboratories, allowing unbiased profiling of cellular or tissue transcriptomes in normal tissues and/or in disease states. In the pain field, comprehensive resources are now available that have profiled mouse DRG neurons at the single cell level [82<sup>••</sup>,83,84<sup>•</sup>,85] and similar resources are published for many other cell types in the periphery and in the CNS [86,87]. Human DRG [42<sup>••</sup>,88] and trigeminal ganglion

(TG) transcriptomes [89,90] have been published allowing for rapid comparison of RNA expression levels between preclinical models and people. A rapidly growing number of transcriptomic studies have been published at various times after nerve injury, or in other neuropathic pain models [91]. Most of these studies have failed to find major transcriptome differences after nerve injury, although some of these studies have detected sex differences that may be related to altered immune cell infiltration [45<sup>•</sup>]. A possible explanation for the relatively small number of transcriptomic changes found in these studies is that single cell sequencing technologies need to be applied to detect transcriptomic changes in neuronal populations brought about by neuropathic pain, with one promising study already performed on a rodent injury

model [84\*]. Improvement of sensitivity is also the focus of research efforts in computational genomics, based on frequentist approaches for large scale testing of correlated hypotheses [92], and Bayesian approaches to identifying gene expression changes in unlabeled single cell samples [93]. A final possibility is that profiling techniques that assess translation of mRNAs, like the cell-type-specific translating ribosome affinity purification (TRAP) or ribosome profiling technologies, can provide greater insight into changes in gene expression brought about by injury. In support of this idea, two recent studies using these techniques demonstrate widespread changes in gene expression at the translational level that are not reflected in bulk transcriptomes [46\*,94]. The application of TRAP sequencing to a CIPN model reveals that many previously described CIPN targets are regulated at the translational level. This study also demonstrates that MNK-eIF4E signaling is a key driver of chronic pain caused by chemotherapy in male and female mice [46\*].

Many groups are now using RNA-seq to gain insight into pain mechanisms in patient populations. We have recently done this on patients with neuropathic pain who are having their DRGs removed during vertebrectomy. Approximately 20% of DRG neurons taken from patients with neuropathic pain show ectopic activity, drawing a striking parallel to findings from preclinical models [42\*\*]. Moreover, changes in transcriptomes are readily observed between chronic pain and non-pain samples. In male patients these transcriptomic changes seem to reflect infiltration and/or phenotypic changes in macrophages that may drive chronic pain. In women transcriptomic changes in pain samples did not parallel findings in men, and may reflect altered gene expression in neurons rather than immune cells [42\*\*]. These findings, while not precisely paralleling findings from preclinical models, suggest conservation of some core mechanisms of acute to chronic pain transition discussed above in patients with neuropathic pain. Importantly, the unprecedented public availability of these RNA-seq datasets creates rich possibilities for data-mining projects aimed at discovery of new pain mechanisms and targets.

### Therapeutics targeting transition resolution mechanisms

An emerging trend in the study of the acute to chronic pain transition is the concept of pain resolution mechanisms [95]. Initial work in this area focused on interleukin 10 (IL10) and resolvins. While there is compelling evidence for both of these mechanisms, the case for IL10 as an endogenous chronic pain resolution mechanism had progressed substantially recently. Several groundbreaking studies have demonstrated that IL10 from T-cells is a key driver of neuropathic pain resolution [71,72]. IL10 also likely plays a role in resolution of other types of pain, like inflammatory pain. Therapeutic approaches employing this mechanism are under clinical

development. They include IL10 gene therapy approaches [96] and IL10-IL4 synerkine [97] as a biologic to accelerate pain resolution.

Exercise has long been recognized as one of the most effective ways to reduce chronic pain. Mechanisms involved in this effect have recently been described and also involve IL10. A history of previous exercise can prevent development of chronic muscle pain brought about by mimicking repeated ischemic insults. This occurs via anti-inflammatory macrophages that increase IL10 synthesis and is effective in males and females [98\*\*]. Previous exercise history also enhances resolution of neuropathic pain and this effect is likewise driven by an IL10-dependent mechanism [99]. These and other studies make the strong case for IL10 as a key chronic pain resolution mechanism that is apparently effective in male and female rodents.

### Putting scientific advances into action

Remarkable gains are happening in our understanding of how acute pain becomes chronic. These advances in basic science need to begin to influence new ways of thinking about developing drugs for and treating chronic pain. First, the temporal definition of chronic pain, while convenient, is outdated. An operational definition of chronic pain would benefit patients. Second, it is time to recognize that sex differences in chronic pain mechanisms are pervasive and that we have little understanding of how chronic pain develops in females. This is particularly unfortunate given that many forms of chronic pain disproportionately affect females [1]. Preclinical studies that focus on male animals out of convenience rather than scientific rationale should be recognized as incomplete [100]. Finally, it is time that we start implementing what we have learned into drug development and clinical trial design. The FDA has long recognized that biological sex differences may guide decisions about the course of drug testing and approval. As a field we should focus on how mechanisms of acute to chronic pain transition can improve the probability that new drugs for this terrible disease will be approved.

### Conflict of interest statement

Nothing declared.

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