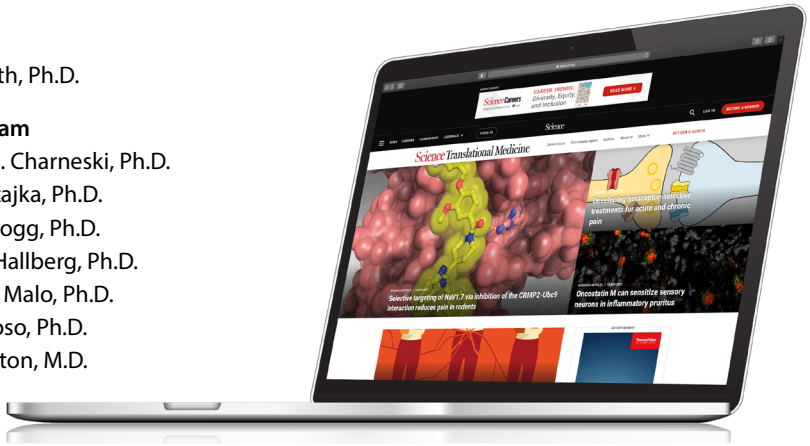
SPECIAL EDITION: **PAIN** | ScienceTranslationalMedicine.org

Science Translational Medicine

AAAS

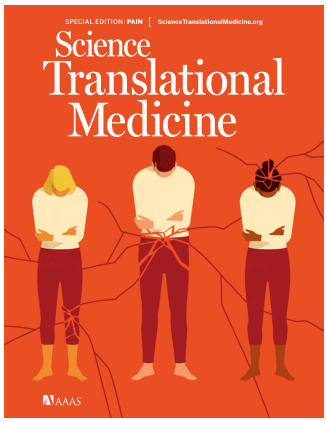
Science Translational Medicine | AAAS

Science Translational Medicine is an interdisciplinary journal that publishes translational research with impact for human health that fills the knowledge gaps between preclinical studies and clinical applications.



Scientific Advisory Board

SPECIAL EDITION: PAIN



Pain, Pain Go Away. The cover image symbolizes the shattering experience of different types of pain in the human body. In this Special Issue, which is designed to showcase the latest advances in pain research, Review articles discuss the principal brain circuits mediating pain (Lindsay *et al.*) and insights and challenges associated with the development of analgesic drugs (Jayakar *et al.*). In addition, three Viewpoint articles highlight promising areas of pain research including inflammatory mechanisms of pain (Kavelaars *et al.*), sexual dimorphism in pain sensitivity (Navratilova *et al.*), and development of imaging-based biomarkers for measuring pain (Tracey). Two Research Articles complete the Special Issue, presenting research findings on a new small molecule with analgesic effects in mouse models of pain (Cai *et al.*) and a cytokine that contributes to inflammatory itch (Tseng and Hoon). These Special Issue articles showcase the encouraging progress made in the field of pain research and the challenges still to be overcome.

Credit: *Science Translational Medicine*

Publisher/*Science* family of journals: **Bill Moran**
AD/Business Development: **Justin Sawyers**
Marketing Manager: **Shawana Arnold**
Layout/Design: **Kim Huynh**

IN THIS BOOKLET

VIEWPOINTS

REVIEWS

RESEARCH ARTICLES

PAIN

Sexual dimorphism in functional pain syndromes

Edita Navratilova[1], Roger B. Fillingim[2], Frank Porreca[1,3]*

Injury-free pain conditions, defined as functional pain syndromes, are more prevalent and more disabling in women. Mechanisms of sexual dimorphism in functional pain are now emerging from preclinical studies, suggesting an opportunity to advance the development of sex-specific therapies that may improve treatment of pain in women.

INTRODUCTION

Chronic pain is estimated to affect nearly 100 million Americans. Among these, 11 million experience high-impact pain that directly impedes daily activities. Both chronic pain and high-impact pain syndromes are more prevalent in women (*1*). Pain can be classified as nociceptive, neuropathic, and, more recently, nociplastic (*2*). Pain conditions with nociplastic features have altered nociception, even though they are not easily associated with tissue injury or lesions of the somatosensory system and are often described as functional (fibromyalgia, temporomandibular disorder, migraine, and irritable bowel syndrome are a few examples). These conditions, more common and disabling in women, are inadequately treated and remain a major unmet medical need. Furthermore, many of these pain disorders often co-occur, resulting in their designation as chronic overlapping pain conditions that are also more common in women (*3*) and result in increased suffering and risk of disability. Here, we highlight mechanisms of sexual dimorphism in functional pain syndromes that are emerging from preclinical studies. We also suggest approaches that can help to bridge preclinical and clinical investigations of functional pain, with the goal of developing sex-specific therapies for these pain disorders.

SEXUAL DIMORPHISM IN FUNCTIONAL PAIN

Pain-related sex differences can be quantitative and qualitative (*4*). Quantitative sex differences are present when the pain response shows a different magnitude in females versus males, whereas qualitative sex differences emerge when the mediators of the pain response differ fundamentally across sexes. Qualitative differences may explain some of the quantitative sex differences but importantly can also emerge in the absence of quantitative sexual dimorphism. Qualitative sex differences are translationally relevant and suggest that the mechanisms driving pain-related endpoints differ by sex and that treatments targeting these mechanisms could have widely divergent efficacy in females and males.

Quantitative sensory testing studies in healthy adults consistently indicate greater sensitivity to experimentally induced pain across multiple stimulus modalities among women compared to men (*1*, *4*). Additionally, healthy females show greater temporal summation of pain, a mechanism whereby each subsequent nociceptive stimulus elicits a greater response, suggesting enhanced pain facilitation (*1*). Thus, women suffer from a greater burden of clinical pain, and even healthy women experience enhanced pain sensitivity.

Among the overlapping biopsychosocial mechanisms that contribute to quantitative and qualitative sex differences in clinical pain and pain sensitivity, sex hormones have received the most attention (*1*, *3*). The most robust sex differences in prevalence and severity of functional pain syndromes and in experimental pain sensitivity emerge during the reproductive years (*1*). Moreover, clinical and experimental pain responses vary across the female menstrual cycle and are influenced by pregnancy and exogenous hormone use (*1*). The key role of sex hormones is supported by increased incidence of migraine in transgender women who are on antiandrogens and receive high doses of estrogen (*5*). Many functional pain syndromes can improve after menopause, supporting a causal link to gonadal hormones.

Psychosocial stress is an important sex-related risk factor for functional pain given that hormonal and behavioral stress responses differ considerably by sex (*6*), and stress is strongly implicated in functional pain conditions (*7*). Stress increases risk for development of female-prevalent functional pain conditions and can contribute to pain exacerbations and outcomes in people with existing pain conditions (*7*). Psychosocial stress is also a major risk factor for development of chronic overlapping pain conditions (COPCs) (*8*). Headache is associated with greater perceived stress, and stress is the most frequently reported trigger of both migraine and tension-type headache (*9*). Adverse childhood experiences (such as abuse) strongly predict the development of adult functional pain syndromes including migraine, and this correlation is much more prevalent in women (*10*, *11*).

SEXUAL DIMORPHISM IN PRECLINICAL MODELS OF FUNCTIONAL PAIN SYNDROMES

Preclinical studies are beginning to unravel sexually dimorphic pain mechanisms that might contribute to the high prevalence of functional pain disorders in women. Sex differences have now been identified in nociceptors, in ascending and descending pain modulatory pathways and stress-related neurohormonal modulation of nociception (*12*). Whereas sensory thresholds generally change to a similar degree following injury in male and female animals, the biological mechanisms promoting pain and analgesia are different [see (*13*) for review]. Sexual dimorphism has been observed in a rodent model of the pain phase of migraine produced by engagement of meningeal nociceptors following minimally invasive application of inflammatory substances onto the dura mater (*14*). Studies modeling the features of the migraine syndrome with two-hit priming strategies including medications promoting hyperalgesia, stress or a prior stimulus activating nociceptors have demonstrated sexual dimorphic mechanisms in the triggering of pain by normally innocuous stimuli (*15*, *16*).

Calcitonin gene-related peptide (CGRP) is causally implicated in the pathophysiology of migraine, and inhibition of CGRP signaling

[1]Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA. [2]University of Florida, Gainesville, FL 32611, USA. [3]Mayo Clinic, Scottsdale, AZ 85259, USA.
*Corresponding author. Email: frankp@arizona.edu

is clinically effective for both acute treatment and prevention of migraine (*17*). Recent preclinical studies have demonstrated that application of CGRP to the dura mater produced periorbital cutaneous allodynia, a surrogate readout of migraine-like pain, at much lower doses in female than in male rats (*18*). Furthermore, following priming with stress, a CGRP antagonist or anti-CGRP monoclonal antibodies were more effective in preventing periorbital allodynia, elicited by trigeminal stimulation with a TRPA1 agonist or with further exposure to stress, in female compared to male mice (*19*, *20*). Neuroanatomical differences in CGRP-related nociceptive pathways from the meninges may therefore contribute to increased sensitivity of female dural nociceptors with possible implications relevant to the increased prevalence of migraine in women. Although CGRP-based therapies, including monoclonal antibodies and CGRP receptor antagonists, have dramatically impacted the lives of many, a large group of people with migraine do not receive benefit from these treatments (*21*). CGRP can signal through both the CGRP receptor and the amylin receptor (*21*, *22*), and provocative studies have recently demonstrated that an amylin analog could elicit migraine in humans. Whether amylin effects or CGRP signaling at amylin receptors may be sexually dimorphic and whether such effects would be female selective are currently being investigated along with potential therapeutic implications.

In an important early study, prolactin was demonstrated to increase capsaicin-evoked release of CGRP in female, but not male, rodent trigeminal ganglion neurons (*23*). The sexually dimorphic effects of prolactin appear to depend on differences in expression of prolactin receptor (PRLR) isoforms in sensory neurons. In rodents, female ganglion neurons express higher quantities of the PRLR long isoform (PRLR-L) than do males (*24*, *25*). Preclinical studies suggest that signaling via the homodimers of the short isoform of PRLR (PRLR-S) increases neuronal excitability and promotes pain, whereas PRLR-L expression and formation of heterodimers interfere with PRLR-S signaling, providing protection against nociceptive sensitization in females (*24*). Down-regulation of PRLR-L and increased signaling via the PRLR-S are therefore a likely mechanism of selective female nociceptor sensitization. In mice, priming with medications known to promote hyperalgesia in both sexes, including opioids (*24*), promotes PRLR-L down-regulation only in neurons from female animals. The down-regulation of PRLR-L results in increased prolactin signaling at PRLR-S and subsequent increased pain responses in female animals.

The dysregulation of PRLR isoforms also provides a neuroendocrine link to stress-related female-prevalent pain conditions. Serum concentrations of prolactin are higher in women and in female rodents and are further increased by stressful events including trauma or injury. Repeated priming events (medications, stress) elicit widespread hypersensitivity to normally innocuous mechanical stimulation in both male and female animals but promote down-regulation of PRLR-L selectively in female animals (*24*, *25*). Moreover, repeated stress-induced pain was blocked by inhibition of prolactin release only in female animals. Thus, whereas hypersensitivity is observed in both sexes, the underlying mechanisms differ and, in females, depend on nociceptor sensitization established by changes in expression of PRLR isoforms following increased release of prolactin. Patients with hyperprolactinemia are more likely to have frequent or chronic migraine (*26*), raising the possibility that prolactin may be in part responsible for the transformation, chronification, or progression of migraine from a paroxysmal/episodic to a chronic disorder with frequent or persistent headache pain that is more common in women. The sexually dimorphic nociceptor sensitization resulting from prolactin receptor isoform dysregulation may also be relevant to other functional pain conditions, a possibility that requires further investigation.

BRIDGING THE KNOWLEDGE GAP BETWEEN PRECLINICAL STUDIES AND HUMAN FUNCTIONAL PAIN DISORDERS

Although substantial advances have been made in revealing the mechanisms that may drive acute and chronic pain following injuries, knowledge of mechanisms promoting injury-free functional pain conditions and their prevalence in females remains rudimentary. Functional pain syndromes have unique underlying pathologies, but they also share many biological features including (i) episodic attacks with frequency determining the likelihood of transformation to a chronic state, (ii) sensitivity to presumed exteroceptive or interoceptive triggering events such as stress, and (iii) hormonal links with pain in women that begin around the time of menarche and diminish at menopause. These overlapping features suggest shared genetic and neural mechanisms that amplify nociception in ascending nociceptive pathways and promote defects in neuronal circuits that project from the brain to modulate nociception (i.e., descending pain modulation) (Table 1). Preclinical investigation of these common mechanisms may therefore provide a path forward for the development of improved and sex-specific therapies for functional pain syndromes.

Most functional pain disorders have a complex yet unknown genetic contribution. Relevant genes for some disorders such as familial hemiplegic migraine have been identified (*27*), permitting mechanistic investigation of the disease preclinically. Preclinical studies of functional pain disorders, however, are commonly limited to investigation of mechanisms by modeling a particular clinical symptom rather than the disease itself. Methodological advances that now permit transcriptomic and functional analysis of human dorsal root ganglia and other tissues at the single-cell level may help to bridge the gap between the laboratory and human pain syndromes (*28*). Increasingly, investigations of inducible pluripotent stem cells (iPSCs) from phenotyped patients with pain syndromes are allowing identification of genetic contributions to these diseases and are additionally likely to reveal sex-specific genetic contributions.

A prominent brain region that serves sexually dimorphic functions is the hypothalamus. Recent experiments involving daily imaging for 1 month of a single female patient with episodic migraine led to the remarkable insight that the hypothalamus was active in the premonitory phase, some 24 to 72 hours before the pain phase of the migraine attack. Hypothalamic activation prior to headache has now been confirmed in a larger group of mainly female patients with episodic migraine, and functional connectivity studies have revealed temporal associations with brainstem circuits (*29*). These clinical insights can guide mechanistic studies in preclinical models to reveal hypothalamic influences on pain circuits and to uncover possible sexually dimorphic mediators in the hypothalamus that can initiate the pain attacks of migraine and other functional pain disorders.

Despite advantages in genetic characterization of human tissues from patients and

Table 1. Potential mechanisms contributing to enhanced pain sensitivity and greater burden of clinical pain in women.

Site	Effect	References
Neuroendocrine responses	Serum prolactin is higher in women and in female rodents and increased by stress. Following priming, rodents show down-regulation of PRLR-L linking the hypothalamus and nociceptors to promote functional pain syndromes.	(*24*, *25*)
Nociceptors	Rodents exhibit sexually dimorphic responses of sensory neurons to prolactin. Females express more PRLR-L. When primed with repeated stress or migraine-inducing drugs, PRLR-L is down-regulated, resulting in increased pain sensitivity in females.	(*23*–*25*)
Ascending nociceptive pathways	Male and female rodents demonstrate neuroanatomical differences in CGRP-related nociceptive pathways. Females show increased sensitivity to dural stimulation with CGRP.	(*18*–*20*)
Descending pain modulation	Healthy women show greater temporal summation of pain, suggesting enhanced pain facilitation. Conditioned pain modulation, a measure of descending inhibition, is variable in humans with some studies suggesting a decrease in women.	(*1*)

imaging studies in humans, preclinical studies in animals remain a necessary systems-level complement that is critical in the discovery of potentially actionable molecular targets. Animal studies are essential in revealing target relevance in the particular pain syndrome and potential side effects that can be anticipated. Dose-response relationships for efficacy and safety of new drugs can also provide early identification of sex differences and help to avoid failures in clinical trials from overdosing or underdosing male or female patients. Behavioral studies can be performed in male and female animals in parallel with investigations in human tissues to identify pharmacokinetic factors that are critical for drug engagement of targets in human trials.

An obvious challenge in preclinical models of functional pain syndromes is that pain does not obviously result from injury. Hyperalgesic priming to induce pain sensitization has been demonstrated to require protein synthesis, suggesting molecular mechanisms that promote the establishment of a pain memory in which each pain attack increases vulnerability to subsequent triggering stimuli, promoting the development of chronic pain (*16*). Sex differences in hyperalgesic priming from application of CGRP or inflammatory mediators such as interleukin-6 have been observed (*18*). Increased investigation of mechanisms that establish and maintain pain memory may allow the discovery of therapies that prevent the transition of episodic pain conditions to their chronic state. Additionally, mechanisms that can promote extinction of established pain memories and reverse treatment-resistant chronic pain conditions may be identified.

Notably, whereas hyperalgesic priming and assessment of sex differences in nociceptors have revealed mechanisms that increase the likelihood of pain in females, few insights have emerged into mechanisms promoting functional pain syndromes in males. Far fewer functional pain syndromes show male prevalence, and developing animal models of disorders such as cluster headache and other trigeminal autonomic cephalalgias that are more common in men remains extremely challenging. Overcoming this knowledge gap is essential and will likely require increased clinical studies to stratify outcomes in male-prevalent and female-prevalent pain disorders. Disaggregating data by sex may permit the discovery of pain promoting or pain protective circuitry and drive preclinical model development and mechanistic investigations.

Clinical trials do not assess nociception but rather evaluate pain, a multidimensional experience arising from integrated activity of multiple brain circuits. Improved alignment of preclinical output measures with those assessed in humans is therefore critical for translational success. In most preclinical studies, output measures are based on sensory features with reflexive motor and vocal responses to noxious stimuli. Although these behavioral manifestations reflect the aversive qualities of pain, they fall far short of the sum of sensory, affective, and cognitive factors reflected in verbal reports from humans (*30*). Recent preclinical efforts have emphasized evaluation of pain based on its physiological role in helping to avoid harm by driving motivation to seek relief (*31*). The relief of pain aversiveness activates brain reward pathways, reinforcing future behaviors. Cognitive resources are also prioritized by pain to allow behavioral choices that promote the most advantageous survival outcomes (*32*). Functional pain disorders alter motivation and impair cognition in humans, and these outcome measures should be assessed in preclinical models to evaluate potential efficacy of pain-relieving targets. The increasing use of operant tasks, where future actions are shaped by outcomes of a behavioral choice, in preclinical research can help to bridge the divide between human self-reporting and indirect animal reporting through motivated behaviors (*31*). Currently, little is known in either preclinical models or in humans about the impact of sex on pain-related motivation and cognition.

Functional pain syndromes are associated with changes in central processing of pain that can be measured through the assessment of pain facilitation and inhibition, for example, by measuring temporal summation and conditioned pain modulation. A composite index of multiple measures of central pain

processing, including conditioned pain modulation, showed high sensitivity and specificity for distinguishing patients with fibromyalgia from healthy controls (*33*). These output measures are highly relevant and likely to be predictive of patients who have the greatest risk for developing chronic pain. Preclinical studies evaluating temporal summation and the diffuse noxious inhibitory control response might provide a direct translational bridge between preclinical and clinical evaluations. At present, there is insufficient information about sex differences in central pain modulation. However, activity in specific brain circuits, including the periaqueductal gray and rostral ventromedial medulla that are key nodes in descending modulation, has shown sexual dimorphism (*34*).

To date, there has been insufficient emphasis on how to bridge knowledge of mechanisms that follow from triggering events such as stress and hormonal events to activation of the nociceptors that can elicit a pain episode. Neurohormonal analysis can be performed in both patients and preclinical animal models to provide information on the consequences of short-term (stress events and menses) and long-term (menopause, neuroendocrine disorders, and transgender transition) pituitary and gonadal hormonal changes that can alter pain in a sex-specific manner. Longitudinal studies of functional pain disorders in women after menopause are currently lacking as are studies evaluating the effectiveness of treatment outcomes in postmenopausal compared to premenopausal women.

A general feature of chronic overlapping pain conditions is central sensitization that results from neural adaptations in central circuits eliciting amplification of sensory inputs. It seems likely, however, that the establishment of central sensitization differs across pain syndromes and between sexes. Central sensitization may be present in multiple forms, possibly resulting from ongoing nociceptor input or by alterations in pain-related brain structure and function. Evidence of the former comes from the finding that coexpression of multiple orofacial pain syndromes involving the trigeminal pathway is greater than the overlap in expression of orofacial syndromes with syndromes affecting other areas of the body (*35*). Divergence of amplification in trigeminal and dorsal root ganglion nociceptive circuits between the sexes has also been observed in preclinical models (*36*). Whether there are sex differences in these mechanisms that may promote increased pain or deficiencies in mechanisms that promote resolution of pain remains to be determined. Most episodic functional pain conditions can become chronic, and whether there are possible sex differences in chronification of injury-free functional pain is also a challenge that must be addressed in future investigations.

CONCLUSION

Clinical investigation and "clinically informed" basic science studies can produce a powerful combination of reverse and forward translation that may lead to the successful bridging of the translational divide in the discovery and development of sex-specific medicines for treating functional pain disorders. Improving knowledge of mechanisms promoting the common features of female-prevalent or male-prevalent functional pain disorders may increase success in the development of medicines that can improve the lives of patients affected by pain.

REFERENCES AND NOTES

1. R. B. Fillingim, C. D. King, M. C. Ribeiro-Dasilva, B. Rahim-Williams, J. L. Riley III, Sex, gender, and pain: A review of recent clinical and experimental findings. *J. Pain* **10**, 447–485 (2009).
2. S. N. Raja, D. B. Carr, M. Cohen, N. B. Finnerup, H. Flor, S. Gibson, F. J. Keefe, J. S. Mogil, M. Ringkamp, K. A. Sluka, X. J. Song, B. Stevens, M. D. Sullivan, P. R. Tutelman, T. Ushida, K. Vader, The revised international association for the study of pain definition of pain: Concepts, challenges, and compromises. *Pain* **161**, 1976–1982 (2020).
3. W. Maixner, R. B. Fillingim, D. A. Williams, S. B. Smith, G. D. Slade, Overlapping chronic pain conditions: Implications for diagnosis and classification. *J. Pain* **17**, T93–T107 (2016).
4. J. S. Mogil, Qualitative sex differences in pain processing: Emerging evidence of a biased literature. *Nat. Rev. Neurosci.* **21**, 353–365 (2020).
5. J. Ailani, Updates on management of headache in women and transgender women. *Curr. Opin. Neurol.* **34**, 339–343 (2021).
6. D. A. Bangasser, B. Wicks, Sex-specific mechanisms for responding to stress. *J. Neurosci. Res.* **95**, 75–82 (2017).
7. P. R. Martin, Stress and primary headache: Review of the research and clinical management. *Curr. Pain Headache Rep.* **20**, 45 (2016).
8. R. B. Fillingim, R. Ohrbach, J. D. Greenspan, A. E. Sanders, N. Rathnayaka, W. Maixner, G. D. Slade, Associations of psychologic factors with multiple chronic overlapping pain conditions. *J. Oral Facial Pain Headache* **34**, s85–s100 (2020).
9. D. P. Turner, A. D. Lebowitz, I. Chtay, T. T. Houle, Forecasting migraine attacks and the utility of identifying triggers. *Curr. Pain Headache Rep.* **22**, 62 (2018).
10. S. Brennenstuhl, E. Fuller-Thomson, The painful legacy of childhood violence: Migraine headaches among adult survivors of adverse childhood experiences. *Headache* **55**, 973–983 (2015).
11. Z. Giano, D. L. Wheeler, R. D. Hubach, The frequencies and disparities of adverse childhood experiences in the U.S. *BMC Public Health* **20**, 1327 (2020).
12. E. J. Bartley, R. B. Fillingim, Sex differences in pain: A brief review of clinical and experimental findings. *Br. J. Anaesth.* **111**, 52–58 (2013).
13. J. S. Mogil, A. L. Bailey, Sex and gender differences in pain and analgesia. *Prog. Brain Res.* **186**, 141–157 (2010).
14. A. Avona, B. N. Mason, C. Burgos-Vega, A. H. Hovhannisyan, S. N. Belugin, J. Mecklenburg, V. Goffin, N. Wajahat, T. J. Price, A. N. Akopian, G. Dussor, Meningeal CGRP-prolactin interaction evokes female-specific migraine behavior. *Ann. Neurol.* **89**, 1129–1144 (2021).
15. D. B. Reichling, J. D. Levine, Critical role of nociceptor plasticity in chronic pain. *Trends Neurosci.* **32**, 611–618 (2009).
16. R. Kandasamy, T. J. Price, The pharmacology of nociceptor priming. *Handb. Exp. Pharmacol.* **227**, 15–37 (2015).
17. D. W. Dodick, CGRP ligand and receptor monoclonal antibodies for migraine prevention: Evidence review and clinical implications. *Cephalalgia* **39**, 445–458 (2019).
18. A. Avona, C. Burgos-Vega, M. D. Burton, A. N. Akopian, T. J. Price, G. Dussor, Dural calcitonin gene-related peptide produces female-specific responses in rodent migraine models. *J. Neurosci.* **39**, 4323–4331 (2019).
19. C. M. Kopruszinski, E. Navratilova, J. Swiokla, D. W. Dodick, I. P. Chessell, F. Porreca, A novel, injury-free rodent model of vulnerability for assessment of acute and preventive therapies reveals temporal contributions of CGRP-receptor activation in migraine-like pain. *Cephalalgia* **41**, 305–317 (2021).
20. A. Avona, B. N. Mason, J. Lackovic, N. Wajahat, M. Motina, L. Quigley, C. Burgos-Vega, C. Moldovan Loomis, L. F. Garcia-Martinez, A. N. Akopian, T. J. Price, G. Dussor, Repetitive stress in mice causes migraine-like behaviors and calcitonin gene-related peptide-dependent hyperalgesic priming to a migraine trigger. *Pain* **161**, 2539–2550 (2020).
21. D. L. Hay, C. S. Walker, CGRP and its receptors. *Headache* **57**, 625–636 (2017).
22. H. Ghanizada, M. A. M. al-Karagholi, C. S. Walker, N. Arngrim, T. Rees, J. Petersen, A. Siow, M. Mørch-Rasmussen, S. Tan, S. J. O'Carroll, P. Harris, L. T. Skovgaard, N. R. Jørgensen, M. Brimble, J. S. Waite, B. J. Rea, L. P. Sowers, A. F. Russo, D. L. Hay, M. Ashina, Amylin analog pramlintide induces migraine-like attacks in patients. *Ann. Neurol.* **89**, 1157–1171 (2021).
23. A. Diogenes, A. M. Patwardhan, N. A. Jeske, N. B. Ruparel, V. Goffin, A. N. Akopian, K. M. Hargreaves, Prolactin modulates TRPV1 in female rat trigeminal sensory neurons. *J. Neurosci.* **26**, 8126–8136 (2006).
24. Y. Chen, A. Moutal, E. Navratilova, C. Kopruszinski, X. Yue, M. Ikegami, M. Chow, I. Kanazawa, S. S. Bellampalli, J. Xie, A. Patwardhan, K. Rice, H. Fields, A. Akopian, V. Neugebauer, D. Dodick, R. Khanna, F. Porreca, The prolactin receptor long isoform regulates nociceptor sensitization and opioid-induced hyperalgesia selectively in females. *Sci. Transl. Med.* **12**, eaay7550 (2020).
25. D. Ikegami, E. Navratilova, X. Yue, A. Moutal, C. M. Kopruszinski, R. Khanna, A. Patwardhan, D. W. Dodick, F. Porreca, A prolactin-dependent sexually dimorphic mechanism of migraine chronification. *Cephalalgia* **Under review**, 033310242110398 (2021).
26. D. Bosco, A. Belfiore, A. Fava, M. de Rose, M. Plastino, C. Ceccotti, P. Mungari, R. Iannacchero, A. Lavano, Relationship between high prolactin levels and migraine attacks in patients with microprolactinoma. *J. Headache Pain* **9**, 103–107 (2008).
27. K. C. Brennan, D. Pietrobon, A systems neuroscience approach to migraine. *Neuron* **97**, 1004–1021 (2018).

28. K. J. A. Johnston, J. Ward, P. R. Ray, M. J. Adams, A. M. McIntosh, B. H. Smith, R. J. Strawbridge, T. J. Price, D. J. Smith, B. I. Nicholl, M. E. S. Bailey, Sex-stratified genome-wide association study of multisite chronic pain in UK Biobank. *PLOS Genet.* **17**, e1009428 (2021).
29. L. H. Schulte, J. Mehnert, A. May, Longitudinal neuroimaging over 30 days: Temporal characteristics of migraine. *Ann. Neurol.* **87**, 646–651 (2020).
30. A. Tappe-Theodor, R. Kuner, Studying ongoing and spontaneous pain in rodents—Challenges and opportunities. *Eur. J. Neurosci.* **39**, 1881–1890 (2014).
31. E. Navratilova, F. Porreca, Reward and motivation in pain and pain relief. *Nat. Neurosci.* **17**, 1304–1312 (2014).
32. C. E. Phelps, E. Navratilova, F. Porreca, Cognition in the chronic pain experience: Preclinical insights. *Trends Cogn. Sci.* **25**, 365–376 (2021).
33. S. Potvin, S. Marchand, Pain facilitation and pain inhibition during conditioned pain modulation in fibromyalgia and in healthy controls. *Pain* **157**, 1704–1710 (2016).
34. C. Linnman, J. C. Beucke, K. B. Jensen, R. L. Gollub, J. Kong, Sex similarities and differences in pain-related periaqueductal gray connectivity. *Pain* **153**, 444–454 (2012).
35. G. D. Slade, J. D. Rosen, R. Ohrbach, J. D. Greenspan, R. B. Fillingim, M. Parisien, S. Khoury, L. Diatchenko, W. Maixner, E. Bair, Anatomical selectivity in overlap of chronic facial and bodily pain. *Pain Rep.* **4**, e729 (2019).
36. A. Avona, T. J. Price, G. Dussor, Interleukin-6 induces spatially dependent whole-body hypersensitivity in rats: Implications for extracephalic hypersensitivity in migraine. *J. Headache Pain* **22**, 70 (2021).

Acknowledgments: We thank W. Schmidt, A. Patwardhan, and D. Dodick for helpful comments on the manuscript.
Competing interests: F.P. has served as a consultant or received research funding from Voyager, Nektar, Amgen, Acadia, Blackthorn, Teva, Eli Lilly, Hoba, Allergan, Abbvie, Ipsen, PeptideLogic, and Proximagen and is a founder of Catalina Pharma. The other authors declare that they have no competing interests.

10.1126/scitranslmed.abj7180

PAIN

Neuroimaging enters the pain biomarker arena

Irene Tracey

Neuroimaging-based pain biomarkers have the potential to deliver objective structural and functional brain-related information about acute and chronic pain states. In this Viewpoint, I describe some of these exciting developments and the challenges ahead.

THE CHALLENGE OF PAIN

Pain is one of our oldest sensory and emotional experiences. It is our body's alarm system and is key to survival. However, pain that persists or recurs for more than 3 months is termed chronic, and it produces untold suffering to patients, carers, and families; it also has a substantial financial cost to society. There are three main types of chronic pain: inflammatory, for example, rheumatoid arthritis; neuropathic, such as painful diabetic neuropathy; and dysfunctional, for example, fibromyalgia. From multiple national surveys, it is estimated that 20% of adults fulfill the criteria for chronic pain; not only is this percentage increasing but also, in a 2016 Global Burden of Disease Study, it was reaffirmed that the high prominence of pain and pain-related diseases is still the leading cause of disability and disease burden globally (*1*–*3*). Comorbid anxiety, depression, insomnia, cognitive dysfunction, and sometimes addiction or substance abuse occur, adding to patient suffering and clinical challenges. The latest International Classification of Diseases (ICD-11) now describes pain as both a health condition (chronic primary pain) and a symptom that is secondary to an underlying disease (chronic secondary pain) (*4*). This update reflects the scientific contributions that have reshaped our thinking about chronic pain. That said, we are still unable to treat patients adequately. Chronic pain remains a major unmet medical need but presents a big therapeutic opportunity (*5*).

The International Association for the Study of Pain (see www.iasp-pain.org) has recently redefined pain to include six additional points, emphasizing, in part, that: "Pain is a personal experience subject to biological, psychological, and social influences and that a person's report of an experience as pain should be respected, but that a verbal description is only one of several behaviors to express pain and an inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain." Here lies the challenge: It is really hard to know someone's pain—a blood test will not tell you. Pain wears many guises: burning, throbbing, shock-like, freezing, pin-pricking, and all these can be phasic, tonic, or sustained yet varying in intensity or unpleasantness. Such pains can be triggered by movement or touch or just occur spontaneously. When it comes to measuring pain clinically, the following are heavily relied upon: (i) self-reporting, through, for example, use of rating scales or questionnaires; (ii) observation of altered behavior, for example, witnessing avoidance of or less movement or even facial grimacing; and (iii) physiology, such as changes in heart or respiration rate and pupil dilation. Experimentally, for humans and nonhuman animals, we have a few more options within these categories. However, sometimes, all fall short of what is needed to objectively, and with less inference or bias, understand at a mechanistic level the pain experienced. In addition, there are challenging situations where pain has to be assessed: comatose and anesthetized patients, neonates and nonverbal subjects, veterinary care, elderly or demented patients, and the law courts (*6*–*9*).

What are the solutions given that one cannot objectify a subjective experience such as pain? The mechanisms underpinning acute and chronic pain, as well as decoding the neural basis for this private experience, can be objectified. This is where tools, such as neuroimaging, might come to the fore and what many laboratories, including my own, have been developing. Biomarkers of pain under development include prognostic, diagnostic, and predictive markers as well as pharmacodynamic markers and markers for monitoring pain and even assessing risk for pain. Other tools exist that are also being applied to biomarker development, and these include psychophysical methods, such as quantitative sensory testing, and electrophysiological tools such as electroencephalography. Biochemical and genetic testing, as well as nerve density quantification using skin biopsies, and the innovative use of patient-derived neurons are all being advanced for biomarker development and are showing some promise (*6*, *8*, *10*). Previously unidentified behavioral metrics are also showing promise, such as more refined or continuous assessment of activity, facial expression, or sleep disturbance. Several reviews on the topic of biomarker development illustrate the growth in this space as well as the hurdles to overcome if we are to be successful (*5*–*14*).

There are many challenges to developing pain biomarkers. Pain is a complex, highly malleable, multidimensional experience that is influenced by and emerges from conscious brain processing. The resultant pain is often nonlinearly related to an originating nociceptive (tissue damaging/threatening) signal because of mechanisms in the peripheral nervous system, spinal cord, brainstem, and brain that powerfully modulate these signals. To think that one neuroimaging biomarker will suffice as a surrogate for something as complex and variable as pain is naïve. Combining different pain biomarkers (neuroimaging, genetics, lifestyle, biochemical, or psychological data) into composite or multimodal biomarkers with high sensitivity and specificity is a more fruitful approach, whether for acute pain (healthy, warning) or for chronic pain (*6*, *11*).

Here, I limit the discussion to neuroimaging using magnetic resonance imaging (MRI) methods, which include functional imaging measures of neural network activity related to evoked, phasic, sustained, or tonic pain, as well as structural imaging measures that assess gray matter volume changes or variations in white matter connections.

Wellcome Centre for Integrative Neuroimaging, Wolfson Building, Nuffield Department Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK. Email: irene.tracey@ndcn.ox.ac.uk

That is not to say other techniques are not as promising, but these are comprehensively covered elsewhere (*5*–*14*). I highlight a few studies to give a flavor of what is possible, noting that, despite the lure of supposedly "seeing pain," none of these approaches are surrogates for the subjective report but should be used as an adjunct, if possible.

NEUROIMAGING AS A BIOMARKER OF PAIN

Whole-brain exploratory approaches

In a marathon set of four repeated imaging studies over the course of 1 year, the Apkarian laboratory followed the journey of patients with subacute back pain (*15*). The pain conditions either resolved or became chronic, and the authors found a potential biomarker that potentially could be prognostic in the clinical setting. Using functional MRI resting-state data that allows for network connectivity strength assessment, the authors found that an increased functional connectivity between the prefrontal cortex and nucleus accumbens during the subacute phase of pain in some patients was predictive of the transition from acute to chronic back pain 1 year later. The specificity and sensitivity throughout the study were impressively high (a receiver operator curve score of 0.81 at 1 year), and findings were replicated in a second validation cohort of patients. A 3-year follow-up study with additional patients included structural brain and genetic metrics alongside functional connectivity measures and, again, emphasized the relevance of these frontostriatal networks as prognostic of chronic pain (*16*). Whether this network is prognostic for other chronic pain conditions remains to be elucidated.

Many research teams are collecting functional blood oxygen level–dependent imaging data (brain responses to evoked pain), resting-state data (network connectivity), or structural neuroimaging data (gray matter volume, cortical thickness, or white matter tract integrity) from many different types of patients with chronic pain. The ambition is to develop not only prognostic biomarkers but also diagnostic and predictive ones too, oftentimes using classification tools or graph theory analyses to aid their development (*17*–*21*). Current outcomes in terms of accuracy, specificity, and sensitivity range from average through good to excellent (*11*, *13*, *17*–*21*). It is too early for there to be agreement as to what might be a generalizable prognostic, predictive, or diagnostic biomarker, not least because of the heterogeneity of these studies. Areas of the brain though that crop up a lot, whether because of response differences or network connectivity or structural changes, include frontostriatal and somatosensory regions. We do not yet know what these changes relate to or whether they are adaptive, maladaptive, or epiphenomenon (*6*, *11*, *13*).

If we are to discover, understand, validate, and build confidence in pain mechanisms and biomarkers, then it will be important, where feasible, to identify them in a cross-species manner. This is now happening with impressive studies that either confirm findings between species or marry data in humans with preclinical animal models in combined studies (*22*–*24*). In a very recent study, the authors verified in humans and rodents that hippocampal impairment occurs in the neuropathic pain state and associated behaviors. To understand the nature of this impairment better, they enhanced hippocampal activity using a range of targeted tools and found that excitation of the dorsal but not ventral hippocampus induced analgesia in two rodent models of neuropathic pain (and in rats and mice) (*24*).

Mechanism-based biomarker developments

Across chronic pain conditions, there are shared mechanisms; therefore, a mechanism-based approach to pain treatment is recommended (*5*, *6*). Biomarkers that reflect important cross-condition and possibly cross-species mechanisms could potentially be very useful for prognosis, diagnosis, and prediction of pain and even determination of pain risk. One such mechanism centers on an imbalance between the facilitatory and inhibitory arms of the descending pain modulatory system (DPMS), a brainstem-subcortical-cortical network that modulates nociceptive processing in the dorsal horn of the spinal cord and controls nociceptive inputs to the brain (*25*). Anatomically, this network includes, among others, brain regions such as the rostroventral medial medulla, periaqueductal gray, amygdala, hypothalamus, anterior cingulate cortex, and other prefrontal and cortical regions that are still being defined. Excessive facilitation and insufficient inhibition are recognized from preclinical models as important in the development and maintenance of persistent pain states (*26*–*28*). Central sensitization is a key mechanism also involved in many chronic pain states and is responsible for key symptoms such as hyperalgesia and allodynia. It is the phenomenon whereby incoming nociceptive signals are amplified because of changes in the dorsal horn of the spinal cord as well as influences from the DPMS (*29*). We have successfully translated these preclinical findings to humans using experimental medicine models of central sensitization and hyperalgesia symptoms (*30*, *31*). Further, we and others have shown that an imbalance toward facilitation is also present across chronic pain conditions (migraine, fibromyalgia, and osteoarthritis) and even correlates with the extent of pain, such as in painful diabetic neuropathy (*32*–*35*). Validation of its utility as a biomarker of pathogenic pain for stratification and prediction comes from surgical outcome studies (*32*). Patients with osteoarthritic pain were stratified using neuroimaging ahead of knee or hip joint replacement on the basis of the presence of DPMS facilitation (that itself was related to scoring as neuropathic on a commonly used questionnaire called PainDETECT). The aim was to determine whether a PainDETECT neuropathic score was predictive of worse pain outcome (with the new joint) 1 year later, which it was. We have suggested that a DPMS imbalance might indicate a vulnerability or risk toward developing chronic pain (*6*, *36*).

Pharmacological imaging studies aimed at determining pharmacodynamic efficacy and drug target engagement in early-phase clinical trials similarly support a mechanism-based approach (*5*, *6*, *37*, *38*). Analgesic drug development requires a major shift in how we conduct these studies, including the use of biomarkers. This is recognized by pharma, academia, funding, and regulatory agencies [see the HEAL (Helping to End Addiction Long-term) initiative (https://heal.nih.gov)].

Biomarkers can be exploited in early drug development to help increase confidence at "go, no-go" decision points and to help stratify patients ahead of the trial on the basis of pathogenic biomarkers that the drug is targeting. We and others have spent many years developing neuroimaging as a powerful tool for analgesic drug development. In various international consortia and collaborations, we have shown the utility of imaging pharmacodynamic effects in this DPMS facilitatory/inhibitory biomarker (as well as other brain regions) as a means to readily disambiguate drug effects from placebo to disambiguating drugs with known efficacy from those without. These studies

can be done in very small subject numbers, and they can display greater sensitivity than analgesic scores (*5*, *6*, *37*, *38*). Other brain regions encoding intensity aspects of phasic or slowly varying pain, particularly the posterior insula cortex, can similarly provide helpful pharmacodynamic readouts of analgesia (induced by drugs or brain stimulation) in healthy volunteers and different patient groups [see (*6*)]. Harris and colleagues (*39*) used magnetic resonance spectroscopy to show that glutamate changes in the posterior insula were both predictive and reflective of analgesic outcomes in patients with fibromyalgia treated with pregabalin. More recently, this team used a machine learning (ML) approach and brain functional connectivity data to predict differential clinical responses to two different drugs used to treat fibromyalgia (*40*). More studies are needed across conditions and analgesic drug classes, as well as a better understanding of how these imaging measures relate to nonimaging measures or biomarkers that reflect similar mechanisms but with potential additive value when combined in a composite manner.

Multivariate pattern analysis and ML approaches

ML exploits the ability of computer algorithms to learn from data and then make predictions about new data. If we create and share more of our neuroimaging datasets through consortia and couple this to using multivariate pattern analysis (MVPA) and ML types of analyses, then we might potentially accelerate neuroimaging biomarker development. Most functional imaging analysis is univariate, which, at a voxel- or cluster-based anatomical level, we interpret meaning from having taken into account prior knowledge. The problem is that this process involves reverse inference, and so, mistakes in interpretation can be made. However, we should remember that this problem of reverse inference is not exclusive to imaging data, as we infer from behavior too and sometimes get that wrong. To improve interpretation, MPVA has been developed. In MVPA studies, a pattern or network of voxel-based activity is assumed to reflect the mental state or perception experienced, such as pain. So, when two different experiences or perceptions, such as having pain or feeling social rejection, appear from a univariate analysis to activate the same brain region, doubt about this region's specificity (and so potential utility as a pain biomarker) comes into question (*7*, *14*). However, MVPA might find that the pattern of activity within that brain region is unique for each task and can be disambiguated. This has helped to distinguish neural patterns resulting from somatic, social, or empathic/vicarious pain (*6*, *11*, *13*). Such clarity is key if one goal is to use neuroimaging data to make decisions about pain, whether from a therapeutic or even legal perspective. However, concerns regarding the use of such data and what is possible flag the need for cautious use, especially if real-life decisions are taken for an individual (*14*).

When ML is combined with MVPA, it is possible to integrate all brain data to create predictive models, or signatures, that, with excellent sensitivity and specificity (so far), can be used to predict components of the pain experience, such as pain intensity. One of the first studies to exploit multiple cohort datasets in this manner was done by Wager and colleagues, drawing data from acute phasic thermal pain studies in healthy volunteers. Their resultant pain model, called the neurological pain signature (NPS), successfully predicted pain (evoked by phasic noxious events judged as painful) with 90 to 100% accuracy (*41*). This signature, which includes activity from the insula, anterior cingulate cortex, secondary sensory cortex, and thalamus, has been shown to be generalizable to similar data from different laboratories. It has been used to distinguish between noxious painful stimuli and other nonpain emotional/aversive experiences, such as those generated through use of negative images, by vicarious pain or anticipating pain, by feeling warmth, by recalling pain, or by social rejection (*42*, *43*). NPS is also generalizable to other types of evoked pain stimuli beyond thermal, such as visceral mechanical distension, mechanical pressure, and electrical (see *13*). The NPS can be modified by opioids and serotonergic drugs, but it does not respond to some forms of placebo and cognitive regulation of pain modulation (*13*, *44*). The NPS is unlikely to be able to predict pain in its entirety, but, rather, the NPS identifies component processes that are yet to be properly understood and questions still remain about the decodability of fMRI data (*7*, *14*, *45*). In addition, recent neuroimaging studies at ultrahigh-field strengths (7 T) examining aversive breathlessness suggest that using the entire NPS network is not necessarily linked to just pain, as breathlessness mapped onto the NPS signature (*46*). However, on further examination, the local NPS responses in the dorsal posterior insula were still able to differentially classify pain from this nonpain-aversive experience, possibly supporting the notion that activity in this brain region is a potential biomarker of peripheral nociceptive inputs, as previously postulated (*46*, *6*). Refinement of the NPS is likely. Other signatures that capture more tonic and sustained pain in healthy controls and that show generalizability to two pain conditions are being developed (*47*). Further work is needed to validate more clinically focused signatures.

This approach can also be used to create "analgesic signatures" as potential biomarkers for drug discovery. We pooled data from multiple drug treatments for analgesic efficacy across cohorts of healthy individuals, experimental human pain models, and patients. Using ML, we identified robust associations between drug-related brain activity modulations and drug efficacy (pharmacodynamics) for phasic noxious stimuli. This general protocol for neuroimaging-based assessment of drug activity in the brain is currently being extended and further refined using new compounds and clinical cohorts and with repeated dosing and long duration of drug administration (*37*).

Validation of neuroimaging pain biomarkers

Biomarkers must be validated before they can be properly exploited in a research or clinical setting. Drawing from other fields where structured frameworks have been developed for precisely this purpose, such as the ACCE framework for genetic testing or the Food and Drug Administration Biomarker Qualification for drug development, we can adapt such frameworks for neuroimaging biomarker validation. The ACCE framework comprises analytic validity, clinical validity, clinical utility, and ethical, legal, and social impacts. A recent review presents an adapted framework based on these four steps for validating neuroimaging pain biomarkers (*11*). The ethical, legal, and societal implications of pain biomarkers are perhaps more challenging to address as discussed in several recent reviews and consensus statements (*7*, *8*). Evidence to support the presence, absence, or magnitude of pain, its cause, and whether it is sufficient to prevent future work is key for personal injury cases, disability benefit claims, or social security support. The lack of evidence beyond self-reporting has, sadly, fueled some companies to offer lawyers or

insurance companies supposed 'personalized neuroimaging pain biomarkers,' jeopardizing the field's reputation. Irrespective of issues related to readiness of neuroimaging pain biomarkers for the clinic or for pharma, there are other questions to be answered: What level of accuracy is needed? Is it appropriate or legal to probe a person's private mental state? What are the long-term consequences on patients' lives, and will there be future biases or discrimination if pain biomarkers appear on a medical record? Biases against and disbelief in patients' pain, particularly for certain groups, are already a fact in some situations, and so, we must develop protections against pain biomarker misuse. This will require constant dialogue between scientists, clinicians, patients, ethicists, and lawyers.

The future: Challenges and opportunities

With national and international funding initiatives now supporting pain biomarker development and with other imaging methods improving, such as spinal cord functional imaging or tonic pain imaging, as well as other techniques being developed to meet the challenge, it will be interesting to see how the pain biomarker field develops in the coming years. Most clinical neuroimaging scanners can generate the datasets described here; however, the analysis steps that produce the pain biomarker signature are less "turn-key." More work is needed if these imaging measures are to be routinely used at scale in a prognostic or diagnostic clinical setting; better still, we neuroimagers need to back-translate our findings to more simple bedside measures that can be rolled out at scale but that are informed by neuroimaging. Big data (that is, large-scale efforts to gather data and curate open-access repositories) provide an additional opportunity for neuroimaging pain biomarker development. This is due to the scale of imaging data collection made possible that no single laboratory or even multiple consortia can generate. The UK Biobank imaging study is acquiring, for example, high-quality structural and functional brain imaging data from 100,000 participants who form part of a larger cohort of 500,000 individuals. The genotype, biological phenotype, lifestyle, and health records of these participants are all linked to the publicly available imaging data. A richer phenotyping of pain within the UK Biobank is ongoing, focusing on neuropathic pain. This will provide an opportunity to validate current neuroimaging pain biomarkers in terms of analytic validity, as well as generate new biomarkers, particularly of a composite nature, which are likely to be more sensitive and specific (*6*, *11*). In summary, recent promising developments in neuroimaging pain biomarkers suggest that they have the potential to deliver objective structural and functional information about acute and chronic pain states.

REFERENCES AND NOTES

1. C. E. Zelaya, J. M. Dahlhamer, J. W. Lucas, E. M. Connor, Chronic pain and high-impact chronic pain among U.S. adults, 2019. NCHS Data Brief, no 390. Hyattsville, MD: National Center for Health Statistics (2020).
2. S. E. E. Mills, K. P. Nicolson, B. H. Smith, Chronic pain: A review of its epidemiology and associated factors in population-based studies. *Br. J. Anaesth.* **123**, e273–e283 (2019).
3. T. Vos, A. A. Abajobir, K. H. Abate *et al.*, Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **390**, 1211–1259 (2017).
4. R. D. Treede, W. Rief, A. Barke, Q. Aziz, M. I. Bennett, R. Benoliel, M. Cohen, S. Evers, N. B. Finnerup, M. B. First, M. A. Giamberardino, S. Kaasa, B. Korwisi, E. Kosek, P. Lavand'homme, M. Nicholas, S. Perrot, J. Scholz, S. Schug, B. H. Smith, P. Svensson, J. W. S. Vlaeyen, S. J. Wang, Chronic pain as a symptom or a disease: The IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain* **160**, 19–27 (2019).
5. D. Borsook, R. Hargreaves, C. Bountra, F. Porreca, Lost but making progress—Where will new analgesic drugs come from? *Sci Transl Med.* **6**, 249sr3 (2014).
6. I. Tracey, C. J. Woolf, N. A. Andrews, Composite pain biomarker signatures for objective assessment and effective treatment. *Neuron* **101**, 783–800 (2019).
7. K. D. Davis, H. Flor, H. T. Greely, G. D. Iannetti, S. Mackey, M. Ploner, A. Pustilnik, I. Tracey, R. D. Treede, T. D. Wager, Brain imaging tests for chronic pain: Medical, legal and ethical issues and recommendations. *Nat. Rev. Neurol.* **13**, 624–638 (2017).
8. K. D. Davis, N. Aghaeepour, A. H. Ahn, M. S. Angst, D. Borsook, A. Brenton, M. E. Burczynski, C. Crean, R. Edwards, B. Gaudilliere, G. W. Hergenroeder, M. J. Iadarola, S. Iyengar, Y. Jiang, J. T. Kong, S. Mackey, C. Y. Saab, C. N. Sang, J. Scholz, M. Segerdahl, I. Tracey, C. Veasley, J. Wang, T. D. Wager, A. D. Wasan, M. A. Pelleymounter, Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: Challenges and opportunities. *Nat. Rev. Neurol.* **16**, 381–400 (2020).
9. F. Moultrie, R. Slater, C. Hartley, Improving the treatment of infant pain. *Curr. Opin. Support. Palliat. Care* **11**, 112–117 (2017).
10. S. M. Smith, R. H. Dworkin, D. C. Turk, R. Baron, M. Polydefkis, I. Tracey, D. Borsook, R. R. Edwards, R. E. Harris, T. D. Wager, L. Arendt-Nielsen, L. B. Burke, D. B. Carr, A. Chappell, J. T. Farrar, R. Freeman, I. Gilron, V. Goli, J. Haeussler, T. Jensen, N. P. Katz, J. Kent, E. A. Kopecky, D. A. Lee, W. Maixner, J. D. Markman, J. C. McArthur, M. P. McDermott, L. Parvathenani, S. N. Raja, B. A. Rappaport, A. S. C. Rice, M. C. Rowbotham, J. K. Tobias, A. D. Wasan, J. Witter, The potential role of sensory testing, skin biopsy, and functional brain imaging as biomarkers in chronic pain clinical trials: IMMPACT considerations. *J. Pain* **18**, 757–777 (2017).
11. S. Mackey, H. T. Greely, K. T. Martucci, Neuroimaging-based pain biomarkers: Definitions, clinical and research applications, and evaluation frameworks to achieve personalized pain medicine. *Pain Rep.* **4**, e762 (2019).
12. D. Reckziegel, E. Vachon-Presseau, B. Petre, T. J. Schnitzer, M. N. Baliki, A. V. Apkarian, Deconstructing biomarkers for chronic pain: Context- and hypothesis- dependent biomarker types in relation to chronic pain. *Pain* **160**, S37–S48 (2019).
13. M. M. van der Miesen, M. A. Lindquist, T. D. Wager, Neuroimaging-based biomarkers for pain: State of the field and current directions. *Pain Rep.* **4**, e751 (2019).
14. A. Mouraux, G. D. Iannetti, The search for pain biomarkers in the human brain. *Brain* **141**, 3290–3307 (2018).
15. M. N. Baliki, B. Petre, S. Torbey, K. M. Herrmann, L. Huang, T. J. Schnitzer, H. L. Fields, A. V. Apkarian, Corticostriatal functional connectivity predicts transition to chronic back pain. *Nat. Neurosci.* **15**, 1117–1119 (2012).
16. E. Vachon-Presseau, P. Tétreault, B. Petre, L. Huang, S. E. Berger, S. Torbey, A. T. Baria, A. R. Mansour, J. A. Hashmi, J. W. Griffith, E. Comasco, T. J. Schnitzer, M. N. Baliki, A. V. Apkarian, Corticolimbic anatomical characteristics predetermine risk for chronic pain. *Brain* **139**, 1958–1970 (2016b).
17. D. E. Harper, Y. Shah, E. Ichesco, G. E. Gerstner, S. J. Peltier, Multivariate classification of pain-evoked brain activity in temporomandibular disorder. *Pain Rep.* **1**, e572 (2016).
18. S. E. Harte, E. Ichesco, J. P. Hampson, S. J. Peltier, T. Schmidt-Wilcke, D. J. Clauw, R. E. Harris, Pharmacologic attenuation of cross-modal sensory augmentation within the chronic pain insula. *Pain* **157**, 1933–1945 (2016).
19. J. J. Kutch, J. S. Labus, R. E. Harris, K. T. Martucci, M. A. Farmer, S. Fenske, C. Fling, E. Ichesco, S. Peltier, B. Petre, W. Guo, X. Hou, A. J. Stephens, C. Mullins, D. J. Clauw, S. C. Mackey, A. V. Apkarian, J. R. Landis, E. A. Mayer; MAPP Research Network, Resting-state functional connectivity predicts longitudinal pain symptom change in urologic chronic pelvic pain syndrome: A MAPP network study. *Pain* **158**, 1069–1082 (2017).
20. P. Tetreault, A. Mansour, E. Vachon-Presseau, T. J. Schnitzer, A. V. Apkarian, M. N. Baliki, Brain connectivity predicts placebo response across chronic pain clinical trials. *PLoS Biol.* **14**, e1002570 (2016).
21. J. S. Labus, J. D. van Horn, A. Gupta, M. Alaverdyan, C. Torgerson, C. Ashe-McNalley, A. Irimia, J. Y. Hong, B. Naliboff, K. Tillisch, E. A. Mayer, Multivariate morphological brain signatures predict patients with chronic abdominal pain from healthy control subjects. *Pain* **156**, 1545–1554 (2015).
22. L. Becerra, E. Navratilova, F. Porreca, D. Borsook, Analogous responses in the nucleus accumbens and cingulate cortex to pain onset (aversion) and offset (relief) in rats and humans. *J. Neurophysiol.* **110**, 1221–1226 (2013).
23. C. S. Hubbard, S. A. Khan, S. Xu, M. Cha, R. Masri, D. A. Seminowicz, Behavioral, metabolic and functional brain changes in a rat model of chronic neuropathic pain: A longitudinal MRI study. *Neuroimage* **107**, 333–344 (2015).
24. X. Wei, M. V. Centeno, W. Ren, A. M. Borruto, D. Procissi, T. Xu, R. Jabakhanji, Z. Mao, H. Kim, Y. Li, Y. Yang,

P. Gutruf, J. A. Rogers, D. J. Surmeier, J. Radulovic, X. Liu, M. Martina, A. V. Apkarian, Activation of the dorsal, but not the ventral, hippocampus relieves neuropathic pain in rodents. *Pain* 10.1097/j.pain.0000000000002279 (2021).
25. M. M. Heinricher, I. Tavares, J. L. Leith, B. M. Lumb, Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res. Rev.* **60**, 214–225 (2009).
26. M. De Felice, R. Sanoja, R. Wang, L. Vera-Portocarrero, J. Oyarzo, T. King, M. H. Ossipov, T. W. Vanderah, J. Lai, G. O. Dussor, H. L. Fields, T. J. Price, F. Porreca, Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. *Pain* **152**, 2701–2709 (2011).
27. R. Wang, T. King, M. de Felice, W. Guo, M. H. Ossipov, F. Porreca, Descending facilitation maintains long-term spontaneous neuropathic pain. *J. Pain* **14**, 845–853 (2013).
28. M. H. Ossipov, K. Morimura, F. Porreca, Descending pain modulation and chronification of pain. *Curr. Opin. Support. Palliat. Care* **8**, 143–151 (2014).
29. C. J. Woolf, Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* **152** (Suppl. 3), S2–S15 (2011).
30. L. Zambreanu, R. G. Wise, J. C. W. Brooks, G. D. Iannetti, I. Tracey, A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. *Pain* **114**, 397–407 (2005).
31. M. C. Lee, L. Zambreanu, D. K. Menon, I. Tracey, Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *J. Neurosci.* **28**, 11642–11649 (2008).
32. A. Soni, V. Wanigasekera, M. Mezue, C. Cooper, M. K. Javaid, A. J. Price, I. Tracey, Central sensitization in knee osteoarthritis: Relating presurgical brainstem neuroimaging and PainDETECT-based patient stratification to arthroplasty outcome. *Arthritis Rheumatol.* **71**, 550–560 (2019).
33. A. R. Segerdahl, A. C. Themistocleous, D. Fido, D. L. Bennett, I. Tracey, A brain-based pain facilitation mechanism contributes to painful diabetic polyneuropathy. *Brain* **141**, 357–364 (2018).
34. D. E. Harper, E. Ichesco, A. Schrepf, J. P. Hampson, D. J. Clauw, T. Schmidt-Wilcke, R. E. Harris, S. E. Harte, Resting functional connectivity of the periaqueductal gray is associated with normal inhibition and pathological facilitation in conditioned pain modulation. *J. Pain* **19**, 635.e1–635.e15 (2018).
35. L. Solstrand Dahlberg, C. N. Linnman, D. Lee, R. Burstein, L. Becerra, D. Borsook, Responsivity of periaqueductal gray connectivity is related to headache frequency in episodic migraine. *Front. Neurol.* **9**, 61 (2018).
36. F. Denk, S. B. McMahon, I. Tracey, Pain vulnerability: A neurobiological perspective. *Nat. Neurosci.* **17**, 192–200 (2014).
37. E. P. Duff, W. Vennart, R. G. Wise, M. A. Howard, R. E. Harris, M. Lee, K. Wartolowska, V. Wanigasekera, F. J. Wilson, M. Whitlock, I. Tracey, M. W. Woolrich, S. M. Smith, Learning to identify CNS drug action and efficacy using multistudy fMRI data. *Sci. Transl. Med.* **7**, 274ra16 (2015).
38. V. Wanigasekera, M. Mezue, J. Andersson, Y. Kong, I. Tracey, Disambiguating pharmacodynamic efficacy from behavior with neuroimaging: Implications for analgesic drug development. *Anesthesiology* **124**, 159–168 (2016).
39. R. E. Harris, V. Napadow, J. P. Huggins, L. Pauer, J. Kim, J. Hampson, P. C. Sundgren, B. Foerster, M. Petrou, T. Schmidt-Wilcke, D. J. Clauw, Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. *Anesthesiology* **119**, 1453–1464 (2013).
40. E. Ichesco, S. J. Peltier, I. Mawla, D. E. Harper, L. Pauer, S. E. Harte, D. J. Clauw, R. E. Harris, Prediction of differential pharmacologic response in chronic pain using functional neuroimaging biomarkers and a support vector machine algorithm: An exploratory study. *Arthritis Rheumatol.* 10.1002/Art.41781 (2021).
41. T. D. Wager, L. Y. Atlas, M. A. Lindquist, M. Roy, C.-W. Woo, E. Kross, An fMRI-based neurologic signature of physical pain. *N. Engl. J. Med.* **368**, 1388–1397 (2013).
42. C. W. Woo, L. Koban, E. Kross, M. A. Lindquist, M. T. Banich, L. Ruzic, J. R. Andrews-Hanna, T. D. Wager, Separate neural representations for physical pain and social rejection. *Nat. Commun.* **5**, 5380 (2014).
43. A. Krishnan, C. W. Woo, L. J. Chang, L. Ruzic, X. Gu, M. López-Solà, P. L. Jackson, J. Pujol, J. Fan, T. D. Wager, Somatic and vicarious pain are represented by dissociable multivariate brain patterns. *eLife* **5**, e15166 (2016).
44. M. Zunhammer, U. Bingel, T. D. Wager; Placebo Imaging Consortium, Placebo effects on the neurologic pain signature: A meta-analysis of individual participant functional magnetic resonance imaging data. *JAMA Neurol.* **75**, 1321–1330 (2018).
45. R. Jabakhanji, A. D. Vigotsky, J. Bielefeld, L. Huang, M. N. Baliki, G. D. Iannetti, A. V. Apkarian, The hard limits of decoding mental states: The decodability of fMRI. *bioRxiv* 2020.12.18.423495 (2020).
46. O. K. Harrison, A. Hayen, T. D. Wager, K. T. S. Pattinson, Investigating the specificity of the neurologic pain signature against breathlessness and finger opposition. *Pain* 10.1097/j.pain.0000000000002327 (2021).
47. J. J. Lee, H. J. Kim, M. Čeko, B. Y. Park, S. A. Lee, H. Park, M. Roy, S. G. Kim, T. D. Wager, C. W. Woo, A neuroimaging biomarker for sustained experimental and clinical pain. *Nat. Med.* **27**, 174–182 (2021).

Funding: I.T. is supported by the Wellcome Trust, the UKRI Medical Research Council, and Merton College, Oxford. **Competing interests:** I.T. was on the scientific advisory board for Amgen (2017–2019); has received honoraria for providing various educational talks and workshops; is a council member of the Medical Research Council of the United Kingdom; is a trustee of MQ, a mental health charity; and is involved with Innovative Medicines Initiative–PainCare (Biopain), a pan pharma-academia consortium.

10.1126/scitranslmed.abj7358

PAIN

Immune regulation of pain: Friend and foe

Annemieke Kavelaars* and Cobi J. Heijnen*

The immune system and the peripheral sensory nervous system communicate through hardwired and humoral routes using shared mediators and receptors. On the basis of studies on pain sensitivity in rodents, the immune system can be viewed as both friend and foe. T cells and macrophages enhance pain via proinflammatory mediators and promote pain resolution via anti-inflammatory mediators and endogenous opioids.

TWO-WAY COMMUNICATION BETWEEN THE IMMUNE SYSTEM AND SENSORY NERVOUS SYSTEM

The immune system and the sensory nervous system communicate via shared mediators and receptors, and the immune system has been described as the sixth or seventh sense (*1*, *2*). Immune cells (leukocytes) are present in the peripheral nervous system, and sensory neurons innervate lymphoid organs providing a hardwired neuroimmune circuit. Peripheral sensory neurons express receptors for a wide array of mediators produced by the immune system in response to infection or tissue damage, including interleukin (IL)1β, tumor necrosis factor (TNF), and IL6; chemokines like CCL3; and inflammatory lipids such as leukotrienes and prostaglandins (*3*, *4*). Leukocytes produce neuropeptides, including pain mediators like substance P and pain-suppressing peptides such as endorphins and enkephalins (*1*). Sensory neurons also express receptors for pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) (*5*). Activation of each of these receptors can increase pain sensitivity and/or induce pain directly through engagement of second messengers that increase neuronal excitability. Activation of receptors for cytokines participating in wound healing, such as IL10 or IL4, on sensory neurons, suppresses pain sensitization. Conversely, the immune and inflammatory response is modulated in response to triggering of receptors for neuropeptides and neurotransmitters on leukocytes (*1*). For example, the local release of calcitonin gene-related peptide (CGRP), a neuropeptide produced by peripheral pain sensing neurons (nociceptors) in response to binding of bacterial determinants to neuronal PAMP receptors, suppresses the antibacterial immune response (*6*). Optogenetic activation of cutaneous sensory neurons activates IL17 production via release of CGRP and augments host defense in the skin (*7*). The hardwired connection and expression of shared mediators and receptors allows for a full circuit of interactions between the peripheral immune system and the peripheral sensory nervous system with potential consequences for nociceptor excitability and thereby for pain signaling (Fig. 1, top).

Tissue damage increases the number and activity of immune cells in the skin, muscle, joint, and other tissues where these cells release proinflammatory mediators. This tissue inflammation has protective effects, including increased pain sensitivity that may result in a guarding response, and will resolve when the tissues heal. In addition, colony-stimulating factor 1 (CSF1) produced by sensory neurons in models of chronic pain promotes expansion of macrophages in the peripheral nervous system (*8*). Deleting the gene encoding CSF1 from sensory neurons or local depletion of all macrophages from the dorsal root ganglia (DRG) (that contain the cell bodies of peripheral sensory neurons) inhibits neuropathic pain (*8*). In addition, microglia and astrocytes in the dorsal horn of the spinal cord release proinflammatory mediators that contribute to nociceptor sensitization in models of chronic pain (*4*).

Recent studies have identified the meningeal immune system as a key regulator of brain activity (*9*). The meninges surround the brain and contain many different immune cells as well as lymphatic vessels draining the brain. The meningeal immune system contributes to normal brain health, neuropathology, and recovery from brain injury, but its role in pain remains to be determined (*9*). The meninges of the brain are contiguous with the spinal cord meninges that extend to the meninges of the dorsal root ganglia and the epi- and perineurium surrounding peripheral nerves. Recent evidence indicates that traumatic nerve injury increases the production of a protein known as PI16 by epi- and perineurial fibroblasts. PI16 promotes influx of leukocytes into damaged nerves resulting in an increase in inflammatory mediators and neuropathic pain (*10*). Mice deficient in PI16 are protected against neuropathic pain induced by nerve injury, and this is associated with reduced macrophage influx into nerve and DRG (*10*).

Peripheral lymph nodes are innervated by sensory neurons that have their cell bodies in the DRG and consist of predominantly peptidergic nociceptors. Optogenetic activation of these nociceptors alters the transcriptome of innate immune cells and stromal cells in the lymph node, demonstrating the existence of a complete neuroimmune circuit (*11*). It is tempting to speculate that activation of lymph node sensory neurons activates neighboring DRG neurons innervating other parts of the body, akin to a phenomenon described as cross-excitation (*12*), which could alter pain sensitivity as a result of immune activity in the lymph node.

The immune system encompasses an adaptive arm consisting of T and B lymphocytes and an innate arm including subsets of granulocytes, mast cells, monocytes, macrophages, dendritic cells, natural killer cells, and innate lymphocytes. All these leukocytes can modulate pain signaling. Here, we will focus on the contribution of macrophages and T cells to neuropathic or inflammatory pain and discuss evidence for a role of these cells in development and resolution of chronic pain (Fig. 1, middle and bottom). Most studies discussed below use mechanical or thermal hypersensitivity as a read out for (chronic) pain. These measures reflect the sensitization of peripheral nociceptors that plays an important role in pain but do not paint the full picture of the experience of pain, which is multidimensional involving the brain.

Laboratories of Neuroimmunology, Department of Symptom Research, Division of Internal Medicine, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.
*Corresponding author. Email: akavelaars@mdanderson.org (A.K.); cjheijnen@mdanderson.org (C.J.H.)

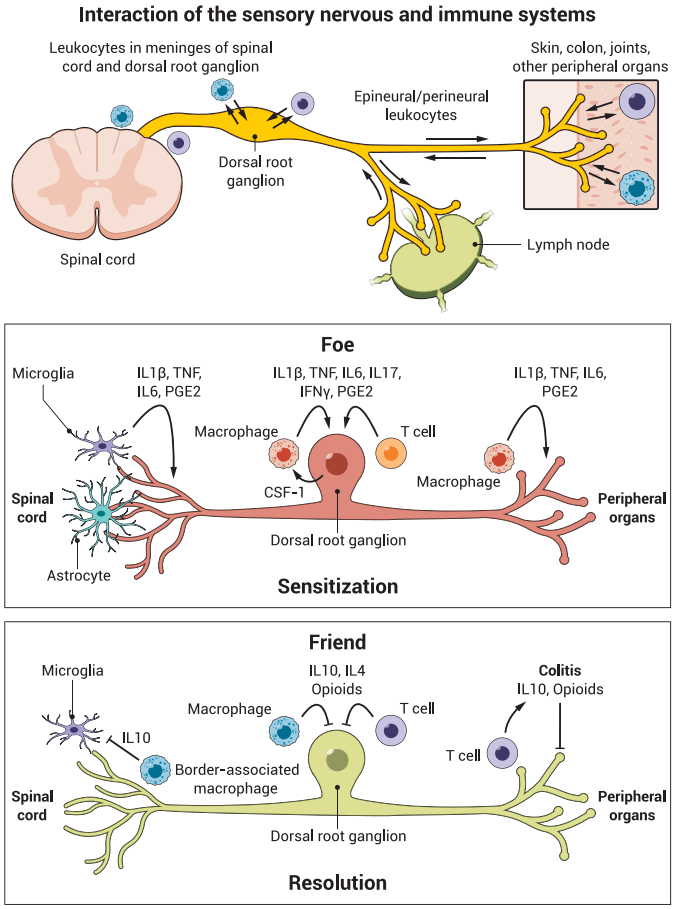


Fig. 1. Interactions between the sensory nervous system and the immune system related to pain. (Top) Shown is a representation of a circuit for bidirectional communication between the peripheral sensory nervous system and the immune system. Middle panel: Microglia, macrophages, and T cells producing proinflammatory cytokines sensitize peripheral sensory neurons and thereby contribute to the onset and maintenance of chronic pain. Bottom Panel: Macrophages and T cells producing IL10, IL4, and endogenous opioids silence sensitized peripheral sensory neurons and suppress microglia activation to promote resolution of pain. mϕ, macrophage; BAM, border-associated macrophage; IFN, interferon; and PGE2, prostaglandin E2.

MACROPHAGES PROMOTE AND RESOLVE PAIN SENSITIZATION

Macrophages comprise a heterogeneous population of phagocytic cells with diverse phenotypic characteristics and functions (*13*). Studies on the regulation of inflammation and tissue repair in the lung, heart, or muscle have shown that macrophages often play a dual role; depending on their activation state and phenotype, they can promote damage and enhance tissue repair. For convenience, macrophages are often divided into two major subsets: proinflammatory M1 macrophages and anti-inflammatory or wound healing M2 macrophages. However, it is becoming increasingly clear that the local microenvironment regulates development and differentiation of macrophages resulting in subtypes releasing specialized sets of cytokines (*13*). In response to tissue damage, macrophages produce high amounts of proinflammatory cytokines, including TNF and IL1. Prolonged activation of these proinflammatory pathways can enhance tissue damage and contribute to chronic pain (*13*, *14*). One of the key functions of macrophages is to clear debris from damaged tissue. Phagocytosis of apoptotic cells, presenting an "eat me" signal such as phosphatidylserine, promotes differentiation of macrophages in a process named efferocytosis. Efferocytosis increases the release of factors that promote resolution of inflammation and wound healing including IL10 (*15*). Insufficient efferocytosis leads to accumulation of DAMPs and persistent proinflammatory activity of macrophages (*15*). Mice with a defect in macrophage phagocytosis due to genetic deletion of the orphan receptor GPR37 display delayed resolution of inflammatory pain. Transfer of wild-type macrophages normalized pain resolution and clearance of cellular debris, indicating a role of efferocytosis in pain resolution (*16*). Although phagocytosing macrophages are present in damaged nerves, damaged cells accumulate, suggesting insufficient efferocytosis associated with the occurrence of neuropathic pain (*17*). Interestingly, macrophages do acquire a wound healing, proresolution phenotype when there is only limited nerve damage (*18*). Mild nerve injury increases a subset of spinal cord border associated macrophages with a tissue repair phenotype (expressing CD163) and production of IL10, and these cells have been shown to promote pain resolution. Depletion of $CD163^+$ macrophages prevented the resolution of pain in mice with mild injury, whereas overexpression of CD163 in macrophages suppressed pain sensitization in mice with severe nerve injury (*18*). Earlier studies had already identified a role of macrophages producing IL10 in the resolution of transient inflammatory pain (*19*).

Thus, there is an emerging picture indicating that macrophages not only promote development and maintenance of pain but also play a key role in pain resolution. Leveraging these dual properties when developing new treatments for pain should lead to more effective interventions.

DUAL ROLE OF T CELLS IN PAIN SENSITIZATION

Macrophages are not the only cells of the immune system with a dual role in models of chronic pain; the same is true for T cells (*20*). Multiple studies [reviewed in (*21*)] demonstrated that mice lacking T cells are protected against pain in models of traumatic nerve injury, whereas pain is reinstated when these mice are reconstituted with $CD4^+$ T cells. This pain promoting effect of $CD4^+$ T cells has been attributed to production of proinflammatory mediators. In contrast, $CD4^+$ effector T cells and T regulatory cells suppress intestinal pain in models of colitis through the release of IL10 and opioid peptides (*20*–*22*).

More recently, a key role of $CD8^+$ T cells in resolution of neuropathic pain induced by chemotherapy has been uncovered (*23*). Pain caused by a short course of chemotherapy resolves within weeks after completion of treatment. However, *Rag2*$^{-/-}$ mice, which do not have T and B cells, transition into chronic

pain without changes in onset and initial severity of chemotherapy-induced pain sensitization. Reconstitution of *Rag2*$^{-/-}$ mice with CD8$^+$ T cells normalizes resolution of chemotherapy-induced pain sensitization and spontaneous pain (*23*). Resolution of inflammatory pain induced by intraplantar complete Freund's adjuvant is also delayed in *Rag2*$^{-/-}$ mice and normalized by reconstitution with CD3$^+$ T cells (*24*).

Together, these data indicate that T cells promote pain in models of traumatic nerve injury, whereas they are essential for pain resolution after transient peripheral inflammation or after completion of chemotherapy. The differences in local environment and type of injury likely govern the differentiation state and function of T cell subsets, thereby defining their pain promoting or suppressing activity. It remains unclear to what extent, if any, T cells contribute to neuropathic pain in the context of diabetes, the most frequent cause of neuropathic pain in humans.

THE IMMUNE SYSTEM AS A TARGET FOR TREATMENT OF CHRONIC PAIN

Inhibition of inflammation using nonsteroidal anti-inflammatory drugs (NSAIDs) is an effective way to manage mild acute pain. Pain management becomes substantially more challenging when pain is chronic, such as in chronic neuropathic pain caused by trauma, diabetes, or chemotherapy or in chronic inflammatory pain, for example, in rheumatoid arthritis. Human and rodent studies indicate key roles for activation of spinal cord glia and immune cells in the peripheral nervous system in chronic neuropathic and inflammatory pain (*4*, *21*). Perhaps counterintuitively, however, anti-inflammatory drugs including NSAIDs, steroids, inhibitors of microglial activity, or inhibitors of cytokine signaling are at best partially successful in managing chronic pain despite promising preclinical findings (*25*, *26*). Species differences in the immune system may be part of the explanation. However, an additional possibility is that these immunosuppressive drugs may also suppress the pain-resolving activity of T cells and macrophages. If so, then the challenge is to develop interventions that suppress the pain-promoting effects of the immune system while, at the same time, stimulating its pain-resolving activity. During evolution, the body has learned to cope with acute non–life-threatening situations but not with chronic diseases including chronic pain because of negative selection pressure. Therefore, the evolutionary utility of shared mediators and receptors by the immune and nervous system as described above may be less well developed for regulation of chronic pain, necessitating active treatment that surpasses the natural resolution mechanisms of acute pain as a result of a short lasting infection, trauma, or sensory stimulus.

CAN WE TREAT PAIN BY ACTIVATING THE PAIN RESOLVING EFFECTS OF MACROPHAGES AND T CELLS?

Expansion of regulatory T cells can suppress pain induced by neuritis or traumatic nerve injury (*21*). In a model of colitis, vaccination with Bacillus Calmette-Guérin followed by reexposure to the immunizing antigen in the gut activates local memory T cells to release opioid peptides and reduce pain (*27*). Other ways to enhance endogenous opioid production by immune cells might be considered as well. Encouraging recent findings demonstrated that endogenous opioids produced by IL10-expressing M2 macrophages mediated the pain-suppressive effects of repeated intrathecal administration of the anti-inflammatory cytokine IL4 (*28*). Seven daily doses of intrathecal IL4 provided sustained pain relief for at least a week after the last dose of IL4, and the prolonged effect was mediated via sustained production of opioids by macrophages (*28*). These findings indicate that it may be possible to develop interventions that stimulate production and release of endogenous opioids by T cells or macrophages as a strategy to promote pain resolution.

CD8$^+$ T cells can be "educated" to promote resolution of chemotherapy-induced peripheral neuropathy. Adoptive transfer of CD8$^+$ T cells from mice that have recovered from cisplatin-induced neuropathy to T cell–deficient mice accelerated resolution of chemotherapy-induced peripheral neuropathy in the recipient animals as compared to mice reconstituted with CD8$^+$ T cells from naïve mice (*23*). Moreover, CD8$^+$ T cells from mice that had recovered from pain induced by either paclitaxel or cisplatin were equally capable of accelerating resolution of cisplatin-induced neuropathy (*23*). It would be interesting to examine whether in vitro exposure of T cells to chemotherapeutics can be used to promote resolution of chemotherapy-induced neuropathy. If so, then one could consider infusing cancer patients with their own educated T cells before chemotherapy to prevent development of persistent peripheral neuropathy.

Multiple studies have identified IL10 as one of the main mediators of the pain suppressing or resolving effects of T cells and macrophages (*18*, *29*–*32*). For example, intrathecal administration of a neutralizing anti-IL10 antibody delays resolution of pain sensitization induced by chemotherapy (*29*). Targeted overexpression of CD163 in spinal cord border-associated macrophages promoted IL10 production and resolution of neuropathic pain (*18*). IL10 production by macrophages may be promoted by activation of efferocytosis pathways. IL10 signaling also mediates the pain resolving effects of intrathecal administration of an adenosine A2A receptor agonist (*32*). The pain suppressing effects of IL10 have mostly been attributed to suppression of spinal cord microglia activity, leading to a reduction in release of proinflammatory cytokines and a subsequent decrease in nociceptor sensitization (*33*). However, IL10 can also suppress pain via a direct effect on nociceptor IL10 receptors. For example, genetic deletion of IL10 receptors from advillin-positive nociceptors delays resolution of chemotherapy-induced peripheral neuropathy (*29*). Deletion of IL10 receptors from peripheral sensory neurons also prevents the accelerated resolution of chemotherapy-induced peripheral neuropathy in mice treated with mesenchymal stem cells (*34*). In vitro, IL10 suppressed the spontaneous activity displayed by DRG neurons from chemotherapy-treated mice (*29*). The pain resolving effect of A3 adenosine receptor agonists in a model of neuropathic pain have recently been attributed to T cell–derived IL10 signaling to DRG neurons, leading to reduced *N*-methyl-D-aspartate receptor phosphorylation and sustained reductions in pain sensitization (*31*).

Administration of IL10 only transiently suppresses pain sensitization, likely due to limited bioavailability (*33*). Sustained pain relief can be achieved by intrathecal injection of IL10-encoding plasmids, providing sustained elevations of local concentrations of IL10 (*33*). An engineered fusion protein consisting of IL4 and IL10 suppressed signs of chemotherapy-induced peripheral neuropathy and accelerated resolution of pain sensitization induced by intraplantar Freund's complete adjuvant. This IL4-IL10 fusion protein resolved inflammatory pain by clustering IL4 and IL10 receptors on nociceptors, leading to recruitment of unique signaling pathways that are not activated by

triggering these receptors after exposure to equimolar doses of the two individual cytokines (*35*). These findings open the possibility to develop therapeutic fusion proteins that cross link cytokine receptors to promote resolution of pain.

Sex differences in the contribution of T cells, microglia, and macrophages to promoting pain have been widely reported (*36*). In contrast, so far, the pain-resolving effects of T cells, microglia, and macrophages do not seem to be sexually dimorphic. Consistently, the studies on the effects of interventions that act through IL10 have not reported sex effects either (*23*, *24*, *29*, *31*, *34*).

CONCLUSION

There is a multilevel circuitry through which the peripheral immune system and the peripheral nervous system communicate to regulate onset, maintenance, and resolution of pain (Fig. 1, top). For a long time, pain researchers have viewed the immune system mainly as the foe (Fig. 1, middle). This is based on the large body of rodent studies demonstrating that prevention of the recruitment or activation of glia, macrophages, or T cells could prevent chronic pain. However, accumulating evidence indicates that macrophages and T cells also play a key role in pain resolution and therefore should be treated as friends (Fig. 1, bottom). The friend-and-foe role of the immune system in the regulation of pain may well explain why immunosuppressive interventions often only partially control pain. Findings summarized above indicate that engaging the immune system as a friend by promoting its pain resolving capacity may provide more effective and, most importantly, persistent pain control. There are still many open questions. For example, how do macrophages acquire the capacity to promote the resolution of pain after mild injury after completion of chemotherapy or after resolution of inflammation? Is the pain resolving activity of macrophages promoted by efferocytosis and the associated metabolic and phenotypic changes? Conversely, is insufficient efferocytosis the driver of the pain-promoting effects of macrophages? Do T cells promote resolution of pain via direct effects of mediators that they release on sensory neurons, or is an additional intermediary role of macrophages required? Do signals provided by the sensory nervous system activate the pain resolving capacity of T cells and macrophages? Answers to these and other open questions should identify potential ways to promote the pain resolving activity of the immune system and uncover new ways to treat chronic pain in a disease-modifying non-addictive way.

REFERENCES AND NOTES

1. J. E. Blalock, The immune system as the sixth sense. *J. Intern. Med.* **257**, 126–138 (2005).
2. J. Kipnis, Immune system: The "seventh sense". *J. Exp. Med.* **215**, 397–398 (2018).
3. R. Raoof, H. Willemen, N. Eijkelkamp, Divergent roles of immune cells and their mediators in pain. *Rheumatology (Oxford)* **57**, 429–440 (2018).
4. G. Chen, Y. Q. Zhang, Y. J. Qadri, C. N. Serhan, R. R. Ji, Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain. *Neuron* **100**, 1292–1311 (2018).
5. C. R. Donnelly, O. Chen, R. R. Ji, How do sensory neurons sense danger signals? *Trends Neurosci.* **43**, 822–838 (2020).
6. I. M. Chiu, B. A. Heesters, N. Ghasemlou, C. A. von Hehn, F. Zhao, J. Tran, B. Wainger, A. Strominger, S. Muralidharan, A. R. Horswill, J. B. Wardenburg, S. W. Hwang, M. C. Carroll, C. J. Woolf, Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* **501**, 52–57 (2013).
7. J. A. Cohen, T. N. Edwards, A. W. Liu, T. Hirai, M. R. Jones, J. Wu, Y. Li, S. Zhang, J. Ho, B. M. Davis, K. M. Albers, D. H. Kaplan, Cutaneous TRPV1$^+$ neurons trigger protective innate type 17 anticipatory immunity. *Cell* **178**, 919–932.e14 (2019).
8. X. Yu, H. Liu, K. A. Hamel, M. G. Morvan, S. Yu, J. Leff, Z. Guan, J. M. Braz, A. I. Basbaum, Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain. *Nat. Commun.* **11**, 264 (2020).
9. K. Alves de Lima, J. Rustenhoven, J. Kipnis, Meningeal immunity and its function in maintenance of the central nervous system in health and disease. *Annu. Rev. Immunol.* **38**, 597–620 (2020).
10. P. Singhmar, R. T. P. Trinh, J. Ma, X. J. Huo, B. Peng, C. J. Heijnen, A. Kavelaars, The fibroblast-derived protein PI16 controls neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 5463–5471 (2020).
11. S. Huang, C. G. K. Ziegler, J. Austin, N. Mannoun, M. Vukovic, J. Ordovas-Montanes, A. K. Shalek, U. H. von Andrian, Lymph nodes are innervated by a unique population of sensory neurons with immunomodulatory potential. *Cell* **184**, 441–459.e25 (2021).
12. V. Shinder, R. Amir, M. Devor, Cross-excitation in dorsal root ganglia does not depend on close cell-to-cell apposition. *Neuroreport* **9**, 3997–4000 (1998).
13. S. P. Nobs, M. Kopf, Tissue-resident macrophages: Guardians of organ homeostasis. *Trends Immunol.* **42**, 495–507 (2021).
14. C. E. A. Silva, R. M. Guimaraes, T. M. Cunha, Sensory neuron-associated macrophages as novel modulators of neuropathic pain. *Pain Rep.* **6**, e873 (2021).
15. D. Panigrahy, M. M. Gilligan, C. N. Serhan, K. Kashfi, Resolution of inflammation: An organizing principle in biology and medicine. *Pharmacol. Ther.* **227**, 107879 (2021).
16. S. Bang, Y. K. Xie, Z. J. Zhang, Z. Wang, Z. Z. Xu, R. R. Ji, GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. *J. Clin. Invest.* **128**, 3568–3582 (2018).
17. D. Kobayashi, N. Kiguchi, F. Saika, S. Kishioka, S. Matsuzaki, Insufficient efferocytosis by M2-like macrophages as a possible mechanism of neuropathic pain induced by nerve injury. *Biochem. Biophys. Res. Commun.* **525**, 216–223 (2020).
18. J. K. Niehaus, B. Taylor-Blake, L. Loo, J. M. Simon, M. J. Zylka, Spinal macrophages resolve nociceptive hypersensitivity after peripheral injury. *Neuron* **109**, 1274–1282.e6 (2021).
19. H. L. D. M. Willemen, N. Eijkelkamp, A. G. Carbajal, H. Wang, M. Mack, J. Zijlstra, C. J. Heijnen, A. Kavelaars, Monocytes/macrophages control resolution of transient inflammatory pain. *J. Pain* **15**, 496–506 (2014).
20. A. Kavelaars, C. J. Heijnen, T cells as guardians of pain resolution. *Trends Mol. Med.* **27**, 302–313 (2021).
21. G. Laumet, J. Ma, A. J. Robison, S. Kumari, C. J. Heijnen, A. Kavelaars, T cells as an emerging target for chronic pain therapy. *Front. Mol. Neurosci.* **12**, 216 (2019).
22. L. Basso, A. Bourreille, G. Dietrich, Intestinal inflammation and pain management. *Curr. Opin. Pharmacol.* **25**, 50–55 (2015).
23. G. Laumet, J. D. Edralin, R. Dantzer, C. J. Heijnen, A. Kavelaars, Cisplatin educates CD8$^+$ T cells to prevent and resolve chemotherapy-induced peripheral neuropathy in mice. *Pain* **160**, 1459–1468 (2019).
24. G. Laumet, J. D. Edralin, R. Dantzer, C. J. Heijnen, A. Kavelaars, CD3(+) T cells are critical for the resolution of comorbid inflammatory pain and depression-like behavior. *Neurobiol. Pain* **7**, 100043 (2020).
25. P. Taylor, B. Manger, J. Alvaro-Gracia, R. Johnstone, J. Gomez-Reino, E. Eberhardt, F. Wolfe, S. Schwartzman, N. Furfaro, A. Kavanaugh, Patient perceptions concerning pain management in the treatment of rheumatoid arthritis. *J. Int. Med. Res.* **38**, 1213–1224 (2010).
26. D. A. Shin, T. U. Kim, M. C. Chang, Minocycline for controlling neuropathic pain: A systematic narrative review of studies in humans. *J. Pain Res.* **Volume 14**, 139–145 (2021).
27. L. Basso, J. Boué, C. Augé, C. Deraison, C. Blanpied, N. Cenac, P. Lluel, N. Vergnolle, G. Dietrich, Mobilization of CD4$^+$ T lymphocytes in inflamed mucosa reduces pain in colitis mice: Toward a vaccinal strategy to alleviate inflammatory visceral pain. *Pain* **159**, 331–341 (2018).
28. M. O. Celik, D. Labuz, J. Keye, R. Glauben, H. Machelska, IL-4 induces M2 macrophages to produce sustained analgesia via opioids. *JCI Insight* **5**, e133093 (2020).
29. G. Laumet, A. Bavencoffe, J. D. Edralin, X. J. Huo, E. T. Walters, R. Dantzer, C. J. Heijnen, A. Kavelaars, Interleukin-10 resolves pain hypersensitivity induced by cisplatin by reversing sensory neuron hyperexcitability. *Pain* **161**, 2344–2352 (2020).
30. K. Krukowski, N. Eijkelkamp, G. Laumet, C. E. Hack, Y. Li, P. M. Dougherty, C. J. Heijnen, A. Kavelaars, CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. *J. Neurosci.* **36**, 11074–11083 (2016).
31. M. Durante, S. Squillace, F. Lauro, L. A. Giancotti, E. Coppi, F. Cherchi, L. di Cesare Mannelli, C. Ghelardini, G. Kolar, C. Wahlman, A. Opejin, C. Xiao, M. L. Reitman, D. K. Tosh, D. Hawiger, K. A. Jacobson, D. Salvemini, Adenosine A3 agonists reverse neuropathic pain via T cell-mediated production of IL-10. *J. Clin. Invest.* **131**, e139299 (2021).
32. A. J. Kwilasz, A. Ellis, J. Wieseler, L. Loram, J. Favret, A. McFadden, K. Springer, S. Falci, J. Rieger, S. F. Maier, L. R. Watkins, Sustained reversal of central neuropathic pain induced by a single intrathecal injection of adenosine A 2A receptor agonists. *Brain Behav. Immun.* **69**, 470–479 (2018).
33. A. J. Kwilasz, P. M. Grace, P. Serbedzija, S. F. Maier, L. R. Watkins, The therapeutic potential of interleukin-10 in neuroimmune diseases. *Neuropharmacology* **96**, 55–69 (2015).
34. N. Boukelmoune, G. Laumet, Y. Tang, J. Ma, I. Mahant, S. K. Singh, C. Nijboer, M. Benders, A. Kavelaars, C. J. Heijnen,

Nasal administration of mesenchymal stem cells reverses chemotherapy-induced peripheral neuropathy in mice. *Brain Behav. Immun.* **93**, 43–54 (2021).
35. J. Prado, R. H. S. Westerink, J. Popov-Celeketic, C. Steen-Louws, A. Pandit, S. Versteeg, W. van de Worp, D. H. A. J. Kanters, K. A. Reedquist, L. Koenderman, C. E. Hack, N. Eijkelkamp, Cytokine receptor clustering in sensory neurons with an engineered cytokine fusion protein triggers unique pain resolution pathways. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2009647118 (2021).
36. S. Rosen, B. Ham, J. S. Mogil, Sex differences in neuroimmunity and pain. *J. Neurosci. Res.* **95**, 500–508 (2017).

Funding: The work of C.J.H. and A.K. is supported by RO1 grants NS073939, NS116704, CA227064, CA230512, and CA208371 from the National Institutes of Health.
Competing interests: The authors declare that they have no competing interests.

10.1126/scitranslmed.abj7152

Science Translational Medicine

PAIN

Developing nociceptor-selective treatments for acute and chronic pain

Selwyn Jayakar[1], Jaehoon Shim[1], Sooyeon Jo[2], Bruce P. Bean[2], Ilyas Singeç[3]*, Clifford J. Woolf[1]*

Despite substantial efforts dedicated to the development of new, nonaddictive analgesics, success in treating pain has been limited. Clinically available analgesic agents generally lack efficacy and may have undesirable side effects. Traditional target-based drug discovery efforts that generate compounds with selectivity for single targets have a high rate of attrition because of their poor clinical efficacy. Here, we examine the challenges associated with the current analgesic drug discovery model and review evidence in favor of stem cell–derived neuronal-based screening approaches for the identification of analgesic targets and compounds for treating diverse forms of acute and chronic pain.

WHAT IS PAIN?

Although we use a single word, pain, to describe a broad range of unpleasant sensations that comprise feelings of soreness coupled with discomfort and unpleasantness, pain itself is complex and can be initiated by a range of quite distinct conditions. It is important to recognize this, as each pain condition may well require different therapeutic interventions (*1*, *2*). There are aspects of pain that are truly physiological; for example, our capacity to detect potentially damaging external stimuli: intense heat or cold, excessive mechanical force, and chemical irritants. This constitutes nociceptive pain, which is driven by the activation of high-threshold nociceptor sensory neurons adapted to transduce such noxious stimuli into an inflow of sensory input into the central nervous system (CNS). The acute pain evoked by the nociceptors through activation of nociceptive circuits helps us learn to avoid environmental danger (*3*–*6*). This pain is highly adaptive and essential for preventing damage in our daily interactions with the external world. Individuals who lack this damage warning system, such as those with congenital insensitivity to pain due to loss-of-function mutations in the voltage-gated sodium channel 1.7 ($Na_v1.7$) or the tropomyosin receptor kinase A (TRKA) receptor, typically damage their tongues and lips when they eat, their toes on walking, and fingers when exploring objects, and have no warning when they break a limb or have appendicitis, which reduces life expectancy (*7*, *8*). Preserving nociceptive pain is essential, therefore, except during or after surgery or immediately after major traumas.

Another adaptive pain is the inflammatory pain that occurs upon tissue injury or when inflammation occurs because of pathogen invasion or pathological inflammation. The consequent activation of the immune system leads to the production of inflammatory mediators that act on nociceptors to both directly activate (*9*–*11*) and sensitize them (*12*, *13*). Consequently, their threshold of activation drops so that low-intensity stimuli, such as light touch or movement of a joint, now activate the sensitized nociceptors, and innocuous stimuli become painful. This pain, at least in an acute setting, is adaptive in that the heightened pain and threshold drop promotes avoiding the use of, or contact with, the inflamed area, which promotes healing and repair. However, this pain typically needs to be clinically reduced to a manageable level. In addition to the pain produced by the activation and sensitization of nociceptors, the augmented sensory inflow triggers use-dependent plasticity in the CNS. This phenomenon of central sensitization amplifies and spreads pain sensitivity beyond the primary area of damage/inflammation to neighboring secondary, noninflamed areas, causing, for example, tenderness around a surgical wound or an inflamed joint, thus prolonging and amplifying the pain (*14*, *15*).

For chronic inflammatory conditions, for example, rheumatoid arthritis, the ongoing inflammatory pain is not adaptive because the inflammation does not resolve and healing does not occur. In these situations, the combined chronic inflammation and pain constitute a major clinical problem to be controlled, and the pain can be considered maladaptive or pathological (*16*). Last, there are two categories of truly maladaptive pain, where the pain is not a reaction to some pathology but a disease state itself. The first of these is neuropathic pain, persistent pain due to lesions, most typically not only of the peripheral nervous system (nerve injuries or neuropathies) (*17*, *18*) but also of the CNS (spinal cord injury or stroke) (*19*) that lead to pathological hyperexcitability of the nociceptive circuits owing to ectopic firing of injured neurons, loss of the normal inhibitory circuits in the CNS (the phenomenon of disinhibition), neuroimmune interactions in the nerve and CNS, and structural and functional connectivity changes in the spinal cord and brain that alter circuit function and output (*20*–*23*).

The last major form of pain is dysfunctional or nociplastic pain, defined as chronic pain in the absence of noxious stimuli, active inflammation, or detectable damage to the nervous system (*24*–*26*). Individuals presenting this type of pain show abnormal functioning of the nervous system such that it amplifies and sustains essentially normal signals to a point at which they are perceived as being painful. The pain may localize to a particular organ, as in tension-type headaches, temporomandibular joint disease, or irritable bowel syndrome, or may be diffuse as in fibromyalgia, a form of chronic widespread pain (*27*).

In addition to these quite distinct types of pain, we need to recognize that pain arising from the somatosensory system (skin/joints/muscle) may differ from that from visceral organs (*28*, *29*), and that acute pain typically differs considerably from chronic pain in terms

[1]F.M. Kirby Neurobiology, Boston Children's Hospital, and Department of Neurology, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA. [3]National Center for Advancing Translational Sciences (NCATS), Stem Cell Translation Laboratory (SCTL), National Institutes of Health (NIH), Rockville, MD 20850, USA.
*Corresponding author. Email: clifford.woolf@childrens.harvard.edu (C.J.W.); ilyas.singec@nih.gov (I.S.)

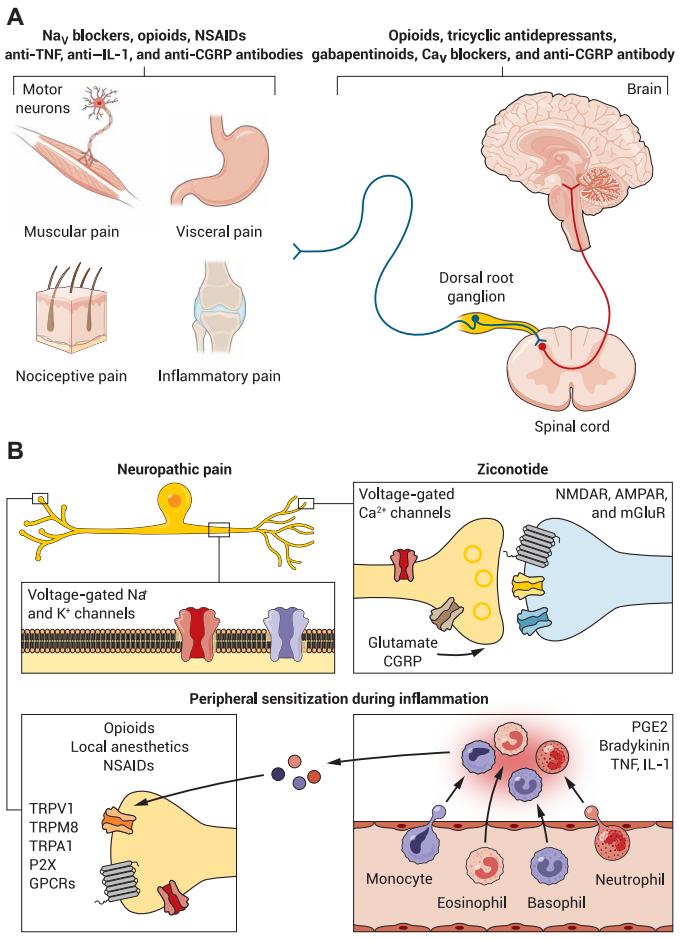


Fig. 1. Clinically used analgesics act on diverse targets in the central and peripheral nervous system. (**A**) Analgesics acting on central and peripheral targets. Opioids act on the brain, spinal cord, nociceptors, and the enteric nervous system, and the debilitating side effects of opioids, such as sedation, respiratory depression, and physical dependence, arise from actions on the brain leading to attempts to develop peripherally restricted opioid ligands (*245*). Tricyclic antidepressants such as amitriptyline, gabapentinoids such as pregabalin, and voltage-gated Ca^{2+} channel (Ca_V) blockers such as ziconotide and anti–calcitonin gene-related peptide (CGRP) receptor analgesics act on the brain and spinal cord. These centrally acting analgesics are used to treat a variety of pain conditions including nociceptive, inflammatory, visceral, and muscular pain. On the other hand, voltage-gated Na^+ channel blockers (Na_V; such as lidocaine) nonsteroidal anti-inflammatory drugs (NSAIDs), anti-TNF, and anti–IL-1 agents, in addition to opioids, act on peripheral nociceptors to mediate analgesia. The broad action of most of these agents on multiple organs or multiple members of a molecular target family, for example, the nonselective actions of lidocaine on all Na_Vs, contributes to their lack of efficacy and numerous side effects, highlighting the need for nociceptor-selective targets and ligands. (**B**) Molecular targets of clinical analgesics and their location within the inflammatory and neuropathic pain or nociception pathway. Inflammatory mediators released from peripheral tissues sensitize nociceptor axon terminals by acting on ion channels and G protein–coupled receptors (GPCRs). NSAIDs, opioids, and local anesthetics all reduce the activation of nociceptor terminals to produce analgesia. Under neuropathic pain conditions, ectopic nociceptor activity leads to both spontaneous pain and central sensitization. Voltage-gated Ca^{2+} channels located at presynaptic terminals control action potential–mediated release of glutamate and neuropeptides from central terminals in the dorsal horn of the spinal cord. In addition to opioids and gabapentinoids, tricyclic antidepressants and antiepileptics are currently prescribed to treat neuropathic pain.

CREDIT: ASHLEY MASTIN/*SCIENCE TRANSLATIONAL MEDICINE*

of mechanisms and response to analgesics (Fig. 1) (*30*). Furthermore, pain has extremely heterogeneous manifestations, likely reflecting different mechanisms. Pain may arise spontaneously in the absence of a stimulus or detectable pathology or may need a stimulus to evoke it. Spontaneous pain is a major element of many clinical conditions, particularly neuropathic pain (*31*). Stimulus-evoked pain includes exaggerated and prolonged pain in response to noxious stimuli, termed hyperalgesia, as well as pain in response to normally innocuous mechanical or thermal stimuli, called tactile or thermal allodynia (*32*). From a treatment perspective, there is a major difference between symptom suppression and disease modification in the setting of pain: The former requires ongoing treatment to reduce the experience of pain, whereas the latter, acting on the pathophysiology of the disease, has the potential to have long-lasting consequences, for example, interventions that can prevent the transition of acute to chronic pain or the development of axonal neuropathy, and hence neuropathic pain. To date, there are only symptom suppressor analgesic interventions.

We need to recognize the diverse nature and the multiple distinct mechanisms responsible for different pain conditions to be able to select those interventions with the greatest chance of efficacy. Building an algorithm that enables identification of the most likely responders to particular therapies that act on distinct mechanisms, rather than relying on an empirical trial and error approach, could be an effective approach for developing better treatments for specific pain conditions. Such a precision pain medicine approach requires parallel efforts to understand and fully reveal all the mechanisms underlying different pains, identify specific biomarkers for the mechanisms, and develop therapies that interact specifically with each of the distinct mechanisms. The goal of developing analgesics with defined selective actions in particular patients will require an approach substantially different from that used conventionally for the development of analgesic therapies. The nature of such a drug development approach, and how to realize it using human stem cell–derived neuronal assays and screens, specifically for selectively targeting nociceptors, is the focus of this Review.

CURRENT PHARMACOLOGICAL TREATMENTS FOR PAIN

The current repertoire of analgesics (Fig. 1) is limited, providing treatment for only a few of the defined types of acute or chronic pain. Existing analgesics have several major problems including generally low efficacy and multiple adverse effects, including induction of tolerance requiring increasing doses, dependency upon withdrawal or discontinuation, and abuse liability (*33*–*35*).

Available analgesics operate through multiple mechanisms. Nonselective sodium channel blockers, acting either as local anesthetics such as lidocaine or bupivacaine or as systemic sodium channel blockers, like carbamazepine, mexiletine, lacosamide, and amitriptyline, reduce electrical activity in neurons (*36*). The lack of selectivity means that all excitable cells exposed to the blockers will be affected, limiting the therapeutic index and triggering adverse effects due to activity on non-nociceptor neurons/cells. Cyclooxygenase (COX)–inhibiting nonsteroidal anti-inflammatory drugs (NSAIDs), which block prostaglandin E2 (PGE_2) synthesis (*37*, *38*), as well as anti–tumor necrosis factor (TNF) or anti–interleukin-1 (IL-1) antibodies in particular inflammatory conditions (*12*, *39*), block the action of inflammatory mediators on nociceptors. COX2 is induced in macrophages during inflammation and therefore COX inhibitors

are only effective in reducing the sensitization of nociceptors in those conditions where COX activity determines pain (*40*–*42*). In the context of postoperative pain relief, the NSAIDs diclofenac, ibuprofen, and naproxen only provide at best 50% maximum pain relief for 4 to 6 hours (*43*) and reduce opioid use only with moderate success (*44*–*46*). In addition, inhibiting PGE_2 production during inflammation, although a widely used analgesic strategy, has several disadvantages on prolonged use, including bleeding, gastrointestinal symptoms, and cyclic vomiting syndrome (*47*). Combination therapy using lower doses of NSAIDs together with acetaminophen has proven beneficial (*48*). TNF-α is elevated in many inflammatory conditions and anti-TNF therapies can be used for treatment of rheumatoid arthritis, Crohn's disease, and psoriasis (*49*). Anti-TNF antibodies reduce cytokine production in joints, particularly IL-1, IL-6, and TNF itself (*50*). Injectable therapies include soluble TNF receptors such as etanercept and PEG-sTNFR1, or TNF-α antibodies such as infliximab, adalimumab, and CDP-870. Small-molecule inhibitors of TNF-α synthesis, such as p38 mitogen-activated protein kinase and PDE4 inhibitors, are additional alternatives (*51*, *52*). However, all of these options are associated with side effects (*51*), including increased risk of congestive heart failure and resurgence of latent tuberculosis. IL-1 blockers, such as anakinra, rilonacept, or canakinumab, provide relief from broad-spectrum inflammatory diseases including rheumatoid arthritis, osteoarthritis, osteomyelitis, traumatic joint injury, systemic juvenile idiopathic arthritis, and gout. However, suppressing innate immune activity increases the risk of bacterial infections (*53*). Reducing synaptic transmission from nociceptors to dorsal horn neurons is another mechanism by which current analgesics act, but the action of the drugs used is not restricted to communication between these cell types, and blocking transmission broadly in the CNS causes adverse effects. Such blockers include mu opiate receptor opioid agonists (*54*), α2δ calcium channel subunit ligands, e.g., the gabapentinoids (*55*, *56*), the $Ca_v2.2$ inhibitor ziconotide (*57*, *58*), and, for migraine, calcitonin gene-related peptide (CGRP) receptor antagonists (*59*, *60*). Although most options for CGRP are monoclonal antibodies, a small-molecule antagonist for its receptor is also proving to be equally effective (*61*). Expression of the α2δ calcium channel subunit increases substantially in dorsal root ganglion (DRG) neurons after nerve injury (*62*, *63*), explaining why gabapentin and pregabalin, which bind to it, are effective in reducing neuropathic pain. Pregabalin is also used to treat nociplastic pain (*64*). Promoting inhibition in the CNS by mimicking the activity of inhibitory transmitters is another mechanism by which some analgesics act. However, at present, these treatments lack selectivity of action for nociceptive circuits. Current options include dual amine uptake inhibitors like duloxetine (*64*–*66*), opioids (*67*), and γ-aminobutyric acid type A (GABA-A) receptor modulators, like the benzodiazepines. Specific GABA-A receptor subtypes expressed in lamina II inhibitory interneurons of the spinal dorsal horn have been linked to analgesia. However, the sedative effects of the currently available nonselective allosteric modulators, such as benzodiazepines, diminish therapeutic potential as analgesics (*68*–*72*). The highly conserved nature of GABA receptor transmembrane and extracellular domain drug-binding pockets where most GABA receptor allosteric modulators bind pose a problem for identifying subtype-selective molecules. Developing efficacy-selective compounds that bind to a particular binding site in different receptor subtypes with equivalent affinity but with selective efficacy in only a small subpopulation of subtypes once bound might offer a partial solution to this problem by minimizing the broad, nonselective drug action of molecules whose affinity for a conserved binding site determines their efficacy (*73*). Last, there are drugs that reduce use-dependent plasticity in the CNS, particularly NMDAR antagonists such as ketamine, which diminishes synaptic plasticity (*74*); once again, the actions are not limited to changes in nociceptive circuits, leading to adverse events (*75*). Timing of treatment needs to be considered, given that in some situations, preemptive treatment can be more effective than treating established pain (*76*, *77*). Thus, the two major problems with existing analgesic therapies are their nonselectivity and the fact that available drugs often only block one of many parallel pain triggering signals. There is, therefore, an enormous need to both improve selectivity for pain mechanisms and broaden action to improve efficacy and reduce adverse effects.

There are targets for analgesic drug development that have shown promise and are moving toward clinical development. These include TRPV1 and TRPA1 channel antagonists to block nociceptor transduction (*78*, *79*), inhibitors of acid-sensitive ion channels (*80*), or purinergic receptors (*81*, *82*); anti–nerve growth factor (NGF) antibodies to neutralize the pain-promoting actions of NGF produced by immune cells (*83*, *84*); JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway inhibitors as nociceptive cytokine signaling inhibitors (*85*); K channel openers to suppress neuronal excitability (*86*, *87*); Na_v selective blockers that act only on sodium channels expressed by nociceptors ($Na_v1.7$ and $Na_v1.8$ blockers) (*88*, *89*); GABA receptor subunit–specific potentiators (*90*, *91*), inhibitors of tetrahydrobiopterin synthesis (*92*), and angiotensin type 2 receptor inhibitors (*93*); and attempts to modify opioid action on opiate receptors to retain analgesia but reduce side effects such as euphoria, tolerance, dependence, and respiratory depression (*94*–*97*). Although some of these efforts have been abandoned because of failure in the clinic, others, for example, biased opioid receptor ligands, and GABAR subtype selective modulators, are still progressing (*34*), but none have resulted in a new analgesic yet.

CURRENT OBSTACLES IN PAIN TREATMENT

The obstacles for the development of effective pain treatments that have not been overcome include the very limited range of targets and mechanisms that current analgesics act upon. This results in pharmacoresistance, a phenomenon where administered analgesic drugs are commonly ineffective in patients either at the start of their use or on chronic administration. Indeed, current U.S. Food and Drug Administration–approved analgesic drugs typically are clinically effective (>50% reduction in pain) in only a minority of patients with chronic pain. A useful metric of this phenomenon is the number needed to treat, which defines how many patients need to be given a particular treatment to see a clinically meaningful reduction in pain. Ideally, this should be 1, but usually it ranges from 5 to 20 for chronic pain conditions (*98*, *99*). Apart from low efficacy across the pain spectrum, most available analgesics also have a low persistence of activity when used chronically, including both opioids and NSAIDs (*100*, *101*).

TARGETING NOCICEPTORS FOR CELL SELECTIVITY

Like all neurons, nociceptors express multiple types of sodium, potassium, and calcium ion channels that together regulate their excitability. However, the expression levels of particular ion channels in

primary mouse nociceptors are substantially different from those in other neurons, even when compared with other primary sensory neurons (*102*). The unique pattern of expression of ion channels in nociceptors results in distinct intrinsic electrical properties compared to other neurons. It should be possible to exploit these differences to differentially inhibit/modulate the excitability of nociceptors, with minimal inhibition of other kinds of neurons. So far, such efforts have largely focused on a "magic bullet" approach of developing highly selective blockers for either $Na_v1.7$ or $Na_v1.8$ channels, which are predominantly expressed in nociceptors. Selective blockers for $Na_v1.7$ channels have been effective in rodent models of pain but so far have been disappointing in human trials. Although the reasons for the failure are still under investigation, it remains possible that it partially reflects problems with pharmacokinetics and target engagement of particular compounds (*103*). A $Na_v1.8$ inhibitor has shown efficacy against some means of inducing pain in healthy volunteers but not others (*104*). It may be that selectively targeting any single ion channel will only have limited efficacy given that excitability is driven by activity of multiple channels, and the capacity of neurons for compensation in response to inhibition of a single channel. It is noteworthy that almost all ion channel–targeted drugs currently effective in clinical practice are not extremely selective, in the sense that they inhibit many different ion channels; for example, carbamazepine and phenytoin used to control hyperexcitability in epilepsy not only target almost all subtypes of Na_vs with similar efficacy but also have effects on many kinds of calcium and potassium channels (*105*). The efficacy of these drugs was established without knowledge of their full target profile, but we now realize that the electrical activity of neurons has a complex nonlinear dependence on the dozens of different ion channel types they express, which are present in different combinations and proportions in different neurons. With this knowledge, we can now set out to analyze and model the overall excitability of different neurons with the goal of rationally identifying the particular combination of targets required for optimal differential cell-selective effects. It should be possible to screen for compounds with polypharmacological actions only on those multiple specific targets present in nociceptors—an approach similar to that proposed for improved G protein–coupled receptor (GPCR)–targeted drugs for mood disorders (*106*).

An additional consideration is that many ion channels have distinct patterns of expression in different regions of nociceptors—peripheral axon terminals, axons in the nerve or dorsal root, and central axon terminals—than in the cell bodies in the DRG, where electrical properties are commonly studied. Our knowledge of the expression of individual ion channels in nerve endings, axons, and central terminals is unfortunately very limited, because even substantial channel densities are difficult to detect using immunocytochemistry. The most detailed knowledge of regional expression in the case of sodium channels is currently based on the differential sensitivity of transduction, action potential propagation, and transmission in response to local tetrodotoxin (TTX) administration, revealing that specific populations of nociceptors differ widely in the extent to which TTX or more specific sodium channel blockers inhibit transduction at terminals, the propagation of action potentials, or central terminal activation. This has led to the conclusion that because $Na_v1.7$ and $Na_v1.8$ channels are differentially important for the generation and the propagation of action potentials, respectively, in various nociceptors, "a dual-targeting strategy of peripheral $Na_v1.7$ and $Na_v1.8$ inhibition would be more effective in providing analgesia" than either alone (*107*). Although this could be done by combining two selective $Na_v1.7$ and $Na_v1.8$ inhibitors, an alternative approach would be to find single compounds with such dual-target action, and with minimal, or no effects on other neuronal, cardiac, or skeletal muscle sodium channels, or even compounds with a broader profile on more/different ion channels.

Identification of the cell region–specific expression of ion channels in nociceptors will be valuable, particularly for the many types of potassium channels that regulate the excitability of nociceptors. There are compounds that can both inhibit sodium channels and enhance several types of potassium channels, like riluzole, used for treating amyotrophic lateral sclerosis (ALS) (*108*, *109*), so it is not far-fetched to search for, and generate single agents that could simultaneously target specifically those multiple sodium, potassium, or calcium channels important for different aspects of nociceptor function, to restore the changes that occur during inflammation or after nerve lesions. Potassium channels with substantial expression in nociceptors include $K_v3.4$, BK, K_v7, and TREK channels (*102*, *110*), and K_v7-enhancing compounds are effective in multiple pain models (*110*, *111*). However, in general, the pharmacology of potassium channel enhancement is an underdeveloped area, but one of future promise for targeting neurons in a cell-selective way, especially because of the widely different patterns of potassium channel expression in different neurons. Overall, a polypharmacology approach toward acting on that set of ion channels that determine nociceptor activity in health or disease should both increase efficacy over single target-selective drugs and, because of cell selectivity, reduce the adverse effects inherent in nonselective drugs.

There are two potential approaches to reach this goal, either identify compounds with the desired activity on the identified set of targets by parallel screening of each channel separately or screen directly for compounds (single-agent or in a combinatorial fashion) that have the desired modulatory action on nociceptors, but not on other excitable cells, using induced pluripotent stem cell (iPSC)–derived neuronal phenotypic screens. Both have advantages and some limitations and ideally a combination of both may be best.

Analgesic development is increasingly being based on the innovative approach of the optimal utilization of human stem cell–derived neurons for disease modeling, cell type– and disease state–specific screening, and identification of patient susceptibility to particular disease states, like neuropathy, or responsiveness to particular treatments (*112*–*119*). Three therapeutic approaches based on human neuron assays are possible. First, blocking inputs to nociceptors to reduce either the activation or the sensitization of nociceptors in health, inflammatory conditions, or after nerve lesions. Second, blocking action potential transmission selectively only in nociceptors, using drugs that silence these neurons with no or minimal activity on other cells, to block pain-triggering signals entering the CNS. The strategy of using permanently charged sodium channel blockers that only enter neurons through activated large pore ion channels, which are primarily only expressed by nociceptors, generates long-duration selective silencing of nociceptors, but can only be administered topically (*120*, *121*). Third, selectively blocking activity only in nociceptive circuits in the CNS, by either reducing excitation or increasing inhibition and preventing/reversing maladaptive plasticity only in these circuits in the spinal cord or brain (*15*, *122*–*124*). However, given the similarity of the cellular nature of the nociceptive circuits to many others in the CNS, it is likely the third approach will be the most challenging, and we will therefore focus here on exploring

how human nociceptors differentiated from stem cells can be used for reducing the activation/sensitization of the nociceptors and selectively silencing them.

HUMAN IPSCS, A RELIABLE SOURCE OF HUMAN CELLULAR MATERIAL

Human PSCs, such as iPSCs, are defined by their unlimited self-renewal capacity in the absence of senescence, with high telomerase activity maintaining telomere length and stability, and the potential to differentiate into all somatic cell types (*125*). Yamanaka and colleagues (*126*, *127*) established the first iPSC lines by reprogramming skin fibroblasts to a pluripotent state by transient expression of four transcription factors (OCT4, SOX2, KLF4, and C-MYC). Given that iPSC lines can be established in a patient- and disease-specific fashion, they offer unprecedented opportunities for translational research and provide the large cell numbers required for phenotypic and genetic screenings that are otherwise unattainable, together with a promise of personalized therapy.

Progress has been made recently in generating and culturing iPSCs using bioreactors, automated liquid handlers, and robotic cell culture systems (*128*–*130*). For instance, it is now possible to automate all essential steps of iPSC culture and directed differentiation into neurons, cardiomyocytes, and hepatocytes (*130*). This approach allows culturing of up to 90 iPSC lines in parallel, which is typically not possible by traditional manual cell culture. Robotic cell culture can support the standardized manufacturing of billions of human cells, which can be cryopreserved and then used for high-throughput and high-content screening of chemical libraries, natural products, and biologics.

DIFFERENTIATING IPSCS INTO FUNCTIONAL CELL TYPES

Converting iPSCs into specialized functional cell types entails stepwise differentiation following the principles of developmental biology. By modulating cell signaling pathways with small molecules and recombinant proteins, iPSCs are directed into lineage-committed precursor cells and then further patterned and differentiated into more specialized cell types that demonstrate gene expression signatures and functional properties similar to their in vivo counterpart, particularly the expression of marker proteins, cell type–specific transcriptomes, active intracellular signaling pathways, response to natural ligands, and electrophysiological properties. However, because most of our developmental biology knowledge is derived from animal models, not all available information on pathways controlling cell differentiation can be directly applied to human cells because of substantial species-specific differences (*131*–*134*). Reproducible protocols need, therefore, to be carefully developed and optimized for human iPSCs followed by comprehensive functional and molecular characterization of differentiated cell types to assess validity, maturation state, and usefulness for translational applications. Currently, a major challenge for using iPSC technology for translational research and therapeutic development is the lack of highly efficient, reproducible, and scalable cell differentiation protocols. Other hurdles for iPSC application are cell line–to–cell line variability and technical variation of cell culture conditions that strongly influence the differentiation process (*135*–*137*). Nevertheless, progress has been made over the past decade in improving cell differentiation protocols. For instance, iPSCs can be converted into neural crest cells expressing the transcription factor SOX10, which then can be further differentiated into nociceptors by simultaneously manipulating specific cell signaling pathways. These iPSC-derived nociceptors express typical transcription factors including BRN3A, ISL1, and PRDM12; neuropeptides such as Substance P and CGRP; transmembrane receptors such as TRPV1, TRKA, TRKB, and P2RX3; and sodium channels particularly $Na_v1.7$, 1.8, and 1.9, and display cell type–specific features of peripheral sensory neurons including response to specific ligands such as capsaicin, menthol, mustard oil, and ATP (adenosine triphosphate) (*115*, *132*, *138*–*142*). Various other cell types relevant for pain research have been derived from iPSCs, including spinal cord neurons (*143*), Schwann cells (*144*, *145*), microglia (*146*), astrocytes (*147*–*149*), GABAergic neurons (*150*), and cortical neurons (*151*).

A thorough characterization of iPSC-derived cells and comparisons with native DRG neurons is necessary before using cells in phenotypic screens. Current differentiation protocols are generally inadequate in achieving the necessary similarity between the stem cell-derived and primary sensory neurons. New sensory neuron differentiation protocols are though being developed by our colleagues at the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) that generate nociceptors with high efficiency and with more mature functional features than current protocols, and with a closer molecular and functional resemblance of the differentiated cells with primary human nociceptors, than existing protocols (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020219811&_cid=P21-KUMWLF-29547-1).

DEVELOPING NOCICEPTOR-SPECIFIC THERAPIES BY IDENTIFYING TARGETS AND COMPOUNDS

We propose two complementary approaches to identify novel pain targets: (i) use differential gene and protein expression analyses to identify and characterize targets selectively expressed in nociceptors or those cells linked to increased excitability in sensory neurons, such as immune cells or glia, and (ii) use phenotypic screens of human iPSC–derived excitable cells against annotated libraries of bioactive compounds whose mechanism of action (MoA) and targets are known, to identify those that selectively inhibit nociceptor excitability while sparing other excitable cells—such hits will reveal targets driving nociceptor-specific function (Fig. 2A).

The first approach will yield a list of gene transcripts and proteins selectively expressed in nociceptors and not in other neurons or excitable cells—using both human tissue samples and iPSC-derived cells (Fig. 2B). Nociceptor selective proteins can then be assembled into causal networks that reveal signaling pathways that modulate excitability (*152*). The best combination of network nodes predicted to diminish or block nociceptor activity can then be determined and tested for selective modulation of nociceptor excitability. Targeting selective pathways instead of a single protein could provide the basis for a polypharmacology approach to modulating nociceptor excitability (*153*–*156*). Single-cell/nuclei RNA sequencing enables the determination of the repertoire of genes expressed in nociceptors under physiological conditions, in response to disease-based manipulations or the action of a drug. Although some attempts have been made to identify targets enriched in primary human DRGs (*131*) relative to those expressed in other human cell types (*157*), single-cell profiling analyses are required to define exactly the human nociceptor transcript profile. Resources exist to combine nociceptor-selective gene sets into biological pathways that modulate

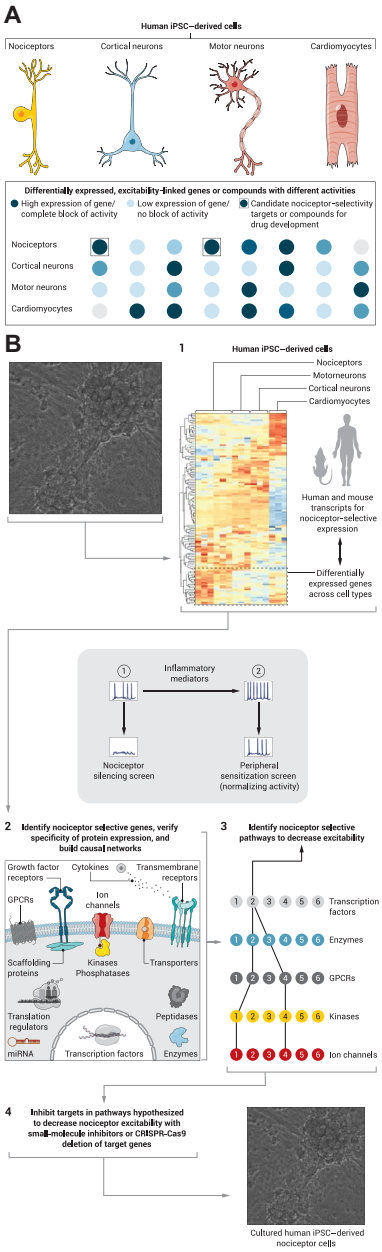


Fig. 2. Identification of candidate analgesics and targets. (**A**) Two approaches for identifying novel analgesic targets using human iPSC–derived nociceptors. In one approach, differential gene expression in nociceptors is identified. In the second, an annotated library of bioactive compounds, whose primary targets of action are known, are screened for silencing electrical activity using multiple cell types. The annotated targets of those compounds that selectively only inhibit nociceptor activity are potential pain targets. (**B**) Identifying and validating candidate nociceptor-selective targets and pathways. Identify genes selectively expressed in iPSC-derived nociceptors (1). A similar comparison of primary mouse and human tissues will yield sets of genes selectively expressed in nociceptors for each species. These can be assembled into signaling pathways (2) predicted to affect nociceptor excitability selectively (3) and represent potential translatable target sets. Nociceptor-selective targets can be validated using small molecules that act on the targets or through CRISPR-Cas9 editing (4). The process can be repeated in the presence of inflammatory mediators, chemotherapeutic agents, or after axon injury, to discover selective targets for different pain conditions. Reporters of intracellular signaling can be used to garner information on pathways activated by disease perturbations.

excitability such as the Integrated Network and Dynamical Reasoning Assembler (INDRA) (*158*) and Ingenuity Pathway Analysis (Qiagen). Pathways hypothesized to selectively modulate nociceptors can then be tested in these neurons and a set of iPSCs derived other cell types such as cortical and motor neurons, and cardiomyocytes, for counter screens.

In the second approach, a phenotypic screen of bioactive compounds to identify hits that selectively only modulate nociceptor excitability will also enable the deconvolution of a set of nociceptor selective pain targets. These targets can then be screened for modulators using a standard approach. Compounds that selectively block action potential generation only in nociceptors will reveal targets for managing nociceptive pain. Compounds that decrease action potential firing only when nociceptors are treated with inflammatory mediators will reveal targets useful in managing inflammatory pain. Targets for neuropathic conditions can be identified by identifying compounds that diminish nociceptor excitability on laser cutting of axons, treatment with chemotherapeutic agents, or other forms of axon injury. Genome-wide genetic editing screens in iPSC-derived neurons could provide an alternative way to identify targets, providing the false-positive and -negative rate is small, and gene editing does not affect iPSC function or their differentiation into nociceptors (*115*, *159*–*164*).

Once suitable targets are identified, their activity profile needs to be verified, either using available small molecules selective for these targets or by knockout/knockdown approaches, looking for nociceptor selectivity and disease normalization with no activity in counter screens on other neurons/cells. After such validation, heterologous expression systems can then be used to identify chemical entities that selectively modulate the function of the validated target in cell lines. Large chemical libraries can be screened against selected ion channel, GPCR, or kinase targets to identify chemical scaffolds as starting material for medicinal chemistry campaigns for structure-activity relationship studies and ensuring drug-like properties. Target-based screening has the technical advantage that the screening assay is typically relatively straightforward with robust signal yielding high Z-prime values (Z', a measure of the separation between positive and negative controls in a high-throughput screening assay). A limitation is that the activity of a target in a non-neuronal heterologous expression system may not match that in nociceptors, because of a lack of posttranslational processing or absence of interacting proteins that create a particular functional molecular architecture in native cells.

CELL- AND DISEASE-BASED PHENOTYPIC SCREENING USING IPSC-DERIVED NOCICEPTORS

Unbiased phenotypic screens can also be designed to identify compounds that act selectively only on a particular cell or change a specific disease-related phenotype (reduce inflammatory sensitization or neuropathic ectopic firing in nociceptors). It is possible that hits identified in such assays may act on multiple targets and signal pathways, and such polypharmacology might show better efficacy in vivo than a compound that acts exclusively on one target, although establishing structure-activity relationships for medicinal chemistry would be challenging. Target-annotated, MoA, library compounds often demonstrate promiscuous pharmacological activity, acting on more than one target, with differing potencies (*165*, *166*). Measuring the activity of a MoA library of compounds on nociceptors and other

excitable cells differentiated from human iPSC–derived cell lines might be able to reveal compounds with differential effects on the excitability of the different cell types. The annotated targets of those compounds that selectively suppress nociceptor excitability could reveal those combinations of excitability-related targets that can be pursued further in a discovery pipeline. Deep annotations of the hit compounds could identify not only their nominal (known/expected) targets but also other unexpected targets (*165*). Such a screen can serve as an entry point to a screening funnel specifically designed to identify potential targets or target combinations with no prior link to pain, in an unbiased, target-agnostic fashion. In addition, this approach can potentially identify compounds for drug repurposing or as a source of lead chemical scaffolds, for medicinal chemistry optimization for improved analgesic efficacy without target identification. Such a phenotypic screen will prioritize the selection of hits with analgesic efficacy, while mitigating undesired side-effect profiles. However, several challenges must be overcome before such a phenotypic screen can be conducted and novel pain targets can be identified by this approach.

Phenotypic readouts for such screens could include changes in excitability, cell morphology, reporter-based interrogation of signaling pathways, and changes in protein expression/function. Currently, there are several image-based assays that can be used to identify compounds that act on nociceptors in high-content screens. Nociceptor activity can be measured using genetically encoded calcium indicators (GECIs) or voltage indicators. The primary advantage of measuring calcium signals as a surrogate for electrical activity, for example, with GCaMP (a GECI that is a fusion of green fluorescent protein, calmodulin, and a myosin light-chain peptide), is the strong magnitude of the signal, whereas a limitation is the slow kinetics of calcium entry (*167*). However, nociceptors are adapted to respond only to the specific activation of their peripheral terminals by noxious stimuli and therefore have low spontaneous activity, which means that it is difficult to directly screen for nociceptor silencing compounds when there is little basal activity to inhibit. One way to circumvent the problem is to increase activity of the nociceptors by, for example, enhancing sodium currents with veratridine (*168*) or reducing potassium flux with barium. Another more physiological way is to incubate the iPSC-derived nociceptors with those inflammatory mediators that sensitize TRPV1 channels, increase the current density of $Na_v1.8$, and enhance the trafficking of $Na_v1.7$ channels to the membrane, mimicking inflammation-induced peripheral sensitization (*169*). Well-known mediators of inflammation such as PGE_2, serotonin, and histamine along with other agents such as NGF can be used to sensitize neurons (*170*). Screening for a reduction of such inflammatory mediator–triggered activity will identify compounds that either silence the nociceptors, for example, by blocking action potential firing, or reduce the peripheral sensitization, by mechanisms such as inhibiting kinases activated by the inflammatory mediators, and secondary screens will be required to identify which specific action the compounds have. Alternatives to genetically encoded excitability reporters are automated patch clamp and multi-well multielectrode array recordings. They have a lower-throughput screening capability and higher cost compared to the previous approach, but the direct measurement of membrane current or voltage makes them a useful option for secondary validation assays. Automated neurite imaging systems in multi-well assay format can, for example, detect compounds that rescue chemotherapy-induced axon degeneration or promote the regeneration of axons after axon injury (*171*). Last, spatially multiplexed imaging of signaling reporter islands as indicators of second messengers [calcium and cAMP (cyclic adenosine monophosphate)] and kinases [PKA (cyclic adenosine monophosphate–dependent protein kinase), PKC (protein kinase C), and phospho-ERK (extracellular signal-regulated kinases)] in neuronal culture can unveil which signaling pathways drive changes in excitability. Such assays can reveal the specific pathway a hit compound acts on and the changes that lead to pathological alteration such as sensitization/ectopic activity in nociceptors (*172*, *173*).

A crucial element of phenotypic screening strategies is to run parallel counter screens. If the aim is to find compounds that act selectively on nociceptors, for example, then a clear demonstration of a lack of activity on other excitable cells is required; if the aim is to reduce the spontaneous firing that drives neuropathic pain, then there must be no effect on noninjured nociceptors; if the goal is to develop a drug that reduces inflammatory pain, then specific disruption of immune cell interaction with nociceptors is required. Cortical and motor neurons are representative of CNS excitable cells, whose activity when affected by analgesics will contribute to major CNS side effects (for example, addiction and sedation). In addition, compounds that act on cardiomyocytes will also be unsuitable leads. Counter phenotypic screens are therefore as important as the primary screen.

A direct comparison of target-based versus phenotypic strategies reveals that phenotypic screens are successful in developing first-in-class drugs (*174*), and combining network-based pharmacology with phenotypic screens increases hit rates (*153*). Screening chemical libraries in miniaturized 384- and 1536-well plates using different compound concentrations can establish valuable dose-response curves and the use of multiple replicates will minimize false-positive and false-negative hits (*175*, *176*). Similarly, taking advantage of fast and precise low-volume liquid dispensing using acoustic sound energy allows for flexible testing of multiple small molecules in combinatorial drug screens (*175*, *177*).

As an example of the promise of nociceptor phenotypic screens, Studer and colleagues (*178*) screened iPSC-derived neural crest precursors from patients with familial dysautonomia against 6912 small-molecule compounds and identified compounds that induce expression of *IKBKAP*, the gene affected in this rare disease that leads to insensitivity to pain. In another example, iPSC-derived motor neurons from patients with ALS harboring the superoxide dismutase 1 (SOD1) mutation display a hyperexcitability similar to that observed in patients with ALS, whereas a genetically corrected line does not. This study also identified the capacity of retigabine, a K_v7 voltage-gated potassium channel opener, to reverse this hyperexcitability (*179*). Screens based on assaying hyperexcitability of the SOD1 mutant lines led to the identification of 13 potential targets that modulate excitability of motor neurons, including K_v7 channels (*180*). iPSC technology does enable screening at different stages of cell differentiation or disease state, and it might be possible, therefore, to elucidate temporal features of pathological changes and perform chemical screens accordingly. For instance, precisely characterizing the transition from acute to chronic pain could lead to treatment options that prevent or reverse the development or maintenance of cellular pathology and aberrant cellular plasticity. Although the standardized use of human iPSC–derived neural cell types generated by stepwise differentiation for high-throughput screening is still in its infancy,

more efficient and scalable cell differentiation protocols, together with improved cryoprotection and viability (*176*), should help overcome technical challenges such as batch-to-batch differences and establish satisfactory Z' values for defined pain-related phenotypes.

An iPSC-based phenotypic screen could be designed as follows. First, human iPSCs are differentiated into desired cell types, for example, sensory, cortical, and motor neurons, and cardiomyocytes, ideally from the same starting iPSC line. Transcriptome-based molecular measurements of a set of known marker genes over a defined period of in vitro maturation of the iPSC-derived neurons can determine the time window for conducting the phenotypic screens (*181*). Furthermore, transcript abundances alone must not dictate the readiness of derived receptors for use in phenotypic screens, complementary approaches measuring protein product expression and distribution, and cellular function must guide assay development. For example, TRPV1 expression and response to capsaicin should determine when nociceptors are ready to be used in phenotypic screens in addition to detecting *TRPV1* transcripts. Nociceptors suitable for screens must also replicate sensitization on application of suitable inflammatory mediators (*119*), for example, with higher Ca^{2+} responses on treatment with PGE_2. Baseline measurements of activity can be recorded with confocal imaging systems with liquid handling capacity, followed by brief incubation with drugs of interest, to detect changes in activity. A compound that selectively silences nociceptor activity while sparing those of motor and cortical neurons as well as cardiomyocytes would be considered a hit compound and advanced to measuring its effects over a range of concentrations to determine half-maximal inhibitory concentration, $IC_{50,}$ of its selective effect on nociceptors. Judgment should be exercised in the initial choice of drug concentration to prevent a universal block of activity in all cell types due to high off-target effects. Bioactive compounds with well-annotated targets that have a reasonable difference in IC_{50} for activity on nociceptors as opposed to other cell types can be deconvoluted to reveal the targets responsible, such as a selective $Na_v1.8$ blocker.

iPSC-derived cell types can also be exploited for disease modeling in response to neurotoxicants, chemotherapeutic drugs, metabolites, and pathogens. Chemotherapy-induced polyneuropathy (CIPN) is a common side effect of cancer treatment, including numbness and loss of proprioception as well as hyperalgesia or allodynia (*182*). To model CIPN, patient-derived iPSC neurons can be treated with paclitaxel, cisplatin, and vincristine (*183*, *184*). Such studies will provide invaluable information on disease mechanisms and interindividual susceptibility as well as generating novel neuroprotectant therapies.

In addition to using phenotypic screening with annotated bioactive compound libraries to identify targets, derived nociceptor phenotypic screening can be performed using large chemically diverse libraries, whose compounds have no identified target. If a compound/chemical scaffold has the desired selectivity profile, for example, only silencing nociceptors and not other excitable cells, then, although how it acts will not be known without further study, the scaffold can be used for a target-agnostic medicinal chemistry campaign program where potency, selectivity, and drug-like properties are improved, as assessed in repeated derived and primary cellular assays and in vivo testing. This strategy has challenges relative to target-based drug discovery approaches but is an option that needs exploration as it has high potential to identify candidates with the desired activity profile, which target-based approaches may not have.

DISEASE MODELING USING IPSC-DERIVED NEURONS

Disease modeling and identification of underlying cellular and molecular disease mechanisms is an important recent opportunity enabled by iPSC technology, constituting "disease-in-a-dish" or "patient-in-a-dish" for probing relevant phenotypes in cell types under precisely controlled laboratory conditions (Fig. 3).

Over the past decade, protocols have been developed to differentiate human iPSCs into a variety of neuronal (sensory, spinal motor, cortical, dopaminergic, and cholinergic neurons) and non-neuronal cell types (oligodendrocytes, astrocytes, and microglia) (*119*, *147*, *185*–*194*). These cells offer unique advantages over use of primary murine neurons and are particularly well suited for screening efforts. Also, primary neurons dissociated from mouse or rat DRGs represent injured neurons and typically must be used within 1 day of plating to preserve molecular similarity to naïve, intact in vivo neurons. However, important questions remain as to whether these derived neurons serve as good models of primary human neurons. Do derived cells fully capture the essential features of primary cells, which phenotypic determinants need to be used for screens and are these surrogates of patient conditions, and do hits have in vivo translational potential?

A protocol developed by Chambers *et al.* (*138*) has been widely used to generate nociceptors to model pain conditions, including using iPSCs from patient with inherited erythromelalgia (*140*, *141*) and migraine (*114*). Using five small molecules that regulate BMP (bone morphogenetic protein), TGF-β (transforming growth factor–β), WNT, Notch, and VEGF/FGF (vascular endothelial growth factor/fibroblast growth factor) signaling, iPSCs are differentiated into sensory neurons with >75% efficiency and express nociceptor markers such as NTRK1 (neurotrophic tyrosine kinase receptor-1), CGRP, and Substance P. The differentiated sensory neurons also express key ion channels ($Na_v1.7$, $Na_v1.8$, and $Na_v1.9$) and transducers (TRPV1, TRPM8, and P2RX3) associated with detecting noxious stimuli and action potential generation. However, the Chambers protocol generates only a few $TRPV1^+$ neurons (1 to 2%), requires extended maturation, and does not fully capture the molecular diversity of DRG sensory neurons. Recent protocols can generate mixed populations of sensory neurons (*195*–*197*). Transdifferentiation protocols that start with fibroblasts have been characterized but have low efficiency (*119*, *198*). Ongoing efforts at NCATS (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020219811&_cid=P21-KUMWLF-29547-1) and other laboratories (*197*) are focused on developing improved iPSC differentiation protocols that are highly efficient and scalable for translational projects and that could produce mature cells without the need to culture them for extended periods of time.

Although the transcriptional profile of iPSC-derived neurons resembles human DRGs and iPSC-derived nociceptors from patients with chronic pain display hyperexcitability (*199*), the extent to which the cell fully captures the functional and disease profile of human nociceptors remains to be determined. Studies have compared primary rodent DRG neurons and human neurons, highlighting similarities and differences in intrinsic electrical properties and synaptic properties, such as resting membrane potential, action potential threshold, amplitude, rise time, after-hyperpolarization magnitude, and spontaneous activity (*199*). Comparisons between primary human DRG neurons and iPSC-derived sensory neurons are required too.

Three key criteria need to be fulfilled before iPSC-derived neurons can be used for phenotypic screens:

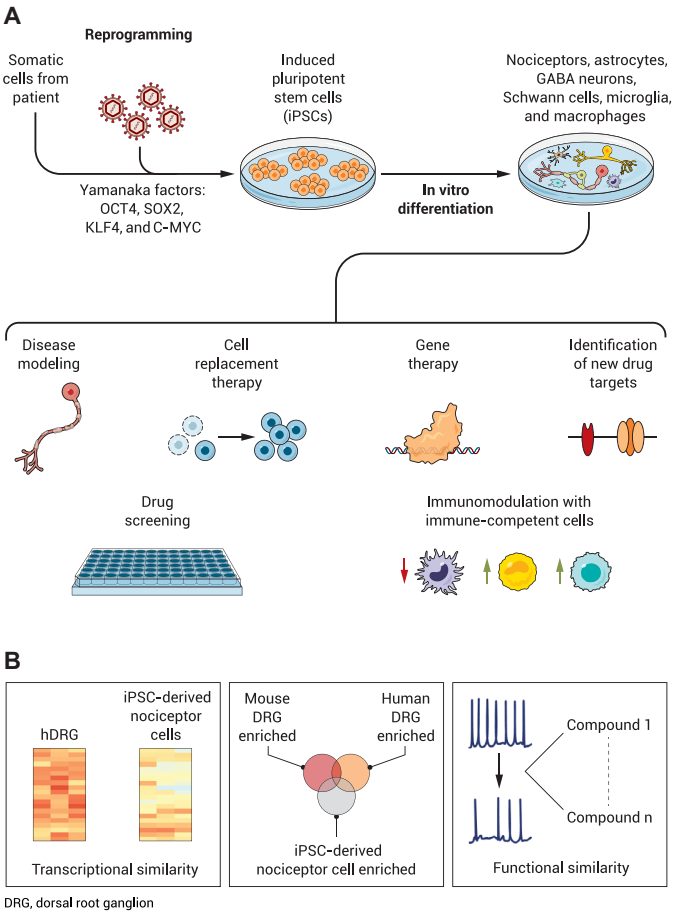


Fig. 3. Human iPSC technology for target discovery and translational applications. (**A**) Patient-derived somatic cells such as skin or blood cells can be reprogrammed into iPSCs using four defined transcription factors (OCT4, SOX2, KLF4, and C-MYC). Once pluripotent stem cell lines are established, they can be expanded indefinitely and differentiated into nociceptors and other cell types relevant to pain, particularly astrocytes, microglia, and macrophages, or for counter screens using cortical and motor neurons, enabling a broad range of applications for translational pain research including disease modeling, identification of novel targets, drug screening, and cell/gene therapy. (**B**) Similarity of human iPSC–derived cells to human primary cells. Neurons and other cell types derived from iPSCs have to be verified for similarity with in vivo counterparts before they can serve as a suitable model for the translational research outlines in (A). Key criteria need to be a transcriptional, translational, and posttranslational similarity between the derived and primary human nociceptors and the expected differences with other derived or primary cell types, and a functional similarity tested for example, with compounds that modulate a particular signaling pathway.

1) Establish transcriptional similarity: Performing RNA-seq experiments comparing iPSC-derived neurons and human and murine primary tissues to identify similarities and differences in gene expression is a critical step to establishing suitability for phenotypic screens. Relative abundance of gene transcripts and fold differences in key marker genes need to be evaluated. Ratios between key ion channel transcript abundance can serve as measures of similarity in membrane conductance levels (*102*, *152*). Mass spectrometry data can reveal presence of specific proteins and post-translational modifications, which reflect active signaling pathways. The rank orders of protein and gene transcript abundance can serve as useful determinant of translational similarity and signaling pathway fidelity (*200*). Antibody staining of key marker gene products can compare subcellular localization.

2) Establish similarity of nociceptor-enriched genes: The utility of phenotypic screens will depend on the presence of target genes enriched in nociceptors when compared to other cell types. A high overlap between genes enriched in human DRGs and genes enriched in differentiated nociceptors, when compared with other derived cells and primary tissues, is an important measure. Recent (*201*–*204*) and prior reports (*131*) provide a wealth of information on transcripts expressed in the human DRG and other tissues (*157*).

3) Establish functional similarity: This is a key consideration for translational potential. On the basis of a particular phenotypic activity profile of a compound on a set of iPSC-derived cell types, can we predict whether it will act as an analgesic with the desired efficacy and side-effect profiles? One approach to establishing a translational matrix to compare functional similarity between human and iPSC-derived nociceptors would be to measure phenotypic effects using a set of curated test compounds on human primary and iPSC-derived sensory neurons. Electrophysiological, Ca^{2+}, and reporter-based intracellular signaling assays can determine whether the cells behave similarly in response to inhibiting or activating certain signaling pathways, including ion channels, GPCRs, and kinases.

Establishing genotype-phenotype relationships and charting disease signatures that accurately reflect disease phenotypes represent a key strategy for translating preclinical research findings into clinical applications (*174*, *205*). Genome engineering using CRISPR-Cas9 allows site-specific and precise manipulation of normal and disease alleles and expands the translational toolbox of iPSCs (*159*, *206*). For modeling monogenic diseases and target identification, the utilization of isogenic iPSC lines genetically matched with the parental cell line is valuable. Use of isogenic iPSC lines enables accurate comparisons between normal and diseased cellular phenotypes and reduces technical and biological variability as potential experimental confounders (*136*, *159*, *207*).

Genome-wide association studies and study of rare familial disorders have provided new insight into the genetics and epigenetics of acute and chronic pain, including susceptibility and interindividual differences, and have identified potential targets (*208*–*212*). For instance, gain-of-function mutations of $Na_v1.7$ lead to erythromelalgia, paroxysmal extreme pain disorder, and small fiber neuropathy (*213*, *214*). By investigating iPSC-derived nociceptors of a family with the same $Na_v1.7$ mutation (S241T) but different pain severity (mother with moderate pain and son with severe pain), correlations between in vitro excitability of nociceptors with the clinical presentation can be made. Further analysis using whole-exome sequencing identified a variant in the potassium channel *KCNQ2* that conferred the resilience to pain in the mother (*142*). Interindividual differences in pain phenotypes and the underlying molecular mechanism(s) can therefore be identified using patient-derived nociceptors. In vitro drug testing in iPSC-derived nociceptors from a patient with small fiber neuropathy also predicted that the anticonvulsant lacosamide alleviates pain in the patient (*116*). Testing patient-derived cells can serve, therefore, as a precision medicine approach to develop personalized treatments. Whereas iPSC-derived nociceptors preserve features on $Na_v1.7$ gain-of-function mutations, such as enhanced activity and temperature dependence, these mutations in mice do not produce a pain phenotype (*215*), suggesting that mice may not be useful for modeling some human pain conditions.

ORGANOIDS AND COMPLEX TISSUE MODELS

Under two-dimensional (2D) cell culture conditions, cells grow attached to flat cell culture plates and therefore do not fully recapitulate in vivo physiology. In contrast, 3D models such as organoids or mini-organs more closely model dynamic in vivo cell function and behavior, including cell-cell interactions and biomechanical forces within the appropriate extracellular matrix (*216*). Use of 3D models can also help enhance cell maturation. Organoid models with relevance for pain research have been developed for skin (*217*), DRG (*218*), and thalamus (*219*) and will provide future opportunities to interrogate nociceptive circuits and test neuroactive drugs. However, the reproducibility and consistency of randomly self-organizing organoid models need to be improved before they can be used as robust assays for drug discovery. Technologies based on advances in bioengineering and biomaterials could lead to standardized microphysiological assays, organs-on-chips, and bioprinted tissues (*220*, *221*). These complex systems are also being developed as platforms for ADME (absorption, distribution, metabolism, and excretion) of small molecules, which may provide human pharmacokinetic predictions. Another model is the "nerve-on-a-chip," generated by culturing Schwann cells with human motor or sensory neurons to establish long axonal projections for measuring electrophysiological properties, drug effects, and response to injury (*222*).

CELL THERAPY

One of the great promises of iPSCs is development of personalized cell-based therapies (Fig. 3). Autologous nonimmunogenic cell therapies, unlike allogeneic approaches, do not require long-term immunosuppression or donors with a matching human leukocyte antigen signature. Although cell products generated from human pluripotent cell lines are being tested in clinical trials (*206*), none target chronic pain. However, cell therapy may become an option for certain pains, because human iPSC–derived GABAergic neurons injected into the spinal cord of mice with chronic neuropathic pain reduces the pain phenotype for up to 2 months after transplantation (*223*). This approach was based on the observation that grafting GABAergic precursors isolated from fetal rodent brains or human embryonic stem cell (ESC)–derived GABAergic neurons mitigated pain after spinal cord or peripheral injury by overcoming disinhibition (*224*, *225*). These studies are consistent with the observation that intrathecal application of the GABA agonist baclofen provides analgesia in patients with chronic pain (*226*). Central neuropathic pain may also benefit from grafting neuronal cells with other inhibitory neurotransmitter phenotypes, such as glycine, oligodendrocytes (*227*), and astrocytes (*148*, *149*), but more research is needed to define optimal cell therapeutic interventions. iPSC-based cell therapies may prove useful for treatment of peripheral neuropathy and neuropathic pain, because the complex interplay of multiple cell types including sensory neurons, Schwann cells, satellite glia, and immune cells contributes to axon loss, demyelination, ectopic firing, and pathological pain (*228*). Grafting undifferentiated neural crest stem cells supports axonal regrowth and myelination after sciatic nerve injury (*229*, *230*). Although progress has been made in differentiating iPSC-derived neural crest stem cells into Schwann cells (*144*, *145*, *231*), more efficient and clinically relevant protocols are needed to generate large quantities of cells with myelination capacity. Satellite glia are specialized support cells that closely envelop the cell bodies of sensory neurons in the DRG and are activated during inflammation and nerve injury (*232*). Despite this, human satellite glia are understudied, and derivation of satellite glia from iPSCs could provide new opportunities. There is also interest in immune cells such as microglia and DRG-resident macrophages, which contribute to the induction and maintenance of chronic pain (*233*, *234*). Derivation of microglia from human iPSCs (*146*) should enable better understanding of their biology, immunomodulatory function, and therapeutic potential. Production of all disease-relevant cell types from iPSCs (neuronal and non-neuronal) would allow the systematic testing of the cells in animal models of central and peripheral neuropathic pain. However, stem cell–derived products will require extensive safety measures to ensure that grafted cells are karyotypically normal and properly differentiated; otherwise, in vivo tumor formation may occur. Use of undifferentiated or proliferative cells is risky as they can continue to grow upon transplantation and form teratomas or other tumors (*235*).

GENE THERAPY

The rationale for gene therapy includes replacing or correcting a disease-causing mutation in a specific cell type, overexpression of neurotransmitters or ligands to modulate specific receptors, expression of transcription factors to drive change in function, neuroprotection, and change of cellular identity or recruitment of endogenous cells for repair or replacement. Relevant technologies for gene delivery use replication-incompetent viruses such as herpes simplex viral vector and adeno-associated viral vector, including in the context of pain (*236*). Other gene therapy strategies are RNA interference and antisense oligonucleotide technology that can degrade or inhibit the processing of specific mRNA molecules, thereby modulating or silencing expression of a protein of interest (*237*–*239*). Application of CRISPR-Cas9 methods can correct genetic defects, overexpress genes, or fine-tune gene expression, via inducible systems that increase the level of control and avoid/reverse unwanted effects. More research is warranted though to understand off-target effects, neurotoxicity, and long-term consequences (*240*). iPSC-derived cell types in the context of gene therapy could become a reality in two scenarios: First, gene therapy therapeutic strategies could be directly tested in relevant human cell types expressing the target gene, as part of a predictive in vitro platform. For instance, modulating the expression of specific ion channels in human nociceptors and characterizing the resulting functional consequences would provide valuable information before moving into application. Second, combined use of gene and cell therapies could expand the therapeutic armamentarium of personalized medicine. In theory, any iPSC-derived somatic cell and gene could be manipulated and given back to the patient donor. Using chemogenetic or optogenetic cell engineering approaches, only the genetically modified cells could be selectively activated or inhibited in the patient. Neuromodulation could also be achieved using prodrugs that only become pharmacologically active after being metabolized in vivo or on photostimulation (*241*–*244*).

PERSPECTIVES AND TRANSLATIONAL OPPORTUNITIES

To develop effective analgesic drugs with no abuse liability or other side effects, it is important to acknowledge pain as a major and diverse systems-level challenge and identify optimal strategies across the preclinical and clinical spectrum. The wealth of recent bioengineering technologies, multiomic platforms, bioinformatics and

machine learning, and artificial intelligence will certainly aid in generating and integrating information derived from genomics, iPSC-derived human cells, and animal models, with a better representation of human disease and predictor of outcome in clinical studies. As a major frontier in therapeutics development, iPSC technology can bridge the gap between genomic data and personalized medicine by enabling disease modeling and phenotype characterization, new target, and clinical candidate identification through drug screening and experimental validation (Fig. 3). The development of such therapeutic strategies requires running trials in a dish and using iPSC lines from large patient cohorts (including different genders, ethnic backgrounds, and disease histories) to identify novel therapeutics and patient responders and nonresponders. Mechanism-based scientific findings using human cellular models can, we argue because of the technical advances in the field, be efficiently translated into clinical applications that improve the management of chronic pain and avoid adverse effects, including dependence and addiction.

Crucial to translating preclinical findings to clinical use is to understand exactly which conclusions drawn from preclinical studies apply to clinical settings. Until recently, preclinical studies largely only used rodent models, although important species differences exist (*203*, *204*), and pain cannot be directly measured in animals. The introduction of human iPSC–derived models provides an opportunity to begin to overcome these difficulties, although challenges remain in establishing which preclinical parameters positively correlate with clinical efficacy. Phenotypic screenings allow for the determination of the specific action of compounds on particular cells and on defined disease phenotypes, as well as exploitation of polypharmacology opportunities. Counter screening provides a means of removing compounds with nonspecific/undesired effects early in the discovery process. Overall, prospects using iPSC-derived neurons are now higher for the development of effective nociceptor selective silencers suitable for managing surgical or post-traumatic pain, and eliminators of either the immune-mediated sensitization that drives inflammatory pain hypersensitivity or the development of ectopic activity in injured nociceptors that triggers neuropathic pain.

A powerful approach to the discovery, validation, and efficacy testing of novel pain targets/analgesic compounds will be a combined, complementary approach, incorporating major facets of the traditional target-based approach along with phenotypic screening. The preservation of intact, functional, cellular components provides the means for a new phenotypic approach for target discovery. Once targets are discovered, traditional target-based approaches can be pursued to identify clinical candidates. Safety profiles can be addressed by the complementary phenotypic screening of appropriate differentiated cell lines and combined with in vivo rodent or other suitable preclinical models.

Information flow between human iPSC–derived cellular phenotypic assays and single target-based assays can certainly contribute to successful drug discovery programs. Target identification outcomes of phenotypic assays from bioactive compound screenings will help inform which target-based assays to run, which, in turn, can determine structure-activity relationships and the high-resolution structural information that can improve affinity and selectivity. However, screening of large chemical diversity libraries against functional or disease phenotypes in derived neurons will likely identify promising lead compounds with a desired selective activity profile. However, it may not be possible to identify which target(s) these lead compounds act on. In this scenario, structure-activity relationships cannot be used to improve the potency or selectivity of a hit compound and medicinal chemistry may need to be driven by machine learning–based algorithms analyzing the different effects of distinct compounds on the phenotype rather than on target-binding. This is the challenge for drug discovery that now flows from the enormous opportunities that human derived cellular phenotypic screening provides. Phenotypic assays in derived human cells will contribute to the analgesic drug discovery process through accessing the molecular architecture of healthy or diseased nociceptors, or other cell types used for counter screens, serving as a pivot to guide target discovery and a means of discovering effective compounds without necessarily identifying targets, which will require a different drug discovery path forward for generating suitable clinical candidates.

REFERENCES AND NOTES

1. L. Colloca, T. Ludman, D. Bouhassira, R. Baron, A. H. Dickenson, D. Yarnitsky, R. Freeman, A. Truini, N. Attal, N. B. Finnerup, C. Eccleston, E. Kalso, D. L. Bennett, R. H. Dworkin, S. N. Raja, Neuropathic pain. *Nat. Rev. Dis. Primers* **3**, 17002 (2017).
2. S. P. Cohen, L. Vase, W. M. Hooten, Chronic pain: An update on burden, best practices, and new advances. *Lancet* **397**, 2082–2097 (2021).
3. C. J. Woolf, Q. Ma, Nociceptors—Noxious stimulus detectors. *Neuron* **55**, 353–364 (2007).
4. M. R. Rubin, M. C. Nguyen-Huu, Alternatively spliced Hox-1.7 transcripts encode different protein products. *DNA Seq.* **1**, 115–124 (1990).
5. M. Wang, B. Thyagarajan, Pain pathways and potential new targets for pain relief. *Biotechnol. Appl. Biochem.* , (2020).
6. A. Khan, S. Khan, Y. S. Kim, Insight into pain modulation: Nociceptors sensitization and therapeutic targets. *Curr. Drug Targets* **20**, 775–788 (2019).
7. J. Sun, L. Li, L. Yang, G. Duan, T. Ma, N. Li, Y. Liu, J. Yao, J. Y. Liu, X. Zhang, Novel SCN9A missense mutations contribute to congenital insensitivity to pain: Unexpected correlation between electrophysiological characterization and clinical phenotype. *Mol. Pain* **16**, 1744806920923881 (2020).
8. I. Drissi, W. A. Woods, C. G. Woods, Understanding the genetic basis of congenital insensitivity to pain. *Br. Med. Bull.* **133**, 65–78 (2020).
9. T. T. Pan, F. Pan, W. Gao, S. S. Hu, D. Wang, Involvement of macrophages and spinal microglia in osteoarthritis pain. *Curr. Rheumatol. Rep.* **23**, 29 (2021).
10. C. C. Shu, S. Zaki, V. Ravi, A. Schiavinato, M. M. Smith, C. B. Little, The relationship between synovial inflammation, structural pathology, and pain in post-traumatic osteoarthritis: Differential effect of stem cell and hyaluronan treatment. *Arthritis Res. Ther.* **22**, 29 (2020).
11. A. Kawabata, Prostaglandin E2 and pain—An update. *Biol. Pharm. Bull.* **34**, 1170–1173 (2011).
12. S. F. de Magalhaes, L. P. Manzo, F. M. de Faria, M. C. de Oliveira-Fusaro, C. M. Nishijima, W. F. Vieira, I. J. M. Bonet, G. G. Dos Santos, C. H. Tambeli, C. A. Parada, Inflammatory pain in peripheral tissue depends on the activation of the TNF-α type 1 receptor in the primary afferent neuron. *Eur. J. Neurosci.* **53**, 376–389 (2021).
13. M. Matsuda, Y. Huh, R. R. Ji, Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. *J. Anesth.* **33**, 131–139 (2019).
14. C. J. Woolf, Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* **152**, S2–S15 (2011).
15. C. J. Woolf, M. W. Salter, Neuronal plasticity: Increasing the gain in pain. *Science* **288**, 1765–1769 (2000).
16. D. Minhas, D. J. Clauw, Pain mechanisms in patients with rheumatic diseases. *Rheum. Dis. Clin. N. Am.* **47**, 133–148 (2021).
17. M. Costigan, J. Scholz, C. J. Woolf, Neuropathic pain: A maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* **32**, 1–32 (2009).
18. N. B. Finnerup, R. Kuner, T. S. Jensen, Neuropathic pain: From mechanisms to treatment. *Physiol. Rev.* **101**, 259–301 (2021).
19. C. Hunt, R. Moman, A. Peterson, R. Wilson, S. Covington, R. Mustafa, M. H. Murad, W. M. Hooten, Prevalence of chronic pain after spinal cord injury: A systematic review and meta-analysis. *Reg. Anesth. Pain Med.* **46**, 328–336 (2021).
20. C. A. von Hehn, R. Baron, C. J. Woolf, Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. *Neuron* **73**, 638–652 (2012).
21. M. Malcangio, Role of the immune system in neuropathic pain. *Scand J Pain* **20**, 33–37 (2019).
22. S. Ashina, D. D. Mitsikostas, M. J. Lee, N. Yamani, S. J. Wang, R. Messina, H. Ashina, D. C. Buse, P. Pozo-Rosich, R. H. Jensen, H. C. Diener, R. B. Lipton, Tension-type headache. *Nat. Rev. Dis. Primers* **7**, 24 (2021).
23. D. Vardeh, R. J. Mannion, C. J. Woolf, Toward a mechanism-based approach to pain diagnosis. *J. Pain* **17**, T50–T69 (2016).
24. M. A. Fitzcharles, S. P. Cohen, D. J. Clauw, G. Littlejohn, C. Usui, W. Hauser, Nociplastic pain: Towards an understanding of prevalent pain conditions. *Lancet* **397**, 2098–2110 (2021).

25. R. Siracusa, R. D. Paola, S. Cuzzocrea, D. Impellizzeri, Fibromyalgia: Pathogenesis, mechanisms, diagnosis and treatment options update. *Int. J. Mol. Sci.* **22**, 3891 (2021).
26. E. Kosek, D. Clauw, J. Nijs, R. Baron, I. Gilron, R. E. Harris, J. A. Mico, A. S. Rice, M. Sterling, Chronic nociplastic pain affecting the musculoskeletal system: Clinical criteria and grading system. *Pain* **162**, 2629–2634 (2021).
27. F. Creed, A review of the incidence and risk factors for fibromyalgia and chronic widespread pain in population-based studies. *Pain* **161**, 1169–1176 (2020).
28. A. M. Drewes, A. E. Olesen, A. D. Farmer, E. Szigethy, V. Rebours, S. S. Olesen, Gastrointestinal pain. *Nat. Rev. Dis. Primers* **6**, 1 (2020).
29. Y. Chen, J. Mu, M. Zhu, A. Mukherjee, H. Zhang, Transient receptor potential channels and inflammatory bowel disease. *Front. Immunol.* **11**, 180 (2020).
30. W. H. van der Gaag, P. D. Roelofs, W. T. Enthoven, M. W. van Tulder, B. W. Koes, Non-steroidal anti-inflammatory drugs for acute low back pain. *Cochrane Database Syst. Rev.* **4**, CD013581 (2020).
31. R. Bouali-Benazzouz, M. Landry, A. Benazzouz, P. Fossat, Neuropathic pain modeling: Focus on synaptic and ion channel mechanisms. *Prog. Neurobiol.* **201**, 102030 (2021).
32. T. S. Jensen, N. B. Finnerup, Allodynia and hyperalgesia in neuropathic pain: Clinical manifestations and mechanisms. *Lancet Neurol.* **13**, 924–935 (2014).
33. J. A. Carter, L. K. Black, D. Sharma, T. Bhagnani, J. S. Jahr, Efficacy of non-opioid analgesics to control postoperative pain: A network meta-analysis. *BMC Anesthesiol.* **20**, 272 (2020).
34. C. J. Woolf, Capturing novel non-opioid pain targets. *Biol. Psychiatry* **87**, 74–81 (2020).
35. A. S. Yekkirala, D. P. Roberson, B. P. Bean, C. J. Woolf, Breaking barriers to novel analgesic drug development. *Nat. Rev. Drug Discov.* **16**, 810 (2017).
36. M. Alsaloum, G. P. Higerd, P. R. Effraim, S. G. Waxman, Status of peripheral sodium channel blockers for non-addictive pain treatment. *Nat. Rev. Neurol.* **16**, 689–705 (2020).
37. H. G. Schaible, A. Ebersberger, G. S. Von Banchet, Mechanisms of pain in arthritis. *Ann. N. Y. Acad. Sci.* **966**, 343–354 (2002).
38. C. F. Villarreal, M. I. Funez, Q. Cunha Fde, C. A. Parada, S. H. Ferreira, The long-lasting sensitization of primary afferent nociceptors induced by inflammation involves prostanoid and dopaminergic systems in mice. *Pharmacol. Biochem. Behav.* **103**, 678–683 (2013).
39. F. Richter, G. Natura, S. Loser, K. Schmidt, H. Viisanen, H. G. Schaible, Tumor necrosis factor causes persistent sensitization of joint nociceptors to mechanical stimuli in rats. *Arthritis Rheum.* **62**, 3806–3814 (2010).
40. E. Pogatzki-Zahn, C. Chandrasena, S. A. Schug, Nonopioid analgesics for postoperative pain management. *Curr. Opin. Anaesthesiol.* **27**, 513–519 (2014).
41. L. L. Mazaleuskaya, V. R. Muzykantov, G. A. FitzGerald, Nanotherapeutic-directed approaches to analgesia. *Trends Pharmacol. Sci.* **42**, 527–550 (2021).
42. P. Jones, S. R. Dalziel, R. Lamdin, J. L. Miles-Chan, C. Frampton, Oral non-steroidal anti-inflammatory drugs versus other oral analgesic agents for acute soft tissue injury. *Cochrane Database Syst. Rev.* , CD007789 (2015).
43. R. A. Moore, S. Derry, D. Aldington, P. J. Wiffen, Single dose oral analgesics for acute postoperative pain in adults—An overview of Cochrane reviews. *Cochrane Database Syst. Rev.* **2015**, CD008659 (2015).
44. E. D. McNicol, M. C. Ferguson, R. Schumann, Single-dose intravenous ketorolac for acute postoperative pain in adults. *Cochrane Database Syst. Rev.* **5**, CD013263 (2021).
45. S. Derry, S. M. Karlin, R. A. Moore, Single dose oral ibuprofen plus codeine for acute postoperative pain in adults. *Cochrane Database Syst. Rev.* **2015**, CD010107 (2015).
46. S. Derry, C. J. Derry, R. A. Moore, Single dose oral ibuprofen plus oxycodone for acute postoperative pain in adults. *Cochrane Database Syst. Rev.* **2013**, CD010289 (2013).
47. L. Laine, Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. *Gastroenterology* **120**, 594–606 (2001).
48. C. J. Derry, S. Derry, R. A. Moore, Single dose oral ibuprofen plus paracetamol (acetaminophen) for acute postoperative pain. *Cochrane Database Syst. Rev.* **2013**, CD010210 (2013).
49. E. Sbidian, A. Chaimani, I. Garcia-Doval, L. Doney, C. Dressler, C. Hua, C. Hughes, L. Naldi, S. Afach, L. Le Cleach, Systemic pharmacological treatments for chronic plaque psoriasis: A network meta-analysis. *Cochrane Database Syst. Rev.* **4**, CD011535 (2021).
50. M. Feldmann, R. N. Maini, Anti-TNF α therapy of rheumatoid arthritis: What have we learned? *Annu. Rev. Immunol.* **19**, 163–196 (2001).
51. M. A. Palladino, F. R. Bahjat, E. A. Theodorakis, L. L. Moldawer, Anti-TNF-α therapies: The next generation. *Nat. Rev. Drug Discov.* **2**, 736–746 (2003).
52. D. I. Jang, A. H. Lee, H. Y. Shin, H. R. Song, J. H. Park, T. B. Kang, S. R. Lee, S. H. Yang, The role of tumor necrosis factor α (TNF-α) in autoimmune disease and current TNF-α Inhibitors in Therapeutics. *Int. J. Mol. Sci.* **22**, 2719 (2021).
53. C. A. Dinarello, A. Simon, J. W. van der Meer, Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat. Rev. Drug Discov.* **11**, 633–652 (2012).
54. J. Sun, S. R. Chen, H. Chen, H. L. Pan, μ-Opioid receptors in primary sensory neurons are essential for opioid analgesic effect on acute and inflammatory pain and opioid-induced hyperalgesia. *J. Physiol.* **597**, 1661–1675 (2019).
55. H. McAnally, U. Bonnet, A. D. Kaye, Gabapentinoid benefit and risk stratification: Mechanisms over myth. *Pain Ther.* **9**, 441–452 (2020).
56. A. C. Dolphin, Voltage-gated calcium channels: Their discovery, function and importance as drug targets. *Brain Neurosci. Adv.* **2**, 2398212818794805 (2018).
57. T. R. Deer, J. E. Pope, M. C. Hanes, G. C. McDowell, Intrathecal therapy for chronic pain: A review of morphine and ziconotide as firstline options. *Pain Med.* **20**, 784–798 (2019).
58. F. Wang, Z. Yan, Z. Liu, S. Wang, Q. Wu, S. Yu, J. Ding, Q. Dai, Molecular basis of toxicity of N-type calcium channel inhibitor MVIIA. *Neuropharmacology* **101**, 137–145 (2016).
59. C. I. Deligianni, D. D. Mitsikostas, M. Ashina, Safety and tolerability evaluation of erenumab for the preventive treatment of migraine. *Expert Opin. Drug Saf.* **20**, 867–876 (2021).
60. A. T. Masoud, M. T. Hasan, A. Sayed, H. N. Edward, A. M. Amer, A. E. Naga, M. Elfil, B. S. Alghamdi, A. Perveen, G. M. Ashraf, E. I. Bahbah, Efficacy of calcitonin gene-related peptide (CGRP) receptor blockers in reducing the number of monthly migraine headache days (MHDs): A network meta-analysis of randomized controlled trials. *J. Neurol. Sci.* **427**, 117505 (2021).
61. E. Popoff, K. Johnston, R. Croop, A. Thiry, L. Harris, L. Powell, V. Coric, G. L'Italien, J. Moren, Matching-adjusted indirect comparisons of oral rimegepant versus placebo, erenumab, and galcanezumab examining monthly migraine days and health-related quality of life in the treatment of migraine. *Headache* **61**, 906–915 (2021).
62. A. Kukkar, A. Bali, N. Singh, A. S. Jaggi, Implications and mechanism of action of gabapentin in neuropathic pain. *Arch. Pharm. Res.* **36**, 237–251 (2013).
63. A. Boroujerdi, J. Zeng, K. Sharp, D. Kim, O. Steward, D. Z. Luo, Calcium channel α-2-delta-1 protein upregulation in dorsal spinal cord mediates spinal cord injury-induced neuropathic pain states. *Pain* **152**, 649–655 (2011).
64. E. Serra, Duloxetine and pregabalin: Safe and effective for the long-term treatment of fibromyalgia? *Nat. Clin. Pract. Neurol.* **4**, 594–595 (2008).
65. A. B. Waitekus, P. Kirkpatrick, Duloxetine hydrochloride. *Nat. Rev. Drug Discov.* **3**, 907–908 (2004).
66. M. W. Branton, T. J. Hopkins, E. C. Nemec, Duloxetine for the reduction of opioid use in elective orthopedic surgery: A systematic review and meta-analysis. *Int. J. Clin. Pharmacol.* **43**, 394–403 (2021).
67. B. P. Roques, M. C. Fournie-Zaluski, M. Wurm, Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nat. Rev. Drug Discov.* **11**, 292–310 (2012).
68. A. J. Todd, Neuronal circuitry for pain processing in the dorsal horn. *Nat. Rev. Neurosci.* **11**, 823–836 (2010).
69. J. Knabl, R. Witschi, K. Hosl, H. Reinold, U. B. Zeilhofer, S. Ahmadi, J. Brockhaus, M. Sergejeva, A. Hess, K. Brune, J. M. Fritschy, U. Rudolph, H. Mohler, H. U. Zeilhofer, Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature* **451**, 330–334 (2008).
70. W. T. Ralvenius, D. Benke, M. A. Acuna, U. Rudolph, H. U. Zeilhofer, Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. *Nat. Commun.* **6**, 6803 (2015).
71. G. Munro, P. K. Ahring, N. R. Mirza, Developing analgesics by enhancing spinal inhibition after injury: GABAA receptor subtypes as novel targets. *Trends Pharmacol. Sci.* **30**, 453–459 (2009).
72. H. U. Zeilhofer, W. T. Ralvenius, M. A. Acuna, Restoring the spinal pain gate: GABA(A) receptors as targets for novel analgesics. *Adv. Pharmacol.* **73**, 71–96 (2015).
73. U. Rudolph, F. Knoflach, Beyond classical benzodiazepines: Novel therapeutic potential of GABAA receptor subtypes. *Nat. Rev. Drug Discov.* **10**, 685–697 (2011).
74. T. J. de Geus, J. Patijn, E. A. J. Joosten, Qualitative review on N-methyl-D-aspartate receptor expression in rat spinal cord during the postnatal development: Implications for central sensitization and pain. *Dev. Neurobiol.* **80**, 443–455 (2020).
75. K. Caruso, D. Tyler, A. Lyden, Ketamine for pain management: A review of literature and clinical application. *Orthop. Nurs.* **40**, 189–193 (2021).
76. C. Wang, H. Fu, J. Wang, F. Huang, X. Cao, Preemptive analgesia using selective cyclooxygenase-2 inhibitors alleviates postoperative pain in patients undergoing total knee arthroplasty: A protocol for PRISMA guided meta-analysis of randomized controlled trials. *Medicine (Baltimore)* **100**, e24512 (2021).
77. S. D. Raja, A. P. Shetty, B. Subramanian, R. M. Kanna, S. Rajasekaran, A prospective randomized study to analyze the efficacy of balanced pre-emptive analgesia in spine surgery. *Spine J.* **19**, 569–577 (2019).
78. D. S. M. de Araujo, R. Nassini, P. Geppetti, F. De Logu, TRPA1 as a therapeutic target for nociceptive pain. *Expert Opin. Ther. Targets* **24**, 997–1008 (2020).
79. M. Gao, Y. Wang, L. Liu, Z. Qiao, L. Yan, A patent review of transient receptor potential vanilloid type 1 modulators (2014-present). *Expert Opin. Ther. Pat.* **31**, 169–187 (2021).
80. J. S. Dulai, E. S. J. Smith, T. Rahman, Acid-sensing ion channel 3: An analgesic target. *Channels* **15**, 94–127 (2021).
81. K. Inoue, M. Tsuda, Nociceptive signaling mediated by P2X3, P2X4 and P2X7 receptors. *Biochem. Pharmacol.* **187**, 114309 (2021).
82. T. H. Tam, M. W. Salter, Purinergic signalling in spinal pain processing. *Purinergic Signal* **17**, 49–54 (2021).

83. B. L. Wise, M. F. Seidel, N. E. Lane, The evolution of nerve growth factor inhibition in clinical medicine. *Nat. Rev. Rheumatol.* **17**, 34–46 (2021).
84. A. M. Malfait, R. E. Miller, R. J. Miller, Basic mechanisms of pain in osteoarthritis: Experimental observations and new perspectives. *Rheum. Dis. Clin. N. Am.* **47**, 165–180 (2021).
85. N. Crispino, F. Ciccia, JAK/STAT pathway and nociceptive cytokine signalling in rheumatoid arthritis and psoriatic arthritis. *Clin. Exp. Rheumatol.* **39**, 668–675 (2021).
86. P. A. Smith, K^+ channels in primary afferents and their role in nerve injury-induced pain. *Front. Cell. Neurosci.* **14**, 566418 (2020).
87. L. Huang, G. Xu, R. Jiang, Y. Luo, Y. Zuo, J. Liu, Development of non-opioid analgesics targeting two-pore domain potassium channels. *Curr. Neuropharmacol.* , (2021).
88. X. Cheng, J. S. Choi, S. G. Waxman, S. D. Dib-Hajj, Mini-review—Sodium channels and beyond in peripheral nerve disease: Modulation by cytokines and their effector protein kinases. *Neurosci. Lett.* **741**, 135446 (2021).
89. R. Chen, O. J. M. Coppes, R. D. Urman, Receptor and molecular targets for the development of novel opioid and non-opioid analgesic therapies. *Pain Physician* **24**, 153–163 (2021).
90. A. Di Lio, D. Benke, M. Besson, J. Desmeules, Y. Daali, Z. J. Wang, R. Edwankar, J. M. Cook, H. U. Zeilhofer, HZ166, a novel GABAA receptor subtype-selective benzodiazepine site ligand, is antihyperalgesic in mouse models of inflammatory and neuropathic pain. *Neuropharmacology* **60**, 626–632 (2011).
91. L. E. Lorenzo, A. G. Godin, F. Ferrini, K. Bachand, I. Plasencia-Fernandez, S. Labrecque, A. A. Girard, D. Boudreau, I. Kianicka, M. Gagnon, N. Doyon, A. Ribeiro-da-Silva, Y. De Koninck, Enhancing neuronal chloride extrusion rescues α/α GABAA-mediated analgesia in neuropathic pain. *Nat. Commun.* **11**, 869 (2020).
92. A. Latremoliere, A. Latini, N. Andrews, S. J. Cronin, M. Fujita, K. Gorska, R. Hovius, C. Romero, S. Chuaiphichai, M. Painter, G. Miracca, O. Babaniyi, A. P. Remor, K. Duong, P. Riva, L. B. Barrett, N. Ferreiros, A. Naylor, J. M. Penninger, I. Tegeder, J. Zhong, J. Blagg, K. M. Channon, K. Johnsson, M. Costigan, C. J. Woolf, Reduction of neuropathic and inflammatory pain through inhibition of the tetrahydrobiopterin pathway. *Neuron* **86**, 1393–1406 (2015).
93. L. Pulakat, C. Sumners, Angiotensin type 2 receptors: Painful, or not? *Front. Pharmacol.* **11**, 571994 (2020).
94. H. Nagase, A. Saitoh, Research and development of kappa opioid receptor agonists and delta opioid receptor agonists. *Pharmacol. Ther.* **205**, 107427 (2020).
95. L. Gottesman-Katz, R. Latorre, S. Vanner, B. L. Schmidt, N. W. Bunnett, Targeting G protein-coupled receptors for the treatment of chronic pain in the digestive system. *Gut* **70**, 970–981 (2021).
96. S. W. Kaski, A. N. White, J. D. Gross, D. P. Siderovski, Potential for Kappa-opioid receptor agonists to engineer nonaddictive analgesics: A narrative review. *Anesth. Analg.* **132**, 406–419 (2021).
97. T. Che, H. Dwivedi-Agnihotri, A. K. Shukla, B. L. Roth, Biased ligands at opioid receptors: Current status and future directions. *Sci. Signal.* **14**, eaav0320 (2021).
98. M. R. Kolber, J. Ton, B. Thomas, J. Kirkwood, S. Moe, N. Dugre, K. Chan, A. J. Lindblad, J. McCormack, S. Garrison, G. M. Allan, C. S. Korownyk, R. Craig, L. Sept, A. N. Rouble, D. Perry, PEER systematic review of randomized controlled trials: Management of chronic low back pain in primary care. *Can. Fam. Physician* **67**, e20–e30 (2021).
99. J. Falk, B. Thomas, J. Kirkwood, C. S. Korownyk, A. J. Lindblad, J. Ton, S. Moe, G. M. Allan, J. McCormack, S. Garrison, N. Dugre, K. Chan, M. R. Kolber, A. Train, L. Froentjes, L. Sept, M. Wollin, R. Craig, D. Perry, PEER systematic review of randomized controlled trials: Management of chronic neuropathic pain in primary care. *Can. Fam. Physician* **67**, e130–e140 (2021).
100. D. Gregori, G. Giacovelli, C. Minto, B. Barbetta, F. Gualtieri, D. Azzolina, P. Vaghi, L. C. Rovati, Association of pharmacological treatments with long-term pain control in patients with knee osteoarthritis: A systematic review and meta-analysis. *JAMA* **320**, 2564–2579 (2018).
101. J. W. Busse, L. Wang, M. Kamaleldin, S. Craigie, J. J. Riva, L. Montoya, S. M. Mulla, L. C. Lopes, N. Vogel, E. Chen, K. Kirmayr, K. De Oliveira, L. Olivieri, A. Kaushal, L. E. Chaparro, I. Oyberman, A. Agarwal, R. Couban, L. Tsoi, T. Lam, P. O. Vandvik, S. Hsu, M. M. Bala, S. Schandelmaier, A. Scheidecker, S. Ebrahim, V. Ashoorion, Y. Rehman, P. J. Hong, S. Ross, B. C. Johnston, R. Kunz, X. Sun, N. Buckley, D. I. Sessler, G. H. Guyatt, Opioids for chronic noncancer pain: A systematic review and meta-analysis. *JAMA* **320**, 2448–2460 (2018).
102. Y. Zheng, P. Liu, L. Bai, J. S. Trimmer, B. P. Bean, D. D. Ginty, Deep sequencing of somatosensory neurons reveals molecular determinants of intrinsic physiological properties. *Neuron* **103**, 598–616.e7 (2019).
103. R. L. Kraus, F. Zhao, P. S. Pall, D. Zhou, J. D. Vardigan, A. Danziger, Y. Li, C. Daley, J. E. Ballard, M. K. Clements, R. M. Klein, M. A. Holahan, T. J. Greshock, R. M. Kim, M. E. Layton, C. S. Burgey, J. Serra, D. A. Henze, A. K. Houghton, Nav1.7 target modulation and efficacy can be measured in nonhuman primate assays. *Sci. Transl. Med.* **13**, eaay1050 (2021).
104. H. J. Hijma, P. S. Siebenga, M. L. de Kam, G. J. Groeneveld, A phase 1, randomized, double-blind, placebo-controlled, crossover study to evaluate the pharmacodynamic effects of VX-150, a highly selective NaV1.8 inhibitor, in healthy male adults. *Pain Med.* **22**, 1814–1826 (2021).
105. G. J. Sills, M. A. Rogawski, Mechanisms of action of currently used antiseizure drugs. *Neuropharmacology* **168**, 107966 (2020).
106. B. L. Roth, D. J. Sheffler, W. K. Kroeze, Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.* **3**, 353–359 (2004).
107. G. Goodwin, S. B. McMahon, The physiological function of different voltage-gated sodium channels in pain. *Nat. Rev. Neurosci.* **22**, 263–274 (2021).
108. M. Dimitriadi, M. J. Kye, G. Kalloo, J. M. Yersak, M. Sahin, A. C. Hart, The neuroprotective drug riluzole acts via small conductance Ca2+–activated K+ channels to ameliorate defects in spinal muscular atrophy models. *J. Neurosci.* **33**, 6557–6562 (2013).
109. F. Duprat, F. Lesage, A. J. Patel, M. Fink, G. Romey, M. Lazdunski, The neuroprotective agent riluzole activates the two P domain K(+) channels TREK-1 and TRAAK. *Mol. Pharmacol.* **57**, 906–912 (2000).
110. C. Tsantoulas, S. B. McMahon, Opening paths to novel analgesics: The role of potassium channels in chronic pain. *Trends Neurosci.* **37**, 146–158 (2014).
111. X. Du, H. Gao, D. Jaffe, H. Zhang, N. Gamper, M-type K^+ channels in peripheral nociceptive pathways. *Br. J. Pharmacol.* **175**, 2158–2172 (2018).
112. P. La Montanara, A. Hervera, L. L. Baltussen, T. H. Hutson, I. Palmisano, F. De Virgiliis, G. Kong, J. Chadwick, Y. Gao, K. Bartus, Q. A. Majid, N. Gorgoraptis, K. Wong, J. Downs, T. Pizzorusso, S. K. Ultanir, H. Leonard, H. Yu, D. S. Millar, N. Istvan, N. D. Mazarakis, S. Di Giovanni, Cyclin-dependent-like kinase 5 is required for pain signaling in human sensory neurons and mouse models. *Sci. Transl. Med.* **12**, eaax4846 (2020).
113. C. L. Schoepf, M. Zeidler, L. Spiecker, G. Kern, J. Lechner, K. K. Kummer, M. Kress, Selected ionotropic receptors and voltage-gated ion channels: More functional competence for human induced pluripotent stem cell (iPSC)-derived nociceptors. *Brain Sci.* **10**, 344 (2020).
114. P. Pettingill, G. A. Weir, T. Wei, Y. Wu, G. Flower, T. Lalic, A. Handel, G. Duggal, S. Chintawar, J. Cheung, K. Arunasalam, E. Couper, L. M. Haupt, L. R. Griffiths, A. Bassett, S. A. Cowley, M. Z. Cader, A causal role for TRESK loss of function in migraine mechanisms. *Brain* **142**, 3852–3867 (2019).
115. L. A. McDermott, G. A. Weir, A. C. Themistocleous, A. R. Segerdahl, I. Blesneac, G. Baskozos, A. J. Clark, V. Millar, L. J. Peck, D. Ebner, I. Tracey, J. Serra, D. L. Bennett, Defining the functional role of NaV1.7 in human nociception. *Neuron* **101**, 905–919.e8 (2019).
116. B. Namer, D. Schmidt, E. Eberhardt, M. Maroni, E. Dorfmeister, I. P. Kleggetveit, L. Kaluza, J. Meents, A. Gerlach, Z. Lin, A. Winterpacht, E. Dragicevic, Z. Kohl, J. Schuttler, I. Kurth, T. Warncke, E. Jorum, B. Winner, A. Lampert, Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. *EBioMed.* **39**, 401–408 (2019).
117. K. Schrenk-Siemens, C. Rosseler, A. Lampert, Translational model systems for complex sodium channel pathophysiology in pain. *Handb. Exp. Pharmacol.* **246**, 355–369 (2018).
118. C. Sommer, Exploring pain pathophysiology in patients. *Science* **354**, 588–592 (2016).
119. B. J. Wainger, E. D. Buttermore, J. T. Oliveira, C. Mellin, S. Lee, W. A. Saber, A. J. Wang, J. K. Ichida, I. M. Chiu, L. Barrett, E. A. Huebner, C. Bilgin, N. Tsujimoto, C. Brenneis, K. Kapur, L. L. Rubin, K. Eggan, C. J. Woolf, Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. *Nat. Neurosci.* **18**, 17–24 (2015).
120. I. Tochitsky, S. Jo, N. Andrews, M. Kotoda, B. Doyle, J. Shim, S. Talbot, D. Roberson, J. Lee, L. Haste, S. M. Jordan, B. D. Levy, B. P. Bean, C. J. Woolf, Inhibition of inflammatory pain and cough by a novel charged sodium channel blocker. *Br. J. Pharmacol.* **178**, 3905–3923 (2021).
121. D. P. Roberson, A. M. Binshtok, F. Blasl, B. P. Bean, C. J. Woolf, Targeting of sodium channel blockers into nociceptors to produce long-duration analgesia: A systematic study and review. *Br. J. Pharmacol.* **164**, 48–58 (2011).
122. R. Kuner, T. Kuner, Cellular circuits in the brain and their modulation in acute and chronic pain. *Physiol. Rev.* **101**, 213–258 (2021).
123. K. Bannister, A. H. Dickenson, Central nervous system targets: Supraspinal mechanisms of analgesia. *Neurotherapeutics* **17**, 839–845 (2020).
124. D. I. Hughes, A. J. Todd, Central nervous system targets: Inhibitory interneurons in the spinal cord. *Neurotherapeutics* **17**, 874–885 (2020).
125. Y. Shi, H. Inoue, J. C. Wu, S. Yamanaka, Induced pluripotent stem cell technology: A decade of progress. *Nat. Rev. Drug Discov.* **16**, 115–130 (2017).
126. K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006).
127. K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872 (2007).
128. D. Paull, A. Sevilla, H. Zhou, A. K. Hahn, H. Kim, C. Napolitano, A. Tsankov, L. Shang, K. Krumholz, P. Jagadeesan, C. M. Woodard, B. Sun, T. Vilboux, M. Zimmer, E. Forero, D. N. Moroziewicz, H. Martinez, M. C. Malicdan, K. A. Weiss, L. B. Vensand,

C. R. Dusenberry, H. Polus, K. T. Sy, D. J. Kahler, W. A. Gahl, S. L. Solomon, S. Chang, A. Meissner, K. Eggan, S. A. Noggle, Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nat. Methods* **12**, 885–892 (2015).
129. M. Daniszewski, D. E. Crombie, R. Henderson, H. H. Liang, R. C. B. Wong, A. W. Hewitt, A. Pebay, Automated cell culture systems and their applications to human pluripotent stem cell studies. *SLAS Technol.* **23**, 315–325 (2018).
130. C. A. Tristan, P. Ormanoglu, J. Slamecka, C. Malley, P. H. Chu, V. M. Jovanovic, Y. Gedik, C. Bonney, E. Barnaeva, J. Braisted, S. K. Mallanna, D. Dorjsuren, M. J. Iannotti, T. C. Voss, S. Michael, A. Simeonov, I. Singec, Robotic high-throughput biomanufacturing and functional differentiation of human pluripotent stem cells. *bioRxiv*, 2020.08.03.235242 (2020).
131. P. Ray, A. Torck, L. Quigley, A. Wangzhou, M. Neiman, C. Rao, T. Lam, J. Y. Kim, T. H. Kim, M. Q. Zhang, G. Dussor, T. J. Price, Comparative transcriptome profiling of the human and mouse dorsal root ganglia: An RNA-seq-based resource for pain and sensory neuroscience research. *Pain* **159**, 1325–1345 (2018).
132. S. Davidson, B. A. Copits, J. Zhang, G. Page, A. Ghetti, R. W. Gereau IV, Human sensory neurons: Membrane properties and sensitization by inflammatory mediators. *Pain* **155**, 1861–1870 (2014).
133. J. Rossant, Mouse and human blastocyst-derived stem cells: Vive les differences. *Development* **142**, 9–12 (2015).
134. C. Rostock, K. Schrenk-Siemens, J. Pohle, J. Siemens, Human vs. mouse nociceptors - similarities and differences. *Neuroscience* **387**, 13–27 (2018).
135. K. Osafune, L. Caron, M. Borowiak, R. J. Martinez, C. S. Fitz-Gerald, Y. Sato, C. A. Cowan, K. R. Chien, D. A. Melton, Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat. Biotechnol.* **26**, 313–315 (2008).
136. J. Choi, S. Lee, W. Mallard, K. Clement, G. M. Tagliazucchi, H. Lim, I. Y. Choi, F. Ferrari, A. M. Tsankov, R. Pop, G. Lee, J. L. Rinn, A. Meissner, P. J. Park, K. Hochedlinger, A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. *Nat. Biotechnol.* **33**, 1173–1181 (2015).
137. J. Schwartzentruber, S. Foskolou, H. Kilpinen, J. Rodrigues, K. Alasoo, A. J. Knights, M. Patel, A. Goncalves, R. Ferreira, C. L. Benn, A. Wilbrey, M. Bictash, E. Impey, L. Cao, S. Lainez, A. J. Loucif, P. J. Whiting; HIPSCI Consortium, A. Gutteridge, D. J. Gaffney, Molecular and functional variation in iPSC-derived sensory neurons. *Nat. Genet.* **50**, 54–61 (2018).
138. S. M. Chambers, Y. Qi, Y. Mica, G. Lee, X. J. Zhang, L. Niu, J. Bilsland, L. Cao, E. Stevens, P. Whiting, S. H. Shi, L. Studer, Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat. Biotechnol.* **30**, 715–720 (2012).
139. E. Eberhardt, S. Havlicek, D. Schmidt, A. S. Link, C. Neacsu, Z. Kohl, M. Hampl, A. M. Kist, A. Klinger, C. Nau, J. Schuttler, C. Alzheimer, J. Winkler, B. Namer, B. Winner, A. Lampert, Pattern of functional TTX-resistant sodium channels reveals a developmental stage of human iPSC- and ESC-derived nociceptors. *Stem Cell Rep.* **5**, 305–313 (2015).
140. L. Cao, A. McDonnell, A. Nitzsche, A. Alexandrou, P. P. Saintot, A. J. Loucif, A. R. Brown, G. Young, M. Mis, A. Randall, S. G. Waxman, P. Stanley, S. Kirby, S. Tarabar, A. Gutteridge, R. Butt, R. M. McKernan, P. Whiting, Z. Ali, J. Bilsland, E. B. Stevens, Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. *Sci. Transl. Med.* **8**, 335ra356 (2016).
141. J. E. Meents, E. Bressan, S. Sontag, A. Foerster, P. Hautvast, C. Rosseler, M. Hampl, H. Schuler, R. Goetzke, T. K. C. Le, I. P. Kleggetveit, K. Le Cann, C. Kerth, A. M. Rush, M. Rogers, Z. Kohl, M. Schmelz, W. Wagner, E. Jorum, B. Namer, B. Winner, M. Zenke, A. Lampert, The role of Nav1.7 in human nociceptors: Insights from human induced pluripotent stem cell-derived sensory neurons of erythromelalgia patients. *Pain* **160**, 1327–1341 (2019).
142. M. A. Mis, Y. Yang, B. S. Tanaka, C. Gomis-Perez, S. Liu, F. Dib-Hajj, T. Adi, R. Garcia-Milian, B. R. Schulman, S. D. Dib-Hajj, S. G. Waxman, Resilience to pain: A peripheral component identified using induced pluripotent stem cells and dynamic clamp. *J. Neurosci.* **39**, 382–392 (2019).
143. E. S. Lippmann, C. E. Williams, D. A. Ruhl, M. C. Estevez-Silva, E. R. Chapman, J. J. Coon, R. S. Ashton, Deterministic HOX patterning in human pluripotent stem cell-derived neuroectoderm. *Stem Cell Rep.* **4**, 632–644 (2015).
144. H. S. Kim, J. Lee, D. Y. Lee, Y. D. Kim, J. Y. Kim, H. J. Lim, S. Lim, Y. S. Cho, Schwann cell precursors from human pluripotent stem cells as a potential therapeutic target for myelin repair. *Stem Cell Rep.* **8**, 1714–1726 (2017).
145. B. Mukherjee-Clavin, R. Mi, B. Kern, I. Y. Choi, H. Lim, Y. Oh, B. Lannon, K. J. Kim, S. Bell, J. K. Hur, W. Hwang, Y. H. Che, O. Habib, R. H. Baloh, K. Eggan, G. Brandacher, A. Hoke, L. Studer, Y. J. Kim, G. Lee, Comparison of three congruent patient-specific cell types for the modelling of a human genetic Schwann-cell disorder. *Nat. Biomed. Eng.* **3**, 571–582 (2019).
146. J. Hasselmann, M. Blurton-Jones, Human iPSC-derived microglia: A growing toolset to study the brain's innate immune cells. *Glia* **68**, 721–739 (2020).
147. R. Krencik, J. P. Weick, Y. Liu, Z. J. Zhang, S. C. Zhang, Specification of transplantable astroglial subtypes from human pluripotent stem cells. *Nat. Biotechnol.* **29**, 528–534 (2011).
148. J. Tcw, M. Wang, A. A. Pimenova, K. R. Bowles, B. J. Hartley, E. Lacin, S. I. Machlovi, R. Abdelaal, C. M. Karch, H. Phatnani, P. A. Slesinger, B. Zhang, A. M. Goate, K. J. Brennand, An efficient platform for astrocyte differentiation from human induced pluripotent stem cells. *Stem Cell Rep.* **9**, 600–614 (2017).
149. J. Tchieu, E. L. Calder, S. R. Guttikonda, E. M. Gutzwiller, K. A. Aromolaran, J. A. Steinbeck, P. A. Goldstein, L. Studer, NFIA is a gliogenic switch enabling rapid derivation of functional human astrocytes from pluripotent stem cells. *Nat. Biotechnol.* **37**, 267–275 (2019).
150. A. M. Maroof, S. Keros, J. A. Tyson, S. W. Ying, Y. M. Ganat, F. T. Merkle, B. Liu, A. Goulburn, E. G. Stanley, A. G. Elefanty, H. R. Widmer, K. Eggan, P. A. Goldstein, S. A. Anderson, L. Studer, Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell* **12**, 559–572 (2013).
151. Y. Zhang, C. Pak, Y. Han, H. Ahlenius, Z. Zhang, S. Chanda, S. Marro, C. Patzke, C. Acuna, J. Covy, W. Xu, N. Yang, T. Danko, L. Chen, M. Wernig, T. C. Sudhof, Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron* **78**, 785–798 (2013).
152. T. O'Leary, A. H. Williams, A. Franci, E. Marder, Cell types, network homeostasis, and pathological compensation from a biologically plausible ion channel expression model. *Neuron* **82**, 809–821 (2014).
153. B. Sidders, A. Karlsson, L. Kitching, R. Torella, P. Karila, A. Phelan, Network-based drug discovery: Coupling network pharmacology with phenotypic screening for neuronal excitability. *J. Mol. Biol.* **430**, 3005–3015 (2018).
154. D. G. Jamieson, A. Moss, M. Kennedy, S. Jones, G. Nenadic, D. L. Robertson, B. Sidders, The pain interactome: Connecting pain-specific protein interactions. *Pain* **155**, 2243–2252 (2014).
155. P. Csermely, V. Agoston, S. Pongor, The efficiency of multi-target drugs: The network approach might help drug design. *Trends Pharmacol. Sci.* **26**, 178–182 (2005).
156. J. S. Brodie, V. Di Marzo, G. W. Guy, Polypharmacology shakes hands with complex aetiopathology. *Trends Pharmacol. Sci.* **36**, 802–821 (2015).
157. M. Mele, P. G. Ferreira, F. Reverter, D. S. DeLuca, J. Monlong, M. Sammeth, T. R. Young, J. M. Goldmann, D. D. Pervouchine, T. J. Sullivan, R. Johnson, A. V. Segre, S. Djebali, A. Niarchou; GTEx Consortium, F. A. Wright, T. Lappalainen, M. Calvo, G. Getz, E. T. Dermitzakis, K. G. Ardlie, R. Guigo, Human genomics. The human transcriptome across tissues and individuals. *Science* **348**, 660–665 (2015).
158. P. V. Todorov, B. M. Gyori, J. A. Bachman, P. K. Sorger, INDRA-IPM: Interactive pathway modeling using natural language with automated assembly. *Bioinformatics* **35**, 4501–4503 (2019).
159. D. Hendriks, H. Clevers, B. Artegiani, CRISPR-cas tools and their application in genetic engineering of human stem cells and organoids. *Cell Stem Cell* **27**, 705–731 (2020).
160. H. Koike-Yusa, Y. Li, E. P. Tan, C. Velasco-Herrera Mdel, K. Yusa, Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. *Nat. Biotechnol.* **32**, 267–273 (2014).
161. M. A. Mandegar, N. Huebsch, E. B. Frolov, E. Shin, A. Truong, M. P. Olvera, A. H. Chan, Y. Miyaoka, K. Holmes, C. I. Spencer, L. M. Judge, D. E. Gordon, T. V. Eskildsen, J. E. Villalta, M. A. Horlbeck, L. A. Gilbert, N. J. Krogan, S. P. Sheikh, J. S. Weissman, L. S. Qi, P. L. So, B. R. Conklin, CRISPR interference efficiently induces specific and reversible gene silencing in human iPSCs. *Cell Stem Cell* **18**, 541–553 (2016).
162. A. V. Anzalone, P. B. Randolph, J. R. Davis, A. A. Sousa, L. W. Koblan, J. M. Levy, P. J. Chen, C. Wilson, G. A. Newby, A. Raguram, D. R. Liu, Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* **576**, 149–157 (2019).
163. R. Tian, M. A. Gachechiladze, C. H. Ludwig, M. T. Laurie, J. Y. Hong, D. Nathaniel, A. V. Prabhu, M. S. Fernandopulle, R. Patel, M. Abshari, M. E. Ward, M. Kampmann, CRISPR interference-based platform for multimodal genetic screens in human iPSC-derived neurons. *Neuron* **104**, 239–255.e12 (2019).
164. J. B. Black, S. R. McCutcheon, S. Dube, A. Barrera, T. S. Klann, G. A. Rice, S. S. Adkar, S. H. Soderling, T. E. Reddy, C. A. Gersbach, Master regulators and cofactors of human neuronal cell fate specification identified by CRISPR gene activation screens. *Cell Rep.* **33**, 108460 (2020).
165. N. Moret, N. A. Clark, M. Hafner, Y. Wang, E. Lounkine, M. Medvedovic, J. Wang, N. Gray, J. Jenkins, P. K. Sorger, Cheminformatics tools for analyzing and designing optimized small-molecule collections and libraries. *Cell Chem. Biol.* **26**, 765–777.e3 (2019).
166. C. H. Arrowsmith, J. E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P. E. Brennan, P. J. Brown, M. E. Bunnage, C. Buser-Doepner, R. M. Campbell, A. J. Carter, P. Cohen, R. A. Copeland, B. Cravatt, J. L. Dahlin, D. Dhanak, A. M. Edwards, M. Frederiksen, S. V. Frye, N. Gray, C. E. Grimshaw, D. Hepworth, T. Howe, K. V. Huber, J. Jin, S. Knapp, J. D. Kotz, R. G. Kruger, D. Lowe, M. M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Muller, R. C. O'Hagan, J. P. Overington, D. R. Owen, S. H. Rosenberg, B. Roth, R. Ross, M. Schapira, S. L. Schreiber, B. Shoichet, M. Sundstrom, G. Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M. A. Walters, T. M. Willson, P. Workman, R. N. Young,

W. J. Zuercher, The promise and peril of chemical probes. *Nat. Chem. Biol.* **11**, 536–541 (2015).
167. M. Z. Lin, M. J. Schnitzer, Genetically encoded indicators of neuronal activity. *Nat. Neurosci.* **19**, 1142–1153 (2016).
168. P. Stacey, A. M. Wassermann, L. Kammonen, E. Impey, A. Wilbrey, D. Cawkill, Plate-based phenotypic screening for pain using human iPSC-derived sensory neurons. *SLAS Discov.* **23**, 585–596 (2018).
169. E. J. Akin, G. P. Higerd, M. A. Mis, B. S. Tanaka, T. Adi, S. Liu, F. B. Dib-Hajj, S. G. Waxman, S. D. Dib-Hajj, Building sensory axons: Delivery and distribution of NaV1.7 channels and effects of inflammatory mediators. *Sci. Adv.* **5**, eaax4755 (2019).
170. R. R. Ji, Z. Z. Xu, Y. J. Gao, Emerging targets in neuroinflammation-driven chronic pain. *Nat. Rev. Drug Discov.* **13**, 533–548 (2014).
171. R. O. Hughes, T. Bosanac, X. Mao, T. M. Engber, A. DiAntonio, J. Milbrandt, R. Devraj, R. Krauss, Small molecule SARM1 inhibitors recapitulate the SARM1(−/−) phenotype and allow recovery of a metastable pool of axons fated to degenerate. *Cell Rep.* **34**, 108588 (2021).
172. C. Linghu, S. L. Johnson, P. A. Valdes, O. A. Shemesh, W. M. Park, D. Park, K. D. Piatkevich, A. T. Wassie, Y. Liu, B. An, S. A. Barnes, O. T. Celiker, C. C. Yao, C. J. Yu, R. Wang, K. P. Adamala, M. F. Bear, A. E. Keating, E. S. Boyden, Spatial multiplexing of fluorescent reporters for imaging signaling network dynamics. *Cell* **183**, 1682–1698.e24 (2020).
173. O. A. Shemesh, C. Linghu, K. D. Piatkevich, D. Goodwin, O. T. Celiker, H. J. Gritton, M. F. Romano, R. Gao, C. J. Yu, H. A. Tseng, S. Bensussen, S. Narayan, C. T. Yang, L. Freifeld, C. A. Siciliano, I. Gupta, J. Wang, N. Pak, Y. G. Yoon, J. F. P. Ullmann, B. Guner-Ataman, H. Noamany, Z. R. Sheinkopf, W. M. Park, S. Asano, A. E. Keating, J. S. Trimmer, J. Reimer, A. S. Tolias, M. F. Bear, K. M. Tye, X. Han, M. B. Ahrens, E. S. Boyden, Precision calcium imaging of dense neural populations via a cell-body-targeted calcium indicator. *Neuron* **107**, 470–486.e11 (2020).
174. D. C. Swinney, J. Anthony, How were new medicines discovered? *Nat. Rev. Drug Discov.* **10**, 507–519 (2011).
175. J. Inglese, D. S. Auld, A. Jadhav, R. L. Johnson, A. Simeonov, A. Yasgar, W. Zheng, C. P. Austin, Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 11473–11478 (2006).
176. Y. Chen, C. A. Tristan, L. Chen, V. M. Jovanovic, C. Malley, P. H. Chu, S. Ryu, T. Deng, P. Ormanoglu, D. Tao, Y. Fang, J. Slamecka, H. Hong, C. A. LeClair, S. Michael, C. P. Austin, A. Simeonov, I. Singec, A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells. *Nat. Methods* **18**, 528–541 (2021).
177. L. A. M. Griner, R. Guha, P. Shinn, R. M. Young, J. M. Keller, D. Liu, I. S. Goldlust, A. Yasgar, C. McKnight, M. B. Boxer, D. Y. Duveau, J. K. Jiang, S. Michael, T. Mierzwa, W. Huang, M. J. Walsh, B. T. Mott, P. Patel, W. Leister, D. J. Maloney, C. A. Leclair, G. Rai, A. Jadhav, B. D. Peyser, C. P. Austin, S. E. Martin, A. Simeonov, M. Ferrer, L. M. Staudt, C. J. Thomas, High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 2349–2354 (2014).
178. G. Lee, C. N. Ramirez, H. Kim, N. Zeltner, B. Liu, C. Radu, B. Bhinder, Y. J. Kim, I. Y. Choi, B. Mukherjee-Clavin, H. Djaballah, L. Studer, Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression. *Nat. Biotechnol.* **30**, 1244–1248 (2012).
179. B. J. Wainger, E. Kiskinis, C. Mellin, O. Wiskow, S. S. Han, J. Sandoe, N. P. Perez, L. A. Williams, S. Lee, G. Boulting, J. D. Berry, R. H. Brown Jr., M. E. Cudkowicz, B. P. Bean, K. Eggan, C. J. Woolf, Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Rep.* **7**, 1–11 (2014).
180. X. Huang, K. C. D. Roet, L. Zhang, A. Brault, A. P. Berg, A. B. Jefferson, J. Klug-McLeod, K. L. Leach, F. Vincent, H. Yang, A. J. Coyle, L. H. Jones, D. Frost, O. Wiskow, K. Chen, R. Maeda, A. Grantham, M. K. Dornon, J. R. Klim, M. T. Siekmann, D. Zhao, S. Lee, K. Eggan, C. J. Woolf, Human amyotrophic lateral sclerosis excitability phenotype screen: Target discovery and validation. *Cell Rep.* **35**, 109224 (2021).
181. K. D. Kaya, H. Y. Chen, M. J. Brooks, R. A. Kelley, H. Shimada, K. Nagashima, N. de Val, C. T. Drinnan, L. Gieser, K. Kruczek, S. Erceg, T. Li, D. Lukovic, Y. K. Adlakha, E. Welby, A. Swaroop, Transcriptome-based molecular staging of human stem cell-derived retinal organoids uncovers accelerated photoreceptor differentiation by 9-cis retinal. *Mol. Vis.* **25**, 663–678 (2019).
182. J. R. Brewer, G. Morrison, M. E. Dolan, G. F. Fleming, Chemotherapy-induced peripheral neuropathy: Current status and progress. *Gynecol. Oncol.* **140**, 176–183 (2016).
183. M. Komatsu, H. E. Wheeler, S. Chung, S. K. Low, C. Wing, S. M. Delaney, L. K. Gorsic, A. Takahashi, M. Kubo, D. L. Kroetz, W. Zhang, Y. Nakamura, M. E. Dolan, Pharmacoethnicity in paclitaxel-induced sensory peripheral neuropathy. *Clin. Cancer Res.* **21**, 4337–4346 (2015).
184. H. E. Wheeler, C. Wing, S. M. Delaney, M. Komatsu, M. E. Dolan, Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells. *PLOS ONE* **10**, e0118020 (2015).
185. M. Caiazzo, M. T. Dell'Anno, E. Dvoretskova, D. Lazarevic, S. Taverna, D. Leo, T. D. Sotnikova, A. Menegon, P. Roncaglia, G. Colciago, G. Russo, P. Carninci, G. Pezzoli, R. R. Gainetdinov, S. Gustincich, A. Dityatev, V. Broccoli, Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476**, 224–227 (2011).
186. U. Pfisterer, A. Kirkeby, O. Torper, J. Wood, J. Nelander, A. Dufour, A. Bjorklund, O. Lindvall, J. Jakobsson, M. Parmar, Direct conversion of human fibroblasts to dopaminergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 10343–10348 (2011).
187. E. Y. Son, J. K. Ichida, B. J. Wainger, J. S. Toma, V. F. Rafuse, C. J. Woolf, K. Eggan, Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**, 205–218 (2011).
188. M. L. Liu, T. Zang, Y. Zou, J. C. Chang, J. R. Gibson, K. M. Huber, C. L. Zhang, Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. *Nat. Commun.* **4**, 2183 (2013).
189. S. Chanda, C. E. Ang, J. Davila, C. Pak, M. Mall, Q. Y. Lee, H. Ahlenius, S. W. Jung, T. C. Sudhof, M. Wernig, Generation of induced neuronal cells by the single reprogramming factor ASCL1. *Stem Cell Rep.* **3**, 282–296 (2014).
190. Z. P. Pang, N. Yang, T. Vierbuchen, A. Ostermeier, D. R. Fuentes, T. Q. Yang, A. Citri, V. Sebastiano, S. Marro, T. C. Sudhof, M. Wernig, Induction of human neuronal cells by defined transcription factors. *Nature* **476**, 220–223 (2011).
191. T. Vierbuchen, A. Ostermeier, Z. P. Pang, Y. Kokubu, T. C. Sudhof, M. Wernig, Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041 (2010).
192. B. Y. Hu, Z. W. Du, S. C. Zhang, Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat. Protoc.* **4**, 1614–1622 (2009).
193. L. Emdad, S. L. D'Souza, H. P. Kothari, Z. A. Qadeer, I. M. Germano, Efficient differentiation of human embryonic and induced pluripotent stem cells into functional astrocytes. *Stem Cells Dev.* **21**, 404–410 (2012).
194. E. M. Abud, R. N. Ramirez, E. S. Martinez, L. M. Healy, C. H. H. Nguyen, S. A. Newman, A. V. Yeromin, V. M. Scarfone, S. E. Marsh, C. Fimbres, C. A. Caraway, G. M. Fote, A. M. Madany, A. Agrawal, R. Kayed, K. H. Gylys, M. D. Cahalan, B. J. Cummings, J. P. Antel, A. Mortazavi, M. J. Carson, W. W. Poon, M. Blurton-Jones, iPSC-derived human microglia-like cells to study neurological diseases. *Neuron* **94**, 278–293.e9 (2017).
195. I. Jones, T. D. Yelhekar, R. Wiberg, P. J. Kingham, S. Johansson, M. Wiberg, L. Carlsson, Development and validation of an in vitro model system to study peripheral sensory neuron development and injury. *Sci. Rep.* **8**, 15961 (2018).
196. A. J. Alshawaf, S. Viventi, W. Qiu, G. D'Abaco, B. Nayagam, M. Erlichster, G. Chana, I. Everall, J. Ivanusic, E. Skafidas, M. Dottori, Phenotypic and functional characterization of peripheral sensory neurons derived from human embryonic stem cells. *Sci. Rep.* **8**, 603 (2018).
197. K. Saito-Diaz, J. R. Street, H. Ulrichs, N. Zeltner, Derivation of peripheral nociceptive, mechanoreceptive, and proprioceptive sensory neurons from the same culture of human pluripotent stem cells. *Stem Cell Rep.* **16**, 446–457 (2021).
198. J. W. Blanchard, K. T. Eade, A. Szucs, V. Lo Sardo, R. K. Tsunemoto, D. Williams, P. P. Sanna, K. K. Baldwin, Selective conversion of fibroblasts into peripheral sensory neurons. *Nat. Neurosci.* **18**, 25–35 (2015).
199. S. J. Middleton, A. M. Barry, M. Comini, Y. Li, P. R. Ray, S. Shiers, A. C. Themistocleous, M. L. Uhelski, X. Yang, P. M. Dougherty, T. J. Price, D. L. Bennett, Studying human nociceptors: From fundamentals to clinic. *Brain* **144**, 1312–1335 (2021).
200. C. Vogel, E. M. Marcotte, Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **13**, 227–232 (2012).
201. M. Q. Nguyen, L. J. von Buchholtz, A. N. Reker, N. J. P. Ryba, S. Davidson, Single nucleus transcriptomic analysis of human dorsal root ganglion neurons. *bioRxiv*, 2021.2007.2002.450845 (2021).
202. B. E. Hall, E. Macdonald, M. Cassidy, S. Yun, M. R. Sapio, P. Ray, M. Doty, P. Nara, M. D. Burton, S. Shiers, A. Ray-Chaudhury, A. J. Mannes, T. J. Price, M. J. Iadarola, A. B. Kulkarni, Transcriptomic analysis of human sensory neurons in painful diabetic neuropathy reveals inflammation and neuronal loss. *bioRxiv*, 2021.2007.2023.453576 (2021).
203. S. Shiers, R. M. Klein, T. J. Price, Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. *bioRxiv*, 2020.2003.2006.981597 (2020).
204. D. Tavares-Ferreira, S. Shiers, P. R. Ray, A. Wangzhou, V. Jeevakumar, I. Sankaranarayanan, A. Cervantes, J. C. Reese, A. Chamessian, B. A. Copits, P. M. Dougherty, R. W. Gereau, M. D. Burton, G. Dussor, T. J. Price, Spatial transcriptomics reveals unique molecular fingerprints of human nociceptors. *bioRxiv*, 2021.2002.2006.430065 (2021).
205. V. K. Gribkoff, L. K. Kaczmarek, The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. *Neuropharmacology* **120**, 11–19 (2017).
206. E. A. Kimbrel, R. Lanza, Next-generation stem cells—Ushering in a new era of cell-based therapies. *Nat. Rev. Drug Discov.* **19**, 463–479 (2020).
207. D. Ortmann, L. Vallier, Variability of human pluripotent stem cell lines. *Curr. Opin. Genet. Dev.* **46**, 179–185 (2017).

208. A. G. Madian, H. E. Wheeler, R. B. Jones, M. E. Dolan, Relating human genetic variation to variation in drug responses. *Trends Genet.* **28**, 487–495 (2012).
209. M. J. Peters, L. Broer, H. L. Willemen, G. Eiriksdottir, L. J. Hocking, K. L. Holliday, M. A. Horan, I. Meulenbelt, T. Neogi, M. Popham, C. O. Schmidt, A. Soni, A. M. Valdes, N. Amin, E. M. Dennison, N. Eijkelkamp, T. B. Harris, D. J. Hart, A. Hofman, F. J. Huygen, K. A. Jameson, G. T. Jones, L. J. Launer, H. J. Kerkhof, M. de Kruijf, J. McBeth, M. Kloppenburg, W. E. Ollier, B. Oostra, A. Payton, F. Rivadeneira, B. H. Smith, A. V. Smith, L. Stolk, A. Teumer, W. Thomson, A. G. Uitterlinden, K. Wang, S. H. van Wingerden, N. K. Arden, C. Cooper, D. Felson, V. Gudnason, G. J. Macfarlane, N. Pendleton, P. E. Slagboom, T. D. Spector, H. Volzke, A. Kavelaars, C. M. van Duijn, F. M. Williams, J. B. van Meurs, Genome-wide association study meta-analysis of chronic widespread pain: Evidence for involvement of the 5p15.2 region. *Ann. Rheum. Dis.* **72**, 427–436 (2013).
210. K. Zorina-Lichtenwalter, M. Parisien, L. Diatchenko, Genetic studies of human neuropathic pain conditions: A review. *Pain* **159**, 583–594 (2018).
211. W. Meng, M. J. Adams, C. N. A. Palmer; 23andMe Research, J. Shi, A. Auton, K. A. Ryan, J. M. Jordan, B. D. Mitchell, R. D. Jackson, M. S. Yau, A. M. McIntosh, B. H. Smith, Genome-wide association study of knee pain identifies associations with GDF5 and COL27A1 in UK Biobank. *Commun. Biol.* **2**, 321 (2019).
212. Z. Naureen, L. Lorusso, P. Manganotti, P. Caruso, G. Mazzon, S. Cecchin, G. Marceddu, M. Bertelli, Genetics of pain: From rare Mendelian disorders to genetic predisposition to pain. *Acta Biomed* **91**, e2020010 (2020).
213. D. L. Bennett, A. J. Clark, J. Huang, S. G. Waxman, S. D. Dib-Hajj, The role of voltage-gated sodium channels in pain signaling. *Physiol. Rev.* **99**, 1079–1151 (2019).
214. Y. Yang, Y. Wang, S. Li, Z. Xu, H. Li, L. Ma, J. Fan, D. Bu, B. Liu, Z. Fan, G. Wu, J. Jin, B. Ding, X. Zhu, Y. Shen, Mutations in SCN9A, encoding a sodium channel α subunit, in patients with primary erythermalgia. *J. Med. Genet.* **41**, 171–174 (2004).
215. L. Chen, N. K. Wimalasena, J. Shim, C. Han, S. I. Lee, R. Gonzalez-Cano, M. Estacion, C. G. Faber, G. Lauria, S. D. Dib-Hajj, C. J. Woolf, S. G. Waxman, Two independent mouse lines carrying the Nav1.7 I228M gain-of-function variant display dorsal root ganglion neuron hyperexcitability but a minimal pain phenotype. *Pain* **162**, 1758–1770 (2021).
216. R. G. Rowe, G. Q. Daley, Induced pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Genet.* **20**, 377–388 (2019).
217. J. Lee, C. C. Rabbani, H. Gao, M. R. Steinhart, B. M. Woodruff, Z. E. Pflum, A. Kim, S. Heller, Y. Liu, T. Z. Shipchandler, K. R. Koehler, Hair-bearing human skin generated entirely from pluripotent stem cells. *Nature* **582**, 399–404 (2020).
218. P. G. Mazzara, S. Muggeo, M. Luoni, L. Massimino, M. Zaghi, P. T. Valverde, S. Brusco, M. J. Marzi, C. Palma, G. Colasante, A. Iannielli, M. Paulis, C. Cordiglieri, S. G. Giannelli, P. Podini, C. Gellera, F. Taroni, F. Nicassio, M. Rasponi, V. Broccoli, Frataxin gene editing rescues Friedreich's ataxia pathology in dorsal root ganglia organoid-derived sensory neurons. *Nat. Commun.* **11**, 4178 (2020).
219. Y. Xiang, Y. Tanaka, B. Cakir, B. Patterson, K. Y. Kim, P. Sun, Y. J. Kang, M. Zhong, X. Liu, P. Patra, S. H. Lee, S. M. Weissman, I. H. Park, hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. *Cell Stem Cell* **24**, 487–497.e (2019).
220. X. Liu, S. Michael, K. Bharti, M. Ferrer, M. J. Song, A biofabricated vascularized skin model of atopic dermatitis for preclinical studies. *Biofabrication* **12**, 035002 (2020).
221. L. A. Low, C. Mummery, B. R. Berridge, C. P. Austin, D. A. Tagle, Organs-on-chips: Into the next decade. *Nat. Rev. Drug Discov.* **20**, 345–361 (2021).
222. A. D. Sharma, L. McCoy, E. Jacobs, H. Willey, J. Q. Behn, H. Nguyen, B. Bolon, J. L. Curley, M. J. Moore, Engineering a 3D functional human peripheral nerve in vitro using the Nerve-on-a-Chip platform. *Sci. Rep.* **9**, 8921 (2019).
223. J. Manion, T. Khuong, D. Harney, J. B. Littleboy, T. Ruan, L. Loo, M. Costigan, M. Larance, L. Caron, G. G. Neely, Human induced pluripotent stem cell-derived GABAergic interneuron transplants attenuate neuropathic pain. *Pain* **161**, 379–387 (2020).
224. T. M. Fandel, A. Trivedi, C. R. Nicholas, H. Zhang, J. Chen, A. F. Martinez, L. J. Noble-Haeusslein, A. R. Kriegstein, Transplanted human stem cell-derived interneuron precursors mitigate mouse bladder dysfunction and central neuropathic pain after spinal cord injury. *Cell Stem Cell* **19**, 544–557 (2016).
225. J. M. Braz, A. Etlin, D. Juarez-Salinas, I. J. Llewellyn-Smith, A. I. Basbaum, Rebuilding CNS inhibitory circuits to control chronic neuropathic pain and itch. *Prog. Brain Res.* **231**, 87–105 (2017).
226. R. E. Zuniga, C. R. Schlicht, S. E. Abram, Intrathecal baclofen is analgesic in patients with chronic pain. *Anesthesiology* **92**, 876–880 (2000).
227. S. A. Goldman, N. J. Kuypers, How to make an oligodendrocyte. *Development* **142**, 3983–3995 (2015).
228. J. Scholz, C. J. Woolf, The neuropathic pain triad: Neurons, immune cells and glia. *Nat. Neurosci.* **10**, 1361–1368 (2007).
229. C. W. Huang, W. C. Huang, X. Qiu, F. F. F. da Silva, A. Wang, S. Patel, L. J. Nesti, M. M. Poo, S. Li, The differentiation stage of transplanted stem cells modulates nerve regeneration. *Sci. Rep.* **7**, 17401 (2017).
230. H. Kimura, T. Ouchi, S. Shibata, T. Amemiya, N. Nagoshi, T. Nakagawa, M. Matsumoto, H. Okano, M. Nakamura, K. Sato, Stem cells purified from human induced pluripotent stem cell-derived neural crest-like cells promote peripheral nerve regeneration. *Sci. Rep.* **8**, 10071 (2018).
231. G. Lee, S. M. Chambers, M. J. Tomishima, L. Studer, Derivation of neural crest cells from human pluripotent stem cells. *Nat. Protoc.* **5**, 688–701 (2010).
232. M. Hanani, D. C. Spray, Emerging importance of satellite glia in nervous system function and dysfunction. *Nat. Rev. Neurosci.* **21**, 485–498 (2020).
233. K. Inoue, M. Tsuda, Microglia in neuropathic pain: Cellular and molecular mechanisms and therapeutic potential. *Nat. Rev. Neurosci.* **19**, 138–152 (2018).
234. X. Yu, H. Liu, K. A. Hamel, M. G. Morvan, S. Yu, J. Leff, Z. Guan, J. M. Braz, A. I. Basbaum, Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain. *Nat. Commun.* **11**, 264 (2020).
235. P. W. Andrews, U. Ben-David, N. Benvenisty, P. Coffey, K. Eggan, B. B. Knowles, A. Nagy, M. Pera, B. Reubinoff, P. J. Rugg-Gunn, G. N. Stacey, Assessing the safety of human pluripotent stem cells and their derivatives for clinical applications. *Stem Cell Rep.* **9**, 1–4 (2017).
236. J. M. Guedon, S. Wu, X. Zheng, C. C. Churchill, J. C. Glorioso, C. H. Liu, S. Liu, L. Vulchanova, A. Bekker, Y. X. Tao, P. R. Kinchington, W. F. Goins, C. A. Fairbanks, S. Hao, Current gene therapy using viral vectors for chronic pain. *Mol. Pain* **11**, 27 (2015).
237. W. Cai, J. Cao, X. Ren, L. Qiao, X. Chen, M. Li, W. Zang, shRNA mediated knockdown of Nav1.7 in rat dorsal root ganglion attenuates pain following burn injury. *BMC Anesthesiol.* **16**, 59 (2016).
238. C. Rinaldi, M. J. A. Wood, Antisense oligonucleotides: The next frontier for treatment of neurological disorders. *Nat. Rev. Neurol.* **14**, 9–21 (2018).
239. A. Vega-Loza, C. Van, A. M. Moreno, F. Aleman, Gene therapies to reduce chronic pain: Are we there yet? *Pain Manag.* **10**, 209–212 (2020).
240. D. Wang, F. Zhang, G. Gao, CRISPR-based therapeutic genome editing: Strategies and in vivo delivery by AAV vectors. *Cell* **181**, 136–150 (2020).
241. K. L. Montgomery, S. M. Iyer, A. J. Christensen, K. Deisseroth, S. L. Delp, Beyond the brain: Optogenetic control in the spinal cord and peripheral nervous system. *Sci. Transl. Med.* **8**, 337rv335 (2016).
242. B. L. Roth, DREADDs for neuroscientists. *Neuron* **89**, 683–694 (2016).
243. G. A. Weir, S. J. Middleton, A. J. Clark, T. Daniel, N. Khovanov, S. B. McMahon, D. L. Bennett, Using an engineered glutamate-gated chloride channel to silence sensory neurons and treat neuropathic pain at the source. *Brain* **140**, 2570–2585 (2017).
244. F. Michoud, C. Seehus, P. Schonle, N. Brun, D. Taub, Z. Zhang, A. Jain, I. Furfaro, O. Akouissi, R. Moon, P. Meier, K. Galan, B. Doyle, M. Tetreault, S. Talbot, L. E. Browne, Q. Huang, C. J. Woolf, S. P. Lacour, Epineural optogenetic activation of nociceptors initiates and amplifies inflammation. *Nat. Biotechnol.* **39**, 179–185 (2021).
245. R. Seth, S. S. Kuppalli, D. Nadav, G. Chen, A. Gulati, Recent advances in peripheral opioid receptor therapeutics. *Curr. Pain Headache Rep.* **25**, 46 (2021).

Acknowledgments: We thank our funding sources for support, all members of the DARPA Panacea Safe Therapeutic Options for Pain and Inflammation consortium for many discussions, and the NIH Medical Illustration Team and BioRender. **Funding:** The authors are supported by DARPA (HR0011-19-2-0022 to B.P.B. and C.J.W.); NIH (R35NS105076-01 and R01AT011447 to C.J.W. and NS036855 to B.P.B.); Regenerative Medicine Program (RMP) of the NIH Common Fund (to I.S.); NIH HEAL Initiative (to I.S.); Intramural Research Program, NCATS, NIH (to I.S.); and a National Research Foundation Fellowship (NRF-2018R1A6A3A03012431 to J.S.). **Competing interests:** C.J.W. and B.P.B. are cofounders of Nocion Therapeutics. C.J.W. is a founder of QurAlis. I.S. is coinventor on a patent for an iPSC-derived nociceptor differentiation protocol. All other authors declare that they have no competing interests.

Submitted 14 June 2021
Resubmitted 16 September 2021
Accepted 19 October 2021
Published 10 November 2021
10.1126/scitranslmed.abj9837

Science Translational Medicine

PAIN

Brain circuits for pain and its treatment

Nicole Mercer Lindsay[1,2], Chong Chen[1], Gadi Gilam[3], Sean Mackey[3]*, Grégory Scherrer[1,4]*

Pain is a multidimensional experience with sensory-discriminative, affective-motivational, and cognitive-evaluative components. Pain aversiveness is one principal cause of suffering for patients with chronic pain, motivating research and drug development efforts to investigate and modulate neural activity in the brain's circuits encoding pain unpleasantness. Here, we review progress in understanding the organization of emotion, motivation, cognition, and descending modulation circuits for pain perception. We describe the molecularly defined neuron types that collectively shape pain multidimensionality and its aversive quality. We also review how pharmacological, stimulation, neurofeedback, surgical, and cognitive-behavioral interventions alter activity in these circuits to relieve chronic pain.

INTRODUCTION

Chronic pain conditions are leading causes of disability and suffering

Chronic pain affects about 20% of the human population worldwide (*1*). Although chronic pain conditions do not directly cause death, they are major sources of disability and suffering. The Global Burden of Disease Study 2019 revealed that chronic low back pain was the single greatest cause of years lived with disability (YLDs) worldwide and that several other chronic pain conditions contribute as major sources of YLDs, including neck pain, migraine, osteoarthritis, other musculoskeletal disorders, and medication overuse headache (*2*, *3*). Furthermore, for patients affected by intractable conditions, the emotional burden associated with the prospect of living with daily pain and suffering can lead to mental disorders (*4*) and even suicide (*5*). In fact, chronic pain is considered both a symptom and a primary disease that brings about other illnesses such as depression (*6*, *7*). Because of this immense medical, economic, and social burden, achieving a better understanding of pain biology to develop targeted, novel, safe, and effective treatments has become a worldwide priority.

Pain mechanisms and treatment: peripheral divergence and central convergence

Most research and drug development efforts to discover effective analgesics focus on peripheral nervous system (PNS) and spinal mechanisms and targets. This strategy is motivated by the relative simplicity of this approach, given the pharmacological challenges associated with efficiently engaging brain targets without generating side effects, and the early description of a PNS cell type dedicated to generating pain, the primary afferent nociceptor (*8*). Successful identification of molecules either selectively expressed by nociceptors or that influence their function has been the main driver of analgesic drug development in recent decades (*9*), such as for ion channel transducers of the transient receptor potential channel family (TRP channels) (*10*, *11*). The recent resolution of dorsal root ganglion (DRG) neuron transcriptomes by RNA sequencing (*12*–*16*) and the discovery of additional potential drug targets, including in other DRG neuron types compared to nociceptors (for example, mechanosensory DRG neurons), as suggested by the role of the ion channel Piezo2 in mechanical allodynia (pain in response to light touch) (*17*, *18*), bring hope for the development of additional pain treatments targeting the PNS.

A complementary approach aims to identify analgesic targets that could directly act on the main concern of individuals living with pain—pain unpleasantness, suffering, and loss of control—by leveraging the latest knowledge of pain brain circuits. Indeed, peripheral nociception's cellular and molecular mechanisms are diverse and complex, corresponding to the function of the PNS to precisely detect, for each individual organ, a multitude of threatening environmental stimuli and/or internal dysfunctions. Notably, RNA sequencing studies also revealed dozens of DRG neuron types capable of generating pain (*12*–*16*); these studies and others have found that the mechanisms of function and molecular repertoires of these cells are dynamic and evolve considerably in an injury- and disease-specific manner as chronic pain develops. Considering a few common types of chronic pain conditions such as low back pain, osteoarthritic pain, migraine, cancer pain, or neuropathic pain (which on its own represents a broadly diverse group of conditions with diverse symptoms such as spontaneous pain and allodynia) illustrates that, for each condition, unique peripheral biological processes engage one or several distinct classes of molecularly defined primary afferent neurons to cause pain. Thus, the treatment of certain pain types by targeting nociceptors could prove exceptionally challenging, with each pain condition requiring specific research and drug development efforts, and the difficulty to faithfully model in animals some of the most prevalent human chronic pain conditions such as low back pain. Further complicating the targeting of peripheral biological processes for individual pain conditions is the growing recognition that these conditions frequently co-occur. This phenomenon, referred to as chronic overlapping pain conditions (COPCs), includes painful conditions such as temporomandibular disorder (TMD), fibromyalgia (FM), irritable bowel syndrome (IBS), vulvodynia, myalgic encephalomyelitis/chronic fatigue syndrome, interstitial cystitis/painful bladder syndrome, endometriosis, chronic tension-type headache, migraine headache, and chronic low back pain (*19*, *20*). Therefore, overcoming the

[1]Department of Cell Biology and Physiology, UNC Neuroscience Center, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. [2]Department of Biology, CNC Program, Department of Applied Physics, Stanford University, Stanford, CA 94305, USA. [3]Division of Pain Medicine, Department of Anesthesiology, Perioperative, and Pain Medicine, Stanford University School of Medicine, Palo Alto, CA 94304, USA. [4]New York Stem Cell Foundation–Robertson Investigator, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
*Corresponding author. Email: smackey@stanford.edu (S.M.); gregory_scherrer@med.unc.edu (G.S.)

emergent peripheral divergence of chronic pain mechanisms and COPCs represents an exciting challenge for the pain field (Fig. 1).

In contrast, after neurons of the trigeminal and spinal anterior/dorsal horn (DH) across all segmental levels process and transmit these diverse peripheral nociceptive signals to the brain, the brain's emotional circuits generate the unpleasant quality of pain across acute and chronic pain types, including COPCs. Thus, this convergent mechanistic organization of pain brain circuits, combined with the development of preclinical assays to interrogate the affective-motivational dimension of pain, provides an opportunity to develop treatments capable of limiting pain suffering and improving the quality of life of broad patient populations, regardless of their primary condition. In this review, we discuss the neural circuits that generate the emotional responses and negative affect during pain perception, and the therapeutic approaches that target these circuits to relieve pain suffering.

THE NEURAL BASIS OF PAIN

Pain multidimensional perceptions and behaviors

Pain is both a sensory and emotional experience. Philosophers have long debated how pain relates to the perception of noxious stimuli. Some argued that pain is the representation of a noxious object/event (representationalist approach, as for vision when we see an object), whereas others characterized pain as a feeling or experience with subjective properties (qualia) that are not necessarily related to that object/event (as is the case for referred pain) (*21*, *22*).

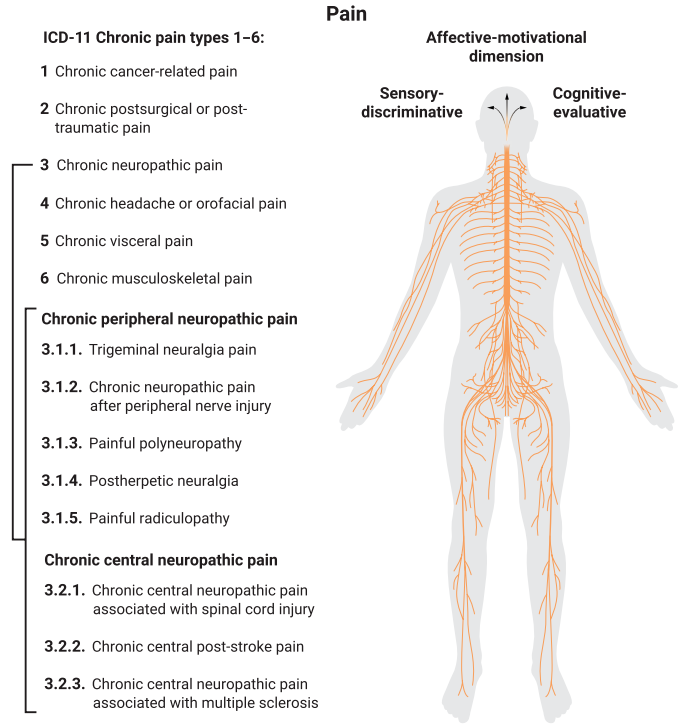


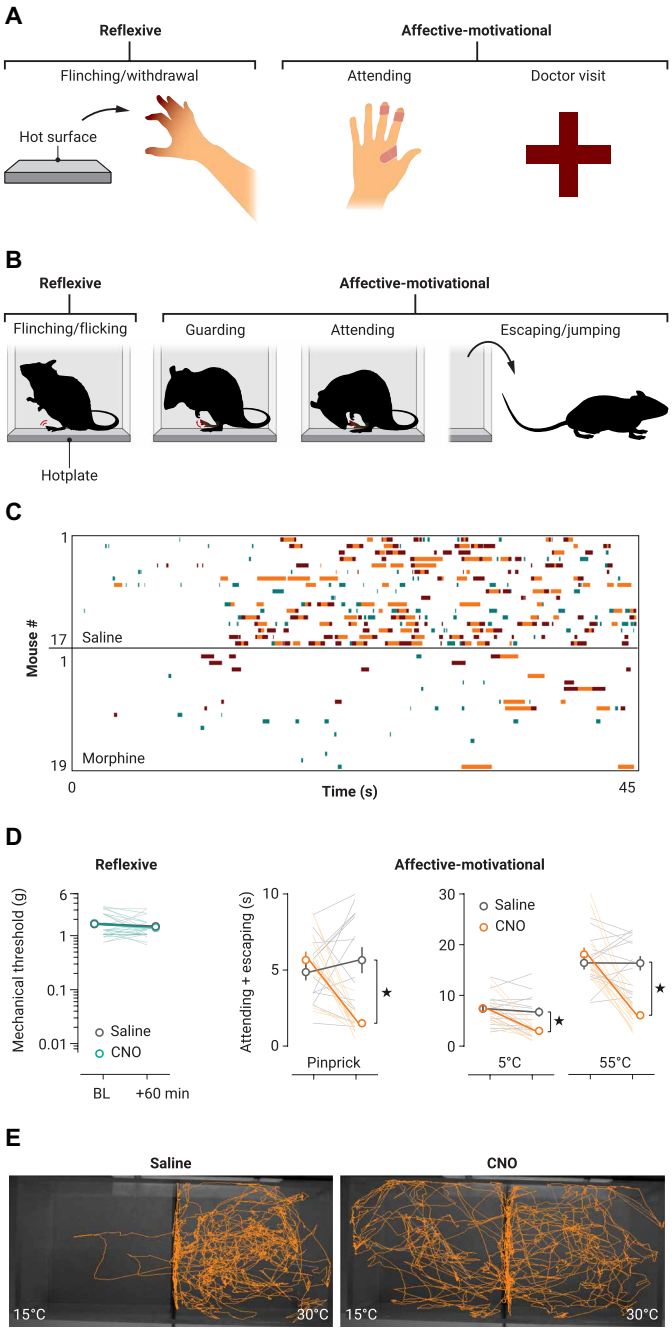
Fig. 1. Peripheral divergence and central convergence in pain mechanisms. The recent International Classification of Diseases (ICD-11) adopted by the World Health Organization describes chronic pain both as a primary disease and as a symptom of other illnesses, and divides it into six main categories. This figure illustrates the multitude of pain types (using neuropathic pain subtypes as an example) that can originate from various organs and tissues of the human body. In each case, nociception is initiated through a variety of complex cellular and molecular mechanisms. Acting on the common brain mechanisms that generate pain unpleasantness raises the possibility of treating chronic pain suffering across all pain categories at once.

CREDIT: ASHLEY MASTIN/*SCIENCE TRANSLATIONAL MEDICINE*

To reconcile these views, pain can be described as a complex multidimensional experience that includes sensory-discriminative, affective-motivational, and cognitive-evaluative components (*23*, *24*). Pain multidimensionality integrates (i) the somatosensory perception of the noxious object/event's features (such as location, temperature, and pressure), (ii) the encoding, within emotional and motivational circuits, of negative affect and the drive to halt the unpleasant percept, and (iii) an evaluation and modulation of pain experience by cognitive circuits. All three components are necessary to optimally select actions that limit exposure to noxious stimuli and pain experience.

As previously discussed for the field of emotions (*4*, *25*, *26*), understanding and treating pain affect require operational definitions that enable mechanistic studies. Pain includes both pre-cognitive physiological and behavioral responses (for example, withdrawal reflex and increase in heart and breathing rates) and cognitive processing of nociceptive information that leads to pain perception and affect; both are important for people living with chronic pain and can be defined and studied in animal models of pain (Fig. 2). First, primary afferent nociceptors [and, in the case of allodynia, non-nociceptive afferents (*27*)] engage motor and autonomic spinal/brainstem circuits to produce fast reflex responses, including withdrawal reflexes (Fig. 2A) (*28*). These stereotyped nocifensive responses, which persist in decerebrated animals (*29*, *30*), limit exposure to noxious stimuli and injury while nociceptive information is transmitted to and processed in the forebrain (*31*–*33*). The multidimensional pain perception is then generated and enables the selection of more complex adaptive behaviors. Specific behaviors are chosen from a panoply of possible nocifensive responses based on the features of the noxious event (sensory-discriminative component) and the expectation—derived from recalling previous experiences and an understanding of the context that led to and accompanies pain perception—that this action is the most likely to relieve pain unpleasantness and promote positive outcomes and survival (for example, attending, cooling down and putting a bandage on the affected body part in response to a mild burn injury; Fig. 2A). During this process, changes in pain perception and its context are continuously monitored and evaluated (cognitive-evaluative dimension). If the selected nocifensive behavior fails to relieve pain unpleasantness, another nocifensive behavior is selected (for example, planning a doctor visit to treat burn pain; Fig. 2A). In fact, in high-order species, conflicting needs can lead individuals not to engage in adaptive behaviors and instead to endure pain, when considering this action beneficial to achieve superiorly important or longer-term goals, at least when the pain condition is perceived as benign (for example, deciding not to go to the doctor and instead prioritizing participation in a work activity).

The temporal logic of nociceptive behavior organization during acute pain perception, with pain-limiting reflexive behaviors exhibited first, followed by reflective and voluntary nocifensive behaviors, is largely conserved between humans and rodents (Fig. 2, A and B). These behaviors can be studied in detail in mice experiencing pain during the hotplate test, if this assay is used to comprehensively analyze mouse behavior (Fig. 2, B to D), rather than scoring only the latency for the first nocifensive reflex. In an opioid pharmacology



CREDIT: ASHLEY MASTIN/*SCIENCE TRANSLATIONAL MEDICINE*

Fig. 2. Categorization of reflexive versus affective-motivational nocifensive behaviors to interrogate pain affect in rodents. (**A**) Examples of human responses to noxious stimulation, which include reflexive and affective-motivational behaviors. (**B**) Mouse responses to noxious stimulation, such as with the hotplate test, also include reflexive and affective-motivational behaviors like protective responses (such as guarding and licking of an affected paw) and escape seeking (for example, rearing and jumping). (**C**) Raster plots showing the nocifensive behavioral responses of individual mice in the hotplate assay and the reduction in both reflexive (green) and affective-motivational (orange, brown) pain behaviors after morphine administration. (**D**) In contrast to the effect of morphine (C), inhibition of nociceptive BLA neurons with hM4Di after injection of clozapine-*N*-oxide (CNO) reduces affective-motivational pain behaviors in the hotplate assay, but not reflexive withdrawal. (**E**) In a two-plate preference assay, CNO also decreases nerve injury-induced aversion to innocuous cool stimuli in the setting of neuropathic allodynia. Adapted from (*37*–*39*).

study that took advantage of single-, double-, and triple-knockout mice for opioid receptor subtypes, after opioid agonist administration intracerebroventricularly, only mu opioid receptor (μ or MOP receptor), but not delta opioid receptor (δ or DOP receptor), activation suppressed reflexive nocifensive withdrawal from noxious heat; however, activation of either μ or δ could result in antinociception if paw licking and jumping on the hotplate was measured to evaluate pain perception (*34*). Given the known differential expression of δ and μ receptors in the brain's pain pathways (*35*, *36*), these results suggested that distinct circuits (and molecules in these circuits) control different nocifensive behaviors during the hotplate pain experience. By annotating video recordings of mice exposed to noxious stimuli, raster plots can be generated to categorize and quantify the rapid and stereotyped reflexive paw withdrawal and flicks/flinches, versus the delayed, reflective, voluntary, and more variable behaviors aimed to minimize pain unpleasantness, which include attending to the affected paw (such as lifting, guarding, licking, and biting) and escape behaviors (searching for an escape route via exploration, rearing, and jumping; Fig. 2B) (*37*–*39*). Thus, each mouse displays a unique sequence of attending and escape behaviors (Fig. 2C), indicating that this method can also be used to study the mechanisms that underlie the idiosyncrasies of both the experience of pain and the expected efficacy of individual actions to provide pain relief. Given this variability, attending and escape behaviors can be grouped and labeled as affective-motivational behaviors (Fig. 2D). This categorization, which can be automated using deep learning approaches such as DeepLabCut or MoSeq, described elsewhere (*40*–*42*), complements other approaches such as conditioned place preference or avoidance paradigms (*43*, *44*), grimace scoring (*45*, *46*), and wheel running monitoring (*47*) to provide a more complete description of pain experience in nonverbal animals, which, combined with rigorous experimental design (*48*), may better predict the clinical efficacy of treatments than when relying solely on reflexive behavior–based measurements (*49*).

Brain circuits for pain experience (Fig. 3A)

Neuroimaging and neurophysiological studies in humans have shown that noxious stimuli elicit neural activation and connectivity patterns within and between numerous brain areas, including the somatosensory cortex, insular cortex (IC), various regions of the prefrontal cortex (PFC), anterior cingulate cortex (ACC), thalamus, periaqueductal gray (PAG), and cerebellum (*50*–*52*). Additional regions, including the basal ganglia, parabrachial complex, posterior cingulate, amygdala, hypothalamus, and supplementary motor area, show less consistent and more context-dependent responses to noxious stimuli. Earlier studies demonstrated a relatively consistent noxious stimuli–evoked response in some of these structures that correlated with the perceived intensity of pain, leading to the hypothesis of a specific network for pain perception, the "pain neuromatrix" (*50*, *51*). More recent evidence has refuted this hypothesis by challenging the notion that pain can be uniquely associated with a specific pattern of activated brain regions (*53*, *54*). Instead, it seems that pain perception engages brain regions that tend to coalesce in networks associated with the multidimensional components of pain experience and broader functionality related to multisensory integration, emotion regulation, general cognitive and attention processing, self-referential processing, and other functions (*55*, *56*). At the same time, a growing number of studies have used multivariate pattern analysis tools to capture, even within the same brain

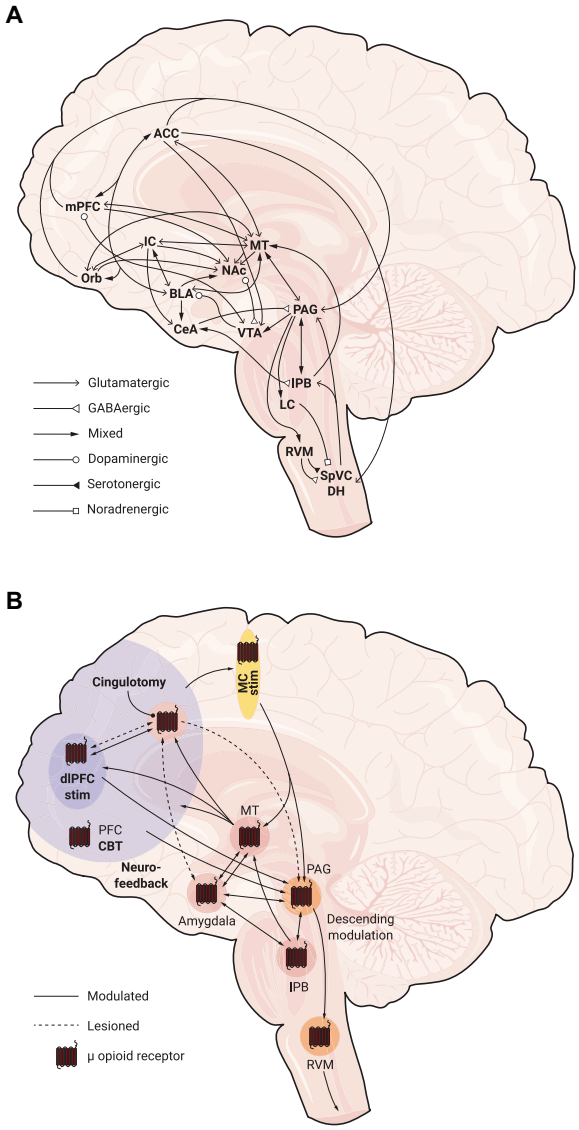


Fig. 3. Pain emotional and cognitive networks and treatments that can ameliorate chronic pain affect. (**A**) Primary afferent neurons synapse onto second-order neurons in the spinal dorsal horn (DH) or the trigeminal nucleus caudalis (SpVC). These neurons, in turn, project to the lateral parabrachial nucleus (lPB) and the periaqueductal gray (PAG), which then connect with the anterior cingulate, insular, and prefrontal cortices, medial thalamus, amygdala, nucleus accumbens, and hypothalamus to generate and modulate pain experience. Note, mixed arrows indicate glutamatergic and GABAergic pathways. (**B**) Prevalent treatments for pain commonly use opioid receptor signaling to induce a prominent action on pain affect circuits. Investigative treatments include motor cortex stimulation (MC stim), dlPFC stimulation (dlPFC stim), neurofeedback, and cognitive behavior therapy (CBT) that act on frontal cortex circuits to modulate pain. In severe cases of intractable pain, cingulotomy reduces chronic pain. Frontal cortex modulation is hypothesized to relieve pain through descending pain control in the PAG, but notable connections to the medial and intralaminar thalamus (MT) and to the parabrachial nucleus could also play a role. ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central amygdala; IC, insular cortex; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; Orb, orbitofrontal cortex; RVM, rostromedial ventral medulla; VTA, ventral tegmental area.

regions, fine-grained differential activation patterns between the distinct components or modalities of pain experience, in healthy individuals versus patients with chronic pain (*57*–*60*). These studies have produced interesting findings; for example, although the amygdala is thought to critically contribute to the affective component of pain experience, these experiments found no specific role for this brain region in the encoding of thermal pain (*58*). Together, these findings suggest that the experience of pain involves numerous interconnected brain structures working together, whereas more domain-general features of the underlying experience may have distinct neural coding through more specific pathways. Pain research in animals offers unique opportunities because it allows characterization (for example, genes and proteins expressed, electrophysiological properties, and connectivity) and causal determination of the function of individual neurons in some of the regions described above. We describe here some of the rodent studies exemplifying the utility of this approach. Nevertheless, we should acknowledge the debate regarding the degree of neurophysiological and anatomical congruence between the rodent and human brain. For example, there are inconsistencies regarding the function and anatomy of the PFC subregions and thalamic nuclei across species (*61*–*63*).

In rodents, as is the case in primates, DH nociceptive projection neurons, which comprise distinct populations located predominantly in lamina I and, to a lesser extent, in deeper DH laminae [there are also, in fact, a number of nociceptive projection neurons in the intermediate and ventral horn that remain understudied (*64*–*66*)], directly transmit nociceptive information to several brain regions in the medulla [nucleus of the solitary tract (NTS), inferior olive, and reticular formation], pons [parabrachial nucleus (PB) and reticular formation], midbrain (PAG, superior colliculus, and reticular formation), and forebrain (thalamus), with the two most thoroughly studied outputs being the PB and thalamus. Activity in these ascending pathways elicits sensory-discriminative and affective-motivational pain perceptions and the array of autonomic physiological responses (for example, increase in breathing rate and grimace) and nocifensive voluntary behaviors (such as attending and escape) that characterize pain experience in most mammals. Regarding the sensory-discriminative aspect of pain, we recommend other readings that describe the function in the representation and discrimination of noxious stimuli of the lateral thalamus [ventral posteromedial (VPM), ventral posterolateral (VPL), and posterior (Po) nuclei], zona incerta (ZI), primary and secondary somatosensory cortices (S1 and S2), PFC, and posterior insular cortex (pIC) (*67*–*69*). In this translational review, we describe recent advances regarding the organization of brain circuits that shape the affective-motivational and cognitive-evaluative dimensions of pain.

Transmission of nociceptive information to the forebrain: spino-parabrachial-amygdalar and spino-thalamo-cortical circuits

Parabrachial nucleus

The PB receives diverse interoceptive and exteroceptive sensory information and plays a vital role in generating a wide array of autonomic responses, such as for pain, respiration, or thermoregulation (*70*–*72*). The lateral PB (lPB) has long been known to receive inputs from nociceptive projection neurons of the contra- and ipsilateral spinal cord (SC) DH and spinal trigeminal nucleus caudalis (SpVC; the neuroanatomical name for the trigeminal DH)

and, to a lesser extent, from neurons located in deeper spinal laminae (*73*–*75*). Recently, the identification of marker genes that define distinct populations of spino-parabrachial and lPB neurons enabled detailed studies of lPB connectivity and function in pain. DH projection neuron populations characterized by the expression of distinct molecular markers [*Tac1*, *Tac1r*, *Gpr83*, or *Phox2a* (*31*, *76*–*78*)] differentially innervate the external, dorsal, and superior (or internal) subdivisions of the lPB (lPBe,d,s/l), with the lPBe additionally receiving inputs from *Trpv1*$^+$ trigeminal ganglion nociceptors (*79*, *80*). Ablation of *Tac1*$^+$ DH projection neurons, which innervate the lPBs/i, abolishes paw licking and conditioned avoidance, but not reflexive nocifensive behaviors, in response to sustained noxious stimulation. Optogenetic stimulation in lPB of axon terminals either from *Tac1*$^+$ (*78*), *Tacr1*$^+$, or *Gpr83*$^+$ (*31*) DH projection neurons or from *Trpv1*$^+$ nociceptors (*80*) drove acute and conditioned avoidance. The nocifensive responses engaged after activation of these different pathways are, however, distinct; for example, stimulation of *Gpr83*$^+$ lPB inputs induced forward locomotion, whereas stimulation of *Tacr1*$^+$ lPB inputs caused backward locomotion and jumping (*31*). Interestingly, in the setting of facial pain induced by capsaicin injection, optogenetic silencing of *Trpv1*$^+$ nociceptor axon terminals in the lPB not only produced preference for the light-paired compartment in a real-time place preference assay but also diminished brisk head withdrawal after stimulation with a von Frey filament, suggesting an action both on pain affect and on reflexive withdrawal, presumably through descending control of nociception in the trigeminal DH (*80*). However, as is typical for optogenetically or chemogenetically driven place aversion or preference experiments in the pain field, although avoidance or preference indicates the aversive versus rewarding quality of the manipulation, whether the percept that motivates the animal's avoidance or preference behavior resembles experiencing authentic pain or analgesia, respectively, requires further clarification. This question can be resolved by comparing neural dynamics (*37*). Remarkably, low-intensity optogenetic stimulation of *Gpr83*$^+$ DH projection neurons, which predominantly receive input from primary afferent mechanosensory neurons and not *Trpv1*$^+$ nociceptors, can also, in contrast to the *Tac1r*$^+$ projection neuron population, promote place preference, suggesting a dual function in generating rewarding or aversive somatosensory experiences (*31*). This result also illustrates the importance of mimicking physiological firing patterns in optogenetic sufficiency experiments. Together, these studies support the idea that the lPB nociceptive circuits are essential for the expression of pain precognitive emotional physiological responses and behaviors. Furthermore, PB neurons integrate competitive signals that modulate pain, such as hunger, which inhibits nociception through inputs from hypothalamic agouti-related protein (*Agrp*$^+$)-expressing neurons and neuropeptide Y signaling in the PB (*81*).

Amygdala

Although the amygdala is prominently considered a key brain region involved in emotional experiences, research has shown that it plays a broader role, including processing and coding the biological value of various types of salient stimuli (*82*–*85*). Basic pain research on the amygdala first identified nociceptive neurons in the central amygdala (CeA), a predominantly gamma-aminobutyric acid–ergic (GABAergic) nucleus, and examined their physiological properties and connectivity, including with the lPB (*86*–*90*). These CeA GABAergic neurons include a distinct ensemble of neurons that are activated by general anesthesia and inhibit pain (*91*). Recent studies have begun to investigate distinct populations of lPB neurons, defined by expression of *Calca*, *Tac1*, *Nts1*, *Pdyn*, *Sst*, and/or *Tac1r* (*29*, *70*–*72*, *92*–*95*). Together, these studies suggest that lPB neurons that receive monosynaptic input from DH projection neurons transmit nociceptive information to the lateral subdivisions of the CeA (CeL) [and the laterocapsular subdivision (CeCL), often referred to as the "nociceptive amygdala"] through two populations of *Slc17a6*$^+$ (VGLUT2$^+$) neurons: (i) *Calca*$^+$ *Slc17a6*$^+$ lPBe neurons, via *Pdyn*$^+$ lPB neurons (*92*, *93*), and (ii) intralaminar (ILN) and midline thalamic (MThal) neurons, via *Tac1r*$^+$ lPB neurons (*94*, *95*). In addition to the CeA, ILN, and MThal, these molecularly defined populations of lPB neurons differentially project to the bed nucleus of the stria terminalis (BNST), ventromedial hypothalamus (VMH) and lateral hypothalamus/parasubthalamic nucleus, lateral and ventrolateral PAG (lPAG and vlPAG), superior colliculus, MThal, medial PFC (mPFC), and insular cortex (IC) (*29*, *93*–*96*). Functionally, silencing *Calca*$^+$ lPBe-to-CeA neurons with the light chain of tetanus toxin (TetTox) inhibited footshock-induced immediate locomotor response and nocifensive jump response in the hotplate test, without altering the latency for reflexive withdrawal from noxious heat or the force of mechanical stimuli necessary to elicit a withdrawal reflex (*92*). These findings indicate the necessity of *Calca*$^+$ lPBe-to-CeA neurons for innate escape behaviors after noxious stimulation, consistent with the essential function of the lPB for a variety of interoceptive and exteroceptive autonomic responses to threat (*71*). However, another study comparing the behavioral effects resulting from optogenetic stimulation of distinct lPB outputs found that activation of the lPBe-to-CeA pathway caused no substantial movement, whereas activation of either lPBd-to-VMH or lPBd-to-PAG neurons increased locomotion and jumping (*93*). Both studies provide evidence that the lPB-to-CeA circuit is necessary for aversive memories, albeit by manipulating different populations of neurons using dissimilar protocols: *Calca*$^+$ lPBe-to-CeA neurons in a footshock-based fear conditioning assay (*92*) or *Pdyn*$^+$ lPBd neurons in an intraplantar formalin-induced conditioned place avoidance assay (*93*). *Tac1r*$^+$ lPB neurons receive ipsi- and contralateral monosynaptic inputs from DH projection neurons and are activated in response to noxious stimuli (*95*). Chemogenetic activation of *Tac1r*$^+$ lPB neurons, which can disynaptically relay nociceptive information to the CeA, facilitated jumping in the hotplate test (*95*), as well as escape responses and nocifensive behaviors (for example, licking) in response to tail clip and after intraplantar injection of the TRPA1 agonist allyl isothiocyanate (AITC) (*95*) or formalin (*94*). Formalin-induced flinching (*94*) and the latency of the first nocifensive response on the hotplate (*95*) remained unaffected. Silencing of *Tac1r*$^+$ lPBs almost completely eliminated licking induced by tail clip or AITC (*95*). *Tac1*$^+$ lPB neurons include a subset of *Calca*$^+$ lPBe-to-CeA neurons, as well as a different population of neurons that project to the medullary reticular formation region (MdD), which contains forelimb premotor neurons (*29*). Remarkably, in the hotplate test, either chemogenetic or optogenetic stimulation of *Tac1*$^+$ lPB neurons resulted in immediate and repetitive jumping behavior and decreased licking. Complex CeA microcircuits, composed of multiple molecularly defined populations (such as *Sst*$^+$, *Pkcd*$^+$, and *Crh*$^+$) with distinct connectivity and functions (*97*–*101*), process nociceptive information, which is transmitted from the CeL to the medial subdivision (CeM), the major output region of the CeA, and then to

brainstem structures such as the PAG (*99*, *102*) that mediate defensive behaviors. Physiological studies have demonstrated that calcitonin gene-related peptide (CGRP), which is encoded by *Calca*, facilitates *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic transmission at these lPB-to-CeL synapses (*103*), which show postsynaptic neuron type–specific (*Som*$^+$ versus *Crh*$^+$) alterations in synaptic transmission after sciatic nerve injury (SNI) (*104*). It is worth noting that *Pdyn*$^+$, *Sst*$^+$, and/or *Crh*$^+$ GABAergic CeA neurons project back to the lPB. This inhibitory pathway normally inhibits nocifensive behaviors; however, CeA-to-lPB inhibitory inputs are reduced after infraorbital nerve injury (*105*). A systematic comparison between the different lPB and CeA outputs, using the same silencing/activating tools and behavioral assays to interrogate distinct aspects of the pain experience, could further clarify the contributions of the lPBe-to-CeA and other lPB output circuits to pain. Optogenetic manipulation of the CeA and connected descending circuits in the lPAG and downstream reticular formation motor networks [the dorsal and ventral medullary reticular formation (MdD and MdV), sometimes called the magnocellular reticular nucleus (Mc)] can produce freezing/immobility and/or flight behaviors in the absence of noxious stimulus or conditioning (*106*). In the same study, the authors reported that optogenetic activation of *Slc17a6*$^+$ (VGLUT2$^+$) lPAG neurons increased withdrawal latency in the tail immersion test. In another study, photostimulation of *Pdyn*$^+$/*Penk*$^+$/*Slc17a6*$^+$ lPB neurons that project to the hypothalamus preoptic area (POA) could induce hypothermia, aversion, and suppression of locomotion (*107*). Disentangling effects on movement from those on nociception and pain experience may not be trivial. If changes in reflexive nocifensive responses after lPB, CeA, and lPAG neuron manipulations result from descending inhibition of nociception in the DH [presumably via the rostral ventromedial medulla (RVM)], one would expect to observe an antinociceptive effect that reduces not only withdrawal reflexes but also affective-motivational pain behaviors. Crucially, the maladaptive nocifensive responses observed when manipulating activity in lPB and CeA circuits (such as immediate and repetitive jumping upon *Tac1*$^+$ lPB neuron activation in the hotplate assay or absence of jumping when silencing *Calca*$^+$ lPBe-to-CeA neurons) illustrate the critical control function of cortical and subcortical structures. In these optogenetic and chemogenetic experiments, cortical and subcortical cognitive inputs are shunted during pain experience, resulting in failure to compute a wealth of information necessary to conceive plans (understanding current context, recalling memories from previous painful experiences, and formulating expectations) to select, among a wide variety of choices, the antinociceptive behaviors that are most likely to succeed, attempt them, and, in case of failure, adjust by selecting other behaviors (expectation violation and reformulation). Together, these results support the idea that lPB and CeA circuits mediate the expression of autonomic physiological effects and behaviors in response to noxious stimuli through connections with brainstem and hypothalamic effectors.

To be useful as a learning signal, the negative valence of acute (nociceptive) pain must be contextualized. Only then can an animal learn and thereby improve its ability to both avoid and respond to noxious stimuli in a context-specific manner to halt pain. For patients with chronic pain, the contextualization and constant evaluation of pain affect through cognitive circuits seem to drive emotional suffering and pain catastrophizing. Catastrophizing reflects maladaptive cognitions in response to actual or anticipated pain and has been associated with poor and deteriorating outcomes for people with chronic pain (*108*–*110*). A recent systematic review (*111*) in both healthy individuals and patients with chronic pain indicates that the brain regions most commonly linked to pain catastrophizing are those consistently active during pain processing and associated with the multidimensionality of pain, including the somatosensory cortex, thalamus, IC, ACC, and medial and dorsolateral PFC (dlPFC). The amygdala was also shown to play a role, although to a lesser extent. In healthy participants, during moderate pain, catastrophizing was negatively associated with neural activation in the amygdala (*112*). Compared to healthy controls, patients with chronic pain exhibited greater connectivity between the amygdala and a network of regions involved in cognitive processing, which was strongest in patients with the highest tendency to catastrophize (*113*). Moreover, patients showed decreased basolateral amygdala (BLA) connectivity to a network of regions involved in self-referential compared to healthy controls. Combatting this deleterious process is a major therapeutic goal. The BLA, unlike the CeA, is densely connected with cortical and subcortical cognitive circuits that process and contextualize affective information. Rodent studies have established that the BLA contains predominantly *Slc17a7*$^+$ (VGLUT1$^+$) pyramidal neurons that project to the CeA and the striatum, particularly to the nucleus accumbens (NAc) (*98*). Over the course of evolution, the size of the BLA versus the CeA within the amygdaloid complex markedly increased (*84*), evincing the critical importance of the BLA in human emotions and presumably in pain affect. However, considerably fewer rodent mechanistic studies have interrogated the contribution of BLA neurons to pain experience. Although footshock has been used extensively in the learning and memory field to investigate BLA function, the representation in the BLA of footshock and that of purely noxious stimuli considerably differ (*37*), presumably because the footshock generates activity in non-nociceptive primary afferents (such as mechanoreceptors and proprioceptors), producing an experience that is unquestionably aversive for the animal, but that does not precisely mimic pain experience. On the other hand, patient H.M., who had a temporal lobectomy that ablated most of the amygdala, including the BLA, but preserved the centromedial nucleus, could detect thermal noxious stimuli and report their intensity, but neither characterized them as painful nor showed motivation to avoid them (*114*, *115*). Recently, in vivo optical recordings of about 17,000 neurons in freely behaving mice encountering noxious stimuli, combined with the chemogenetic manipulation of BLA neurons active during pain, enabled the identification of a distinct neural ensemble of *CamkIIa*$^+$ *Rspo2*$^+$ BLA neurons that specifically encodes the negative affective valence of noxious stimuli across pain modalities (heat, cold, and mechanical) and is necessary for the behavioral manifestation of pain affect (*37*). Inhibition of this nociceptive ensemble using genetic tagging in TRAP mice and $G_{i/o}$ protein–coupled DREADDs (hM4Di) alleviated pain affective-motivational behaviors (attending and escape) without altering withdrawal reflexes, anxiety, or reward. Moreover, functional studies of this nociceptive ensemble revealed a causal neural basis for allodynia. Specifically, after peripheral nerve injury, innocuous stimuli begin to activate this nociceptive ensemble to drive dysfunctional perceptual changes associated with neuropathic pain, including aversion to light mechanical and cool stimuli, as reported in patients. Interestingly, this recoding phenomenon resembles that which occurs during fear conditioning, when the representation of the conditioned

stimulus (CS) becomes more similar to that of the unconditioned stimulus (US) (*116*), suggesting that pain chronification and associative learning share common BLA mechanisms, consistent with the view that aspects of the chronic pain disease state result from maladaptive plasticity in learning circuits. Neuroanatomical and electrophysiological studies have revealed the extensive connectivity of this nociceptive ensemble, including monosynaptic inputs from cortical areas such as the ACC and IC, MThal and hypothalamus, and projections to numerous regions such as the ACC, CeA, and NAc (*117*). In these pathways, altered activity in the BLA during chronic pain, including in arthritis pain models, results in enhanced feedforward inhibition both of mPFC pyramidal neurons, impairing decision-making, and of CeA and intercalated cell (ITC) masses (*101*, *118*), which are small clusters of tightly packed GABAergic neurons that receive BLA inputs and synapse onto CeA neurons.

Thalamus

In human functional magnetic resonance imaging (fMRI) studies, the thalamus is one of the brain regions most consistently activated by painful stimuli (*119*). The ILN and MThal, the latter of which includes the mediodorsal (MD) thalamus, are the major thalamic nuclei involved in pain affect and cognitive-evaluative processing of pain (*120*). As part of the dorsal thalamus, the ILN and MThal regions are composed almost entirely of *Slc17a6*$^+$ (VGLUT2$^+$) glutamatergic, excitatory neurons and are modulated by inhibitory neurons in the thalamic reticular nucleus and ZI. This region of the thalamus receives a confluence of nociceptive, arousal, and visceral information, notably not only from the *Tacr1*$^+$ neurons in the lPB (*94*), the NTS (*121*), and the PAG, but also from brainstem arousal nuclei like the pedunculopontine nucleus, locus coeruleus, and various parts of the reticular formation (*122*, *123*), as well as sparse inputs directly from the SC DH and SpVC (*124*). These diverse ascending signals are integrated with forebrain thalamo-amygdalar, thalamo-striatal, and thalamo-cortical loops (*125*–*127*). The ILN and MThal are composed of many small nuclei including the central medial (CM), parafascicular (Pf), central lateral (CL), reunions (Re), and submedius (Sm) (*119*). Sequencing data and axon morphology stratify the ILN and MThal neurons into two classes: The ILN and MThal nuclei excluding MD show similar RNA profiles, whereas the MD thalamus more closely resembles so-called higher-order processing thalamic nuclei like the posterior (Po) thalamus for somatosensation and the lateral posterior (LP) thalamus for vision (*128*). Each of the small nuclei in the ILN and MThal has distinct connections with the PFC (*122*, *123*, *129*, *130*), and some are known to play a specific role in processing pain affect (*126*, *131*, *132*). One well-studied example shows that the Sm nucleus, through its prime prefrontal partner, the ventrolateral orbitofrontal cortex (Orb), engages the vlPAG descending pain control circuits using opioid peptides, serotonin, dopamine, and glutamate (*131*). Furthermore, separate modulation of MD pathways to either the BLA or ACC was found to inversely modulate pain-related aversion (*127*). As a final example, a recent study showed that, when the CM nucleus is lesioned before nerve injury, mechanical hyperalgesia failed to develop, and revealed that the CM receives vlPAG inputs and sends outputs to excitatory neurons in the BLA that could mediate this effect (*132*). Although there is evidence for the specific roles of the ILN and MThal in acute and chronic pain, more emphasis must be placed on specific pathways to fully dissect the role of the thalamus in pain affect, particularly circuits connecting the ILN, BLA, and cortical hubs for pain affect such as the ACC and IC, which themselves have dense reciprocal connections with the BLA (*133*, *134*).

Cortical circuits involved in the affective-motivational and cognitive-evaluative dimensions of pain

The insular, anterior cingulate, and prefrontal cortices play important roles in mediating the cortical components of the affective-motivational and cognitive-evaluative aspects of pain experience. Human imaging studies performed during acute pain have specifically identified that the IC-to-PFC pathway is activated by discrimination of pain intensity, whereas the dlPFC is activated during spatial discrimination of pain (*135*).

The IC is one of the brain regions most consistently activated in fMRI during pain (*56*, *136*) and while observing others in pain (*137*), and is the only cortical region that can be stimulated to induce pain experience (*138*). The anterior IC (aIC) and pIC receive visceral and nociceptive information through reciprocal connections with the PB, NTS, and ILN/MThal (*139*) and integrate this information with sensory and cognitive cues to generate internal and emotional states (*140*). The IC is thought to serve as a bridge for the exchange of pain affective and sensory-discriminative signals through reciprocal connections between the pIC, which connects to S1, S2, and lateral thalamus, and the aIC, which connects with the Orb, NAc, and ILN/MThal (*139*). Optogenetic inhibition of *CamkIIa*$^+$ neurons in the pIC of mice and transcranial magnetic stimulation (TMS) of the pIC in humans lead to enhanced a decrease in capsaicin-induced mechanical hypersensitivity and increased heat pain thresholds, respectively (*141*, *142*). Lesions of the pIC, but not of the ACC, prevent long-term mechanical hypersensitivity in sciatic nerve–injured mice (*143*). Together, these studies suggest that the pIC modulates the sensory-discriminative component of pain (*141*, *142*). In contrast, the aIC is thought to be important for pain affect and for its relief, including via μ opioid receptors (*144*, *145*). Injections of morphine into the aIC resulted in reduced nocifensive behaviors after hindpaw formalin injection (*144*).

Although the IC reciprocally connects to the BLA, these inputs display topographical patterns. The aIC preferentially targets excitatory outputs to the anterior BLA, the region preferentially associated with positive-valence neurons (*146*). In contrast, the pIC sends dense excitatory outputs to the posterior BLA, which is thought to be involved in negative valence processing. The entirety of the IC also sends excitatory projections to the CeA, which can drive descending circuits that mediate nocifensive behaviors. How these pathways encode pain affect and aversion during painful situations remains unexplored; however, conditioned taste aversion assays have implicated the necessity of the IC-to-amygdalar pathways (*133*, *147*). Activation of IC-to-BLA projection neurons during a pleasurable consumption (saccharin) induced aversion to an otherwise positive cue (*133*). These studies suggest an important role for these reciprocal IC-amygdalar connections in generating the negative valence of pain (*148*).

The ACC contributes to numerous functions related to cognition (such as attention or learning), socio-emotional processes (like reward or empathy), and somatosensation, and although it is undoubtedly engaged during pain, there remains an ongoing debate as to the precise nature of its contribution (*149*–*151*). The ACC described here is distinct from the more caudal midcingulate cortex (MCC); these cingulate regions contribute differently to nocifensive behaviors (*142*, *152*–*154*). In humans with ACC cingulotomies and

animal models involving lesions to the ACC, pain aversiveness is often diminished, with minimal impacts on executive, cognitive, or motor functions (*155*, *156*); however, this decrease in pain affect may be disorder- and/or context-specific, as shown by a case study in which a patient with schizoaffective disorder reported increased pain after cingulotomy (*157*). Structural changes have been observed in layer 2/3 (L2/3) of the ACC after induction of chronic pain in rodent models [recently reviewed here (*69*, *158*)]. After the development of chronic pain in mice with SNI, L5 pyramidal neurons in the ACC have increased dendritic integration due to a decrease in hyperpolarization-activated cyclic nucleotide-regulated (HCN) channels, which is reversed by the serotonergic agonist 5-HT_7 (*159*). A second study found HCN channel dysfunction in L2/3 pyramidal neurons in the ACC and mPFC developed after SNI in rats (*160*), further suggesting HCN channel function in the ACC changes during chronic pain.

Optogenetic activation of pyramidal neurons in the rodent ACC increases pain-related aversive behaviors. Optogenetic stimulation of pyramidal *CamkIIa*$^+$ ACC neurons abolishes ketamine-induced reductions in aversion to a pinprick-paired chamber in a conditioned place preference assay (*161*). Optogenetic inhibition of ACC neurons in rats with either chronic constriction of the trigeminal nerve or SNI resulted in a reduction of cold hypersensitivity, similar to what is observed after ACC lesion in rodents or cingulotomy in humans (*162*, *163*).

Bidirectional modulation of the ACC in the context of chronic pain can induce or abolish negative pain affect, resulting in secondary effects on mood, such as anxiodepressive phenotypes similar to those observed in patients with chronic pain. Lesions of the ACC abolish anxiodepressive-like behaviors in mice with SNI, including immobility during the forced swimming test and aberrant grooming behavior observed after splash (*143*). Conversely, optogenetic activation of predominantly *Thy1*$^+$ pyramidal neurons in L2/3 and L5 of the ACC induces anxiodepressive phenotypes in healthy mice, consistent with nerve-injured mice (*143*). Slice electrophysiology studies revealed presynaptic and postsynaptic long-term potentiation mechanisms in the ACC that have been associated with chronic pain and comorbid anxiety (*164*).

The ACC input and output circuits regulating pain affect are being explored in rodents using electrophysiology, calcium imaging, and manipulation of subcircuits with opto- and chemogenetics. Experiments examining the relationship between the ACC and MD thalamus show that noxious stimulus-evoked activity in acute and chronic pain states transmits through the MD thalamus before reaching the ACC and that lesioning the MD thalamus abolishes aberrant spiking in the ACC (*165*). This study reported that the MD thalamus inputs to ACC L2/3 are responsible for transmitting aberrant spiking activity to L5 neurons that, in turn, project to the BLA and dorsolateral PAG (dlPAG) as well as back to the MD thalamus (*127*, *165*, *166*). Optogenetic activation of the ACC-to-MD pathway was mildly aversive, as evidenced by a slight avoidance of the side paired with optogenetic stimulation in a place preference assay (*127*). In contrast, optogenetic activation of the ACC-to-BLA pathway reduces SNI-associated aversion for the optogenetic stimulation-paired chamber (*127*). fMRI studies in humans show ACC activation during pain or pain relief, as well as when observing another human in pain (*167*, *168*). A meta-analysis of fMRI during pain empathy consistently observed activation of the posterior ACC/anterior MCC border region and aIC and hypothesized an instrumental role for these two regions in empathy (*137*). Recent studies have shown that rodents likewise respond to social contagion with prosocial behaviors (*169*). Mice observing other mice with an acute inflammatory injury have decreased nocifensive thresholds; furthermore, this social transfer of pain is dependent on a pathway from the ACC to the NAc (*170*).

Although both the human and rodent PFC are similarly involved in decision-making, identification of reward, and executive functions, the rodent PFC differs in important ways from the human PFC. The most functionally analogous rodent structure to the human dlPFC lies within the rodent mPFC. Furthermore, the entire rodent PFC is agranular, whereas in humans, the mPFC, dlPFC, and most of Orb are granular (in other words, the mPFC and Orb lack a cortical L4 in rodents) (*171*–*173*). Although rodents might lack complex abstract thought, they show affective-motivational and cognitive-evaluative behaviors in response to painful stimuli not altogether dissimilar from those of humans (attending to injury, avoidance, etc.) (Fig. 2, A and B).

The mPFC, composed of the infralimbic (IL) and prelimbic (PL) cortical regions, and Orb are particularly well studied for their roles in Pavlovian and instrumental conditioning (*174*, *175*), both of which are driven by reward or punishment (for example, pain relief or pain). As previously discussed, the Orb receives input from the Sm nucleus of the ILN and receives notable inputs from the IC and ACC that create associations between pain and environmental cues conveyed from secondary somatosensory cortex or other higher-order sensory cortices (*176*). The Orb responds to a diverse set of nociceptive stimuli (cutaneous, visceral, and thermal) and can act on descending pain control through its direct output to the vlPAG (*131*).

The mPFC plays a key role in generating complex associations using working and long-term memory. A gradient has been observed from the ACC ventrally through the PL and IL that demonstrates the importance of the more dorsal ACC (dACC) and PL for memory retrieval, whereas the ventral IL is important for working memory (*177*). Mice with SNI exhibit altered mPFC-to-hippocampus oscillation patterns and decreased working memory (*178*). The PL and IL regions change distinctively during chronic pain. The PL had no change in density of FOS protein (an immediate early gene that reports recent neural activity) in mice observing a cagemate in pain; however, there was an increase in FOS expression after observing a stranger in pain (*179*). Acute blockade of the glucocorticoid stress response in the PL induces a social transfer of pain for stranger mice similar to that for cagemates, whereas injection of corticosterone in the PL reduces the social transfer of pain for cagemates (*179*). Inputs to the PL region from the ventral tegmental area (VTA) release dopamine, which induces antinociception in a mouse model of chronic pain by activating PL-to-dlPAG neurons (*180*). Bilateral lesions of the PL, but not IL, result in heat hypersensitivity and anxiety-like behaviors (*181*). Optogenetic inhibition of PL pyramidal *CamkIIa*$^+$ neurons induces anxiety-like behaviors, suggesting that the PL is involved in the regulation of social context and anxiety related to pain (*181*). The IL tends to show less distinctive changes during acute or chronic pain; however, BDNF protein decreases in the IL after peripheral inflammatory injury, and infusion of BDNF in the IL reverses inflammatory hypersensitivity (*182*). Further discussion of the distinct roles of the mPFC in chronic pain can be found elsewhere (*183*).

The PFC and ACC play critical roles in modulating pain experience based on the expectation of pain or pain relief. In humans, this

effect is often associated with the expectation of treatment. Human fMRI and positron emission tomography scans have paved the way to understanding the brain circuits underlying this phenomenon. Across the entire brain, fMRI studies have associated placebo analgesia, a phenomenon in which pain perception is shaped by expectation, with correlated activity in the PFC, ACC, hippocampus, PAG, pons, and cerebellum (*184*–*188*). Placebo analgesia in humans has recently been reviewed (*135*). Recent and ongoing work in rodents has used operant conditioning, which allows more precise circuit dissections to understand the precise pathways that mediate placebo or nocebo effects. For example, pairing opioids or aspirin with a CS cue showed that rodents can anticipate analgesia (*189*). Further work is needed to fully establish rodent models of placebo analgesia to take full advantage of the genetic and circuit dissection tools available.

PFC outputs to the PAG are believed to play a critical role in modulating pain by activating the descending pain control pathways from the PAG to the RVM and are discussed later in this review. Together, the PFC consolidates pain affective information and sensory features, evaluates motivational factors, and computes a course of action, effected through motor circuits, to halt or choose to endure pain.

Midbrain circuits for reward and aversion, and the motivation-decision model of pain

Pain is aversive, whereas pain relief is rewarding. The motivation to avoid pain and seek pain relief is generated through dopaminergic VTA and substantia nigra compacta (SNc) outputs, particularly to the NAc (mesolimbic dopaminergic system) (*190*). Human fMRI studies have revealed the involvement of the VTA and NAc both during pain and when anticipating pain or its relief, as well as altered functionality during chronic pain (*191*–*197*), consistent with the dual function of the VTA-to-NAc pathway in processing both rewarding and aversive stimuli. Rodent studies have shown NAc responses analogous to those in humans during pain onset and offset (*198*) and have enabled investigation of the anatomy and function of discrete VTA and NAc cell types and circuits in aversion and reward (*190*, *199*–*203*). For example, in a mouse model of nerve injury–induced neuropathic pain, increased excitability of NAc indirect pathway medium spiny projection neurons (MSNs) increased mechanical allodynia (*204*). In a rat model of migraine, vlPAG inputs to the VTA are required to generate conditioned place avoidance (*205*, *206*). Remarkably, dysfunction in the mesolimbic dopaminergic system during chronic pain also involves non-neuronal cells, including activated microglia in the VTA that can alter dopamine release in the NAc (*207*). In addition, the decreased motivational drive that can accompany chronic pain has been associated with galanin receptor 1–induced depression of excitatory synaptic transmission in NAc indirect pathway MSNs (*208*). Inhibition of κ opioid receptor signaling in the NAc using the selective antagonist NorBNI or chemogenetic inhibition of NAc dynorphin-expressing (*Pdyn*$^+$) MSNs restored normal motivation in a model of chronic inflammatory pain (*209*). Note that alongside this mesolimbic dopaminergic pathway, which mediates learning and anticipation of pain, the mesocortical dopamine system entrains the relative reward value (*190*), both systems defining the aversiveness of the situation and urgency to respond during pain. Crucially, pain aversiveness is often perceived in the context of other conflicting goals; cortical inputs to the NAc resolve these motivational conflicts and implement action decisions based on predictions (*210*, *211*). Glutamatergic projections from the ACC, IL, and PL regions to the NAc and VTA regulate approach-avoidance behaviors (*212*–*214*). Chemogenetic inhibition and optogenetic excitation of the IL-to-NAc pathway revealed an essential role for determining the approach-avoid balance in response to a pain-predictive cue (*212*). Pairing chemogenetic inhibition of either the ACC-to-NAc and ACC-to-VTA (*214*) or PL-to-NAc (*213*) projections with a chamber in a conditioned place paradigm led to chamber preference in chronic injury rats, but not controls. The importance of reward circuits and motivation in the context of pain has been thoroughly reviewed elsewhere (*192*, *210*, *211*, *215*, *216*).

Descending circuits for pain modulation

Activity in forebrain and midbrain circuits can profoundly influence nociception at the spinal level through direct cortico-spinal connections or medullary relays (*217*–*222*). For example, ACC neuron axon terminals, which can be observed in the SC DH, facilitate spinal excitatory transmission and behavioral hypersensitivity (*223*). Furthermore, neurons of the somatosensory cortex also innervate the DH, control tactile sensitivity, and contribute to tactile allodynia during neuropathic pain (*224*). The PAG critically contributes to descending pain modulation by integrating forebrain and midbrain inputs and, through neurons located predominantly in its lateral and ventral quadrants (vlPAG), by engaging distinct populations of RVM neurons that project to the DH and facilitate or inhibit nociception. Three populations of nociceptive RVM neurons have been defined: (i) On-cells show a burst in firing rate before a nociceptive withdrawal reflex and facilitate pain; (ii) off-cells fire tonically, pause during withdrawal reflexes, and inhibit pain; and (iii) neutral cells show no alteration in firing pattern during a nociceptive reflex, and their role remains less well understood (*218*, *219*, *225*). Recent studies have begun to elucidate the molecular identity of some of these RVM-to-SC neurons, their connectivity, and modulatory function in distinct pain modalities (*218*, *226*). A population of dual GABAergic and enkephalinergic (*Penk*$^+$) RVM-to-SC neurons reduces behavioral sensitivity to both heat and mechanical stimuli (*227*). In contrast, another population of GABAergic, but Penk-negative, RVM-to-SC neurons facilitates mechanical pain by inhibiting spinal GABAergic and enkephalinergic (*Penk*$^+$) neurons that normally presynaptically inhibit mechanosensitive primary afferent DRG neurons via $GABA_A$ and opioid receptors located on their central terminals (*228*). These RVM-to-SC neurons express the μ opioid receptor and represent a class of RVM on-cells. Alternatively, RVM neurons can modulate nociception by synapsing directly onto the central terminals of nociceptors and controlling their release of glutamate. Thus, RVM-to-SC serotonergic neurons release serotonin (5-HT) onto 5-HT3A- and TRPV1-expressing nociceptors, which sensitizes TRPV1 and causes hyperalgesia (*229*). In addition to GABA and 5-HT, another neurotransmitter, noradrenaline (NA) from the locus coeruleus (LC), critically contributes to descending pain modulation. Remarkably, activation of LC neurons that project to the SC inhibits nociception and can relieve neuropathic pain, whereas activating forebrain-projecting LC neurons increases spontaneous pain (*230*). This engagement of LC neurons for descending antinociception may depend on phospholipase Cβ4 (PLCβ4) signaling in PAG-to-LC neurons (*231*). These descending pain control systems show considerable sexual dimorphism (*232*), as well as modulation by

additional antinociceptive and pronociceptive endogenous molecules and drugs such as hormones, neuropeptides, cannabinoids, and nicotine (*233*–*237*). Last, note that the vlPAG also contains ascending pain modulatory neurons; a recent study described a class of vlPAG/dorsal raphe nucleus dopamine antinociceptive neurons that project to the BNST (*238*). Remarkably, this cell population shows sexual dimorphism; its optogenetic activation inhibited nocifensive behaviors resulting from inflammatory pain in male, but not female, mice.

MANIPULATING THE BRAIN'S AFFECTIVE PATHWAYS TO PROVIDE PAIN RELIEF (FIG. 3B)

Pharmacology (opioids)

Long-term opioid use is associated with harmful side effects, as well as risk of misuse, abuse, and opioid use disorder (*239*). However, clinical experience suggests that, in a subgroup of patients with chronic pain, stable doses of opioids can provide durable pain relief with limited side effects. This section focuses on opioids because longstanding evidence indicates a direct action on affective-motivational and cognitive-evaluative networks (*240*, *241*). Furthermore, the identification of the μ opioid receptor as the molecular target of clinical opioids like morphine (*242*) has enabled detailed mechanistic studies of neurotransmission modulation by opioids (*243*–*245*), including in affective circuits. Both clinical and rodent studies support the idea that opioids preferentially decrease the affective component of pain (*246*–*248*). For example, moderate activation of μ opioid receptors with a low dose of the biased and partial agonist PZM21 reduced affective-motivational nocifensive behaviors, without altering reflexive withdrawal from noxious stimuli in rodents (*39*). These features separate opioids from other common analgesic drugs that limit the production of pronociceptive mediators [for example, non-steroidal anti-inflammatory drugs (NSAIDs) (*249*)] or affect the function of primary afferent nociceptors (including sodium channel or calcium channel blockers such as lidocaine and ziconotide, respectively) or for which the molecular and circuit mechanisms of action remain unclear (such as gabapentinoids, anticonvulsants, and antidepressants). For example, activation of the gabapentin receptor α2δ-1, in addition to its effects on ion channels (*250*, *251*), can promote excitatory synaptogenesis in response to thrombospondin released by astrocytes in the SC DH (*252*, *253*). Gabapentin blocks this synaptogenesis mechanism, which may contribute to central sensitization during chronic neuropathic pain. However, it remains unclear whether gabapentinoids also influence remodeling of brain synapses of the pain affect circuitry via the same mechanisms to produce pain relief (*254*).

At the circuit level, μ opioid receptor distribution is prominent in emotional and cognitive brain circuits (*255*). The study of these opioidergic circuits has been facilitated by the generation of mutant mouse lines in which individual opioid receptor or peptide genes have been either targeted to express fluorescent receptors or DNA recombinases or flanked by loxP sites for conditional deletion experiments (*117*, *228*, *256*–*262*). Remarkably, μ opioid receptor–expressing neurons are found in lPB, ILN/MThal, and PAG, the three major output regions by which nociceptive DH projection neurons connect with emotional and cognitive circuits (*35*, *36*, *257*, *263*, *264*).

In the lPB, μ opioid receptors are expressed by *Calca*$^+$ lPBe neurons, in which μ receptor activation decreases glutamate release onto CeL neurons. In the dorsomedial/midline thalamus (dMT), μ receptors are present in thalamo-cortical (ACC), thalamo-striatal [dorsomedial striatum (DMS)], and thalamo-amygdalar (CeL and BA) circuits. In the dMT, including the paraventricular (PVT) and paratenial (PT) thalamic nuclei, μ receptor activation decreases glutamatergic transmission between dMT neurons and basal amygdala (BA) and CeL amygdala neurons, resulting in an overall reduction in feedforward activation of CeM neurons (*265*). μ receptors are also expressed by several classes of amygdalar neurons: by some BLA neurons (*266*) and, more abundantly, by ITC masses and populations of CeA GABAergic neurons, including *Cck*$^+$ neurons and neurons that project to the PAG, in which μ receptors regulate both the flow of information within the amygdaloid complex through G protein-coupled inwardly rectifying K+ (GIRK)-mediated hyperpolarization and the release of GABA in downstream targets (*36*, *267*–*270*). Note that μ receptor expression in the PVT might also mediate the expression of opioid withdrawal symptoms and aversive memory through a PVT-to-NAc circuit (*271*). A recent study demonstrated that μ opioid receptor$^+$ dMT neurons project to the dorsomedial, rather than the ventral, region of the striatum, where they synapse onto MSNs that receive convergent, μ opioid receptor–negative [although, see also (*272*)] input from ACC pyramidal neurons. Interestingly, these μ opioid receptor$^+$ thalamostriatal neurons also project back onto L5 ACC pyramidal neurons, and μ opioid receptor agonists can presynaptically decrease glutamate release onto both DMS MSNs and L5 ACC pyramidal neurons. The latter synaptic mechanism of function of μ opioid receptors may contribute to the antinociceptive effect of intracerebral ACC morphine injections on the affective component of pain, without influencing withdrawal reflexes (*247*, *273*). Because glutamatergic thalamic μ opioid receptor$^+$ neurons predominantly express VGLUT2, these synaptic mechanisms could underlie the reduced opioid antinociception in mice with a conditional deletion of μ receptors in *Slc17a6*$^+$ neurons (*274*). However, aside from regulating excitability and transmitter release, including via several forms of synaptic plasticity in multiple types of pyramidal neurons, such as in the mPFC and insula (*126*, *272*), μ receptors are also thought to be expressed by multiple populations of GABAergic cortical interneurons such as *Lamp5*$^+$, *Sst*$^+$, *Vip*$^+$, and *Pvalb*$^+$ neurons (*275*). Precise genetic strategies may be required to resolve the contribution of these distinct populations of cortical μ opioid receptor-expressing neurons to opioid analgesia. Although intracerebral injection of μ opioid receptor agonist into the vlPAG has long been known to produce antinociception (*276*), the identity and connectivity of μ opioid receptor–expressing neurons in the vlPAG remain less well understood (*218*). We know, however, that these vlPAG neurons regulate the activity of several classes of spinally projecting neurons in the RVM, including μ receptor–expressing on-cells (*217*–*222*, *227*, *228*, *277*). Identifying the precise contribution to these different processes of the molecularly and pharmacologically diverse types of receptors activated by morphine-like opioids represents an exciting challenge (*278*–*282*).

Note that the expression patterns of δ and κ opioid receptors in pain circuits profoundly differ from that of μ receptors (*36*, *255*, *257*, *259*, *283*, *284*), consistent with the divergent properties of their selective agonists. For example, in the cortex, δ receptors are predominantly expressed by *Pvalb*$^+$ (PV) inhibitory interneurons rather than by thalamic μ-expressing glutamatergic inputs to the ACC, where δ enhances the glutamatergic, excitatory input from the MThal to the pyramidal neurons in the ACC by disinhibiting local

feed-forward inhibition mediated by *Pvalb*$^+$ interneurons (*126*). Note that these PV inhibitory interneurons represent the class of cortical and hippocampal neurons that abundantly coexpresses δ and μ receptors, a rare feature in the nervous system (*36*, *257*, *275*, *283*–*285*). In the amygdala, δ receptors are predominantly found in the BLA, on the soma and axon terminals (*258*), in contrast to μ receptor distribution in ITC and CeA neurons. κ receptors are also present in affective and valence circuits, but generally in different cell types compared to μ, consistent with the diverging properties of their selective agonists (*209*, *286*, *287*). Last, although μ opioid receptors are expressed by nociceptors and spinal projection neurons (*36*, *283*), conditional deletion studies suggest that μ receptors in nociceptors are dispensable for morphine analgesia [(*38*); however, see also (*288*)].

Stimulation

The first documented use of stimulation intended specifically to alleviate chronic pain was performed in the 1960s, targeting deep brain electrical stimulation (DBS) to the thalamus (*185*). In the 1980s, TMS was developed as an alternative to electrical stimulation (ES) (*289*). TMS uses magnetic induction to generate macroscopic electrical currents in the brain (*289*). A shift from invasive to noninvasive forms of stimulation like TMS has made stimulation and modulation of brain circuits available to a broader patient group. Today, TMS and transcranial direct current stimulation (tDCS) are the most commonly used methods for noninvasive modulation of brain circuits to alleviate chronic pain (*290*).

How do TMS, ES, and tDCS work?

ES and TMS drive action potential firing by exciting neurons and passing axons and backfiring input terminals at the site of stimulation (*291*). In contrast, tDCS is less temporally and spatially specific than ES and TMS and acts by hyperpolarizing the resting membrane potential, making the anode region more excitable and the cathode less (*292*). Studies in human subjects and animal models both show that high-frequency stimulus trains excite the target more efficiently than low-frequency trains (*293*–*297*). Theta burst stimulation, a TMS protocol commonly used for cortical stimulation, uses three pulse bursts delivered at high frequency (for example, 50 Hz), repeated every 200 ms (5 Hz) (*293*). Theta burst protocols are a compromise aimed to capture the advantages of high-frequency stimulation while limiting the risk of inducing seizures (*294*, *298*). Examination of fMRI interleaved between TMS pulse trains shows a stimulus target volume of 5 to 10 cm^3 (*299*). In vivo voltage dye imaging in cat cortex found a progressive rise in excitation at the targeted region throughout a 10-Hz electrical stimulus train (*300*), supporting previous slice electrophysiology studies that had similarly concluded that high-frequency stimulation in cortical tissue preferentially activates excitatory neurons (*301*). Furthermore, a recent calcium imaging study in mice showed that excitatory neurons in L2/3 activate in response to specific stimulation frequencies, similar to visual cortical neurons tuned to a specific direction of drifting grating stimuli (*296*). Although our understanding of the biophysics underlying brain stimulation is evolving, many important questions remain to be explored.

MCS for pain relief and as a model for understanding TMS and tDCS

The motor cortex (MC) is a common target of studies attempting to determine the neurobiology of cortical stimulation. MC stimulation (MCS) results in motor activity that enables confirmation of correct targeting. The muscle end-plate potential (MEP), which can be performed in humans and in animal models, enables examination of resting motor threshold, amplitude of stimulus-evoked responses, and long-term changes in muscle tone (*299*, *302*). Performing MCS with MEP as the readout reveals long-term changes in MC excitability after stimulation (*303*, *304*).

MCS was first used to reduce chronic pain in 1991, when Tsubokawa and colleagues (*305*) implanted electrodes into the primary MC of patients with refractory central pain. Nine of 12 patients described their pain relief as good or excellent on the days when stimulation occurred, and 8 of these patients continued the therapy with reduced chronic pain after 1 year of treatment (*305*). In the intervening 30 years, hundreds of patients have received MCS. A meta-analysis across studies reported that 64% of patients experienced a favorable response after MCS and 45 to 75% of patients reported a decrease of at least 5 points on a 0 to 10 visual analog scale (VAS) of pain intensity (*297*). Furthermore, case studies applying MCS to patients with severe, otherwise untreatable, pain showed remarkable pain relief (*295*, *305*, *306*).

The mechanisms underlying MCS efficacy remain poorly understood. fMRI imaging in human subjects identified MCS-induced hotspots of activity in descending pain control regions that correlated with suppression of acute secondary hyperalgesia (*307*). Intracranial injection of GABAergic or glycinergic antagonists into the PAG of SNI rats before MCS prevented MCS-induced antinociception, providing further evidence for the involvement of descending pain control (*308*). In contrast, MCS increased the density of FOS$^+$ neurons in the ACC and BLA, and lesions of the aIC enhanced MCS-induced antinociception in a chronic pain rat model (*309*–*311*), together suggesting that pain affective circuits are involved in MCS analgesia as well.

Noninvasive targeting of pain-related brain regions

In addition to MC, noninvasive brain stimulation has been targeted to many pain-related brain areas with the intention of reducing chronic pain, including the ACC, IC, somatosensory cortex, and dlPFC, with varying degrees of success (*312*). Stimulation of S1 and/or S2 has been found to modulate perception of sensory features of pain without providing the clinically necessary reduction in pain affect (*313*–*315*). In contrast, a study using noninvasive stimulation of the ACC or IC in patients with central chronic pain found that neither target evoked a measurable effect on chronic pain scores in patients, although the ACC stimulation decreased anxiety and the IC stimulation increased heat pain thresholds (*141*). The most promising alternative, to the MC for noninvasive stimulation is the dlPFC, the stimulation of which reduces acute pain in healthy volunteers and decreases chronic pain scores in patients (*290*, *316*–*318*).

DBS for chronic pain relief

Many regions critical for pain processing are difficult to effectively stimulate noninvasively. The ACC, VP thalamus, and PAG have all been identified as promising DBS targets for reducing chronic pain; the literature for these methods has recently been reviewed (*319*).

Neurofeedback

Given the crucial role of the brain in the experience of pain and its modulation, researchers have hypothesized that direct manipulation of one or more brain regions could enhance pain modulatory systems and thereby reduce the underlying central nervous system (CNS) abnormalities associated with chronic pain. In addition to the pharmacological, direct stimulation (TMS, DBS, and tDCS), and surgical techniques discussed in this review, researchers have

developed neurofeedback techniques that teach individuals to self-regulate brain functionality. Neurofeedback is a noninvasive therapy that directly targets brain activity and/or connectivity patterns and uses either electroencephalograph (EEG) recordings or fMRI signals to provide individuals with real-time visual and/or auditory feedback reflective of the targeted brain functionality (*320*, *321*).

EEG neurofeedback is used more frequently than fMRI because of its greater accessibility and lower cost. Typically, EEG neurofeedback targets a change in a specific oscillatory bandwidth, most often the alpha band (8 to 13 Hz) (*322*). In contrast to EEG neurofeedback, fMRI neurofeedback measures and feeds back information from specific brain regions or networks using fMRI's higher spatial resolution. The lower temporal resolution of fMRI seems to benefit the learning of self-regulating brain functionality.

An example from one of the earliest studies of fMRI neurofeedback fed back brain signals from the dACC (*323*). In healthy volunteers given an evoked thermal stimulus, neurofeedback training led to increased control over brain activity and an associated increase in control over pain intensity. In a single training session, patients with chronic pain noted reduced pain that correlated with the degree of brain control over the dACC. Similarly, Guan *et al.* (*324*) modulated the rostral ACC (rACC) in a group of patients with postherpetic neuralgia (PHN). Patients learned to modulate their rACC signal and their pain perception. Using an fMRI-to-EEG amygdala fingerprint, Goldway *et al.* (*325*) conducted a neurofeedback trial in which they taught patients with FM to modulate their own amygdalar activity using a single EEG channel. Patients demonstrated improvements in objective measures of sleep and follow-up improvements in pain, demonstrating the benefit of this approach combining fMRI and EEG neurofeedback (*325*).

More recently, Zhang *et al.* (*326*) illustrated the potential of implicit learning strategies to modulate pain. Specifically, they used real-time decoded fMRI signals from the IC integrated into a closed-loop feedback control system and found that decoding the brain patterns without the participant's volitional control leads to adaptive changes in the brain. These results demonstrate the need to account for these adaptive changes in the design of future systems intended to direct brain control. Although neurofeedback using fMRI and EEG is a promising avenue for therapeutic interventions, researchers must still identify the optimal brain targets, patterns, frequency bands, and networks for manipulation; demonstrate that neurofeedback training leads to learning; ensure that neurofeedback leads to measurable changes in behavior (examples include pain relief, coping, pain catastrophizing, and fear avoidance); and develop appropriate controls and clinical trial designs (*327*, *328*). For additional information on neurofeedback in the context of pain, we direct the reader to the following reviews (*320*, *321*, *329*).

Cognitive behavioral therapy

Cognitive behavioral therapy (CBT) is a psychotherapeutic treatment encompassing a set of techniques and approaches, ranging from structured psychotherapies to self-help materials, that helps individuals learn to identify and change destructive and/or disturbing thought patterns that may negatively influence behavior and emotions (*330*–*333*). Key processes for pain management include relaxation training, cognitive restructuring, and exposure techniques. In addition to pain, CBT is used to treat a wide variety of mental health conditions including addiction (*334*, *335*), anxiety (*336*, *337*), depression (*338*, *339*), and personality disorders (*340*). It has also proved helpful for patients with chronic pain (*340*, *341*).

Although we refer here and below to CBT (and its neural correlates) as a singular therapy, it represents a family of psychological treatments that has evolved over time. The first generation of CBT applied learning principles intended to change overt behavior. Classic CBT (second generation) was introduced in the late 1970s and focuses on the role of maladaptive thought processes in emotion, behavior, and pain. More recently, a third generation of CBT places more emphasis on themes such as acceptance, mindfulness, values, metacognition, and interpersonal relationships, giving rise to therapies such as acceptance and commitment therapy, mindfulness-based cognitive therapy, and several others. This section will focus on classic CBT and review its neural correlates.

CBT draws on cognitive and behavioral strategies to improve pain-related functioning and help patients cope with pain (*341*). After CBT treatment, patients with chronic pain report reduced pain, distress, nocebo hyperalgesia, and pain catastrophizing, as well as improvement of their daily functioning (*342*–*344*). CBT-induced pain relief is highly variable between patients, and the improvement correlates strongly with the patient's attitude: Distressed patients who see their pain as an uncontrollable and highly negative life event benefit less, whereas patients with low perceived disability and high orientation toward self-management during CBT treatment benefit more (*342*, *343*). These observations support the hypothesis that the outcomes of multidisciplinary pain treatment correlate with the individual patient's cognitions and coping responses (*343*).

Although CBT continues to be widely used for pain management, the neural mechanisms that mediate analgesia during CBT remain unclear. Human functional neuroimaging studies dominate CBT research related to pain perception. Given the relatively limited literature investigating brain activation changes during CBT treatment in patients with pain, it is helpful to first understand how CBT impacts an individual's psychological state to affect pain processing. Studies examining the effects of distraction on pain processing found that pain-evoked activity in several cortical areas, like the S1, IC, and ACC, is stronger when an individual focuses on pain than when distracted (*345*–*347*). Neuroimaging studies evaluating the effects of emotional states on pain processing found that negative emotional states alter pain-evoked cortical activation in several brain regions, but most consistently in the ACC (*345*, *347*, *348*). Placebo administration has been shown to increase activity in the ACC, PAG, and cerebellum but decrease activity in the S1 and IC (*349*–*351*).

Studies have demonstrated that, for individuals with chronic pain, CBT generates both functional and structural changes in the brain. One of the first studies on chronic pain found that CBT treatment increased pain-evoked activity in the lateral PFC, which subsequently increased its connectivity with the thalamus, compared to controls (*352*). This lateral PFC region contributes to semantic processing and cognitive control, both of which are associated with exposure and cognitive restructuring therapies. Similarly, large brain networks involved in sensorimotor, self-referential, and cognitive control show altered connectivity patterns in patients with chronic pain compared to controls, which return to baseline after CBT (*353*–*355*). These connectivity changes also occurred in healthy controls when receiving CBT-based training to cope with evoked pain (*356*). In another study in patients with chronic pain, CBT led to increased gray matter volume in multiple regions

associated with pain processing, such as the dlPFC, ACC, and S1, some of which correlated with decreased pain catastrophizing (*357*).

A few studies have shown evidence that CBT generally affects neural function in pain networks. Biofeedback relaxation activates the ACC, basal ganglia, S1, inferior parietal cortex, and cerebellar vermis (*358*). Similarly, progressive muscle relaxation, one type of CBT, gradually decreases activity in the superior frontal gyrus (SFG), inferior frontal gyrus (IFG), and posterior cingulate cortex (PCC) (*359*), suggesting that CBT may activate endogenous pain descending modulatory systems. Last, the efficiency of CBT for the treatment of other mental diseases such as anxiety and depression (*68*, *360*) may suggest that the nociceptive neurons within CBT-responsive cognitive and emotional circuits are polymodal neurons that control other functions beyond pain, such as attention and mood.

Finally, researchers have identified that different cognitive strategies to modulate pain evoke distinct brain activity patterns. For example, during focused attention, brain activity localizes to the pre- and postcentral gyrus (the primary motor and somatosensory cortices, respectively), middle occipital gyrus, and inferior parietal lobe, whereas reappraisal of the pain (imaging the painful stimulus alternating between harmful or nonharmful) engaged the thalamus, amygdala, ventral lateral PFC, MCC, and parahippocampal gyrus (*361*). The postcentral gyrus was the only area that overlapped in activation during both strategies. In a more recent study, researchers investigated three distinct pain modulation strategies: (i) non-imaginal distraction by counting backward in steps of seven, (ii) imaginal distraction by imagining a safe place, and (iii) reinterpretation of the pain valence (reappraisal) (*362*). They also identified strategy-dependent activations. Reappraisal and the imaginal distraction (safe place) primarily engaged the anterior insula, whereas the non-imaginal distraction task activated primarily the central operculum. The tasks involving distraction from pain (counting and safe place) modulated activity in the PCC. Together, these findings and others suggest that combining specific strategies with targeted brain stimulation or neurofeedback enhances treatment efficacy.

Surgery

First used in 1962, cingulotomy (lesioning of the ACC or the cingulum bundle white matter) has long been an option for decreasing chronic pain unpleasantness in patients who fail to respond to other interventions (*185*, *363*). In this initial study, 16 patients suffering from debilitating chronic pain were selected for unilateral or bilateral cingulotomies (*363*). The authors classified pain relief as poor, fair, good, or excellent, finding that 12 of the 16 patients experienced good or excellent pain relief and 11 of 16 showed decreases in comorbid psychiatric disorders (*363*). The authors further noted that pain relief was observed immediately after the cingulotomy was performed, while the patient was still in the operating room (*363*). Although use of this technique has decreased in recent years in favor of nondestructive alternatives, a recent meta-analysis comparing data from 11 articles that included 224 patients concluded that, across all studies, more than 60% of patients reported substantial pain relief at least 1 year after the intervention (*364*). In this meta-analysis, the few side effects noted included, in <5% of patients, transient postoperative confusion and/or seizures (*364*). However, a case study of a patient with schizoaffective disorder found that cingulotomy increased pain, the opposite of the expected effect (*157*). Last, note that a number of other surgical procedures are used to treat pain (*365*).

CONCLUSION

Although pharmacotherapy, brain stimulation, neurofeedback, CBT, and surgical protocols used to treat pain continue to improve, research regarding the brain circuits and neuron types that mediate pain affect in animal models is revealing a wealth of candidate molecular targets to develop innovative analgesic drugs that could selectively dampen the unpleasantness of pain, without altering nociception in the circuits that underlie other necessary aspects of pain experience, such as withdrawal reflexes and the sensory-discriminative dimension of pain.

Cell type–specific multiomics is revolutionizing our understanding of neuronal diversity by revealing the molecular content of individual neurons within circuits. In the pain field, single-cell/nucleus RNA sequencing (*366*) can now generate, from each of a subject's nociceptive neurons, comprehensive catalogs of expressed genes that encode proteins with inhibitory functions and potential analgesic capabilities, enabling precision pain medicine. Similarly, although this review focuses on circuits, molecules in non-neuronal cells such as microglia could be targeted for the treatment of pain (*367*, *368*). Optically recording the activity of molecularly defined nociceptive neurons in freely moving mice experiencing chronic pain and administered with candidate analgesics can be used as a screening approach that relies on the normalization of both affective-motivational behaviors and pathological neural codes associated with pain chronification in the amygdala (*37*) and connected regions including the ACC, IC, ILN, MThal, and lPB (*148*). For example, with agonists of neuromodulatory receptors such as inhibitory $G_{i/o}$ protein-coupled receptors ($G_{i/o}$ GPCRs), individualized drug dosage could reduce patients' pain unpleasantness while preserving both withdrawal reflexes and the sensory-discriminative dimension of pain. Such pain asymbolia-like treatments would not only rescue the well-being and function of patients with chronic pain, but also maintain sufficient nociceptive functions necessary to sense and withdraw from noxious stimuli unrelated to their chronic pain condition, a substantial challenge when targeting primary afferent nociceptors or spinal networks and their ascending circuits. As a proof of principle, expressing and activating $G_{i/o}$ protein-coupled DREADDs (hM4Di) (*369*) in BLA nociceptive neurons of mice alleviated pain affective-motivational behaviors across pain modalities (acute heat, cold and mechanical pain, and chronic neuropathic pain) without altering withdrawal reflexes, anxiety, or reward (*37*); one would expect that activating $G_{i/o}$ GPCRs natively expressed in these neurons to have the same effect. Alternatively, by resolving the molecular repertoires both of the μ opioid receptor–expressing neuron types that modulate emotional and cognitive pain circuits to dampen pain affect during opioid analgesia and of the μ opioid receptor–expressing neuron types responsible for deleterious effects such as addiction and opioid-induced respiratory depression, researchers could potentially develop better opioid therapies that mimic the effect of morphine on nociceptive neurons and/or adjuvant therapeutics that oppose deleterious opioid signaling in reward and breathing circuits.

In conclusion, these neural circuit discoveries and translational endeavors, supported by outstanding efforts such as the National Institutes of Health (NIH) Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) (*370*) and Helping to End Addiction Long-term (HEAL) (*371*, *372*) Initiatives and its Preclinical Screening Platform for Pain (PSPP) (*373*), provide an unprecedented opportunity to end the dual public health crises of chronic

pain and opioid use disorders. To fulfill this goal, we will need to use these discoveries to develop better biomarkers to facilitate the development of non-addictive pain therapies. Objective biomarkers can indicate that a therapeutic intervention has reached its central target, predict the response to the therapy, enhance the quality of the clinical trial by allowing clustering of patients by presumed responsiveness, and improve monitoring of safety and efficacy over time. Frameworks for developing and validating neuroimaging-based biomarkers and composite biomarkers have been put forward (*374*, *375*). Programs like the NIH HEAL Initiative are stimulating considerable research efforts to advance the development and translation of biomarkers to yield targeted, safe, and effective therapies for pain.

REFERENCES AND NOTES

1. Global Burden of Disease Study 2013 Collaborators, Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **386**, 743–800 (2015).
2. A. S. C. Rice, B. H. Smith, F. M. Blyth, Pain and the global burden of disease. *Pain* **157**, 791–796 (2016).
3. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **392**, 1789–1858 (2018).
4. G. Gilam, J. J. Gross, T. D. Wager, F. J. Keefe, S. C. Mackey, What is the relationship between pain and emotion? Bridging constructs and communities. *Neuron* **107**, 17–21 (2020).
5. R. Calati, C. Laglaoui Bakhiyi, S. Artero, M. Ilgen, P. Courtet, The impact of physical pain on suicidal thoughts and behaviors: Meta-analyses. *J. Psychiatr. Res.* **71**, 16–32 (2015).
6. R.-D. Treede, W. Rief, A. Barke, Q. Aziz, M. I. Bennett, R. Benoliel, M. Cohen, S. Evers, N. B. Finnerup, M. B. First, M. A. Giamberardino, S. Kaasa, B. Korwisi, E. Kosek, P. Lavand'homme, M. Nicholas, S. Perrot, J. Scholz, S. Schug, B. H. Smith, P. Svensson, J. W. S. Vlaeyen, S.-J. Wang, Chronic pain as a symptom or a disease: The IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain* **160**, 19–27 (2019).
7. Institute of Medicine (US) Committee on Advancing Pain Research, Care, and Education, *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research* (National Academies Press, 2011).
8. S. C. S. Sherrington, *The Integrative Action of the Nervous System* (Yale Univ. Press, 1906).
9. C. J. Woolf, Capturing novel non-opioid pain targets. *Biol. Psychiatry* **87**, 74–81 (2020).
10. A. Patapoutian, S. Tate, C. J. Woolf, Transient receptor potential channels: Targeting pain at the source. *Nat. Rev. Drug Discov.* **8**, 55–68 (2009).
11. A. I. Basbaum, D. M. Bautista, G. Scherrer, D. Julius, Cellular and molecular mechanisms of pain. *Cell* **139**, 267–284 (2009).
12. N. Sharma, K. Flaherty, K. Lezgiyeva, D. E. Wagner, A. M. Klein, D. D. Ginty, The emergence of transcriptional identity in somatosensory neurons. *Nature* **577**, 392–398 (2020).
13. W. Renthal, I. Tochitsky, L. Yang, Y.-C. Cheng, E. Li, R. Kawaguchi, D. H. Geschwind, C. J. Woolf, Transcriptional reprogramming of distinct peripheral sensory neuron subtypes after axonal injury. *Neuron* **108**, 128–144.e9 (2020).
14. P. Ray, A. Torck, L. Quigley, A. Wangzhou, M. Neiman, C. Rao, T. Lam, J.-Y. Kim, T. H. Kim, M. Q. Zhang, G. Dussor, T. J. Price, Comparative transcriptome profiling of the human and mouse dorsal root ganglia: An RNA-seq-based resource for pain and sensory neuroscience research. *Pain* **159**, 1325–1345 (2018).
15. R. Y. North, Y. Li, P. Ray, L. D. Rhines, C. E. Tatsui, G. Rao, C. A. Johansson, H. Zhang, Y. H. Kim, B. Zhang, G. Dussor, T. H. Kim, T. J. Price, P. M. Dougherty, Electrophysiological and transcriptomic correlates of neuropathic pain in human dorsal root ganglion neurons. *Brain* **142**, 1215–1226 (2019).
16. D. Usoskin, A. Furlan, S. Islam, H. Abdo, P. Lönnerberg, D. Lou, J. Hjerling-Leffler, J. Haeggström, O. Kharchenko, P. V. Kharchenko, S. Linnarsson, P. Ernfors, Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat. Neurosci.* **18**, 145–153 (2015).
17. S. E. Murthy, M. C. Loud, I. Daou, K. L. Marshall, F. Schwaller, J. Kühnemund, A. G. Francisco, W. T. Keenan, A. E. Dubin, G. R. Lewin, A. Patapoutian, The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. *Sci. Transl. Med.* **10**, eaat9897 (2018).
18. M. Szczot, J. Liljencrantz, N. Ghitani, A. Barik, R. Lam, J. H. Thompson, D. Bharucha-Goebel, D. Saade, A. Necaise, S. Donkervoort, A. R. Foley, T. Gordon, L. Case, M. C. Bushnell, C. G. Bönnemann, A. T. Chesler, PIEZO2 mediates injury-induced tactile pain in mice and humans. *Sci. Transl. Med.* **10**, eaat9892 (2018).
19. M. J. Barad, J. A. Sturgeon, J. Hong, A. K. Aggarwal, S. C. Mackey, Characterization of chronic overlapping pain conditions in patients with chronic migraine: A CHOIR study. *Headache* **61**, 872–881 (2021).
20. W. Maixner, R. B. Fillingim, D. A. Williams, S. B. Smith, G. D. Slade, Overlapping chronic pain conditions: Implications for diagnosis and classification. *J. Pain* **17**, T93–T107 (2016).
21. M. Auvray, E. Myin, C. Spence, The sensory-discriminative and affective-motivational aspects of pain. *Neurosci. Biobehav. Rev.* **34**, 214–223 (2010).
22. A. D. Craig, How do you feel? Interoception: The sense of the physiological condition of the body. *Nat. Rev. Neurosci.* **3**, 655–666 (2002).
23. R. Melzack, K. L. Casey, Sensory, motivational, and central control determinants of pain: A new conceptual model, in *The Skin Senses*, D. R. Kenshalo, Ed. (Charles C Thomas Publisher, 1968), pp. 423–439.
24. S. N. Raja, D. B. Carr, M. Cohen, N. B. Finnerup, H. Flor, S. Gibson, F. J. Keefe, J. S. Mogil, M. Ringkamp, K. A. Sluka, X.-J. Song, B. Stevens, M. D. Sullivan, P. R. Tutelman, T. Ushida, K. Vader, The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain* **161**, 1976–1982 (2020).
25. J. E. LeDoux, What emotions might be like in other animals. *Curr. Biol.* **31**, R824–R829 (2021).
26. D. J. Anderson, R. Adolphs, A framework for studying emotions across species. *Cell* **157**, 187–200 (2014).
27. C. Peirs, S.-P. G. Williams, X. Zhao, C. M. Arokiaraj, D. W. Ferreira, M.-C. Noh, K. M. Smith, P. Halder, K. A. Corrigan, J. Y. Gedeon, S. J. Lee, G. Gatto, D. Chi, S. E. Ross, M. Goulding, R. P. Seal, Mechanical allodynia circuitry in the dorsal horn is defined by the nature of the injury. *Neuron* **109**, 73–90.e7 (2021).
28. G. Gatto, S. Bourane, X. Ren, S. Di Costanzo, P. K. Fenton, P. Halder, R. P. Seal, M. D. Goulding, A functional topographic map for spinal sensorimotor reflexes. *Neuron* **109**, 91–104.e5 (2021).
29. A. Barik, J. H. Thompson, M. Seltzer, N. Ghitani, A. T. Chesler, A brainstem-spinal circuit controlling nocifensive behavior. *Neuron* **100**, 1491–1503.e3 (2018).
30. C. J. Woolf, Long term alterations in the excitability of the flexion reflex produced by peripheral tissue injury in the chronic decerebrate rat. *Pain* **18**, 325–343 (1984).
31. S. Choi, J. Hachisuka, M. A. Brett, A. R. Magee, Y. Omori, N.-U.-A. Iqbal, D. Zhang, M. M. DeLisle, R. L. Wolfson, L. Bai, C. Santiago, S. Gong, M. Goulding, N. Heintz, H. R. Koerber, S. E. Ross, D. D. Ginty, Parallel ascending spinal pathways for affective touch and pain. *Nature* **587**, 258–263 (2020).
32. J. Braz, C. Solorzano, X. Wang, A. I. Basbaum, Transmitting pain and itch messages: A contemporary view of the spinal cord circuits that generate gate control. *Neuron* **82**, 522–536 (2014).
33. C. Peirs, R. P. Seal, Neural circuits for pain: Recent advances and current views. *Science* **354**, 578–584 (2016).
34. G. Scherrer, K. Befort, C. Contet, J. Becker, A. Matifas, B. L. Kieffer, The delta agonists DPDPE and deltorphin II recruit predominantly mu receptors to produce thermal analgesia: A parallel study of mu, delta and combinatorial opioid receptor knockout mice. *Eur. J. Neurosci.* **19**, 2239–2248 (2004).
35. A. Mansour, H. Khachaturian, M. E. Lewis, H. Akil, S. J. Watson, Anatomy of CNS opioid receptors. *Trends Neurosci.* **11**, 308–314 (1988).
36. D. Wang, V. L. Tawfik, G. Corder, S. A. Low, A. François, A. I. Basbaum, G. Scherrer, Functional divergence of delta and mu opioid receptor organization in CNS pain circuits. *Neuron* **98**, 90–108.e5 (2018).
37. G. Corder, B. Ahanonu, B. F. Grewe, D. Wang, M. J. Schnitzer, G. Scherrer, An amygdalar neural ensemble that encodes the unpleasantness of pain. *Science* **363**, 276–281 (2019).
38. G. Corder, V. L. Tawfik, D. Wang, E. I. Sypek, S. A. Low, J. R. Dickinson, C. Sotoudeh, J. D. Clark, B. A. Barres, C. J. Bohlen, G. Scherrer, Loss of μ opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia. *Nat. Med.* **23**, 164–173 (2017).
39. A. Manglik, H. Lin, D. K. Aryal, J. D. McCorvy, D. Dengler, G. Corder, A. Levit, R. C. Kling, V. Bernat, H. Hübner, X.-P. Huang, M. F. Sassano, P. M. Giguère, S. Löber, D. Duan, G. Scherrer, B. K. Kobilka, P. Gmeiner, B. L. Roth, B. K. Shoichet, Structure-based discovery of opioid analgesics with reduced side effects. *Nature* **537**, 185–190 (2016).
40. A. B. Wiltschko, T. Tsukahara, A. Zeine, R. Anyoha, W. F. Gillis, J. E. Markowitz, R. E. Peterson, J. Katon, M. J. Johnson, S. R. Datta, Revealing the structure of pharmacobehavioral space through motion sequencing. *Nat. Neurosci.* **23**, 1433–1443 (2020).
41. N. T. Fried, A. Chamessian, M. J. Zylka, I. Abdus-Saboor, Improving pain assessment in mice and rats with advanced videography and computational approaches. *Pain* **161**, 1420–1424 (2020).
42. A. Mathis, P. Mamidanna, K. M. Cury, T. Abe, V. N. Murthy, M. W. Mathis, M. Bethge, DeepLabCut: Markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).
43. K. J. Sufka, Conditioned place preference paradigm: A novel approach for analgesic drug assessment against chronic pain. *Pain* **58**, 355–366 (1994).

44. T. King, L. Vera-Portocarrero, T. Gutierrez, T. W. Vanderah, G. Dussor, J. Lai, H. L. Fields, F. Porreca, Unmasking the tonic-aversive state in neuropathic pain. *Nat. Neurosci.* **12**, 1364–1366 (2009).
45. A. H. Tuttle, M. J. Molinaro, J. F. Jethwa, S. G. Sotocinal, J. C. Prieto, M. A. Styner, J. S. Mogil, M. J. Zylka, A deep neural network to assess spontaneous pain from mouse facial expressions. *Mol. Pain* **14**, 174480691876365 (2018).
46. D. J. Langford, A. L. Bailey, M. L. Chanda, S. E. Clarke, T. E. Drummond, S. Echols, S. Glick, J. Ingrao, T. Klassen-Ross, M. L. Lacroix-Fralish, L. Matsumiya, R. E. Sorge, S. G. Sotocinal, J. M. Tabaka, D. Wong, A. M. J. M. van den Maagdenberg, M. D. Ferrari, K. D. Craig, J. S. Mogil, Coding of facial expressions of pain in the laboratory mouse. *Nat. Methods* **7**, 447–449 (2010).
47. E. J. Cobos, N. Ghasemlou, D. Araldi, D. Segal, K. Duong, C. J. Woolf, Inflammation-induced decrease in voluntary wheel running in mice: A nonreflexive test for evaluating inflammatory pain and analgesia. *Pain* **153**, 876–884 (2012).
48. N. A. Andrews, A. Latrémolière, A. I. Basbaum, J. S. Mogil, F. Porreca, A. S. C. Rice, C. J. Woolf, G. L. Currie, R. H. Dworkin, J. C. Eisenach, S. Evans, J. S. Gewandter, T. D. Gover, H. Handwerker, W. Huang, S. Iyengar, M. P. Jensen, J. D. Kennedy, N. Lee, J. Levine, K. Lidster, I. Machin, M. P. McDermott, S. B. McMahon, T. J. Price, S. E. Ross, G. Scherrer, R. P. Seal, E. S. Sena, E. Silva, L. Stone, C. I. Svensson, D. C. Turk, G. Whiteside, Ensuring transparency and minimization of methodologic bias in preclinical pain research: PPRECISE considerations. *Pain* **157**, 901–909 (2016).
49. S. S. Negus, Addressing the opioid crisis: The importance of choosing translational endpoints in analgesic drug discovery. *Trends Pharmacol. Sci.* **39**, 327–330 (2018).
50. M. Ingvar, Pain and functional imaging. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **354**, 1347–1358 (1999).
51. E. A. Moulton, J. D. Schmahmann, L. Becerra, D. Borsook, The cerebellum and pain: Passive integrator or active participator? *Brain Res. Rev.* **65**, 14–27 (2010).
52. R. Peyron, B. Laurent, L. García-Larrea, Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol. Clin.* **30**, 263–288 (2000).
53. G. D. Iannetti, A. Mouraux, From the neuromatrix to the pain matrix (and back). *Exp. Brain Res.* **205**, 1–12 (2010).
54. K. D. Davis, H. Flor, H. T. Greely, G. D. Iannetti, S. Mackey, M. Ploner, A. Pustilnik, I. Tracey, R.-D. Treede, T. D. Wager, Brain imaging tests for chronic pain: Medical, legal and ethical issues and recommendations. *Nat. Rev. Neurol.* **13**, 624–638 (2017).
55. A. Kucyi, K. D. Davis, The dynamic pain connectome. *Trends Neurosci.* **38**, 86–95 (2015).
56. K. T. Martucci, S. C. Mackey, Neuroimaging of pain: Human evidence and clinical relevance of central nervous system processes and modulation. *Anesthesiology* **128**, 1241–1254 (2018).
57. T. D. Wager, L. Y. Atlas, M. A. Lindquist, M. Roy, C.-W. Woo, E. Kross, An fMRI-based neurologic signature of physical pain. *N. Engl. J. Med.* **368**, 1388–1397 (2013).
58. J. E. Brown, N. Chatterjee, J. Younger, S. Mackey, Towards a physiology-based measure of pain: Patterns of human brain activity distinguish painful from non-painful thermal stimulation. *PLOS ONE* **6**, e24124 (2011).
59. M. Liang, A. Mouraux, L. Hu, G. D. Iannetti, Primary sensory cortices contain distinguishable spatial patterns of activity for each sense. *Nat. Commun.* **4**, 1979 (2013).
60. J.-J. Lee, H. J. Kim, M. Čeko, B.-y. Park, S. A. Lee, H. Park, M. Roy, S.-G. Kim, T. D. Wager, C.-W. Woo, A neuroimaging biomarker for sustained experimental and clinical pain. *Nat. Med.* **27**, 174–182 (2021).
61. M. Laubach, L. M. Amarante, K. Swanson, S. R. White, What, if anything, is rodent prefrontal cortex? *eNeuro* **5**, ENEURO.0315-18.2018 (2018).
62. S. van Heukelum, R. B. Mars, M. Guthrie, J. K. Buitelaar, C. F. Beckmann, P. H. E. Tiesinga, B. A. Vogt, J. C. Glennon, M. N. Havenith, Where is cingulate cortex? A cross-species view. *Trends Neurosci.* **43**, 285–299 (2020).
63. A. D. Craig, A rat is not a monkey is not a human: Comment on Mogil (*Nature Rev. Neurosci.* 10, 283–294 (2009)). *Nat. Rev. Neurosci.* **10**, 466 (2009).
64. S. P. Hunt, A. Pini, G. Evan, Induction of c-*fos*-like protein in spinal cord neurons following sensory stimulation. *Nature* **328**, 632–634 (1987).
65. R. W. Presley, D. Menétrey, J. D. Levine, A. I. Basbaum, Systemic morphine suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in the rat spinal cord. *J. Neurosci.* **10**, 323–335 (1990).
66. D. Menétrey, J. de Pommery, J. M. Besson, Electrophysiological characteristics of lumbar spinal cord neurons backfired from lateral reticular nucleus in the rat. *J. Neurophysiol.* **52**, 595–611 (1984).
67. R. Kuner, T. Kuner, Cellular circuits in the brain and their modulation in acute and chronic pain. *Physiol. Rev.* **101**, 213–258 (2021).
68. M. C. Bushnell, M. Čeko, L. A. Low, Cognitive and emotional control of pain and its disruption in chronic pain. *Nat. Rev. Neurosci.* **14**, 502–511 (2013).
69. L. L. Tan, R. Kuner, Neocortical circuits in pain and pain relief. *Nat. Rev. Neurosci.* **22**, 458–471 (2021).
70. C. A. Campos, A. J. Bowen, C. W. Roman, R. D. Palmiter, Encoding of danger by parabrachial CGRP neurons. *Nature* **555**, 617–622 (2018).
71. R. D. Palmiter, The parabrachial nucleus: CGRP neurons function as a general alarm. *Trends Neurosci.* **41**, 280–293 (2018).
72. M. C. Chiang, A. Bowen, L. A. Schier, D. Tupone, O. Uddin, M. M. Heinricher, Parabrachial complex: A hub for pain and aversion. *J. Neurosci.* **39**, 8225–8230 (2019).
73. W. R. Mehler, Some neurological species differences—A posteriori. *Ann. N. Y. Acad. Sci.* **167**, 424–468 (1969).
74. M. Wiberg, A. Blomqvist, The spinomesencephalic tract in the cat: Its cells of origin and termination pattern as demonstrated by the intraaxonal transport method. *Brain Res.* **291**, 1–18 (1984).
75. J. L. Hylden, H. Hayashi, G. J. Bennett, R. Dubner, Spinal lamina I neurons projecting to the parabrachial area of the cat midbrain. *Brain Res.* **336**, 195–198 (1985).
76. D. Cameron, E. Polgár, M. Gutierrez-Mecinas, M. Gomez-Lima, M. Watanabe, A. J. Todd, The organisation of spinoparabrachial neurons in the mouse. *Pain* **156**, 2061–2071 (2015).
77. R. B. Roome, R. Brian Roome, F. B. Bourojeni, B. Mona, S. Rastegar-Pouyani, R. Blain, A. Dumouchel, C. Salesse, W. S. Thompson, M. Brookbank, Y. Gitton, L. Tessarollo, M. Goulding, J. E. Johnson, M. Kmita, A. Chédotal, A. Kania, Phox2a defines a developmental origin of the anterolateral system in mice and humans. *Cell Rep.* **33**, 108425 (2020).
78. T. Huang, S.-H. Lin, N. M. Malewicz, Y. Zhang, Y. Zhang, M. Goulding, R. H. LaMotte, Q. Ma, Identifying the pathways required for coping behaviours associated with sustained pain. *Nature* **565**, 86–90 (2019).
79. D. J. Cavanaugh, A. T. Chesler, A. C. Jackson, Y. M. Sigal, H. Yamanaka, R. Grant, D. O'Donnell, R. A. Nicoll, N. M. Shah, D. Julius, A. I. Basbaum, Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *J. Neurosci.* **31**, 5067–5077 (2011).
80. E. Rodriguez, K. Sakurai, J. Xu, Y. Chen, K. Toda, S. Zhao, B.-X. Han, D. Ryu, H. Yin, W. Liedtke, F. Wang, A craniofacial-specific monosynaptic circuit enables heightened affective pain. *Nat. Neurosci.* **20**, 1734–1743 (2017).
81. A. L. Alhadeff, Z. Su, E. Hernandez, M. L. Klima, S. Z. Phillips, R. A. Holland, C. Guo, A. W. Hantman, B. C. De Jonghe, J. N. Betley, A neural circuit for the suppression of pain by a competing need state. *Cell* **173**, 140–152.e15 (2018).
82. M. Pignatelli, A. Beyeler, Valence coding in amygdala circuits. *Curr. Opin. Behav. Sci.* **26**, 97–106 (2019).
83. L. Pessoa, R. Adolphs, Emotion processing and the amygdala: From a 'low road' to 'many roads' of evaluating biological significance. *Nat. Rev. Neurosci.* **11**, 773–782 (2010).
84. P. H. Janak, K. M. Tye, From circuits to behaviour in the amygdala. *Nature* **517**, 284–292 (2015).
85. J. LeDoux, The amygdala. *Curr. Biol.* **17**, R868–R874 (2007).
86. J. F. Bernard, M. Peschanski, J. M. Besson, A possible spino (trigemino)-ponto-amygdaloid pathway for pain. *Neurosci. Lett.* **100**, 83–88 (1989).
87. J. F. Bernard, J. M. Besson, The spino(trigemino)pontoamygdaloid pathway: Electrophysiological evidence for an involvement in pain processes. *J. Neurophysiol.* **63**, 473–490 (1990).
88. Y. Carrasquillo, R. W. Gereau 4th, Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. *J. Neurosci.* **27**, 1543–1551 (2007).
89. V. Neugebauer, W. Li, G. C. Bird, G. Bhave, R. W. Gereau IV, Synaptic plasticity in the amygdala in a model of arthritic pain: Differential roles of metabotropic glutamate receptors 1 and 5. *J. Neurosci.* **23**, 52–63 (2003).
90. V. Neugebauer, W. Li, Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *J. Neurophysiol.* **87**, 103–112 (2002).
91. T. Hua, B. Chen, D. Lu, K. Sakurai, S. Zhao, B.-X. Han, J. Kim, L. Yin, Y. Chen, J. Lu, F. Wang, General anesthetics activate a potent central pain-suppression circuit in the amygdala. *Nat. Neurosci.* **23**, 854–868 (2020).
92. S. Han, M. T. Soleiman, M. E. Soden, L. S. Zweifel, R. D. Palmiter, Elucidating an affective pain circuit that creates a threat memory. *Cell* **162**, 363–374 (2015).
93. M. C. Chiang, E. K. Nguyen, M. Canto-Bustos, A. E. Papale, A.-M. M. Oswald, S. E. Ross, Divergent neural pathways emanating from the lateral parabrachial nucleus mediate distinct components of the pain response. *Neuron* **106**, 927–939.e5 (2020).
94. J. Deng, H. Zhou, J.-K. Lin, Z.-X. Shen, W.-Z. Chen, L.-H. Wang, Q. Li, D. Mu, Y.-C. Wei, X.-H. Xu, Y.-G. Sun, The parabrachial nucleus directly channels spinal nociceptive signals to the intralaminar thalamic nuclei, but not the amygdala. *Neuron* **107**, 909–923.e6 (2020).
95. A. Barik, A. Sathyamurthy, J. Thompson, M. Seltzer, A. Levine, A. Chesler, A spinoparabrachial circuit defined by Tacr1 expression drives pain. *eLife* **10**, e61135 (2021).
96. F. Grady, L. Peltekian, G. Iverson, J. C. Geerling, Direct parabrachial–cortical connectivity. *Cereb. Cortex* **30**, 4811–4833 (2020).
97. T. D. Wilson, S. Valdivia, A. Khan, H.-S. Ahn, A. P. Adke, S. M. Gonzalez, Y. K. Sugimura, Y. Carrasquillo, Dual and opposing functions of the central amygdala in the modulation of pain. *Cell Rep.* **29**, 332–346.e5 (2019).

98. P. Veinante, I. Yalcin, M. Barrot, The amygdala between sensation and affect: A role in pain. *J. Mol. Psychiatry* **1**, 9 (2013).
99. V. Neugebauer, Amygdala pain mechanisms. *Handb. Exp. Pharmacol.* **227**, 261–284 (2015).
100. A. P. Adke, A. Khan, H.-S. Ahn, J. J. Becker, T. D. Wilson, S. Valdivia, Y. K. Sugimura, S. Martinez Gonzalez, Y. Carrasquillo, Cell-type specificity of neuronal excitability and morphology in the central amygdala. *eNeuro* **8**, ENEURO.0402–ENEU20.2020 (2021).
101. V. Neugebauer, M. Mazzitelli, B. Cragg, G. Ji, E. Navratilova, F. Porreca, Amygdala, neuropeptides, and chronic pain-related affective behaviors. *Neuropharmacology* **170**, 108052 (2020).
102. J.-N. Li, P. L. Sheets, The central amygdala to periaqueductal gray pathway comprises intrinsically distinct neurons differentially affected in a model of inflammatory pain. *J. Physiol.* **596**, 6289–6305 (2018).
103. Y. Okutsu, Y. Takahashi, M. Nagase, K. Shinohara, R. Ikeda, F. Kato, Potentiation of NMDA receptor-mediated synaptic transmission at the parabrachial-central amygdala synapses by CGRP in mice. *Mol. Pain* **13**, 174480691770920 (2017).
104. J.-N. Li, P. L. Sheets, Spared nerve injury differentially alters parabrachial monosynaptic excitatory inputs to molecularly specific neurons in distinct subregions of the central amygdala. *Pain* **161**, 166–176 (2020).
105. C. Jenne, M. Morales, R. Masri, A. Keller, An amygdalo-parabrachial pathway regulates pain perception and chronic pain. *J. Neurosci.* **40**, 3424–3442 (2020).
106. P. Tovote, M. S. Esposito, P. Botta, F. Chaudun, J. P. Fadok, M. Markovic, S. B. E. Wolff, C. Ramakrishnan, L. Fenno, K. Deisseroth, C. Herry, S. Arber, A. Lüthi, Midbrain circuits for defensive behaviour. *Nature* **534**, 206–212 (2016).
107. A. J. Norris, J. R. Shaker, A. L. Cone, I. B. Ndiokho, M. R. Bruchas, Parabrachial opioidergic projections to preoptic hypothalamus mediate behavioral and physiological thermal defenses. *eLife* **10**, e60779 (2021).
108. M. M. Wertli, J. M. Burgstaller, S. Weiser, J. Steurer, R. Kofmehl, U. Held, Influence of catastrophizing on treatment outcome in patients with nonspecific low back pain: A systematic review. *Spine* **39**, 263–273 (2014).
109. P. J. Quartana, C. M. Campbell, R. R. Edwards, Pain catastrophizing: A critical review. *Expert. Rev. Neurother.* **9**, 745–758 (2009).
110. B. D. Darnall, Pain psychology and pain catastrophizing in the perioperative setting: A review of impacts, interventions, and unmet needs. *Hand Clin.* **32**, 33–39 (2016).
111. A. Galambos, E. Szabó, Z. Nagy, A. E. Édes, N. Kocsel, G. Juhász, G. Kökönyei, A systematic review of structural and functional MRI studies on pain catastrophizing. *J. Pain Res.* **12**, 1155–1178 (2019).
112. D. A. Seminowicz, K. D. Davis, Cortical responses to pain in healthy individuals depends on pain catastrophizing. *Pain* **120**, 297–306 (2006).
113. Y. Jiang, D. Oathes, J. Hush, B. Darnall, M. Charvat, S. Mackey, A. Etkin, Perturbed connectivity of the amygdala and its subregions with the central executive and default mode networks in chronic pain. *Pain* **157**, 1970–1978 (2016).
114. N. Hebben, S. Corkin, H. Eichenbaum, K. Shedlack, Diminished ability to interpret and report internal states after bilateral medial temporal resection: Case H.M. *Behav. Neurosci.* **99**, 1031–1039 (1985).
115. J. Annese, N. M. Schenker-Ahmed, H. Bartsch, P. Maechler, C. Sheh, N. Thomas, J. Kayano, A. Ghatan, N. Bresler, M. P. Frosch, R. Klaming, S. Corkin, Postmortem examination of patient HM's brain based on histological sectioning and digital 3D reconstruction. *Nat. Commun.* **5**, 3122 (2014).
116. B. F. Grewe, J. Gründemann, L. J. Kitch, J. A. Lecoq, J. G. Parker, J. D. Marshall, M. C. Larkin, P. E. Jercog, F. Grenier, J. Z. Li, A. Lüthi, M. J. Schnitzer, Neural ensemble dynamics underlying a long-term associative memory. *Nature* **543**, 670–675 (2017).
117. W. M. McCallum, D. Wang, A. Shuster, D. J. Berg, S. A. Low, G. Scherrer, A suite of knockin mouse lines to resolve the functional organization of opioid receptors in neural circuits, in *2019 Neuroscience Meeting Planner* (Society for Neuroscience, 2019).
118. J. M. Thompson, V. Neugebauer, Cortico-limbic pain mechanisms. *Neurosci. Lett.* **702**, 15–23 (2019).
119. C.-T. Yen, P.-L. Lu, Thalamus and pain. *Acta Anaesthesiol. Taiwanica* **51**, 73–80 (2013).
120. Melzack R., K. L. Casey, in *The Skin Senses*, D. R. Kenshalo, Ed. (Charles C Thomas Publisher, 1986), pp. 423–435.
121. G. F. Gebhart, K. Bielefeldt, Physiology of visceral pain. *Compr. Physiol.* **6**, 1609–1633 (2016).
122. K. E. Krout, R. E. Belzer, A. D. Loewy, Brainstem projections to midline and intralaminar thalamic nuclei of the rat. *J. Comp. Neurol.* **448**, 53–101 (2002).
123. K. E. Krout, A. D. Loewy, Parabrachial nucleus projections to midline and intralaminar thalamic nuclei of the rat. *J. Comp. Neurol.* **428**, 475–494 (2000).
124. C. Gauriau, J. F. Bernard, A comparative reappraisal of projections from the superficial laminae of the dorsal horn in the rat: The forebrain. *J. Comp. Neurol.* **468**, 24–56 (2004).
125. H. J. Groenewegen, H. W. Berendse, The specificity of the "nonspecific" midline and intralaminar thalamic nuclei. *Trends Neurosci.* **17**, 52–57 (1994).
126. W. T. Birdsong, B. C. Jongbloets, K. A. Engeln, D. Wang, G. Scherrer, T. Mao, Synapse-specific opioid modulation of thalamo-cortico-striatal circuits. *eLife* **8**, e45146 (2019).
127. K. S. Meda, T. Patel, J. M. Braz, R. Malik, M. L. Turner, H. Seifikar, A. I. Basbaum, V. S. Sohal, Microcircuit mechanisms through which mediodorsal thalamic input to anterior cingulate cortex exacerbates pain-related aversion. *Neuron* **102**, 944–959.e3 (2019).
128. J. W. Phillips, A. Schulmann, E. Hara, J. Winnubst, C. Liu, V. Valakh, L. Wang, B. C. Shields, W. Korff, J. Chandrashekar, A. L. Lemire, B. Mensh, J. T. Dudman, S. B. Nelson, A. W. Hantman, A repeated molecular architecture across thalamic pathways. *Nat. Neurosci.* **22**, 1925–1935 (2019).
129. E. Kuramoto, S. Pan, T. Furuta, Y. R. Tanaka, H. Iwai, A. Yamanaka, S. Ohno, T. Kaneko, T. Goto, H. Hioki, Individual mediodorsal thalamic neurons project to multiple areas of the rat prefrontal cortex: A single neuron-tracing study using virus vectors. *J. Comp. Neurol.* **525**, 166–185 (2017).
130. P. L. A. Gabbott, T. A. Warner, P. R. L. Jays, P. Salway, S. J. Busby, Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *J. Comp. Neurol.* **492**, 145–177 (2005).
131. J. S. Tang, C. L. Qu, F. Q. Huo, The thalamic nucleus submedius and ventrolateral orbital cortex are involved in nociceptive modulation: A novel pain modulation pathway. *Prog. Neurobiol.* **89**, 383–389 (2009).
132. Y. Sun, J. Wang, S. H. Liang, J. Ge, Y. C. Lu, J. N. Li, Y. B. Chen, D. S. Luo, H. Li, Y. Q. Li, Involvement of the ventrolateral periaqueductal gray matter-central medial thalamic nucleus-basolateral amygdala pathway in neuropathic pain regulation of rats. *Front. Neuroanat.* **14**, 32 (2020).
133. H. Kayyal, A. Yiannakas, S. K. Chandran, M. Khamaisy, V. Sharma, K. Rosenblum, Activity of insula to basolateral amygdala projecting neurons is necessary and sufficient for taste valence representation. *J. Neurosci.* **39**, 9369–9382 (2019).
134. S. A. Allsop, R. Wichmann, F. Mills, A. Burgos-Robles, C. J. Chang, A. C. Felix-Ortiz, A. Vienne, A. Beyeler, E. M. Izadmehr, G. Glober, M. I. Cum, J. Stergiadou, K. K. Anandalingam, K. Farris, P. Namburi, C. A. Leppla, J. C. Weddington, E. H. Nieh, A. C. Smith, D. Ba, E. N. Brown, K. M. Tye, Corticoamygdala transfer of socially derived information gates observational learning. *Cell* **173**, 1329–1342.e18 (2018).
135. W.-Y. Ong, C. S. Stohler, D. R. Herr, Role of the prefrontal cortex in pain processing. *Mol. Neurobiol.* **56**, 1137–1166 (2019).
136. A. V. Apkarian, M. C. Bushnell, R.-D. Treede, J.-K. Zubieta, Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain* **9**, 463–484 (2005).
137. C. Lamm, J. Decety, T. Singer, Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. *NeuroImage* **54**, 2492–2502 (2011).
138. L. Mazzola, J. Isnard, R. Peyron, F. Mauguire, Stimulation of the human cortex and the experience of pain: Wilder Penfield's observations revisited. *Brain* **135**, 631–640 (2012).
139. D. A. Gehrlach, C. Weiand, T. N. Gaitanos, E. Cho, A. S. Klein, A. A. Hennrich, K. K. Conzelmann, N. Gogolla, A whole-brain connectivity map of mouse insular cortex. *eLife* **9**, e55585 (2020).
140. V. Picchio, V. Cammisotto, F. Pagano, R. Carnevale, I. Chimenti, The insular cortex and the amygdala: Shared functions and interactions. *Dermatol. Int.*, 1–15 (2012).
141. R. Galhardoni, V. A. Da Silva, L. García-Larrea, C. Dale, A. F. Baptista, L. M. Barbosa, L. M. Bahia Menezes, S. R. D. T. De Siqueira, F. Valério, J. Rosi, A. L. De Lima Rodrigues, D. T. R. Mendes Fernandes, P. M. Lorencini Selingardi, M. A. Marcolin, F. L. De Souza Duran, C. R. Ono, L. T. Lucato, A. M. B. L. Fernandes, F. E. F. Da Silva, L. T. Yeng, A. R. Brunoni, C. A. Buchpiguel, M. J. Teixeira, D. C. De Andrade, Insular and anterior cingulate cortex deep stimulation for central neuropathic pain disassembling the percept of pain. *Neurology* **92**, e2165–e2175 (2019).
142. L. L. Tan, P. Pelzer, C. Heinl, W. Tang, V. Gangadharan, H. Flor, R. Sprengel, T. Kuner, R. Kuner, A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat. Neurosci.* **20**, 1591–1601 (2017).
143. F. Barthas, J. Sellmeijer, S. Hugel, E. Waltisperger, M. Barrot, I. Yalcin, The anterior cingulate cortex is a critical hub for pain-induced depression. *Biol. Psychiatry* **77**, 236–245 (2015).
144. A. R. Burkey, E. Carstens, J. J. Wenniger, J. Tang, L. Jasmin, An opioidergic cortical antinociception triggering site in the agranular insular cortex of the rat that contributes to morphine antinociception. *J. Neurosci.* **16**, 6612–6623 (1996).
145. L. Jasmin, A. Granato, P. T. Ohara, Rostral agranular insular cortex and pain areas of the central nervous system: A tract-tracing study in the rat. *J. Comp. Neurol.* **468**, 425–440 (2004).
146. J. Kim, M. Pignatelli, S. Xu, S. Itohara, S. Tonegawa, Antagonistic negative and positive neurons of the basolateral amygdala. *Nat. Neurosci.* **19**, 1636–1646 (2016).
147. Y. Livneh, A. U. Sugden, J. C. Madara, R. A. Essner, V. I. Flores, L. A. Sugden, J. M. Resch, B. B. Lowell, M. L. Andermann, Estimation of current and future physiological states in insular cortex. *Neuron* **105**, 1094–1111.e10 (2020).
148. D. J. Berg, C. Chen, B. Ahanonu, M. Chen, S. Quake, M. J. Schnitzer, G. Scherrer, Resolving the molecular identity and connectivity of amygdalar neural ensembles active during pain, in *2019 Neuroscience Meeting Planner* (Society for Neuroscience, 2019).
149. B. A. Vogt, Pain and emotion interactions in subregions of the cingulate gyrus. *Nat. Rev. Neurosci.* **6**, 533–544 (2005).

150. T. D. Wager, L. Y. Atlas, M. M. Botvinick, L. J. Chang, R. C. Coghill, K. D. Davis, G. D. Iannetti, R. A. Poldrack, A. J. Shackman, T. Yarkoni, Pain in the ACC? *Proc. Natl. Acad. Sci. U.S.A.* **113**, E2474–E2475 (2016).
151. R. B. Ebitz, B. Y. Hayden, Dorsal anterior cingulate: A Rorschach test for cognitive neuroscience. *Nat. Neurosci.* **19**, 1278–1279 (2016).
152. B. A. Vogt, G. Paxinos, Cytoarchitecture of mouse and rat cingulate cortex with human homologies. *Brain Struct. Funct.* **219**, 185–192 (2014).
153. B. A. Vogt, Midcingulate cortex: Structure, connections, homologies, functions and diseases. *J. Chem. Neuroanat.* **74**, 28–46 (2016).
154. C. A. Mussio, S. E. Harte, G. S. Borszcz, Regional differences within the anterior cingulate cortex in the generation versus suppression of pain affect in rats. *J. Pain* **21**, 121–134 (2020).
155. P. N. Fuchs, Y. B. Peng, J. A. Boyette-Davis, M. L. Uhelski, The anterior cingulate cortex and pain processing. *Front. Integr. Neurosci.* **8**, 35 (2014).
156. I. Strauss, A. Berger, S. Ben Moshe, M. Arad, U. Hochberg, T. Gonen, R. Tellem, Double anterior stereotactic cingulotomy for intractable oncological pain. *Stereotact. Funct. Neurosurg.* **95**, 400–408 (2018).
157. K. D. Davis, W. D. Hutchison, A. M. Lozano, J. O. Dostrovsky, Altered pain and temperature perception following cingulotomy and capsulotomy in a patient with schizoaffective disorder. *Pain* **59**, 189–199 (1994).
158. T. V. P. Bliss, G. L. Collingridge, B.-K. Kaang, M. Zhuo, Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat. Rev. Neurosci.* **17**, 485–496 (2016).
159. M. Santello, T. Nevian, Dysfunction of cortical dendritic integration in neuropathic pain reversed by serotoninergic neuromodulation. *Neuron* **86**, 233–246 (2015).
160. S. C. Matos, Z. Zhang, P. Séguéla, Peripheral neuropathy induces HCN channel dysfunction in pyramidal neurons of the medial prefrontal cortex. *J. Neurosci.* **35**, 13244–13256 (2015).
161. H. Zhou, Q. Zhang, E. Martinez, J. Dale, S. Hu, E. Zhang, K. Liu, D. Huang, G. Yang, Z. Chen, J. Wang, Ketamine reduces aversion in rodent pain models by suppressing hyperactivity of the anterior cingulate cortex. *Nat. Commun.* **9**, 3751 (2018).
162. H. C. Moon, W. I. Heo, Y. J. Kim, D. Lee, S. Y. Won, H. R. Kim, S. M. Ha, Y. J. Lee, Y. S. Park, Optical inactivation of the anterior cingulate cortex modulate descending pain pathway in a rat model of trigeminal neuropathic pain created via chronic constriction injury of the infraorbital nerve. *J. Pain Res.* **10**, 2355–2364 (2017).
163. K. C. Elina, H. C. Moon, J. Islam, H. K. Kim, Y. S. Park, The effect of optogenetic inhibition of the anterior cingulate cortex in neuropathic pain following sciatic nerve injury. *J. Mol. Neurosci.* **71**, 638–650 (2021).
164. K. Koga, G. Descalzi, T. Chen, H. G. Ko, J. Lu, S. Li, J. Son, T. H. Kim, C. Kwak, R. L. Huganir, M. G. Zhao, B. K. Kaang, G. L. Collingridge, M. Zhuo, Coexistence of two forms of LTP in ACC provides a synaptic mechanism for the interactions between anxiety and chronic pain. *Neuron* **85**, 377–389 (2015).
165. H. C. Shih, J. W. Yang, C. M. Lee, B. C. Shyu, Spontaneous cingulate high-current spikes signal normal and pathological pain states. *J. Neurosci.* **39**, 5128–5142 (2019).
166. J. W. Yang, H. C. Shih, B. C. Shyu, Intracortical circuits in rat anterior cingulate cortex are activated by nociceptive inputs mediated by medial thalamus. *J. Neurophysiol.* **96**, 3409–3422 (2006).
167. C. Corradi-Dell'Acqua, A. Tusche, P. Vuilleumier, T. Singer, Cross-modal representations of first-hand and vicarious pain, disgust and fairness in insular and cingulate cortex. *Nat. Commun.* **7**, 10904 (2016).
168. W. D. Hutchison, K. D. Davis, A. M. Lozano, R. R. Tasker, J. O. Dostrovsky, Pain-related neurons in the human cingulate cortex. *Nat. Neurosci.* **2**, 403–405 (1999).
169. K. Z. Meyza, I. B.-A. Bartal, M. H. Monfils, J. B. Panksepp, E. Knapska, The roots of empathy: Through the lens of rodent models. *Neurosci. Biobehav. Rev.* **76**, 216–234 (2017).
170. M. L. Smith, N. Asada, R. C. Malenka, Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia. *Science* **371**, 153–159 (2021).
171. V. J. Brown, E. M. Bowman, Rodent models of prefrontal cortical function. *Trends Neurosci.* **25**, 340–343 (2002).
172. S. Shiers, T. J. Price, Molecular, circuit, and anatomical changes in the prefrontal cortex in chronic pain. *Pain* **161**, 1726–1729 (2020).
173. L. K. Bicks, H. Koike, S. Akbarian, H. Morishita, Prefrontal cortex and social cognition in mouse and man. *Front. Psychol.* **6**, 1805 (2015).
174. S. B. Ostlund, B. W. Balleine, Orbitofrontal cortex mediates outcome encoding in Pavlovian but not instrumental conditioning. *J. Neurosci.* **27**, 4819–4825 (2007).
175. A. B. Mulder, R. E. Nordquist, O. Orgüt, C. M. A. Pennartz, Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. *Behav. Brain Res.* **146**, 77–88 (2003).
176. J. E. Ledoux, R. Brown, A higher-order theory of emotional consciousness. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E2016–E2025 (2017).
177. T. Sigurdsson, S. Duvarci, Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. *Front. Syst. Neurosci.* **9**, 190 (2016).
178. H. Cardoso-Cruz, D. Lima, V. Galhardo, Impaired spatial memory performance in a rat model of neuropathic pain is associated with reduced hippocampus–prefrontal cortex connectivity. *J. Neurosci.* **33**, 2465–2480 (2013).
179. N. K. Lidhar, S. Darvish-Ghane, S. Sivaselvachandran, S. Khan, F. Wasif, H. Turner, M. Sivaselvachandran, N. M. Fournier, L. J. Martin, Prelimbic cortex glucocorticoid receptors regulate the stress-mediated inhibition of pain contagion in male mice. *Neuropsychopharmacology* **46**, 1183–1193 (2021).
180. S. Huang, Z. Zhang, E. Gambeta, S. C. Xu, C. Thomas, N. Godfrey, L. Chen, S. M'Dahoma, S. L. Borgland, G. W. Zamponi, Dopamine inputs from the ventral tegmental area into the medial prefrontal cortex modulate neuropathic pain-associated behaviors in mice. *Cell Rep.* **31**, 107812 (2020).
181. G. Q. Wang, C. Cen, C. Li, S. Cao, N. Wang, Z. Zhou, X. M. Liu, Y. Xu, N. X. Tian, Y. Zhang, J. Wang, L. P. Wang, Y. Wang, Deactivation of excitatory neurons in the prelimbic cortex via Cdk5 promotes pain sensation and anxiety. *Nat. Commun.* **6**, 7660 (2015).
182. L. Yue, L.-Y. Ma, S. Cui, F.-Y. Liu, M. Yi, Y. Wan, Brain-derived neurotrophic factor in the infralimbic cortex alleviates inflammatory pain. *Neurosci. Lett.* **655**, 7–13 (2017).
183. K. K. Kummer, M. Mitrić, T. Kalpachidou, M. Kress, The medial prefrontal cortex as a central hub for mental comorbidities associated with chronic pain. *Int. J. Mol. Sci.* **21**, 3440 (2020).
184. Y.-W. Shih, H.-Y. Tsai, F.-S. Lin, Y.-H. Lin, C.-Y. Chiang, Z.-L. Lu, X. M.-T. Tseng, Effects of positive and negative expectations on human pain perception engage separate but interrelated and dependently regulated cerebral mechanisms. *J. Neurosci.* **39**, 1261–1274 (2019).
185. B. S. Foley, Wall and Melzack's Textbook of Pain, 5th Edition. *Am. J. Phys. Med. Rehabil.* **85**, 581 (2006).
186. S. Geuter, F. Eippert, C. Hindi Attar, C. Büchel, Cortical and subcortical responses to high and low effective placebo treatments. *NeuroImage* **67**, 227–236 (2013).
187. M. Amanzio, F. Benedetti, C. A. Porro, S. Palermo, F. Cauda, Activation likelihood estimation meta-analysis of brain correlates of placebo analgesia in human experimental pain. *Hum. Brain Mapp.* **34**, 738–752 (2012).
188. M. Zunhammer, T. Spisák, T. D. Wager, U. Bingel; Placebo Imaging Consortium, Meta-analysis of neural systems underlying placebo analgesia from individual participant fMRI data. *Nat. Commun.* **12**, 1391 (2021).
189. A. Keller, T. Akintola, L. Colloca, Placebo analgesia in rodents: Current and future research. *Int. Rev. Neurobiol.* , 1–15 (2018).
190. S. i. Kim, Neuroscientific model of motivational process. *Front. Psychol.* **4**, 98 (2013).
191. M. L. Loggia, C. Berna, J. Kim, C. M. Cahalan, R. L. Gollub, A. D. Wasan, R. E. Harris, R. R. Edwards, V. Napadow, Disrupted brain circuitry for pain-related reward/punishment in fibromyalgia. *Arthritis Rheum.* **66**, 203–212 (2014).
192. R. A. Serafini, K. D. Pryce, V. Zachariou, The mesolimbic dopamine system in chronic pain and associated affective comorbidities. *Biol. Psychiatry* **87**, 64–73 (2020).
193. S. Yu, W. Li, W. Shen, R. R. Edwards, R. L. Gollub, G. Wilson, J. Park, A. Ortiz, J. Cao, J. Gerber, I. Mawla, S.-T. Chan, J. Lee, A. D. Wasan, V. Napadow, T. J. Kaptchuk, B. Rosen, J. Kong, Impaired mesocorticolimbic connectivity underlies increased pain sensitivity in chronic low back pain. *NeuroImage* **218**, 116969 (2020).
194. M. Fairhurst, K. Wiech, P. Dunckley, I. Tracey, Anticipatory brainstem activity predicts neural processing of pain in humans. *Pain* **128**, 101–110 (2007).
195. J. Jensen, A. R. McIntosh, A. P. Crawley, D. J. Mikulis, G. Remington, S. Kapur, Direct activation of the ventral striatum in anticipation of aversive stimuli. *Neuron* **40**, 1251–1257 (2003).
196. L. Becerra, H. C. Breiter, R. Wise, R. G. Gonzalez, D. Borsook, Reward circuitry activation by noxious thermal stimuli. *Neuron* **32**, 927–946 (2001).
197. K. Forkmann, K. Wiech, T. Sommer, U. Bingel, Reinstatement of pain-related brain activation during the recognition of neutral images previously paired with nociceptive stimuli. *Pain* **156**, 1501–1510 (2015).
198. L. Becerra, E. Navratilova, F. Porreca, D. Borsook, Analogous responses in the nucleus accumbens and cingulate cortex to pain onset (aversion) and offset (relief) in rats and humans. *J. Neurophysiol.* **110**, 1221–1226 (2013).
199. R. Bourdy, M. Barrot, A new control center for dopaminergic systems: Pulling the VTA by the tail. *Trends Neurosci.* **35**, 681–690 (2012).
200. C. R. Ferrario, G. Labouèbe, S. Liu, E. H. Nieh, V. H. Routh, S. Xu, E. C. O'Connor, Homeostasis meets motivation in the battle to control food intake. *J. Neurosci.* **36**, 11469–11481 (2016).
201. H. Morikawa, C. A. Paladini, Dynamic regulation of midbrain dopamine neuron activity: Intrinsic, synaptic, and plasticity mechanisms. *Neuroscience* **198**, 95–111 (2011).
202. M. Morales, E. B. Margolis, Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nat. Rev. Neurosci.* **18**, 73–85 (2017).
203. K. T. Beier, X. J. Gao, S. Xie, K. E. DeLoach, R. C. Malenka, L. Luo, Topological organization of ventral tegmental area connectivity revealed by viral-genetic dissection of input-output relations. *Cell Rep.* **26**, 159–167.e6 (2019).
204. W. Ren, M. V. Centeno, S. Berger, Y. Wu, X. Na, X. Liu, J. Kondapalli, A. V. Apkarian, M. Martina, D. J. Surmeier, The indirect pathway of the nucleus accumbens shell amplifies neuropathic pain. *Nat. Neurosci.* **19**, 220–222 (2016).
205. R. S. Laurent, V. Martinez Damonte, A. C. Tsuda, J. A. Kauer, Periaqueductal gray and rostromedial tegmental inhibitory afferents to VTA have distinct synaptic plasticity and opiate sensitivity. *Neuron* **106**, 624–636.e4 (2020).

206. M. W. Waung, E. B. Margolis, A. R. Charbit, H. L. Fields, A midbrain circuit that mediates headache aversiveness in rats. *Cell Rep.* **28**, 2739–2747.e4 (2019).
207. A. M. W. Taylor, A. Castonguay, A. J. Taylor, N. P. Murphy, A. Ghogha, C. Cook, L. Xue, M. C. Olmstead, Y. De Koninck, C. J. Evans, C. M. Cahill, Microglia disrupt mesolimbic reward circuitry in chronic pain. *J. Neurosci.* **35**, 8442–8450 (2015).
208. N. Schwartz, P. Temkin, S. Jurado, B. K. Lim, B. D. Heifets, J. S. Polepalli, R. C. Malenka, Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. *Science* **345**, 535–542 (2014).
209. N. Massaly, B. A. Copits, A. R. Wilson-Poe, L. Hipólito, T. Markovic, H. J. Yoon, S. Liu, M. C. Walicki, D. L. Bhatti, S. Sirohi, A. Klaas, B. M. Walker, R. Neve, C. M. Cahill, K. I. Shoghi, R. W. Gereau 4th, J. G. McCall, R. Al-Hasani, M. R. Bruchas, J. A. Morón, Pain-induced negative affect is mediated via recruitment of the nucleus accumbens kappa opioid system. *Neuron* **102**, 564–573.e6 (2019).
210. H. L. Fields, How expectations influence pain. *Pain* **159** (suppl. 1), S3–S10 (2018).
211. B. Seymour, R. J. Dolan, Emotion, motivation, and pain, in *Textbook of Pain* (Elsevier, 2013), pp. 248–255.
212. N. Schwartz, C. Miller, H. L. Fields, Cortico-accumbens regulation of approach-avoidance behavior is modified by experience and chronic pain. *Cell Rep.* **19**, 1522–1531 (2017).
213. M. Lee, T. R. Manders, S. E. Eberle, C. Su, J. D'amour, R. Yang, H. Y. Lin, K. Deisseroth, R. C. Froemke, J. Wang, Activation of corticostriatal circuitry relieves chronic neuropathic pain. *J. Neurosci.* **35**, 5247–5259 (2015).
214. S. H. Gao, L. L. Shen, H. Z. Wen, Y. D. Zhao, P. H. Chen, H. Z. Ruan, The projections from the anterior cingulate cortex to the nucleus accumbens and ventral tegmental area contribute to neuropathic pain-evoked aversion in rats. *Neurobiol. Dis.* **140**, 104862 (2020).
215. B. Ziółkowska, The role of mesostriatal dopamine system and corticostriatal glutamatergic transmission in chronic pain. *Brain Sci.* **11**, 1311 (2021).
216. A. M. W. Taylor, S. Becker, P. Schweinhardt, C. Cahill, Mesolimbic dopamine signaling in acute and chronic pain: Implications for motivation, analgesia, and addiction. *Pain* **157**, 1194–1198 (2016).
217. K. Bannister, A. H. Dickenson, The plasticity of descending controls in pain: Translational probing. *J. Physiol.* **595**, 4159–4166 (2017).
218. E. E. Bagley, S. L. Ingram, Endogenous opioid peptides in the descending pain modulatory circuit. *Neuropharmacology* **173**, 108131 (2020).
219. M. M. Heinricher, I. Tavares, J. L. Leith, B. M. Lumb, Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res. Rev.* **60**, 214–225 (2009).
220. H. L. Fields, Pain modulation: Expectation, opioid analgesia and virtual pain. *Prog. Brain Res.* **122**, 245–253 (2000).
221. B. K. Lau, C. W. Vaughan, Descending modulation of pain: The GABA disinhibition hypothesis of analgesia. *Curr. Opin. Neurobiol.* **29**, 159–164 (2014).
222. V. K. Samineni, J. G. Grajales-Reyes, B. A. Copits, D. E. O'Brien, S. L. Trigg, A. M. Gomez, M. R. Bruchas, R. W. Gereau 4th, Divergent modulation of nociception by glutamatergic and GABAergic neuronal subpopulations in the periaqueductal gray. *eNeuro* **4**, ENEURO.0129-16.2017 (2017).
223. T. Chen, W. Taniguchi, Q.-Y. Chen, H. Tozaki-Saitoh, Q. Song, R.-H. Liu, K. Koga, T. Matsuda, Y. Kaito-Sugimura, J. Wang, Z.-H. Li, Y.-C. Lu, K. Inoue, M. Tsuda, Y.-Q. Li, T. Nakatsuka, M. Zhuo, Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nat. Commun.* **9**, 1886 (2018).
224. Y. Liu, A. Latremoliere, X. Li, Z. Zhang, M. Chen, X. Wang, C. Fang, J. Zhu, C. Alexandre, Z. Gao, B. Chen, X. Ding, J.-Y. Zhou, Y. Zhang, C. Chen, K. H. Wang, C. J. Woolf, Z. He, Touch and tactile neuropathic pain sensitivity are set by corticospinal projections. *Nature* **561**, 547–550 (2018).
225. A. I. Basbaum, H. L. Fields, Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. *Annu. Rev. Neurosci.* **7**, 309–338 (1984).
226. M. M. Morgan, K. L. Whittier, D. M. Hegarty, S. A. Aicher, Periaqueductal gray neurons project to spinally projecting GABAergic neurons in the rostral ventromedial medulla. *Pain* **140**, 376–386 (2008).
227. Y. Zhang, S. Zhao, E. Rodriguez, J. Takatoh, B.-X. Han, X. Zhou, F. Wang, Identifying local and descending inputs for primary sensory neurons. *J. Clin. Invest.* **125**, 3782–3794 (2015).
228. A. François, S. A. Low, E. I. Sypek, A. J. Christensen, C. Sotoudeh, K. T. Beier, C. Ramakrishnan, K. D. Ritola, R. Sharif-Naeini, K. Deisseroth, S. L. Delp, R. C. Malenka, L. Luo, A. W. Hantman, G. Scherrer, A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. *Neuron* **93**, 822–839.e6 (2017).
229. Y. S. Kim, Y. Chu, L. Han, M. Li, Z. Li, P. C. LaVinka, S. Sun, Z. Tang, K. Park, M. J. Caterina, K. Ren, R. Dubner, F. Wei, X. Dong, Central terminal sensitization of TRPV1 by descending serotonergic facilitation modulates chronic pain. *Neuron* **81**, 873–887 (2014).
230. S. Hirschberg, Y. Li, A. Randall, E. J. Kremer, A. E. Pickering, Functional dichotomy in spinal- vs prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from ascending aversion and anxiety in rats. *eLife* **6**, e29808 (2017).
231. J.-H. Kim, G. Gangadharan, J. Byun, E.-J. Choi, C. J. Lee, H.-S. Shin, Yin-and-yang bifurcation of opioidergic circuits for descending analgesia at the midbrain of the mouse. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 11078–11083 (2018).
232. D. L. Averitt, L. N. Eidson, H. H. Doyle, A. Z. Murphy, Neuronal and glial factors contributing to sex differences in opioid modulation of pain. *Neuropsychopharmacology* **44**, 155–165 (2019).
233. M.-H. Li, K. L. Suchland, S. L. Ingram, Compensatory activation of cannabinoid CB2 receptor inhibition of GABA release in the rostral ventromedial medulla in inflammatory pain. *J. Neurosci.* **37**, 626–636 (2017).
234. F. J. Jareczek, S. R. White, D. L. Hammond, Plasticity in brainstem mechanisms of pain modulation by nicotinic acetylcholine receptors in the rat. *eNeuro* **4**, ENEURO.0364-16.2017 (2017).
235. I. C. Umana, C. A. Daniele, B. A. Miller, C. Abburi, K. Gallagher, M. A. Brown, P. Mason, D. S. McGehee, Nicotinic modulation of descending pain control circuitry. *Pain* **158**, 1938–1950 (2017).
236. M. Silva, D. Martins, A. Charrua, F. Piscitelli, I. Tavares, C. Morgado, V. Di Marzo, Endovanilloid control of pain modulation by the rostroventromedial medulla in an animal model of diabetic neuropathy. *Neuropharmacology* **107**, 49–57 (2016).
237. T. M. Marshall, D. S. Herman, T. M. Largent-Milnes, H. Badghisi, K. Zuber, S. C. Holt, J. Lai, F. Porreca, T. W. Vanderah, Activation of descending pain-facilitatory pathways from the rostral ventromedial medulla by cholecystokinin elicits release of prostaglandin-E_2 in the spinal cord. *Pain* **153**, 86–94 (2012).
238. W. Yu, D. Pati, M. M. Pina, K. T. Schmidt, K. M. Boyt, A. C. Hunker, L. S. Zweifel, Z. A. McElligott, T. L. Kash, Periaqueductal gray/dorsal raphe dopamine neurons contribute to sex differences in pain-related behaviors. *Neuron* **109**, 1365–1380.e5 (2021).
239. N. D. Volkow, A. T. McLellan, Opioid abuse in chronic pain–misconceptions and mitigation strategies. *N. Engl. J. Med.* **374**, 1253–1263 (2016).
240. J. K. Zubieta, Y. R. Smith, J. A. Bueller, Y. Xu, M. R. Kilbourn, D. M. Jewett, C. R. Meyer, R. A. Koeppe, C. S. Stohler, Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* **293**, 311–315 (2001).
241. H. L. Fields, The doctor's dilemma: Opiate analgesics and chronic pain. *Neuron* **69**, 591–594 (2011).
242. H. W. Matthes, R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. Le Meur, P. Dollé, E. Tzavara, J. Hanoune, B. P. Roques, B. L. Kieffer, Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**, 819–823 (1996).
243. W. T. Birdsong, J. T. Williams, Recent progress in opioid research from an electrophysiological perspective. *Mol. Pharmacol.* **98**, 401–409 (2020).
244. J. T. Williams, S. L. Ingram, G. Henderson, C. Chavkin, M. von Zastrow, S. Schulz, T. Koch, C. J. Evans, M. J. Christie, Regulation of μ-opioid receptors: Desensitization, phosphorylation, internalization, and tolerance. *Pharmacol. Rev.* **65**, 223–254 (2013).
245. V. C. Dang, M. J. Christie, Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. *Br. J. Pharmacol.* **165**, 1704–1716 (2012).
246. R. C. Kupers, H. Konings, H. Adriaensen, J. M. Gybels, Morphine differentially affects the sensory and affective pain ratings in neurogenic and idiopathic forms of pain. *Pain* **47**, 5–12 (1991).
247. L. Gomtsian, K. Bannister, N. Eyde, D. Robles, A. H. Dickenson, F. Porreca, E. Navratilova, Morphine effects within the rodent anterior cingulate cortex and rostral ventromedial medulla reveal separable modulation of affective and sensory qualities of acute or chronic pain. *Pain* **159**, 2512–2521 (2018).
248. E. Navratilova, C. W. Atcherley, F. Porreca, Brain circuits encoding reward from pain relief. *Trends Neurosci.* **38**, 741–750 (2015).
249. T. A. Samad, K. A. Moore, A. Sapirstein, S. Billet, A. Allchorne, S. Poole, J. V. Bonventre, C. J. Woolf, Interleukin-1β-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature* **410**, 471–475 (2001).
250. L. Li, S.-R. Chen, M.-H. Zhou, L. Wang, D.-P. Li, H. Chen, G. Lee, V. Jayaraman, H.-L. Pan, α2δ-1 switches the phenotype of synaptic AMPA receptors by physically disrupting heteromeric subunit assembly. *Cell Rep.* **36**, 109396 (2021).
251. S. R. A. Alles, S. M. Cain, T. P. Snutch, Pregabalin as a pain therapeutic: Beyond calcium channels. *Front. Cell. Neurosci.* **14**, 83 (2020).
252. D.-S. Kim, K.-W. Li, A. Boroujerdi, Y. P. Yu, C.-Y. Zhou, P. Deng, J. Park, X. Zhang, J. Lee, M. Corpe, K. Sharp, O. Steward, C. Eroglu, B. Barres, F. Zaucke, Z. C. Xu, Z. D. Luo, Thrombospondin-4 contributes to spinal sensitization and neuropathic pain states. *J. Neurosci.* **32**, 8977–8987 (2012).
253. Ç. Eroglu, N. J. Allen, M. W. Susman, N. A. O'Rourke, C. Y. Park, E. Özkan, C. Chakraborty, S. B. Mulinyawe, D. S. Annis, A. D. Huberman, E. M. Green, J. Lawler, R. Dolmetsch, K. C. Garcia, S. J. Smith, Z. D. Luo, A. Rosenthal, D. F. Mosher, B. A. Barres, Gabapentin receptor α2δ-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* **139**, 380–392 (2009).
254. P. J. Wiffen, S. Derry, R. F. Bell, A. S. Rice, T. R. Tölle, T. Phillips, R. A. Moore, Gabapentin for chronic neuropathic pain in adults. *Cochrane Database Syst. Rev.* **6**, CD007938 (2017).

255. G. Corder, D. C. Castro, M. R. Bruchas, G. Scherrer, Endogenous and exogenous opioids in pain. *Annu. Rev. Neurosci.* **41**, 453–473 (2018).
256. J. Bailly, N. Del Rossi, L. Runtz, J.-J. Li, D. Park, G. Scherrer, A. Tanti, M.-C. Birling, E. Darcq, B. L. Kieffer, Targeting morphine-responsive neurons: Generation of a knock-in mouse line expressing Cre recombinase from the mμ-opioid receptor gene locus. *eNeuro* **7**, ENEURO.0433-19.2020 (2020).
257. E. Erbs, L. Faget, G. Scherrer, A. Matifas, D. Filliol, J.-L. Vonesch, M. Koch, P. Kessler, D. Hentsch, M.-C. Birling, M. Koutsourakis, L. Vasseur, P. Veinante, B. L. Kieffer, D. Massotte, A mμ–delta opioid receptor brain atlas reveals neuronal co-occurrence in subcortical networks. *Brain Struct. Funct.* **220**, 677–702 (2015).
258. G. Scherrer, P. Tryoen-Tóth, D. Filliol, A. Matifas, D. Laustriat, Y. Q. Cao, A. I. Basbaum, A. Dierich, J.-L. Vonesh, C. Gavériaux-Ruff, B. L. Kieffer, Knockin mice expressing fluorescent δ-opioid receptors uncover G protein-coupled receptor dynamics invivo. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9691–9696 (2006).
259. L. M. Snyder, M. C. Chiang, E. Loeza-Alcocer, Y. Omori, J. Hachisuka, T. D. Sheahan, J. R. Gale, P. C. Adelman, E. I. Sypek, S. A. Fulton, R. L. Friedman, M. C. Wright, M. G. Duque, Y. S. Lee, Z. Hu, H. Huang, X. Cai, K. A. Meerschaert, V. Nagarajan, T. Hirai, G. Scherrer, D. H. Kaplan, F. Porreca, B. M. Davis, M. S. Gold, H. R. Koerber, S. E. Ross, Kappa opioid receptor distribution and function in primary afferents. *Neuron* **99**, 1274–1288.e6 (2018).
260. C. Chen, A. H. Willhouse, P. Huang, N. Ko, Y. Wang, B. Xu, L. H. M. Huang, B. Kieffer, M. F. Barbe, L.-Y. Liu-Chen, Characterization of a knock-in mouse line expressing a fusion protein of κ opioid receptor conjugated with tdTomato: 3-dimensional brain imaging via CLARITY. *eNeuro* **7**, ENEURO.0028-20.2020 (2020).
261. P. C. S. Chung, H. L. Keyworth, E. Martin-Garcia, P. Charbogne, E. Darcq, A. Bailey, D. Filliol, A. Matifas, G. Scherrer, A.-M. Ouagazzal, C. Gaveriaux-Ruff, K. Befort, R. Maldonado, I. Kitchen, B. L. Kieffer, A novel anxiogenic role for the delta opioid receptor expressed in GABAergic forebrain neurons. *Biol. Psychiatry* **77**, 404–415 (2015).
262. R. Weibel, D. Reiss, L. Karchewski, O. Gardon, A. Matifas, D. Filliol, J. A. J. Becker, J. N. Wood, B. L. Kieffer, C. Gaveriaux-Ruff, Mu opioid receptors on primary afferent nav1.8 neurons contribute to opiate-induced analgesia: Insight from conditional knockout mice. *PLOS ONE* **8**, e74706 (2013).
263. U. Arvidsson, M. Riedl, S. Chakrabarti, J. H. Lee, A. H. Nakano, R. J. Dado, H. H. Loh, P. Y. Law, M. W. Wessendorf, R. Elde, Distribution and targeting of a mu-opioid receptor (MOR1) in brain and spinal cord. *J. Neurosci.* **15**, 3328–3341 (1995).
264. J. Brunton, S. Charpak, μ-opioid peptides inhibit thalamic neurons. *J. Neurosci.* **18**, 1671–1678 (1998).
265. L. Goedecke, X. Bengoetxea, P. Blaesse, H.-C. Pape, K. Jüngling, μ-opioid receptor-mediated downregulation of midline thalamic pathways to basal and central amygdala. *Sci. Rep.* **9**, 17837 (2019).
266. S. Sugita, E. Tanaka, R. A. North, Membrane properties and synaptic potentials of three types of neurone in rat lateral amygdala. *J. Physiol.* **460**, 705–718 (1993).
267. D. G. Standaert, S. J. Watson, R. A. Houghten, C. B. Saper, Opioid peptide immunoreactivity in spinal and trigeminal dorsal horn neurons projecting to the parabrachial nucleus in the rat. *J. Neurosci.* **6**, 1220–1226 (1986).
268. D. Huang, F. S. Grady, L. Peltekian, J. J. Laing, J. C. Geerling, Efferent projections of CGRP/Calca-expressing parabrachial neurons in mice. *J. Comp. Neurol.* **529**, 2911–2957 (2021).
269. S. A. Kissiwaa, S. D. Patel, B. L. Winters, E. E. Bagley, Opioids differentially modulate two synapses important for pain processing in the amygdala. *Br. J. Pharmacol.* **177**, 420–431 (2020).
270. T. F. Finnegan, S.-R. Chen, H.-L. Pan, Effect of the μ opioid on excitatory and inhibitory synaptic inputs to periaqueductal gray-projecting neurons in the amygdala. *J. Pharmacol. Exp. Ther.* **312**, 441–448 (2005).
271. Y. Zhu, C. F. R. Wienecke, G. Nachtrab, X. Chen, A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature* **530**, 219–222 (2016).
272. B. Muñoz, D. L. Haggerty, B. K. Atwood, Synapse-specific expression of mu opioid receptor long-term depression in the dorsomedial striatum. *Sci. Rep.* **10**, 7234 (2020).
273. E. Navratilova, J. Y. Xie, D. Meske, C. Qu, K. Morimura, A. Okun, N. Arakawa, M. Ossipov, H. L. Fields, F. Porreca, Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain. *J. Neurosci.* **35**, 7264–7271 (2015).
274. X.-Y. Zhang, Y.-N. Dou, L. Yuan, Q. Li, Y.-J. Zhu, M. Wang, Y.-G. Sun, Different neuronal populations mediate inflammatory pain analgesia by exogenous and endogenous opioids. *eLife* **9**, e55289 (2020).
275. S. J. Smith, U. Sümbül, L. T. Graybuck, F. Collman, S. Seshamani, R. Gala, O. Gliko, L. Elabbady, J. A. Miller, T. E. Bakken, J. Rossier, Z. Yao, E. Lein, H. Zeng, B. Tasic, M. Hawrylycz, Single-cell transcriptomic evidence for dense intracortical neuropeptide networks. *eLife* **8**, e47889 (2019).
276. F. G. Fang, C. M. Haws, K. Drasner, A. Williamson, H. L. Fields, Opioid peptides (DAGO-enkephalin, dynorphin A (1--13), BAM 22P) microinjected into the rat brainstem: Comparison of their antinociceptive effect and their effect on neuronal firing in the rostral ventromedial medulla. *Brain Res.* **501**, 116–128 (1989).
277. H. Fields, State-dependent opioid control of pain. *Nat. Rev. Neurosci.* **5**, 565–575 (2004).
278. M. Convertino, A. Samoshkin, J. Gauthier, M. S. Gold, W. Maixner, N. V. Dokholyan, L. Diatchenko, μ-Opioid receptor 6-transmembrane isoform: A potential therapeutic target for new effective opioids. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **62**, 61–67 (2015).
279. G. W. Pasternak, Opioids and their receptors: Are we there yet? *Neuropharmacology* **76**, 198–203 (2014).
280. R. M. van Rijn, J. L. Whistler, M. Waldhoer, Opioid-receptor-heteromer-specific trafficking and pharmacology. *Curr. Opin. Pharmacol.* **10**, 73–79 (2010).
281. M. Lemos Duarte, L. A. Devi, Post-translational modifications of opioid receptors. *Trends Neurosci.* **43**, 417–432 (2020).
282. H. L. Fields, E. B. Margolis, Understanding opioid reward. *Trends Neurosci.* **38**, 217–225 (2015).
283. R. Bardoni, V. L. Tawfik, D. Wang, A. François, C. Solorzano, S. A. Shuster, P. Choudhury, C. Betelli, C. Cassidy, K. Smith, J. C. de Nooij, F. Mennicken, D. O'Donnell, B. L. Kieffer, C. Jeffrey Woodbury, A. I. Basbaum, A. B. MacDermott, G. Scherrer, Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn. *Neuron* **81**, 1443 (2014).
284. G. Scherrer, N. Imamachi, Y.-Q. Cao, C. Contet, F. Mennicken, D. O'Donnell, B. L. Kieffer, A. I. Basbaum, Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. *Cell* **137**, 1148–1159 (2009).
285. Z. Yao, C. T. J. van Velthoven, T. N. Nguyen, J. Goldy, A. E. Sedeno-Cortes, F. Baftizadeh, D. Bertagnolli, T. Casper, M. Chiang, K. Crichton, S.-L. Ding, O. Fong, E. Garren, A. Glandon, N. W. Gouwens, J. Gray, L. T. Graybuck, M. J. Hawrylycz, D. Hirschstein, M. Kroll, K. Lathia, C. Lee, B. Levi, D. McMillen, S. Mok, T. Pham, Q. Ren, C. Rimorin, N. Shapovalova, J. Sulc, S. M. Sunkin, M. Tieu, A. Torkelson, H. Tung, K. Ward, N. Dee, K. A. Smith, B. Tasic, H. Zeng, A taxonomy of transcriptomic cell types across the isocortex and hippocampal formation. *Cell* **184**, 3222–3241.e26 (2021).
286. C. Chavkin, G. F. Koob, Dynorphin, dysphoria, and dependence: The stress of addiction. *Neuropsychopharmacology* **41**, 373–374 (2016).
287. R. Al-Hasani, J. G. McCall, G. Shin, A. M. Gomez, G. P. Schmitz, J. M. Bernardi, C.-O. Pyo, S. I. Park, C. M. Marcinkiewcz, N. A. Crowley, M. J. Krashes, B. B. Lowell, T. L. Kash, J. A. Rogers, M. R. Bruchas, Distinct subpopulations of nucleus accumbens dynorphin neurons drive aversion and reward. *Neuron* **87**, 1063–1077 (2015).
288. C. Gaveriaux-Ruff, C. Nozaki, X. Nadal, X. C. Hever, R. Weibel, A. Matifas, D. Reiss, D. Filliol, M. A. Nassar, J. N. Wood, R. Maldonado, B. L. Kieffer, Genetic ablation of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia. *Pain* **152**, 1238–1248 (2011).
289. C. M. Epstein, Physics and biophysics of TMS, in *The Oxford Handbook of Transcranial Stimulation* (Oxford Univ. Press, 2008).
290. H. Knotkova, *Pain: Brain Stimulation in the Treatment of Pain* (Nova Science Publishers, 2010).
291. M. H. Histed, V. Bonin, R. Clay Reid, Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation. *Neuron* **63**, 508–522 (2009).
292. M. A. Nitsche, L. G. Cohen, E. M. Wassermann, A. Priori, N. Lang, A. Antal, W. Paulus, F. Hummel, P. S. Boggio, F. Fregni, A. Pascual-Leone, Transcranial direct current stimulation: State of the art 2008. *Brain Stimul.* **1**, 206–223 (2008).
293. Y.-Z. Huang, M. J. Edwards, E. Rounis, K. P. Bhatia, J. C. Rothwell, Theta burst stimulation of the human motor cortex. *Neuron* **45**, 201–206 (2005).
294. R. Galhardoni, G. S. Correia, H. Araujo, L. T. Yeng, D. T. Fernandes, H. H. Kaziyama, M. A. Marcolin, D. Bouhassira, M. J. Teixeira, D. C. De Andrade, Repetitive transcranial magnetic stimulation in chronic pain: A review of the literature. *Arch. Phys. Med. Rehabil.* **96**, S156–S172 (2015).
295. M. Gaertner, J. T. Kong, K. H. Scherrer, A. Foote, S. Mackey, K. A. Johnson, Advancing transcranial magnetic stimulation methods for complex regional pain syndrome: An open-label study of paired theta burst and high-frequency stimulation. *Neuromodulation* **21**, 409–416 (2018).
296. J. R. Eles, K. C. Stieger, T. D. Y. Kozai, The temporal pattern of intracortical microstimulation pulses elicits distinct temporal and spatial recruitment of cortical neuropil and neurons. *J. Neural Eng.* **18**, 015001 (2021).
297. M. F. DosSantos, N. Ferreira, R. L. Toback, A. C. Carvalho, A. F. DaSilva, Potential mechanisms supporting the value of motor cortex stimulation to treat chronic pain syndromes. *Front. Neurosci.* **10**, 18 (2016).
298. T. Riechert, *Stereotactic Brain Operations: Methods, Clinical Aspects, Indications* (Huber, 1980).
299. J. Reijonen, L. Säisänen, M. Könönen, A. Mohammadi, P. Julkunen, The effect of coil placement and orientation on the assessment of focal excitability in motor mapping with navigated transcranial magnetic stimulation. *J. Neurosci. Methods* **331**, 108521 (2020).
300. V. Kozyrev, U. T. Eysel, D. Jancke, Voltage-sensitive dye imaging of transcranial magnetic stimulation-induced intracortical dynamics. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 13553–13558 (2014).
301. A. T. Hussin, J. A. Boychuk, A. R. Brown, Q. J. Pittman, G. C. Teskey, Intracortical microstimulation (ICMS) activates motor cortex layer 5 pyramidal neurons mainly transsynaptically. *Brain Stimul.* **8**, 742–750 (2015).

302. T. Suzuki, K. Sugawara, K. Ogahara, T. Higashi, Time course of corticospinal excitability and intracortical inhibition just before muscle relaxation. *Front. Hum. Neurosci.* **10**, 1 (2016).
303. M. A. Nitsche, W. Paulus, Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* **57**, 1899–1901 (2001).
304. T. Wu, M. Sommer, F. Tergau, W. Paulus, Lasting influence of repetitive transcranial magnetic stimulation on intracortical excitability in human subjects. *Neurosci. Lett.* **287**, 37–40 (2000).
305. T. Tsubokawa, Y. Katayama, T. Yamamoto, T. Hirayama, S. Koyama, Treatment of thalamic pain by chronic motor cortex stimulation. *Pacing Clin. Electrophysiol.* **14**, 131–134 (1991).
306. D. R. Esfahani, M. T. Pisansky, R. M. Dafer, D. E. Anderson, Motor cortex stimulation: Functional magnetic resonance imaging-localized treatment for three sources of intractable facial pain: Report of 3 cases. *J. Neurosurg.* **114**, 189–195 (2011).
307. T. J. Meeker, M. L. Keaser, S. A. Khan, R. P. Gullapalli, D. A. Seminowicz, J. D. Greenspan, Non-invasive motor cortex neuromodulation reduces secondary hyperalgesia and enhances activation of the descending pain modulatory network. *Front. Neurosci.* **13**, 467 (2019).
308. E. M. De Andrade, R. C. R. Martinez, R. L. Pagano, P. S. S. Lopes, A. V. V. Auada, F. V. Gouveia, G. F. Antunes, D. V. Assis, I. Lebrun, E. T. Fonoff, Neurochemical effects of motor cortex stimulation in the periaqueductal gray during neuropathic pain. *J. Neurosurg.* **132**, 239–251 (2020).
309. H. H. Jung, J. Shin, J. Kim, S. H. Ahn, S. E. Lee, C. S. Koh, J. S. Cho, C. Kong, H. C. Shin, S. J. Kim, J. W. Chang, Rostral agranular insular cortex lesion with motor cortex stimulation enhances pain modulation effect on neuropathic pain model. *Neural Plast.* **2016**, 3898924 (2016).
310. K. Kudo, T. Takahashi, S. Suzuki, The changes of c-Fos expression by motor cortex stimulation in the deafferentation pain model. *Neurol. Med. Chir.* **54**, 537–544 (2014).
311. R. L. Pagano, D. V. Assis, J. A. Clara, A. S. Alves, C. S. Dale, M. J. Teixeira, E. T. Fonoff, L. R. Britto, Transdural motor cortex stimulation reverses neuropathic pain in rats: A profile of neuronal activation. *Eur. J. Pain* **15**, 268.e1–268.e14 (2011).
312. X. Moisset, M. Lanteri-Minet, D. Fontaine, Neurostimulation methods in the treatment of chronic pain. *J. Neural Transm.* **127**, 673–686 (2020).
313. L. K. Case, C. M. Laubacher, H. Olausson, B. Wang, P. A. Spagnolo, M. C. Bushnell, Encoding of touch intensity but not pleasantness in human primary somatosensory cortex. *J. Neurosci.* **36**, 5850–5860 (2016).
314. P. L. Lockwood, G. D. Iannetti, P. Haggard, Transcranial magnetic stimulation over human secondary somatosensory cortex disrupts perception of pain intensity. *Cortex* **49**, 2201–2209 (2013).
315. L. K. Case, C. M. Laubacher, E. A. Richards, P. Spagnolo, H. Olausson, M. C. Bushnell, Inhibitory rTMS of secondary somatosensory cortex reduces intensity but not pleasantness of gentle touch. *Neurosci. Lett.* **653**, 84–91 (2017).
316. Y. Umezaki, B. W. Badran, W. H. DeVries, J. Moss, T. Gonzales, M. S. George, The efficacy of daily prefrontal repetitive transcranial magnetic stimulation (rTMS) for burning mouth syndrome (BMS): A randomized controlled single-blind study. *Brain Stimul.* **9**, 234–242 (2016).
317. D. A. Seminowicz, M. Moayedi, The dorsolateral prefrontal cortex in acute and chronic pain. *J. Pain* **18**, 1027–1035 (2017).
318. F. Brighina, A. Piazza, G. Vitello, A. Aloisio, A. Palermo, O. Daniele, B. Fierro, rTMS of the prefrontal cortex in the treatment of chronic migraine: A pilot study. *J. Neurol. Sci.* **227**, 67–71 (2004).
319. S. G. J. Boccard, E. A. C. Pereira, T. Z. Aziz, Deep brain stimulation for chronic pain. *J. Clin. Neurosci.* **22**, 1537–1543 (2015).
320. R. Roy, R. de la Vega, M. P. Jensen, J. Miró, Neurofeedback for pain management: A systematic review. *Front. Neurosci.* **14**, 671 (2020).
321. K. Patel, H. Sutherland, J. Henshaw, J. R. Taylor, C. A. Brown, A. J. Casson, N. J. Trujillo-Barreton, A. K. P. Jones, M. Sivan, Effects of neurofeedback in the management of chronic pain: A systematic review and meta-analysis of clinical trials. *Eur. J. Pain* **24**, 1440–1457 (2020).
322. M. P. Jensen, M. A. Day, J. Miró, Neuromodulatory treatments for chronic pain: Efficacy and mechanisms. *Nat. Rev. Neurol.* **10**, 167–178 (2014).
323. R. C. decharms, F. Maeda, G. H. Glover, D. Ludlow, J. M. Pauly, D. Soneji, J. D. E. Gabrieli, S. C. Mackey, Control over brain activation and pain learned by using real-time functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18626–18631 (2005).
324. M. Guan, L. Ma, L. Li, B. Yan, L. Zhao, L. Tong, S. Dou, L. Xia, M. Wang, D. Shi, Self-regulation of brain activity in patients with postherpetic neuralgia: A double-blind randomized study using real-time FMRI neurofeedback. *PLOS ONE* **10**, e0123675 (2015).
325. N. Goldway, J. Ablin, O. Lubin, Y. Zamir, J. N. Keynan, A. Or-Borichev, M. Cavazza, F. Charles, N. Intrator, S. Brill, E. Ben-Simon, H. Sharon, T. Hendler, Volitional limbic neuromodulation exerts a beneficial clinical effect on Fibromyalgia. *NeuroImage* **186**, 758–770 (2019).
326. S. Zhang, W. Yoshida, H. Mano, T. Yanagisawa, F. Mancini, K. Shibata, M. Kawato, B. Seymour, Pain control by co-adaptive learning in a brain-machine interface. *Curr. Biol.* **30**, 3935–3944.e7 (2020).
327. L. E. Stoeckel, K. A. Garrison, S. Ghosh, P. Wighton, C. A. Hanlon, J. M. Gilman, S. Greer, N. B. Turk-Browne, M. T. deBettencourt, D. Scheinost, C. Craddock, T. Thompson, V. Calderon, C. C. Bauer, M. George, H. C. Breiter, S. Whitfield-Gabrieli, J. D. Gabrieli, S. M. LaConte, L. Hirshberg, J. A. Brewer, M. Hampson, A. Van Der Kouwe, S. Mackey, A. E. Evins, Optimizing real time fMRI neurofeedback for therapeutic discovery and development. *Neuroimage Clin.* **5**, 245–255 (2014).
328. N. Lubianiker, N. Goldway, T. Fruchtman-Steinbok, C. Paret, J. N. Keynan, N. Singer, A. Cohen, K. C. Kadosh, D. E. J. Linden, T. Hendler, Process-based framework for precise neuromodulation. *Nat. Hum. Behav.* **3**, 436–445 (2019).
329. H. Chapin, E. Bagarinao, S. Mackey, Real-time fMRI applied to pain management. *Neurosci. Lett.* **520**, 174–181 (2012).
330. S. G. Hofmann, A. Asnaani, I. J. J. Vonk, A. T. Sawyer, A. Fang, The efficacy of cognitive behavioral therapy: A review of meta-analyses. *Cogn. Ther. Res.* **36**, 427–440 (2012).
331. H. An, R.-H. He, Y.-R. Zheng, R. Tao, Cognitive-behavioral therapy. *Adv. Exp. Med. Biol.* **1010**, 321–329 (2017).
332. K. Rnic, D. J. A. Dozois, R. A. Martin, Cognitive distortions, humor styles, and depression. *Eur. J. Psychol. Assess.* **12**, 348–362 (2016).
333. A. A. Lazarus, A. Abramovitz, A multimodal behavioral approach to performance anxiety. *J. Clin. Psychol.* **60**, 831–840 (2004).
334. K. M. Carroll, B. D. Kiluk, Cognitive behavioral interventions for alcohol and drug use disorders: Through the stage model and back again. *Psychol. Addict. Behav.* **31**, 847–861 (2017).
335. A. Verdejo-Garcia, G. Garcia-Fernandez, G. Dom, Cognition and addiction. *Dialogues Clin. Neurosci.* **21**, 281–290 (2019).
336. L. Borza, Cognitive-behavioral therapy for generalized anxiety. *Dialogues Clin. Neurosci.* **19**, 203–208 (2017).
337. D. A. Clark, A. T. Beck, Cognitive theory and therapy of anxiety and depression: Convergence with neurobiological findings. *Trends Cogn. Sci.* **14**, 418–424 (2010).
338. A. Zhang, L. A. Borhneimer, A. Weaver, C. Franklin, A. H. Hai, S. Guz, L. Shen, Cognitive behavioral therapy for primary care depression and anxiety: A secondary meta-analytic review using robust variance estimation in meta-regression. *J. Behav. Med.* **42**, 1117–1141 (2019).
339. C. A. Webb, I. M. Rosso, S. L. Rauch, Internet-based cognitive-behavioral therapy for depression: Current progress and future directions. *Harv. Rev. Psychiatry* **25**, 114–122 (2017).
340. S. Marques, D. Barrocas, D. Rijo, Psychological treatments for borderline personality disorder: A review of cognitive-behavioral oriented therapies. *Acta Medica Port.* **30**, 307–319 (2017).
341. N. R. Cunningham, S. Kashikar-Zuck, R. C. Coghill, Brain mechanisms impacted by psychological therapies for pain: Identifying targets for optimization of treatment effects. *Pain Rep.* **4**, e767 (2019).
342. L. M. McCracken, D. C. Turk, Behavioral and cognitive–behavioral treatment for chronic pain: Outcome, predictors of outcome, and treatment process. *Spine* **27**, 2564–2573 (2002).
343. M. P. Jensen, J. A. Turner, J. M. Romano, Changes in beliefs, catastrophizing, and coping are associated with improvement in multidisciplinary pain treatment. *J. Consult. Clin. Psychol.* **69**, 655–662 (2001).
344. B. D. Darnall, L. Colloca, Optimizing placebo and minimizing nocebo to reduce pain, catastrophizing, and opioid use: A review of the science and an evidence-informed clinical toolkit. *Int. Rev. Neurobiol.* **139**, 129–157 (2018).
345. C. Villemure, M. C. Bushnell, Mood influences supraspinal pain processing separately from attention. *J. Neurosci.* **29**, 705–715 (2009).
346. S. E. Longe, R. Wise, S. Bantick, D. Lloyd, H. Johansen-Berg, F. McGlone, I. Tracey, Counter-stimulatory effects on pain perception and processing are significantly altered by attention: An fMRI study. *Neuroreport* **12**, 2021–2025 (2001).
347. M. Ploner, M. C. Lee, K. Wiech, U. Bingel, I. Tracey, Flexible cerebral connectivity patterns subserve contextual modulations of pain. *Cereb. Cortex* **21**, 719–726 (2011).
348. M. L. Phillips, L. J. Gregory, S. Cullen, S. Coen, V. Ng, C. Andrew, V. Giampietro, E. Bullmore, F. Zelaya, E. Amaro, D. G. Thompson, A. R. Hobson, S. C. R. Williams, M. Brammer, Q. Aziz, The effect of negative emotional context on neural and behavioural responses to oesophageal stimulation. *Brain* **126**, 669–684 (2003).
349. T. D. Wager, J. K. Rilling, E. E. Smith, A. Sokolik, K. L. Casey, R. J. Davidson, S. M. Kosslyn, R. M. Rose, J. D. Cohen, Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science* **303**, 1162–1167 (2004).
350. D.-M. Ellingsen, J. Wessberg, M. Eikemo, J. Liljencrantz, T. Endestad, H. Olausson, S. Leknes, Placebo improves pleasure and pain through opposite modulation of sensory processing. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 17993–17998 (2013).
351. F. Eippert, U. Bingel, E. D. Schoell, J. Yacubian, R. Klinger, J. Lorenz, C. Büchel, Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron* **63**, 533–543 (2009).
352. K. B. Jensen, E. Kosek, R. Wicksell, M. Kemani, G. Olsson, J. V. Merle, D. Kadetoff, M. Ingvar, Cognitive behavioral therapy increases pain-evoked activation of the prefrontal cortex in patients with fibromyalgia. *Pain* **153**, 1495–1503 (2012).

353. A. Yoshino, Y. Okamoto, G. Okada, M. Takamura, N. Ichikawa, C. Shibasaki, S. Yokoyama, M. Doi, R. Jinnin, H. Yamashita, M. Horikoshi, S. Yamawaki, Changes in resting-state brain networks after cognitive–behavioral therapy for chronic pain. *Psychol. Med.* **48**, 1148–1156 (2018).
354. A. Lazaridou, J. Kim, C. M. Cahalan, M. L. Loggia, O. Franceschelli, C. Berna, P. Schur, V. Napadow, R. R. Edwards, Effects of cognitive-behavioral therapy (CBT) on brain connectivity supporting catastrophizing in fibromyalgia. *Clin. J. Pain* **33**, 215–221 (2017).
355. M. Shpaner, C. Kelly, G. Lieberman, H. Perelman, M. Davis, F. J. Keefe, M. R. Naylor, Unlearning chronic pain: A randomized controlled trial to investigate changes in intrinsic brain connectivity following cognitive behavioral therapy. *Neuroimage Clin.* **5**, 365–376 (2014).
356. A. Kucyi, T. V. Salomons, K. D. Davis, Cognitive behavioral training reverses the effect of pain exposure on brain network activity. *Pain* **157**, 1895–1904 (2016).
357. D. A. Seminowicz, M. Shpaner, M. L. Keaser, G. M. Krauthamer, J. Mantegna, J. A. Dumas, P. A. Newhouse, C. G. Filippi, F. J. Keefe, M. R. Naylor, Cognitive-behavioral therapy increases prefrontal cortex gray matter in patients with chronic pain. *J. Pain* **14**, 1573–1584 (2013).
358. H. D. Critchley, R. N. Melmed, E. Featherstone, C. J. Mathias, R. J. Dolan, Brain activity during biofeedback relaxation: A functional neuroimaging investigation. *Brain* **124**, 1003–1012 (2001).
359. S. Kobayashi, K. Koitabashi, Effects of progressive muscle relaxation on cerebral activity: An fMRI investigation. *Complement. Ther. Med.* **26**, 33–39 (2016).
360. L. Pessoa, On the relationship between emotion and cognition. *Nat. Rev. Neurosci.* **9**, 148–158 (2008).
361. J. M. Lawrence, F. Hoeft, K. E. Sheau, S. C. Mackey, Strategy-dependent dissociation of the neural correlates involved in pain modulation. *Anesthesiology* **115**, 844–851 (2011).
362. E. Schulz, A. Stankewitz, V. Witkovský, A. M. Winkler, I. Tracey, Strategy-dependent modulation of cortical pain circuits for the attenuation of pain. *Cortex* **113**, 255–266 (2019).
363. E. L. Foltz, L. E. White, Pain "Relief" by frontal cingulumotomy. *J. Neurosurg.* **19**, 89–100 (1962).
364. J. Sharim, N. Pouratian, Anterior cingulotomy for the treatment of chronic intractable pain: A systematic review. *Pain Physician* **19**, 537–550 (2016).
365. M. J. Dorsi, F. A. Lenz, Neurosurgical approaches to the treatment of pain, in *Wall and Melzack's Textbook of Pain* (Elsevier, 2013).
366. J.-F. Poulin, B. Tasic, J. Hjerling-Leffler, J. M. Trimarchi, R. Awatramani, Disentangling neural cell diversity using single-cell transcriptomics. *Nat. Neurosci.* **19**, 1131–1141 (2016).
367. M. W. Salter, B. Stevens, Microglia emerge as central players in brain disease. *Nat. Med.* **23**, 1018–1027 (2017).
368. R.-R. Ji, A. Chamessian, Y.-Q. Zhang, Pain regulation by non-neuronal cells and inflammation. *Science* **354**, 572–577 (2016).
369. B. L. Roth, DREADDs for neuroscientists. *Neuron* **89**, 683–694 (2016).
370. N. S. Hsu, H. Y. Fang, K. K. David, J. W. Gnadt, G. C. Peng, E. M. Talley, J. M. Ward, J. Ngai, W. J. Koroshetz, The promise of the BRAIN initiative: NIH strategies for understanding neural circuit function. *Curr. Opin. Neurobiol.* **65**, 162–166 (2020).
371. F. S. Collins, W. J. Koroshetz, N. D. Volkow, Helping to end addiction over the long-term: The research plan for the NIH HEAL initiative. *JAMA* **320**, 129–130 (2018).
372. N. D. Volkow, W. J. Koroshetz, The role of neurologists in tackling the opioid epidemic. *Nat. Rev. Neurol.* **15**, 301–305 (2019).
373. S. Iyengar, S. A. Woller, R. Hommer, J. Beierlein, C. B. Wright, A. P. Tamiz, B. I. Karp, Critical NIH resources to advance therapies for pain: Preclinical screening program and phase II human clinical trial network. *Neurotherapeutics* **17**, 932–934 (2020).
374. K. D. Davis, N. Aghaeepour, A. H. Ahn, M. S. Angst, D. Borsook, A. Brenton, M. E. Burczynski, C. Crean, R. Edwards, B. Gaudilliere, G. W. Hergenroeder, M. J. Iadarola, S. Iyengar, Y. Jiang, J.-T. Kong, S. Mackey, C. Y. Saab, C. N. Sang, J. Scholz, M. Segerdahl, I. Tracey, C. Veasley, J. Wang, T. D. Wager, A. D. Wasan, M. A. Pelleymounter, Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: Challenges and opportunities. *Nat. Rev. Neurol.* **16**, 381–400 (2020).
375. S. Mackey, H. T. Greely, K. T. Martucci, Neuroimaging-based pain biomarkers: Definitions, clinical and research applications, and evaluation frameworks to achieve personalized pain medicine. *Pain Rep.* **4**, e762 (2019).

Acknowledgments: We thank J. Blair for editing the manuscript. We regret that we could not cite numerous important studies given the reference number limit and broad scope of this review article. We encourage readers to also consider the references in the articles we could cite. **Funding:** The authors' research on the circuits for pain and its treatment is supported by NIH grants (S.M.) R61NS11865, R01NS109450, K24NS126781, (N.M.L.) F32DE030003, (G.S.) R01DA044481, R01NS106301, and R21DA049241. G.S. is a New York Stem Cell Foundation – Robertson Investigator. **Competing interests:** G.S. is a cofounder of Epiodyne, a drug discovery company, an inventor on a patent application related to imaging of neural dynamics to discover analgesics, and a member of the NIH PSPP Preclinical Screening Platform for Pain External Consulting Board.

Submitted 7 July 2021
Accepted 22 October 2021
Published 10 November 2021
10.1126/scitranslmed.abj7360

Science Translational Medicine

Selective targeting of NaV1.7 via inhibition of the CRMP2-Ubc9 interaction reduces pain in rodents

Song Cai[1]†, Aubin Moutal[1]†, Jie Yu[1], Lindsey A. Chew[1], Jörg Isensee[2], Reena Chawla[3], Kimberly Gomez[1], Shizhen Luo[1], Yuan Zhou[1], Aude Chefdeville[1], Cynthia Madura[1], Samantha Perez-Miller[1,4], Shreya Sai Bellampalli[1], Angie Dorame[1], David D. Scott[1], Liberty François-Moutal[1], Zhiming Shan[1], Taylor Woodward[5], Vijay Gokhale[3,6], Andrea G. Hohmann[5], Todd W. Vanderah[1,7], Marcel Patek[8,9], May Khanna[1,3,4,8], Tim Hucho[2], Rajesh Khanna[1,3,4,7,8]*‡

The voltage-gated sodium NaV1.7 channel, critical for sensing pain, has been actively targeted by drug developers; however, there are currently no effective and safe therapies targeting NaV1.7. Here, we tested whether a different approach, indirect NaV1.7 regulation, could have antinociceptive effects in preclinical models. We found that preventing addition of small ubiquitin-like modifier (SUMO) on the NaV1.7-interacting cytosolic collapsin response mediator protein 2 (CRMP2) blocked NaV1.7 functions and had antinociceptive effects in rodents. In silico targeting of the SUMOylation site in CRMP2 (Lys374) identified >200 hits, of which compound 194 exhibited selective in vitro and ex vivo NaV1.7 engagement. Orally administered 194 was not only antinociceptive in preclinical models of acute and chronic pain but also demonstrated synergy alongside other analgesics—without eliciting addiction, rewarding properties, or neurotoxicity. Analgesia conferred by 194 was opioid receptor dependent. Our results demonstrate that 194 is a first-in-class protein-protein inhibitor that capitalizes on CRMP2-NaV1.7 regulation to deliver safe analgesia in rodents.

INTRODUCTION

The NaV1.7 voltage-gated sodium channel defines the threshold of the action potential and amplifies small depolarizing inputs, acting as a sentinel of nociceptive signal propagation (*1*, *2*). The importance of NaV1.7 in nociception is evident from human pain syndromes caused by gain- and loss-of-function mutations in *SCN9A*, the gene encoding human NaV1.7 (*3*). Genetic and pharmacological interference of NaV1.7 renders animals refractory to pain (*4*–*7*). Conversely, up-regulation of NaV1.7 function contributes to the establishment of chronic pain (*8*, *9*). So far, direct blockers of NaV1.7 have been disappointing in clinical trials (*10*, *11*) because of lack of isoform selectivity, insufficient target engagement or off-target side effects [orthostatic hypotension (*12*)], failure to engage spinal NaV1.7, and suboptimal clinical trial design (*13*, *14*). Targeted in vivo epigenetic repression of NaV1.7 has been recently proposed as a potential platform for pain control (*15*). Here, we hypothesized that targeting NaV1.7 indirectly but specifically, via modulation of its interactions with accessory proteins, may be an effective therapeutic approach.

[1]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ 85724, USA. [2]Department of Anesthesiology and Intensive Care Medicine, Translational Pain Research, University Hospital of Cologne, University Cologne, Joseph-Stelzmann-Str 9, Cologne D-50931, Germany. [3]BIO5 Institute, 1657 East Helen Street, Tucson, AZ 85721, USA. [4]Center for Innovation in Brain Sciences, University of Arizona, Tucson, AZ 85721, USA. [5]Department of Psychological and Brain Sciences, Program in Neuroscience and Gill Center for Biomolecular Science, Indiana University, Bloomington, IN 47405-2204, USA. [6]College of Pharmacy, University of Arizona, 1703 East Mabel Street, Tucson, AZ 85721, USA. [7]Comprehensive Pain and Addiction Center, The University of Arizona, Tucson, AZ 85724, USA. [8]Regulonix LLC, 1555 E. Entrada Segunda, Tucson, AZ 85718, USA. [9]Bright Rock Path LLC, Tucson, AZ 85724, USA.
*Corresponding author. Email: rkhanna@arizona.edu, rk4272@nyu.edu
†These authors contributed equally to this work.
‡Present address: Department of Molecular Pathobiology, New York University, College of Dentistry 345 East 24th Street New York, NY 10010, USA.

We previously reported that NaV1.7 surface expression and current density are selectively controlled by SUMOylation of the cytosolic regulator collapsin response mediator protein 2 (CRMP2) at lysine-374 (K374)(Fig. 1A) (*16*–*18*). Restricting CRMP2 SUMOylation promotes recruitment of an endocytic protein complex to NaV1.7 and its subsequent clathrin-mediated internalization, culminating in decreased surface expression and current density (*17*–*19*). We identified an interaction between CRMP2 and the E2 small ubiquitin-like modifier (SUMO)–conjugating enzyme Ubc9 (*20*), uncoupling of which reduced NaV1.7 currents and alleviated experimental neuropathic nociception (*20*). Structural and biophysical studies identified a unique interface between Ubc9 and CRMP2, coordinated by R440 and V371 on CRMP2 (Fig. 1B) (*21*), a regulatory site potentially amenable for small-molecule targeting (Fig. 1B).

To further elucidate the in vivo role of CRMP2 SUMOylation in pain, we generated CRMP2 K374A knock-in ($CRMP2^{K374A/K374A}$) mice in which Lys^{374} was replaced with Ala (*18*). $CRMP2^{K374A/K374A}$ mice had reduced NaV1.7 membrane localization and function in sensory neurons (*18*, *19*). Behavioral evaluation of $CRMP2^{K374A/K374A}$ mice demonstrated no changes in depressive or repetitive, compulsive-like behaviors and a decrease in noxious thermal sensitivity (*18*). No changes were observed in $CRMP2^{K374A/K374A}$ mice to inflammatory, acute, or visceral pain. In contrast, in a neuropathic model, $CRMP2^{K374A/K374A}$ mice failed to develop persistent mechanical allodynia (*18*). Our study suggests that CRMP2 SUMOylation–dependent control of peripheral NaV1.7 is a hallmark of chronic, but not physiological, neuropathic pain.

To leverage this unique pathway for NaV1.7 regulation, a set of diverse 50,000 small molecules were computationally docked to a pocket encompassing the SUMOylated residue K374 (*22*) in CRMP2. Potential hits from the virtual screening were expected to inhibit the Ubc9-CRMP2 interaction, thereby blocking CRMP2 SUMOylation by Ubc9 and ultimately reducing NaV1.7 surface

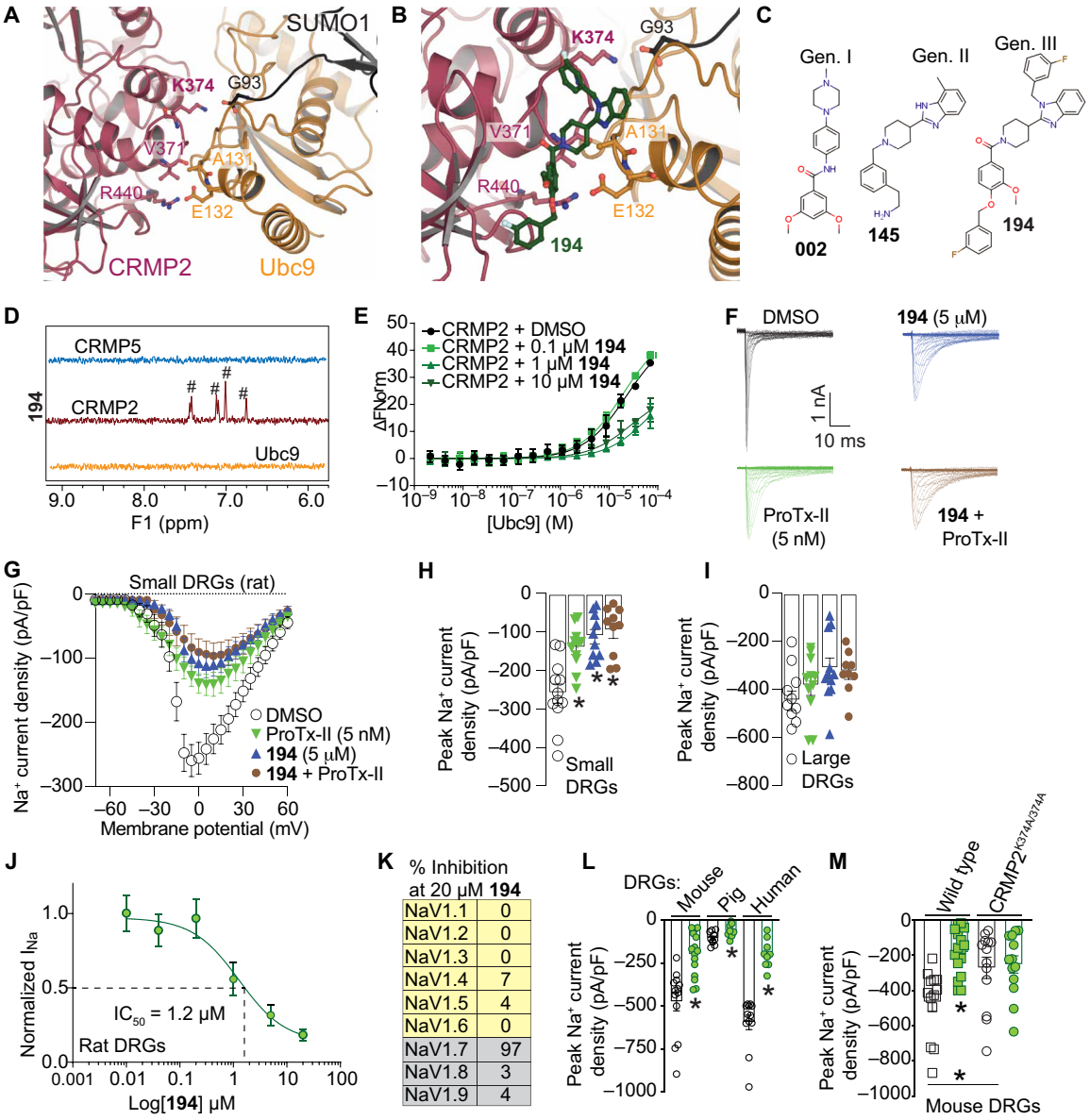


Fig. 1. Identification and characterization of compound 194 as an inhibitor of CRMP2-Ubc9 interaction and inhibitor of NaV1.7 currents in sensory neurons from multiple species. (**A**) Ribbon representation of the tripartite interaction between CRMP2 (dark pink), Ubc9 (orange), and SUMO2 (black). Key residues involved in the interaction are outlined as follows: V371 of CRMP2 interacts with A131 of Ubc9, and R440 of CRMP2 forms a salt bridge with E132 of Ubc9, and the K374 residue of CRMP2 to which the SUMO protein is positioned for covalent attachment. (**B**) Close-up representation of the best docking pose of **194** (green) near the interface between CRMP2 and Ubc9. (**C**) Structure of **002** and **145** and of the lead compound **194**, which presents with features from both core pharmacophores. (**D**) One-dimensional ^{1}H saturation transfer difference nuclear magnetic resonance (STD NMR) showing resonance difference spectrum for CRMP5, CRMP2, and Ubc9 in the presence of **194**. Regions that yielded an STD signal in the presence of **194** are indicated with #. ppm, parts per million. (**E**) Microscale thermophoresis of nitrilotriacetic acid (NTA)–labeled CRMP2 interaction with Ubc9 in the absence or presence of increasing concentrations of **194**. Data were fitted with a one-site binding model. (**F**) Representative sodium current traces recorded from small-sized DRG neurons, incubated overnight with **194** (5 μM) with or without the NaV1.7-selective inhibitor ProTx-II (*24*) (5 nM, 5 to 15 min), in response to depolarization steps from −70 to +60 mV from a holding potential of −60 mV. Summary of current-voltage curves (**G**) and normalized peak (**H**) currents (picoamperes/picoFarads) from small (<20 μm) (i) or large (~38 to 51 μm). p = 0.0020, 0.0005, and 0.0300 for DMSO versus ProTx-II, **194**, and **194** + ProTx-II, respectively (one Way- ANOVA; see table S1). (**I**) DRG neurons as indicated (n = 10 to 12 cells per group for small and n = 9 to 11 cells per group for large). (**J**) Concentration curve illustrating inhibition of NaV1.7 currents by **194** with an inhibitory constant IC_{50} of 1.2 μM (n = 12 to 25 cells per concentration). (**K**) Percentage inhibition of NaV1.x-mediated currents in HEK293 cell lines expressing NaV1.x subunits (yellow boxes) or rat DRGs (NaV1.7–9; gray boxes) after overnight treatment with 20 μM **194**. NaV1.7 was isolated with TTX (*17*), NaV1.8 was isolated with 500 nM selective blocker A803467 (*70*), whereas NaV1.9 was isolated with post hoc subtraction techniques described previously (*17*, *18*). (**L**) Bar graph of normalized peak NaV1.7 current densities of mouse, pig, and human DRG neurons treated overnight with 5 μM **194** (n = 9 to 12 cells per condition). (**M**) Bar graph of normalized peak NaV1.7 current densities of DRGs from wild-type and $CRMP2^{K374A/K374A}$ mice neurons treated overnight with 5 μM **194** (n > 15 cells per condition). Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

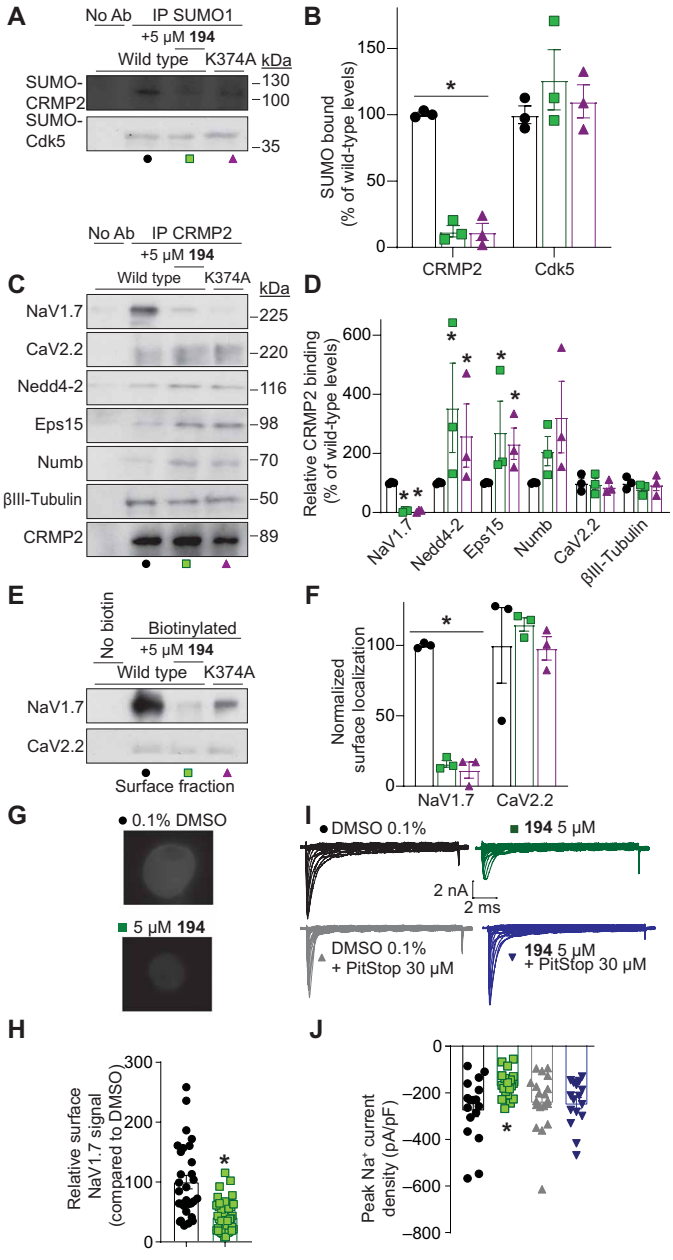


Fig. 2. 194 blocks CRMP2 SUMOylation promoting recruitment of endocytosis-related proteins and internalization of NaV1.7. (**A**) Representative immunoblot and (**B**) quantification of SUMO1 immunoprecipitates from Cath.-a-differentiated (CAD) cells treated overnight with **194** (5 μM) or transfected with the SUMO-null CRMP2-K374A plasmid and probed with anti-CRMP2 or anti-cdk5 antibodies ($n = 3$ independent samples). Ab, antibody. * denotes p = 0.0095 and 0.0047 comparing wildtype to 194 or K374A, respectively (Kruskal-Wallis test). (**C**) Representative immunoblot and (**D**) quantification of CRMP2 immunoprecipitates from CAD cells treated overnight with **194** (5 μM) or transfected with the SUMO-null CRMP2-K374A plasmid and probed with antibodies against NaV1.7, CaV2.2, βIII-tubulin, and endocytic proteins Nedd4–2, Eps15, Numb, and CRMP2 as control ($n = 3$ independent samples). * denotes p = 0.0014 and 0.0242 comparing NaV1.7 binding to CRMP2 between wildtype and 194 or K374A respectively; p = 0.0507 and 0.0188 comparing Nedd4-2 binding to CRMP2 between wildtype and 194 or K374A respectively; and p = 0. 0.0175 and 0.0507 comparing Eps15 binding to CRMP2 between wildtype and 194 or K374A respectively (Kruskal-Wallis test). (**E**) Representative immunoblot and (**F**) quantification of the biotinylated-fraction of NaV1.7 in CAD cells treated overnight with **194** (5 μM) or transfected with CRMP2-K374A construct ($n = 3$ independent samples). * denotes p = 0.0100 and 0.0062 comparing NaV1.7 surface expression between wildtype and 194 or K374A, respectively (Kruskal-Wallis test). (**G**) Representative images of rat DRG cultures labeled with an extracellular epitope antibody against NaV1.7. (**H**) Quantification of normalized surface expression of NaV1.7 per neuron ($n = 37$ to 45 cells). * denotes p <0.0001 between wildtype and 194 (Mann Whitney test). (**I**) Representative traces and (**J**) graph of normalized peak Na^+ current density (pA/pF) from rat DRG neurons treated with **194** (5 μM) overnight with or without pretreatment (30 min before **194** application) with 30 μM CME inhibitor PitStop ($n = 16$ to 19 cells per condition). * denotes p = 0.0108 comparing DMSO and 194 (One-way ANOVA with Tukey's post doc test). Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

expression and current density. Subsequent in vitro screening of over 200 hits led to identification of the hit series—benzoylated 2-(4-piperidinyl)-1,3-benzimidazole analog—compound **194** (Fig. 1C and fig. S1). Here, we demonstrate that **194** (i) specifically inhibits the CRMP2-Ubc9 interaction and SUMOylation of CRMP2, (ii) decreases NaV1.7 currents in sensory neurons from four species, (iii) reduces spinal nociceptive neurotransmission, (iv) decreases mechanical allodynia in six models of neuropathic pain, (v) synergizes with commonly used painkillers, (vi) engages NaV1.7-dependent endogenous opioid signaling, and (vii) has no motor or depressive-like side effect as well as does not exhibit addictive properties.

RESULTS

An analog of benzoylpiperidylbenzimidazole compounds binds to CRMP2 and reduces NaV1.7 currents in sensory neurons across species

To find blockers of the interaction between the E2 SUMO-conjugating enzyme Ubc9 and CRMP2, we computationally docked 50,000 small molecules to a pocket encompassing the SUMOylated residue (K374) in CRMP2 (Fig. 1, A and B). Over 200 hits, across three iterative cycles (Fig. 1C), were screened for their ability to inhibit Ca^{2+} influx triggered by application of the sodium channel activator veratridine (*23*). Of these, the lead compound **194** (fig. S1), a benzoylpiperidylbenzimidazole analog (Fig. 1C), specifically bound to CRMP2, but not the related CRMP5, or Ubc9 (Fig. 1D). **194** inhibited the Ubc9-CRMP2 interaction in a concentration-dependent manner (Fig. 1E). In small- to medium-diameter rat dorsal root ganglion (DRG) neurons, 5 μM **194** inhibited ~57% of the total sodium current (Fig. 1, F to H), with an IC_{50} of inhibition of 1.2 μM (Fig. 1J). Lack of further inhibition upon coapplication of the NaV1.7-specific blocker ProTx-II (*24*) suggests that 100% of functional NaV1.7 was blocked by **194** (Fig. 1, F to H). **194** did not inhibit sodium currents in large-diameter DRGs (>38 to 51 μm), ruling out effects on NaV1.1 and NaV1.6 channels expressed by this neuron size class (Fig. 1I) (*25*). **194** had no effect on activation, steady-state inactivation, recovery from inactivation, or resurgent currents but increased entry into inactivation and slow inactivation (fig. S2). Inhibition by **194** was specific to NaV1.7 as no other NaV1.x channels were inhibited (**194** used at ~16.7-fold its IC_{50} value) in recordings from heterologous cells or DRG neurons (Fig. 1J). Tetrodotoxin-resistant (TTX-R; i.e., NaV1.8 and NaV1.9) currents were not affected by **194** (fig. S3A). **194** inhibited NaV1.7 in DRG

neurons from mouse, pig, and human (Fig. 1L). **194** had no effect on total sodium currents recorded from DRGs of transgenic mice deficient in CRMP2 SUMOylation ($CRMP2^{K374A/K374A}$) mice (Fig. 1M). **194** did not block NaV1.7 currents acutely (5 to 15 min) or after a 3-hour incubation (fig. S3B). **194** did not inhibit the cardiac hERG channel or the presynaptic N-type voltage-gated calcium channel (CaV2.2; a <10% reduction in N-type currents) (fig. S3, C and D). In summary, **194** specifically inhibits the Ubc9-CRMP2 interaction and represents a first-in-class, selective regulator of NaV1.7 across species.

194 reduces NaV1.7 currents by promoting clathrin-mediated internalization of NaV1.7 through blocking SUMOylation of CRMP2

As **194** prevents the binding between Ubc9 and CRMP2 (Fig. 1E), it is expected to block CRMP2 SUMOylation, thereby triggering clathrin-mediated endocytosis (CME) of NaV1.7 by promoting the recruitment of endocytic proteins Numb, Nedd4–2, and Eps15 (*17*–*20*). CRMP2 SUMOylation was abolished by **194** to a magnitude concordant with that of SUMOylation-deficient CRMP2 (Fig. 2, A and B). SUMOylation of another protein—the cyclin-dependent kinase 5 (Cdk5) (*26*)—was not affected by **194** (Fig. 2, A and B). Canonical CRMP2 phosphorylation at Ser^{522} by cdk5 (*27*) was not affected by **194** (fig. S3, E and F). Treatment with **194** phenocopied genetic loss of CRMP2 SUMOylation by promoting CRMP2 association with the endocytic proteins Numb, Eps15, and Nedd4–2 while decreasing its interaction with NaV1.7 but not the CRMP2-interacting proteins CaV2.2 or βIII-tubulin (Fig. 2, C and D). Consequently, cell surface expression of NaV1.7, but not CaV2.2 (Fig. 2, E and F), was decreased (Fig. 2, G and H). Consistent with **194**'s proposed mechanism of action on CRMP2, PitStop2-mediated inhibition of clathrin assembly in rat DRG neurons nullified **194**-mediated reduction of TTX-S Na^+ current density (Fig. 2, I and J) while sparing TTX-R currents (fig. S3G). Thus, **194** reduces Na^+ currents via facilitating the internalization of NaV1.7.

194 inhibits in vivo SUMOylation and spinal neurotransmission

NaV1.7 is expressed in the lamina I of the spinal cord where primary afferent sensory neurons synapse onto second-order neurons (*28*, *29*). As proof of in vivo target engagement, **194** inhibited CRMP2 SUMOylation in the spinal dorsal horn (Fig. 3, A and B). Because **194** decreases NaV1.7 membrane localization in vitro (Fig. 2, F to K), we next determined whether it could affect NaV1.7 presynaptic localization in vivo. **194** decreased the presynaptic expression of NaV1.7 in the spinal dorsal horn (Fig. 3, C and D). To test whether displacement of NaV1.7 from the presynaptic element affected spinal nociceptive neurotransmission, we recorded spontaneous

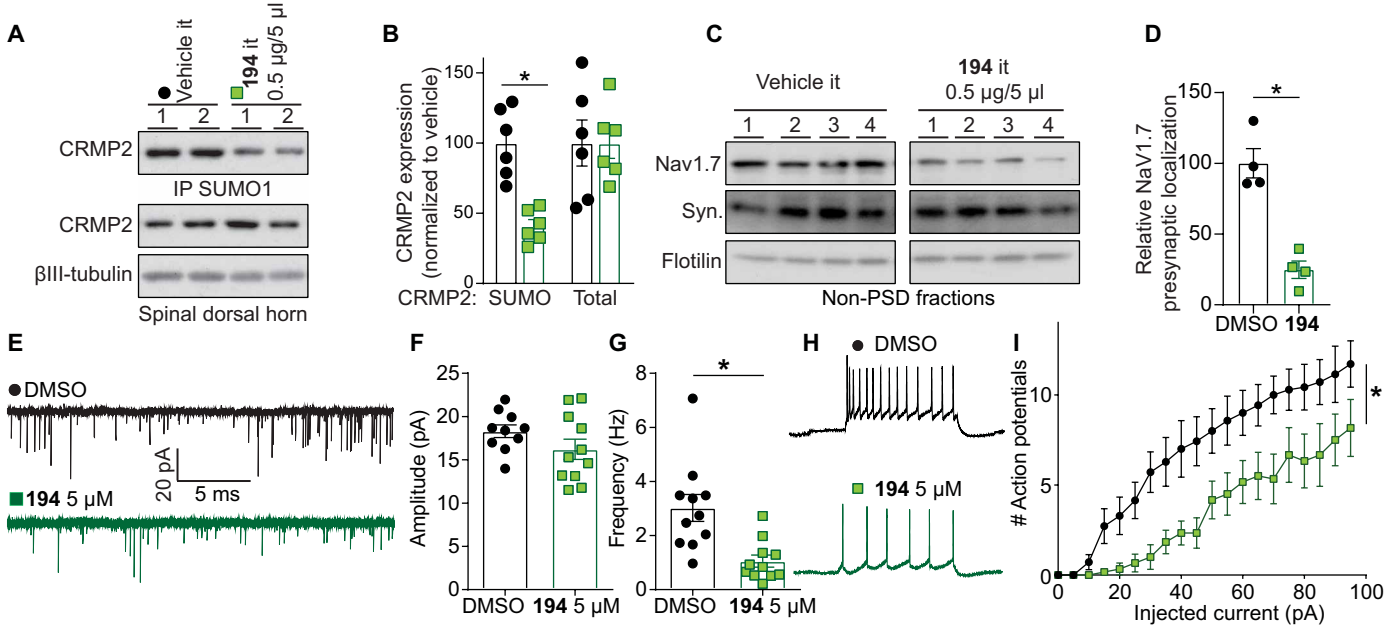


Fig. 3. 194 inhibits CRMP2 SUMOylation, decreases the presynaptic fraction of NaV1.7 in vivo, and reduces sEPSC and action potentials in the lumbar horn of the spinal cord. (**A**) Representative immunoblot and (**B**) quantification of SUMO1 immunoprecipitates from spinal cord lysates of rats prepared 2 hours after intrathecal administration with **194** (0.5 μg in 5 μl) plasmid and probed with anti-CRMP2 antibody ($n = 6$ independent samples). βIII-Tubulin was used as a loading control. * denotes p = 0.0022 comparing SUMO IP between DMSO and 194 (Mann Whitney test). (**C**) Representative immunoblots of presynaptic fractions of lumbar dorsal horn samples from rats after intrathecal administration with **194** (0.5 μg in 5 μl) probed with antibodies against NaV1.7, the presynaptic marker synaptophysin (Syn.), and membrane-associated protein flotillin (loading control). PSD, postsynaptic density. (**D**) Quantification of NaV1.7 in the presynaptic fraction ($n = 4$ rats per condition). * denotes p = 0.0002 comparing relative NaV1.7 presynaptic localization between DMSO and 194 (Mann Whitney test). (**E**) Representative spontaneous excitatory postsynaptic potential (sEPSC) recordings of lumbar dorsal horn neurons perfused with 0.1% dimethyl sulfoxide (DMSO) or **194** (5 μM). Summary of (**F**) amplitudes and (**G**) frequencies of sEPSCs for both groups ($n = 11$ cells per condition). * denotes p = 0.0018 comparing mean frequency between wildtype and 194 (Mann Whitney test). (**H**) Representative action potential recordings of lumbar dorsal horn neurons perfused with 0.1% DMSO or **194** (5 μM). (**I**) Summary of the number of action potentials recorded as a function of current injected ($n = 6$ to 7 cells per condition). * denotes p <0.01 comparing # of action potentials, at current injections of >15 pA, between DMSO and 194 (Mann Whitney test). Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

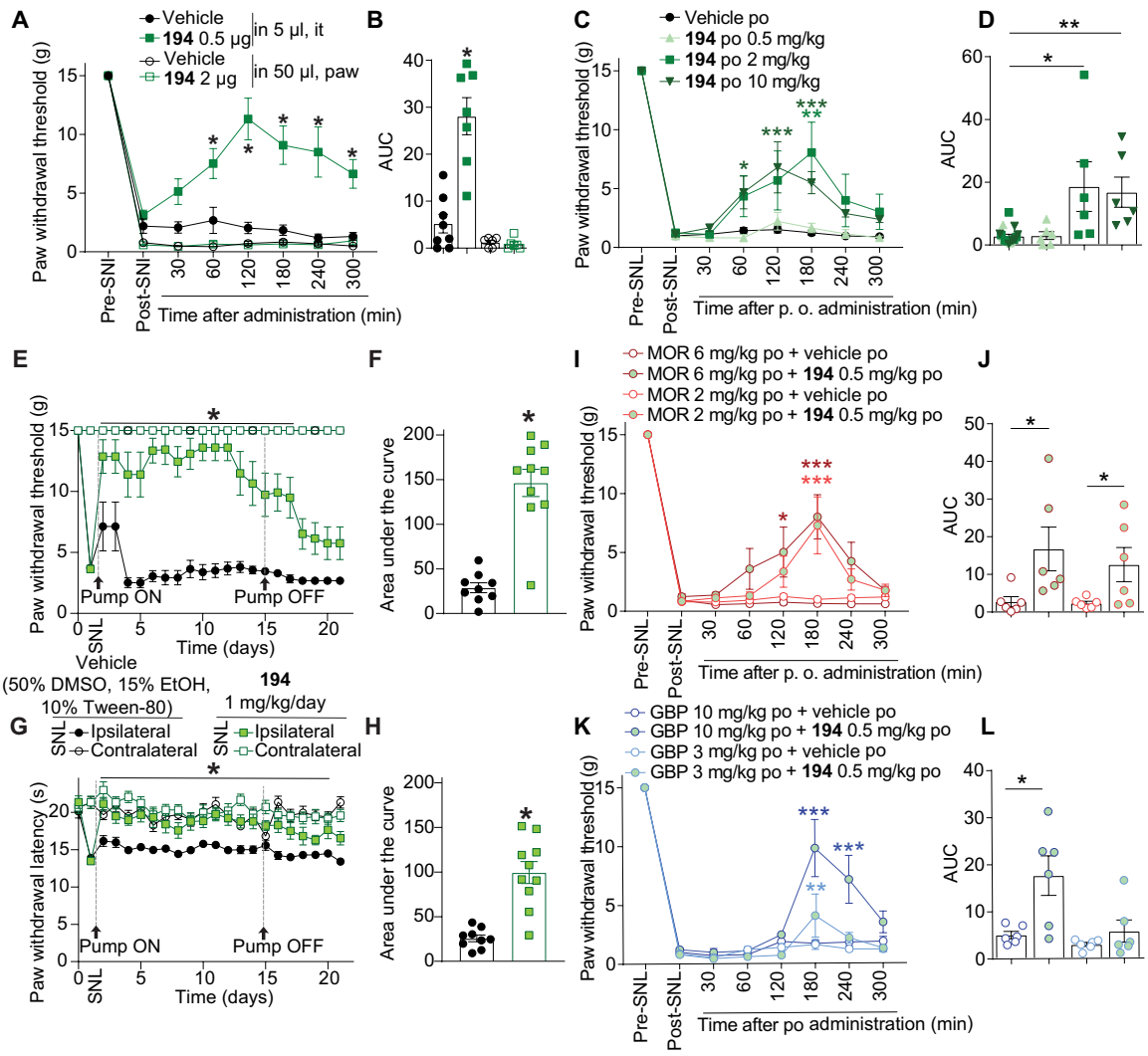


Fig. 4. 194 is antinociceptive when administered intrathecally or orally and produces synergistic antiallodynic effects with other analgesics. Time course (**A**) and quantification (**B**) of paw withdrawal threshold (PWT) of male rats that underwent spared nerve injury (SNI). At 14 days after SNI, **194** was administered either intrathecally (0.5 µg/5 µl) or in the paw (2.0 µg/50 µl, intraplantar). Time course (**C**) and quantification (**D**) of paw withdrawal threshold (PWT) of male rats that underwent spinal nerve ligation (SNL) surgery. At 21 days after SNL injury, decreased PWTs were noted. Rats were orally (po) administered 2 or 10 mg/kg of **194** ($n = 6$ rats per group). Sustained administration of **194** [1 mg/kg per day, subcutaneously (sc)] was achieved via osmotic minipump, for 14 days, and tactile (**E** and **F**) and thermal (**G** and **H**) hypersensitivity induced by SNL was assessed. Behavior was tested at multiple time points indicated in the bar graph. Time course (**I**) and quantification (**J**) of PWTs of male rats that underwent SNL surgery and administered doses of morphine (MOR; 2 or 6 mg/kg, po) alone or in combination with vehicle or **194** (0.5 mg/kg, po) ($n = 6$ rats per group). Time course (**K**) and quantification (**L**) of PWTs of male rats that underwent SNL surgery and administered doses of gabapentin (GBP; 3 or 10 mg/kg, po) alone or in combination with vehicle or **194** (0.5 mg/kg, po) ($n = 6$ rats per group). Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

excitatory postsynaptic currents (sEPSCs) from cells in the substantia gelatinosa layer of the dorsal horn of the spinal cord of rats perfused with **194** (Fig. 3E). **194** had no effect on the amplitude (Fig. 3, E and F) but decreased the frequency of sEPSCs (Fig. 3, E and G). Furthermore, **194** decreased dorsal horn neuronal excitability (Fig. 3, H and I). Therefore, **194** exhibits target engagement in vivo via suppression of CRMP2 SUMOylation and reduction of NaV1.7 trafficking and function.

Behavioral appraisal of 194 reveals efficacy across multiple pain models

We previously reported that genetic (*30*) or peptide-based (*20*, *21*) interference of CRMP2 SUMOylation reversed allodynia associated with the spared nerve injury (SNI) model of experimental pain. Consistent with these studies, central [0.5 µg/5 µl, intrathecally (it)] administration of **194** reversed mechanical allodynia (Fig. 4, A and B). In contrast, peripheral (2.0 µg/50 µl, intraplantar) injection failed to reverse SNI-induced allodynia (Fig. 4, A and B). In addition, spinally delivered **194** (0.5 µg in 5 µl) alleviated mechanical allodynia in rat models of HIV- and chemotherapy-induced sensory neuropathy (fig. S4, A to D). Because pharmacokinetic studies showed **194** to be orally available (half-life ~2 hours; fig. S5), we next tested whether oral administration of **194** would provide pain relief in rat models of chemotherapy- and nerve injury–induced neuropathic pain. **194** at 2 and 10 mg/kg, but not at 0.5 mg/kg, restored mechanical sensitivity

in animals with chemotherapy-induced (fig. S4, F and G) and nerve injury–induced neuropathic nociception (Fig. 4, C and D). **194** (0.5 μg/5 μl, 2 hours after intrathecal injection) produced conditioned place preference (CPP) in rats with nerve injury–induced neuropathic pain (fig. S4E). No tolerance (loss of efficacy) was noted during a 14-day chronic dosing paradigm of **194** (1 mg/kg per day, subcutaneously) (Fig. 4, E to H). In addition, the pain-relieving effect of **194** lasted for 3 days beyond cessation of chronic exposure (Fig. 4, E to H). **194** inhibited formalin-induced defensive behaviors (fig. S6A), as well as nerve-injury (fig. S6, B and C) and postoperative pain behaviors (fig. S6, D and E). Thus, **194** demonstrates efficacy in reversing pain (evoked and affective) in six different models across two species and via four routes of administration.

194 analgesia synergizes with morphine and gabapentin

Given the high prevalence of side effects arising from commonly used painkillers (*31*), a reduction in their use would be beneficial. We observed an enhancement between subtherapeutic doses of **194** and morphine on pain relief in a rat model of neuropathic pain (Fig. 4, I and J). Similarly, a subtherapeutic dose of **194** demonstrated synergy with parallel administration of gabapentin (Fig. 4, K and L). These results indicate that **194** can potentiate with different classes of analgesics to provide pain relief at subtherapeutic doses.

194 increases endogenous opioid signaling

In humans with nonfunctional NaV1.7, insensitivity to pain has been linked not only to the role of NaV1.7 in electrical conduction of nociceptive neuronal sodium influx but also to modulation of nociceptive signaling by regulation of endogenous opioids (*32*). NaV1.7 genetic deficiency in mice has been shown to control the balance of G protein–coupled receptor (GPCR)–mediated pro- and antinociceptive intracellular signaling, which converges on the activity of type II protein kinase A (PKA-II) (*33*). **194** did not change the cell counts, cell size distribution of DRG neurons, or NaV1.7 expression, suggesting that the compound was not cytotoxic (fig. S7, A and B). We therefore tested **194**'s effect on depolarization-induced activation of PKA-II in DRG neurons after application of the NaV1.7 agonist veratridine (Fig. 5A). PKA-II activation by veratridine was blocked by TTX (IC_{50} = 60 nM) and by **194** with an IC_{50} of 2 μM (Fig. 5, B to D). No additional block of PKA-II activation was observed upon cotreatment with **194** and TTX, further supporting the notion that **194** acts on TTX-sensitive sodium channels (Fig. 5B). Treatment with **194** also resulted in a slight increase in the mRNA expression (number, area, and staining intensity of foci) of the μ- and δ-opioid receptor ligand proenkephalin (PENK) in DRG neurons (Fig. 5, E and F, and fig. S7C). To address a possible effect of **194** on opioid receptor signaling in vitro, DRG neurons were pretreated with different doses of **194** overnight and then stimulated with forskolin in the presence of increasing doses of the opioid Met-enkephalin. Forskolin-induced PKA-II activity was dose-dependently inhibited by Met-enkephalin (Fig. 5G). The presence of **194**, however, did not modulate the effect of opioids (Fig. 5G).

If **194** mediates antinociception via opioid receptor signaling at the central terminals of nociceptors, then blocking these receptors with the antagonist naloxone should negate **194**'s pain-relieving effect in vivo. **194** reversed paclitaxel-induced mechanical allodynia (Fig. 5, H and I), which was prevented by coadministration of naloxone (Fig. 5, H and I). Thus, **194**'s antinociceptive mechanism of action likely involves PENK up-regulation and PKA-II activation downstream of opioid receptor engagement.

194 does not cause locomotor deficits and depressive-like behavior and is not addictive

194 was counter-screened in behavioral assays in rodents to assess potential toxicity. No signs of systemic toxicity were observed upon continuous infusion of 100mg/kg per day of **194** for 10 days in rats as a histopathological study of all major organs did not reveal any anomalies (fig. S8). Spinal administration of **194** (0.5 μg, it) did not affect motor performances of rats (Fig. 6A). In mice, spinal administration of **194** increased their latency to lick their hindpaw when placed on a 52°C hot plate compared with vehicle-treated mice (Fig. 6B), whereas tail-flick latency to withdraw their tails from a 52°C water bath was no different between the two groups (Fig. 6C). Systemic administration of **194** [10 mg/kg, intraperitoneally (ip)] in mice did not affect motor performance (open field) (Fig. 6, D and E). **194** had no effect on anxiety (elevated plus maze) (Fig. 6F) or depression (forced swim test) behaviors (Fig. 6G). **194** treatment did not affect the ability of mice to find a hidden piece of food, ruling out any adverse effect on smell as NaV1.7 is also an essential requirement for odor perception (Fig. 6H) (*34*). Last, **194** was screened for addictive potential and induction of physical dependence (*35*). **194** did not induce place preference (Fig. 6I), indicating that the compound is unlikely to be addictive. **194** did not affect naloxone-precipitated jumping behavior, which measures physical signs of opioid withdrawal, in mice rendered dependent to morphine by an escalating dose schedule over 3 days (Fig. 6J). Thus, **194** did not exacerbate opioid-induced physical dependence processes.

DISCUSSION

Previous efforts to target NaV1.7 for pain relief have focused on development of direct channel blockers without success (*11*, *36*, *37*). Lack of selectivity [for example, Biogen's vixotrigine (*38*)], insufficient channel blockade, failure to engage spinal NaV1.7, suboptimal clinical trial design (*13*, *14*), and failure to engage NaV1.7-dependent endogenous opioid signaling (*13*, *14*) have been proposed as potential reasons for clinical failure of direct blockers of NaV1.7 (*14*). Here, we identified a first-in-class small-molecule regulator of NaV1.7 that might overcome these hurdles to deliver safe analgesia.

The compound **194**, a class of benzoylpiperidylbenzimidazole, emerged as a lead series after three generations of optimization to maximally target NaV1.7 inhibition in DRG neurons. **194** blocked in vitro and in vivo SUMOylation of CRMP2 to selectively reduce the amount of surface-expressed NaV1.7. **194** blocked all of the ProTx-II–sensitive (the NaV1.7-sensitive) current in small-diameter DRGs but was ineffective in large-diameter DRGs, thereby ruling out potential effects of **194** on NaV1.1 and NaV1.6. Lack of a functional effect on other NaV1.x channels, the presynaptic N-type voltage-gated calcium channel (CaV2.2), the cardiac hERG channel, and NaV1.7 currents from $CRMP2^{K374A/K374A}$ mice demonstrated the specificity of **194** for CRMP2 SUMOylation-dependent control of NaV1.7. That **194** did not affect binding between established CRMP2 interacting proteins CaV2.2 and βIII-tubulin provides further proof of specificity of the mechanism of action of this compound. These results are congruent with previous reports, obtained using CRMP2 K374A mutants in DRGs or heterologous cells, demonstrating lack of effects on (i) TTX-R currents or heterologously

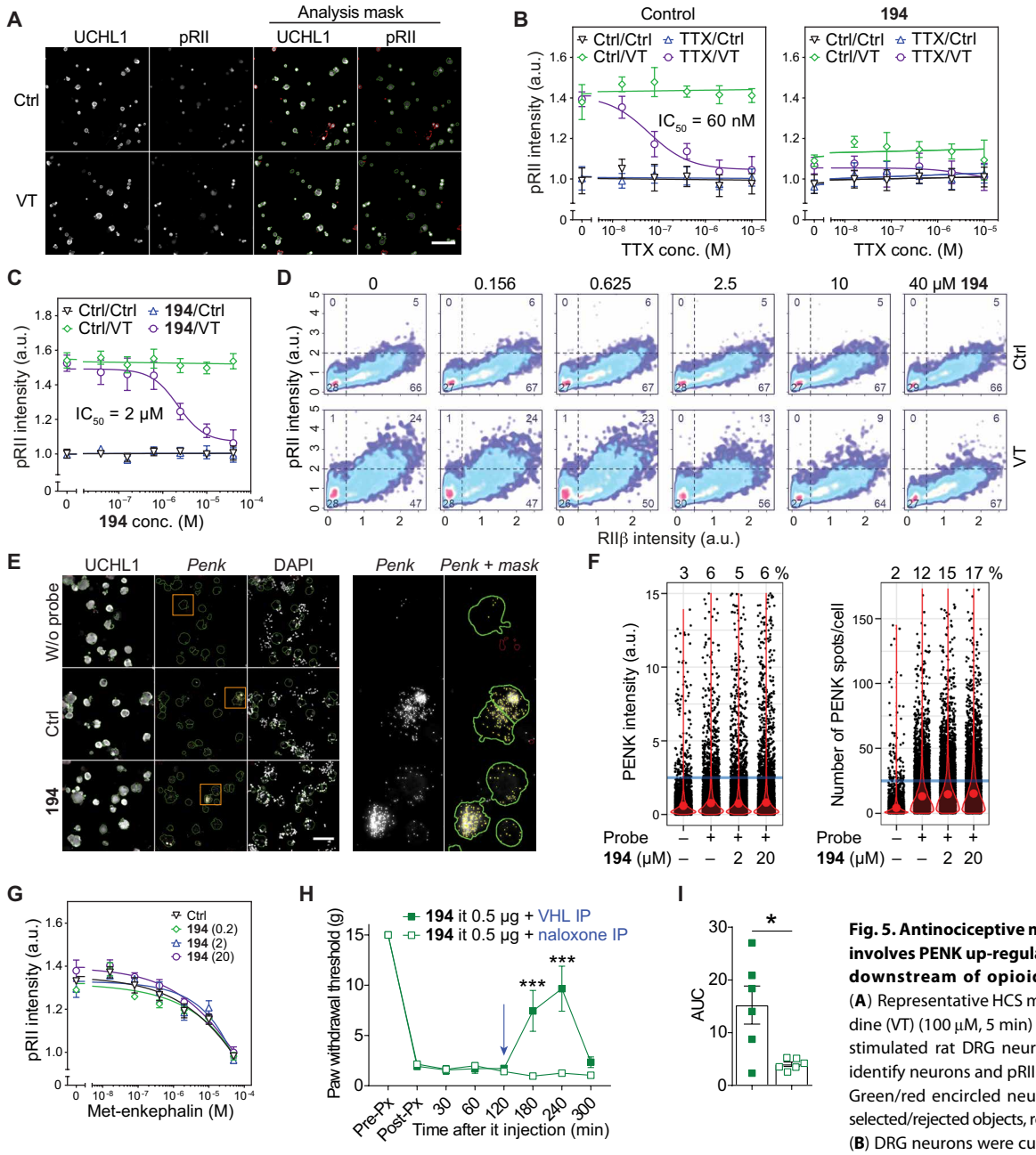


Fig. 5. Antinociceptive mechanism of action of 194 involves PENK up-regulation and PKA-II inhibition downstream of opioid receptor engagement. (**A**) Representative HCS microscopy images of veratridine (VT) (100 μM, 5 min) or control (Ctrl, 0.2% DMSO) stimulated rat DRG neurons labeled with UCHL1 to identify neurons and pRII to quantify PKA-II signaling. Green/red encircled neurons indicate automatically selected/rejected objects, respectively. Scale bar, 200 μm. (**B**) DRG neurons were cultured in the absence (0.2 % DMSO) or presence of **194** (20 μM in 0.2% DMSO). After 12 hours, cells were pretreated with increasing doses of TTX (0 to 10 μM, 5 min) and then tested for veratridine-induced (10 μM, 5 min) PKA-II activity as measured by anti–phospho-RII staining as a proxy for PKA-II activity. a.u., artbitrary units. (**C**) DRG neurons were pretreated for 6 hours with increasing doses of **194** (0 to 40 μM), followed by stimulation with VT (100 μM, 3 min), and again PKA-II activity as measured by anti–phospho-RII staining as a proxy for PKA-II activity. Values represent means ± SEM; $n = 4$ replicate experiments; >2500 neurons per condition. (**D**) Single cell–based density plots of pRII/RIIβ-labeled DRG neurons shown in (C). Dashed lines indicate the gating threshold to discriminate activated (those with increased pRII intensity) and nociceptive [those with increased RIIβ(+)] intensity, neurons with the numbers indicating the relative percentage of cells in the respective quadrant. Combined data of $n = 4$ experiments with a total of >2500 neurons per condition. (**E**) HCS microscopy images of rat DRG neurons treated with 20 μM **194** or its solvent for 12 hours, followed by labeling of the neuronal marker ubiquitin C-terminal hydrolase L1 (UCHL1, formerly PGP9.5) by immunocytochemistry and proenkephalin (*Penk*) mRNA expression by fluorescence in situ hybridization (FISH). The top panel shows controls without PENK probe. Fluorescent foci representing PENK transcripts were quantified within UCHL1-positive neuronal areas (see enlarged sections). (**F**) Single-cell data showing the mean *Penk* intensity and number of *Penk* spots per cell for all analyzed neurons (>8000 per condition). (**G**) Effect of **194** on opioid receptor signaling in vitro. DRG neurons were pretreated for 5 hours after seeding with different doses of **194** (0, 0.2, 2, and 20 μM) or solvent control (0.1% DMSO). The next day, DRG cultures were stimulated with forskolin (3 μM) for 3 min in the presence of increasing doses of the opioid Met-enkephalin (0 to 50 μM). Values represent means ± SEM; $n = 4$ replicate experiments; >2500 neurons per condition. (**H**) Time course and (**I**) area under the curve (AUC) quantification of the paw withdrawal thresholds (PWTs) of male rats treated with paclitaxel (Px). Twenty-eight days after the initial injection of Px, the PWTs of rats were assessed after intrathecal administration of 0.5 μg of **194** in the copresence of vehicle (DMSO) or naloxone (6 mg/kg, ip; indicated by the blue arrow), administered at the 2-hour mark of the 5-hour time course ($n = 6$ rats per group). Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

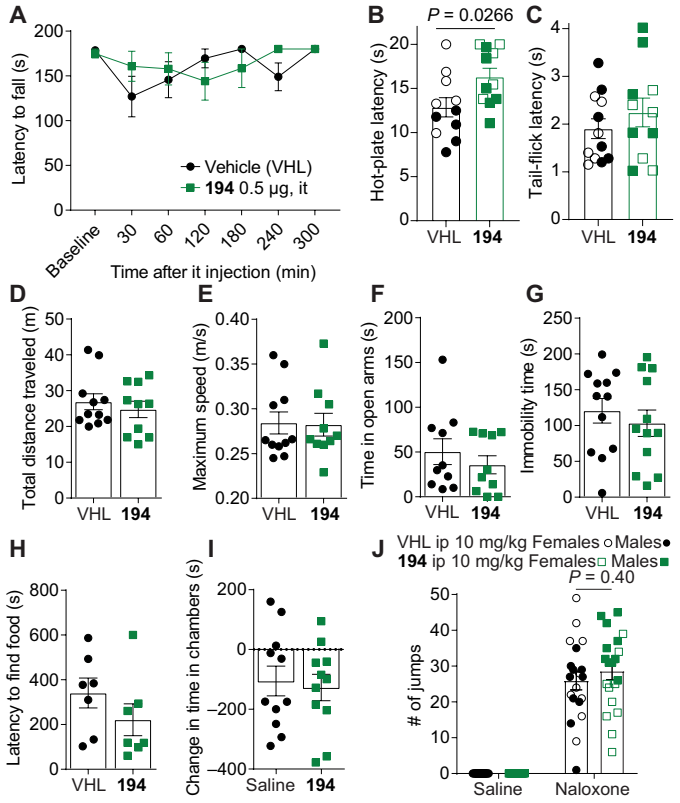


Fig. 6. 194 does not impair motor function, induce anxiety- or depressive-like behavior, or produce reward or physical dependence. (**A**) Time course of the latency of rats to fall off a rotarod after 3 days of training (baseline) and after receiving an intrathecal injection of 0.5 µg of **194** ($n = 8$ rats per group). Latencies to respond to noxious heat (52°C) in the hot plate (**B**) or tail-flick (52°C) (**C**) tests in female (open symbols) and male (closed symbols) in CD1 mice 2 hours after an intrathecal injection of 0.5 µg of **194** ($n = 11$ to 12 mice per group). (**D**) Total distance traveled and (**E**) maximum speed of CD1 male mice in an open field tested at 120 min after intraperitoneal injection of 10 mg/kg of **194** or its vehicle (VHL) were not modified ($n = 10$ to 11 mice per group). (**F**) Time spent exploring the open arms of an elevated plus maze by CD1 male mice was not reduced by intraperitoneal injection of 10 mg/kg of 194 ($n = 10$ to 11 mice per group). (**G**) Immobility time of CD1 male mice in the forced swim test was assessed after intraperitoneal injection of 10 mg/kg of **194** ($n = 12$ mice per group). (**H**) Latency of CD1 male mice to find a hidden piece of food was assessed after intraperitoneal injection of 10 mg/kg of **194** ($n = 7$ mice per group). (**I**) Changes from baseline in the time spent in chambers (seconds) paired with administration of either 194 at 10 mg/kg or vehicle (VHL) in a conditioned place preference assay ($n = 11$ rats per group). (**J**) Number of withdrawal jumps in male and female C57Bl/6J mice rendered dependent to morphine by repeated administration. Mice were challenged with naloxone (10 mg/kg, ip) to precipitate opioid withdrawal and assess physical dependence ($n = 20$ mice per group). Withdrawal jumps were counted after saline challenge in **194**- and vehicle-treated groups. Complete sample size and statistical information are provided in table S1. Error bars indicate mean ± SEM.

expressed NaV1.1, NaV1.3, and NaV1.5 channels (*16*, *17*); (ii) voltage-gated Ca^{2+} and K^+ currents (*18*); (iii) CRMP2 binding to tubulin outgrowth (*16*); (iv) neurite outgrowth—a canonical function of CRMP2 (*16*); or (v) CRMP2 phosphorylation (*17*). As suggested by the complete conservation of the CRMP2 SUMOylation motif (*22*) and its regulation of NaV1.7 (*17*) from rodents to humans, **194** inhibited NaV1.7 currents in mouse, rat, pig, and human sensory neurons, suggesting translational potential of our preclinical lead compound.

A critical step to achieve the on-target specificity involved triaging compounds that bound the E2 SUMO-conjugating enzyme Ubc9. This counterselection was necessary as Ubc9 is broadly distributed (*39*), Ubc9 knockout (KO) mice are embryonic lethal (*40*), and global decreases in SUMOylation are implicated in cognitive dysfunction (*41*); thus, directly targeting Ubc9 is not viable. Only a few small-molecule inhibitors of SUMOylation have been reported to date (*42*–*44*), and these are therapeutically impractical because by targeting the SUMOylation machinery—either E1 or E2—they are likely to be nonselective antagonists of essential biological processes. Experimentally, no binding to Ubc9 (or CRMP5) was noted. Block of SUMOylation was specific to CRMP2, as SUMOylation of Cdk5 (*26*), another Ubc9 client, was unaffected by **194**. Thus, our study gave us a way to uniquely target NaV1.7 with high selectivity.

That the effect of **194** is observed only after an overnight incubation may at first seem at odds with ex vivo/in vivo effects, which develop with much more rapid time scales. The timing of these in vitro versus in vivo effects is likely due to a slower rate of deSUMOylation of CRMP2, which is necessary to observe inhibition of CRMP2 SUMOylation by **194** in vitro. Inhibiting CME with PitStop (30 min) negated the reduction in NaV1.7 currents imposed by **194**, demonstrating that all of NaV1.7 can be recycled within 30 min. These results are consistent with our previous findings showing that inhibiting CME with PitStop reverses the reduction in NaV1.7 currents imposed by genetic block of CRMP2 SUMOylation in DRGs from rats and mice (*17*, *19*). This shows that, in our hands, NaV1.7 recycling occurs in less than 30 min. Of relevance to our observations on **194** are findings that gabapentin, which targets the trafficking of the N-type calcium channel (CaV2.2) by inhibiting the recycling of this channel, decreases DRG Ca^{2+} currents but requires >40 hours of incubation to achieve inhibition (*45*, *46*). This is in contrast with the rapid effects of gabapentin observed in ex vivo and in vivo conditions (*47*, *48*).

$CRMP2^{K374A/K374A}$ mice are refractory to the development of chronic neuropathic pain (*18*), whereas acute nociceptive pain is intact despite pharmacological (**194**) or genetic suppression of CRMP2 SUMOylation (*18*). In peripheral nerve damage, concomitant increases in CRMP2 SUMOylation (*30*) and NaV1.7 localization in the gracile nucleus have been reported (*8*, *49*). Therefore, limiting NaV1.7 function via targeting of CRMP2 SUMOylation is likely to spare acute nociceptive pain while silencing chronic pain and may contribute to the specificity/safety of **194**. In $CRMP2^{K374A/K374A}$ mice, hippocampal long-term potentiation (LTP) was unaltered as evidenced by unchanged basal excitability and presynaptic or postsynaptic hippocampal plasticity in the CA1 circuit between wild-type and transgenic mice (*18*). Hippocampal LTP involves the presynaptic NaV1.1, NaV1.2, and NaV1.6 channels (*50*); the lack of effect of deSUMOylated CRMP2 on LTP (or the lack of effect of **194** on NaV1.1, NaV1.2 and NaV1.6 channels) rules out any potential regulation of these channels by CRMP2.

At present, our assessment of central nervous system (CNS) exposure of **194** is driven by structural considerations, such as the number of hydrogen bond donors (0), pK_a (nonionizable and neutral), total polar surface area (56.5 A^2), and molecular weight. Values of those properties for **194** suggest a rather high chance of passive diffusion through the blood-brain barrier (BBB). We used a BBB score algorithm reported previously (*51*) to calculate a BBB score of 3 for **194**; values of the BBB score in the range of 4 to 6 correctly predict 90% of CNS drugs. However, because **194** reversed

SNI-induced mechanical allodynia when administered spinally but not peripherally, our data support a central mechanism of action of **194**, which is consistent with a recent study reporting a central mechanism of analgesia in mice and humans lacking the sodium channel NaV1.7 (*52*). These recent observations are also in agreement with our data showing that **194** reduces (i) presynaptic NaV1.7, (ii) frequency of spinal EPSCs, and (iii) excitability. To enable full interpretation of pharmacokinetic (PK)/pharmacodynamic (PD) experiments, we intend—in future work —to obtain experimental values for unbound concentration in brain/plasma of **194** and any possible metabolites. Although **194** (at 5 μM) blocks all of NaV1.7 in cultured DRGs, our pharmacokinetic data indicate that a plasma concentration of ~0.1 μM at 1 hour after administration of **194** is still pain relieving. At this sub-IC_{50} dose, only partial inhibition of NaV1.7 is expected. Although the high degree (>90%) of channel block/current (in vitro) has been proposed as a prerequisite for achieving analgesia (in vivo), to our knowledge, no study has proven target engagement and the extent of block in vivo. However, evidence from human genetic studies shows that despite having congenital insensitivity to pain (CIP) due to mutations in NaV1.7, partial channel function was still retained in patients (*53*, *54*). These two reports support the possibility that even partial inhibition of NaV1.7 leads to painlessness.

Our previous work on the $CRMP2^{K374A/K374A}$ mice revealed no changes in depressive or repetitive, compulsive-like behaviors and a decrease in noxious thermal sensitivity. No changes were observed in $CRMP2^{K374A/K374A}$ mice to inflammatory, acute, or visceral pain (*18*). By contrast, in a neuropathic model, $CRMP2^{K374A/K374A}$ mice, irrespective of sex, failed to develop persistent mechanical allodynia (*18*). Collectively, this previous study demonstrated that CRMP2 SUMOylation–dependent control of peripheral NaV1.7 is a hallmark of chronic, but not physiological, neuropathic pain, thus providing strong support in targeting CRMP SUMOylation with a small-molecule approach to inhibit NaV1.7. No effects were noted on excitability of sensory neurons or on hippocampal and spinal cord plasticity (at both presynaptic and postsynaptic sites) in the $CRMP2^{K374A/K374A}$ mice (*18*). Thus, the general lack of behavioral impairments observed in our $CRMP2^{K374A/K374A}$ mice underscores the overall safety of targeting CRMP2 SUMOylation for pain in rodents (*18*).

Appraisal of **194** demonstrated dose-dependent reversal of pain behaviors across six different models (formalin, postsurgical, SNI, spared nerve ligation, paclitaxel-induced peripheral neuropathy, and HIV-induced sensory neuropathy) in two species (mice and rats) via four routes of administration (intrathecal, intraperitoneal, subcutaneous, and oral). **194** relieved affective pain (produced CPP) associated with a nerve injury–induced model. In NaV1.7 KO mice refractory to pain and humans with CIP, the opioid antagonist naloxone precipitates pain (*32*), most likely via up-regulation of endogenous opioids. Similarly, coadministration of naloxone completely reversed the analgesic effects of the tarantula venom–derived NaV1.7 blocking μ-theraphotoxin-Pn3a peptide (*55*). **194** capitalizes on this unique mechanism to provide analgesia.

Conflicting reports both in favor and against a link for up-regulation of endogenous opioid peptides and NaV1.7 inhibition have been reported. It had been suggested that μ- and δ-opioid receptors are involved (*56*), and as early as 1974, it was proposed that sodium concentration is a critical regulator of opioid agonist binding (*57*) at μ- and δ-opioid receptors (*58*–*60*). Further support for this hypothesis was inferred from the following observations: (i) The expression of proenkephalin (PENK) mRNA encoding a precursor to endogenous opioid peptides was increased in conditional NaV1.7 KO mice (*32*); (ii) the opioid receptor antagonist naloxone restored thermal and mechanical sensitivity in NaV1.7 KO mice, whereas infusion of naloxone enabled a patient with CIP to experience a noxious thermal stimulus for the first time (*32*); (iii) naloxone coadministration reversed the analgesia observed for the NaV1.7 selective inhibitor μ-theraphotoxin-Pn3a in a mouse model of postsurgical pain—whole-cell patch-clamp electrophysiology ruled out a direct effect of naloxone on NaV1.7 channels (*55*); and (iv) naloxone coadministration reversed analgesia of GpTx I, a venom-derived gating modifying toxin inhibitor of NaV1.7 channels, in multiple rodent pain models (*61*). However, there are also data challenging the NaV1.7-endogenous opioid link: (i) Bennett and colleagues (*62*) saw no up-regulation of PENK mRNA in NaV1.7 KO human induced pluripotent stem cells (iPSCs) and very little basal expression in healthy control neurons, (ii) in vitro treatment with naloxone failed to normalize hypoexcitability of NaV1.7 KO iPSCs (*62*), and (iii) naloxone failed to restore acute pain sensitivity in NaV1.7 loss-of-function rats (*63*). The presence of **194**, however, did not modulate the effect of opioids in our models. As we see a clear effect of naloxone in vivo, our findings support that the analgesic effect of **194** involves opioid-dependent mechanisms likely at the central terminals of nociceptors (*52*). Thus, our work implicates a role for opioid receptor engagement in the full mechanism of analgesia produced by **194**.

We did not observe tolerance to **194**-mediated pain relief. **194** did not produce CPP in naïve rats, ruling out the possibility that **194** is intrinsically rewarding. This is in contrast to rewarding analgesic drugs such as morphine, which produce CPP in naïve or uninjured subjects (*64*). **194** also did not exacerbate physical dependence to morphine or produce other common unwanted effects of painkillers including motor dysfunction and anxiety. In addition, chronic administration of high doses of **194** did not result in any cytotoxicity in any major organs. Another salient feature of **194** was its ability to potentiate the effect of subtherapeutic morphine or gabapentin, highlighting an opioid/gabapentin-sparing benefit, reminiscent of the analgesic synergy of Pn3a with oxycodone (*55*).

Several limitations of our study and of current knowledge should be noted. First, although we designed **194** to specifically inhibit CRMP2 SUMOylation, we cannot exclude the possibility that other SUMOylated proteins are also affected. As the canonical ψK*X*(E/D) SUMOylation motif maybe found in other proteins, **194** may also limit their SUMOylation. Second, CRMP2 SUMOylation–dependent regulation of NaV1.7 is not recapitulated in human embryonic kidney (HEK) 293 cells overexpressing NaV1.7 because these cells do not have sufficient endogenous levels of the SUMOylation machinery (Ubc9 and SUMO1)(*16*). Although we show that **194** has no effect on NaV1.1–6, these data were acquired in a recombinant system wherein CRMP2-dependent regulation of NaV1.7 was not recapitulated. That **194** did not inhibit sodium currents in large-diameter DRGs (>38 to 51 μm) rules out effects on at least NaV1.1 and NaV1.6 channels expressed by this neuron-size class (*25*). TTX-R NaV1.8 and NaV1.9 currents were also not affected by **194**. Third, given that all recordings are from "axotomized" DRGs and that there is the presence of the pronociceptive agent NGF in the culture media, these DRGs likely represent a "neuropathic" state. This is particularly exciting because these data reinforce the notion that antagonists of CRMP2 SUMOylation, such as **194**,

would spare acute nociceptive pain while silencing chronic pain. Fourth, **194** caused a modest increase in *Penk* mRNA expression in cultured DRGs. If endogenous opioids modulate the effect of **194** in vivo, this likely occurs at the level of the central synapse, which cannot be analyzed in cultured DRG neurons. Further research is required to prove that opioid-receptor engagement is critical for **194**'s full mechanism of action.

Poor design of trials [for example, failure to recognize that heterogeneity of sensory phenotypes of patients may hint at different underlying mechanisms (*65*, *66*) or lack of target modulation assays to enable clinical interpretation of NaV1.7 inhibitors, but see (*67*)] has likely led to faltering of NaV1.7-targeted drugs at the doorstep of the clinical stage (*68*). Future clinical trials for CRMP2 SUMOylation inhibitors should stratify patients with chronic pain based on the presence of SUMOylated CRMP2 in skin biopsies; increased amount of SUMOylated CRMP2 are present in the skin, spinal cord, and sciatic nerves in preclinical models of neuropathic pain (*30*). **194** might benefit patients with chronic pain selected using this precision medicine–style approach. Combining a multidisciplinary approach with a differentiated mode of action, our work identifies **194** as a first-in-class NaV1.7 inhibitor with target specificity to deliver safe analgesia in preclinical models.

MATERIALS AND METHODS

Study design

Our study goal was to harness the mechanism of indirect regulation of the voltage-gated sodium NaV1.7 channel by SUMOylated CRMP2 and to provide potential therapeutic strategies for control of chronic pain. We hypothesized that preventing CRMP2 SUMOylation by the E2 SUMO-conjugating enzyme Ubc9 would reduce NaV1.7 surface expression and currents, lead to a reduction in spinal nociceptive transmission, culminating in normalization of mechanical allodynia in models of neuropathic pain. Thus, compounds with the ability to prevent CRMP2 SUMOylation to indirectly regulate NaV17 activity might provide benefit in preventing neuropathic pain conditions. The research objects were Sprague Dawley rats and $CRMP2^{K374A/K374A}$ mice as well as cultured DRG cells from rats and mice. DRGs from Yucatan miniswine and humans were also used. Calculation of sample size was determined based on previous experiments using G-power statistics, indicating that 6 to 10 animals per group are required for $\alpha < 0.05$. The exact n numbers used in each study are indicated in the respective figure legends. For behavioral experiments, data from animals that died or had severe health problems in the middle of the experiments were excluded (<1%). Experiments were completed in multiple time periods in male and female mice and rats, ensuring that replication was observed. Western blotting, electrophysiology, fluorescence in situ hybridization (FISH), and immunohistochemical imaging data were reproduced in multiple rats/mice. Animals were assigned randomly to experimental and control groups. The experimenters were blinded for animal allocation and behavioral testing until all data collection was complete.

Statistical analysis

Statistical analysis was performed using GraphPad Prism v8/9 (GraphPad). Adherence to Gaussian distribution for each dataset was tested using the d'Agostino-Pearson normality test, and subsequent tests were chosen accordingly. Unless stated otherwise, data are presented at mean ± SEM. Dose-response curves of high-content screening (HCS) microscopy data were generated using nonlinear regression curve fitting (three parameters and standard Hill slope) with Prism (GraphPad). The parameters of the model (top, bottom, or pEC_{50}/pIC_{50} values) were compared using the extra-sum-of-squares F test. HCS experiments of PENK expression were analyzed with R (*69*) using ordinary one-way analysis of variance (ANOVA). Bonferroni's post hoc analysis was applied to determine P values of selected pairs (i.e., control versus treatment at each time point) defined in a contrast matrix using the R library multcomp. Complete sample size and statistical information are provided in table S1.

SUPPLEMENTARY MATERIALS

www.science.org/doi/10.1126/scitranslmed.abh1314
Materials and Methods
Figs. S1 to S8
Tables S1 and S2
Data file S1
References (*69*–*90*)

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. J. E. Meents, E. Bressan, S. Sontag, A. Foerster, P. Hautvast, C. Rösseler, M. Hampl, H. Schüler, R. Goetzke, T. K. C. Le, I. P. Kleggetveit, K. L. Cann, C. Kerth, A. M. Rush, M. Rogers, Z. Kohl, M. Schmelz, W. Wagner, E. Jørum, B. Namer, B. Winner, M. Zenke, A. Lampert, The role of Nav1.7 in human nociceptors: Insights from human induced pluripotent stem cell-derived sensory neurons of erythromelalgia patients. *Pain* **160**, 1327–1341 (2019).
2. S. G. Waxman, G. W. Zamponi, Regulating excitability of peripheral afferents: Emerging ion channel targets. *Nat. Neurosci.* **17**, 153–163 (2014).
3. S. D. Dib-Hajj, Y. Yang, J. A. Black, S. G. Waxman, The Na(V)1.7 sodium channel: From molecule to man. *Nat. Rev. Neurosci.* **14**, 49–62 (2013).
4. M. A. Nassar, L. C. Stirling, G. Forlani, M. D. Baker, E. A. Matthews, A. H. Dickenson, J. N. Wood, Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12706–12711 (2004).
5. M. S. Minett, M. A. Nassar, A. K. Clark, G. Passmore, A. H. Dickenson, F. Wang, M. Malcangio, J. N. Wood, Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. *Nat. Commun.* **3**, 791 (2012).
6. S. D. Shields, L. Deng, R. M. Reese, M. Dourado, J. Tao, O. Foreman, J. H. Chang, D. H. Hackos, Insensitivity to pain upon adult-onset deletion of Nav1.7 or its blockade with selective inhibitors. *J. Neurosci.* **38**, 10180–10201 (2018).
7. J. Gingras, S. Smith, D. J. Matson, D. Johnson, K. Nye, L. Couture, E. Feric, R. Yin, B. D. Moyer, M. L. Peterson, J. B. Rottman, R. J. Beiler, A. B. Malmberg, S. I. McDonough, Global nav1.7 knockout mice recapitulate the phenotype of human congenital indifference to pain. *PLOS ONE* **9**, e105895 (2014).
8. C. J. Laedermann, M. Cachemaille, G. Kirschmann, M. Pertin, R. D. Gosselin, I. Chang, M. Albesa, C. Towne, B. L. Schneider, S. Kellenberger, H. Abriel, I. Decosterd, Dysregulation of voltage-gated sodium channels by ubiquitin ligase NEDD4-2 in neuropathic pain. *J. Clin. Invest.* **123**, 3002–3013 (2013).
9. H. Zhang, P. M. Dougherty, Enhanced excitability of primary sensory neurons and altered gene expression of neuronal ion channels in dorsal root ganglion in paclitaxel-induced peripheral neuropathy. *Anesthesiology* **120**, 1463–1475 (2014).
10. J. M. Zakrzewska, J. Palmer, V. Morisset, G. M. Giblin, M. Obermann, D. A. Ettlin, G. Cruccu, L. Bendtsen, M. Estacion, D. Derjean, S. G. Waxman, G. Layton, K. Gunn, S. Tate; study investigators, Safety and efficacy of a Nav1.7 selective sodium channel blocker in patients with trigeminal neuralgia: A double-blind, placebo-controlled, randomised withdrawal phase 2a trial. *Lancet Neurol.* **16**, 291–300 (2017).
11. A. M. Donnell, S. Collins, Z. Ali, L. Iavarone, R. Surujbally, S. Kirby, R. P. Butt, Efficacy of the Nav1.7 blocker PF-05089771 in a randomised, placebo-controlled, double-blind clinical study in subjects with painful diabetic peripheral neuropathy. *Pain* **159**, 1465–1476 (2018).
12. M. E. Rothenberg, M. Tagen, J. H. Chang, J. Boyce-Rustay, M. Friesenhahn, D. H. Hackos, A. Hains, D. Sutherlin, M. Ward, W. Cho, Safety, tolerability, and pharmacokinetics of GDC-0276, a novel NaV1.7 inhibitor, in a first-in-human, single- and multiple-dose study in healthy volunteers. *Clin. Drug Investig.* **39**, 873–887 (2019).
13. G. Goodwin, S. B. McMahon, The physiological function of different voltage-gated sodium channels in pain. *Nat. Rev. Neurosci.* **22**, 263–274 (2021).

14. D. A. Eagles, C. Y. Chow, G. F. King, Fifteen years of $Na_V1.7$ channels as an analgesic target: Why has excellent in vitro pharmacology not translated into in vivo analgesic efficacy? *Br. J. Pharmacol.* **2020**, 1–20 (2020).
15. A. M. Moreno, F. Alemán, G. F. Catroli, M. Hunt, M. Hu, A. Dailamy, A. Pla, S. A. Woller, N. Palmer, U. Parekh, D. M. Donald, A. J. Roberts, V. Goodwill, I. Dryden, R. F. Hevner, L. Delay, G. G. D. Santos, T. L. Yaksh, P. Mali, Long-lasting analgesia via targeted in situ repression of $Na_V1.7$ in mice. *Sci. Transl. Med.* **13**, eaay9056 (2021).
16. E. T. Dustrude, S. M. Wilson, W. Ju, Y. Xiao, R. Khanna, CRMP2 protein SUMOylation modulates $Na_V1.7$ channel trafficking. *J. Biol. Chem.* **288**, 24316–24331 (2013).
17. E. T. Dustrude, A. Moutal, X. Yang, Y. Wang, M. Khanna, R. Khanna, Hierarchical CRMP2 posttranslational modifications control NaV1.7 function. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E8443–E8452 (2016).
18. A. Moutal, S. Cai, J. Yu, H. J. Stratton, A. Chefdeville, K. Gomez, D. Ran, C. L. Madura, L. Boinon, M. Soto, Y. Zhou, Z. Shan, L. A. Chew, K. E. Rodgers, R. Khanna, Studies on CRMP2 SUMOylation-deficient transgenic mice identify sex-specific $Na_V1.7$ regulation in the pathogenesis of chronic neuropathic pain. *Pain* **161**, 2629–2651 (2020).
19. K. Gomez, D. Ran, C. L. Madura, A. Moutal, R. Khanna, Non-SUMOylated CRMP2 decreases $Na_V1.7$ currents via the endocytic proteins Numb, Nedd4-2 and Eps15. *Mol. Brain* **14**, 20 (2021).
20. L. Francois-Moutal, E. T. Dustrude, Y. Wang, T. Brustovetsky, A. Dorame, W. Ju, A. Moutal, S. Perez-Miller, N. Brustovetsky, V. Gokhale, M. Khanna, R. Khanna, Inhibition of the Ubc9 E2 SUMO-conjugating enzyme-CRMP2 interaction decreases $Na_V1.7$ currents and reverses experimental neuropathic pain. *Pain* **159**, 2115–2127 (2018).
21. L. Francois-Moutal, D. D. Scott, S. Perez-Miller, V. Gokhale, M. Khanna, R. Khanna, Chemical shift perturbation mapping of the Ubc9-CRMP2 interface identifies a pocket in CRMP2 amenable for allosteric modulation of Nav1.7 channels. *Channels* **12**, 219–227 (2018).
22. E. T. Dustrude, S. Perez-Miller, L. François-Moutal, A. Moutal, M. Khanna, R. Khanna, A single structurally conserved SUMOylation site in CRMP2 controls NaV1.7 function. *Channels* **11**, 316–328 (2017).
23. S. Cai, S. S. Bellampalli, J. Yu, W. Li, Y. Ji, E. M. K. Wijeratne, A. Dorame, S. Luo, Z. Shan, M. Khanna, A. Moutal, J. M. Streicher, A. A. L. Gunatilaka, R. Khanna, (–)-Hardwickiic acid and hautriwaic acid induce antinociception via blockade of tetrodotoxin-sensitive voltage-dependent sodium channels. *ACS Chem. Neurosci.* **10**, 1716–1728 (2019).
24. W. A. Schmalhofer, J. Calhoun, R. Burrows, T. Bailey, M. G. Kohler, A. B. Weinglass, G. J. Kaczorowski, M. L. Garcia, M. Koltzenburg, B. T. Priest, ProTx-II, a selective inhibitor of $Na_V1.7$ sodium channels, blocks action potential propagation in nociceptors. *Mol. Pharmacol.* **74**, 1476–1484 (2008).
25. C. Ho, M. E. O'Leary, Single-cell analysis of sodium channel expression in dorsal root ganglion neurons. *Mol. Cell. Neurosci.* **46**, 159–166 (2011).
26. A. Büchner, P. Krumova, S. Ganesan, M. Bähr, K. Eckermann, J. H. Weishaupt, Sumoylation of p35 modulates p35/cyclin-dependent kinase (Cdk) 5 complex activity. *Neuromolecular Med.* **17**, 12–23 (2015).
27. M. Brown, T. Jacobs, B. Eickholt, G. Ferrari, M. Teo, C. Monfries, R. Z. Qi, T. Leung, L. Lim, C. Hall, Alpha2-chimaerin, cyclin-dependent Kinase 5/p35, and its target collapsin response mediator protein-2 are essential components in semaphorin 3A-induced growth-cone collapse. *J.Neurosci.* **24**, 8994–9004 (2004).
28. S. R. A. Alles, F. Nascimento, R. Luján, A. P. Luiz, Q. Millet, M. A. Bangash, S. Santana-Varela, X. Zhou, J. J. Cox, A. L. Okorokov, M. Beato, J. Zhao, J. N. Wood, Sensory neuron-derived $Na_V1.7$ contributes to dorsal horn neuron excitability. *Sci Adv* **6**, eaax4568 (2020).
29. J. Yu, A. Moutal, A. Dorame, S. S. Bellampalli, A. Chefdeville, I. Kanazawa, N. Y. N. Pham, K. D. Park, J. M. Weimer, R. Khanna, Phosphorylated CRMP2 regulates spinal nociceptive neurotransmission. *Mol. Neurobiol.* **56**, 5241–5255 (2019).
30. A. Moutal, E. T. Dustrude, T. M. Largent-Milnes, T. W. Vanderah, M. Khanna, R. Khanna, Blocking CRMP2 SUMOylation reverses neuropathic pain. *Mol. Psychiatry* **23**, 2119–2121 (2018).
31. L. E. Chaparro, P. J. Wiffen, R. A. Moore, I. Gilron, Combination pharmacotherapy for the treatment of neuropathic pain in adults. *Cochrane Database Syst. Rev.* **2012**, Cd008943 (2012).
32. M. S. Minett, V. Pereira, S. Sikandar, A. Matsuyama, S. Lolignier, A. H. Kanellopoulos, F. Mancini, G. D. Iannetti, Y. D. Bogdanov, S. Santana-Varela, Q. Millet, G. Baskozos, R. M. Allister, J. J. Cox, J. Zhao, J. N. Wood, Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel $Na_V1.7$. *Nat. Commun.* **6**, 8967 (2015).
33. J. Isensee, L. Krahé, K. Moeller, V. Pereira, J. E. Sexton, X. Sun, E. Emery, J. N. Wood, T. Hucho, Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in $Na_V1.7$ knockout mice. *Sci. Signal.* **10**, eaah4874 (2017).
34. J. Weiss, M. Pyrski, E. Jacobi, B. Bufe, V. Willnecker, B. Schick, P. Zizzari, S. J. Gossage, C. A. Greer, T. Leinders-Zufall, C. G. Woods, J. N. Wood, F. Zufall, Loss-of-function mutations in sodium channel $Na_V1.7$ cause anosmia. *Nature* **472**, 186–190 (2011).
35. V. Iyer, R. A. Slivicki, A. C. Thomaz, J. D. Crystal, K. Mackie, A. G. Hohmann, The cannabinoid CB2 receptor agonist LY2828360 synergizes with morphine to suppress neuropathic nociception and attenuates morphine reward and physical dependence. *Eur. J. Pharmacol.* **886**, 173544 (2020).
36. E. C. Emery, A. P. Luiz, J. N. Wood, $Na_V1.7$ and other voltage-gated sodium channels as drug targets for pain relief. *Expert Opin. Ther. Targets* **20**, 975–983 (2016).
37. P. Siebenga, G. van Amerongen, J. L. Hay, A. M. Donnell, D. Gorman, R. Butt, G. J. Groeneveld, Lack of detection of the analgesic properties of PF-05089771, a selective $Na_V1.7$ inhibitor, using a battery of pain models in healthy subjects. *Clin. Transl. Sci.* **13**, 318–324 (2019).
38. J. R. Deuis, J. S. Wingerd, Z. Winter, T. Durek, Z. Dekan, S. R. Sousa, K. Zimmermann, T. Hoffmann, C. Weidner, M. A. Nassar, P. F. Alewood, R. J. Lewis, I. Vetter, Analgesic effects of GpTx-1, PF-04856264 and CNV1014802 in a mouse model of $Na_V1.7$-mediated pain. *Toxins* **8**, 78 (2016).
39. M. Watanabe, K. Takahashi, K. Tomizawa, H. Mizusawa, H. Takahashi, Developmental regulation of Ubc9 in the rat nervous system. *Acta Biochim. Pol.* **55**, 681–686 (2008).
40. K. Nacerddine, F. Lehembre, M. Bhaumik, J. Artus, M. Cohen-Tannoudji, C. Babinet, P. P. Pandolfi, A. Dejean, The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. *Dev. Cell* **9**, 769–779 (2005).
41. L. Lee, E. Dale, A. Staniszewski, H. Zhang, F. Saeed, M. Sakurai, M. Fa, I. Orozco, F. Michelassi, N. Akpan, H. Lehrer, O. Arancio, Regulation of synaptic plasticity and cognition by SUMO in normal physiology and Alzheimer's disease. *Sci. Rep.* **4**, 7190 (2014).
42. I. Fukuda, A. Ito, G. Hirai, S. Nishimura, H. Kawasaki, H. Saitoh, K. I. Kimura, M. Sodeoka, M. Yoshida, Ginkgolic acid inhibits protein SUMOylation by blocking formation of the E1-SUMO intermediate. *Chem. Biol.* **16**, 133–140 (2009).
43. I. Fukuda, A. Ito, M. Uramoto, H. Saitoh, H. Kawasaki, H. Osada, M. Yoshida, Kerriamycin B inhibits protein SUMOylation. *J. Antibiot.* **62**, 221–224 (2009).
44. M. Hirohama, A. Kumar, I. Fukuda, S. Matsuoka, Y. Igarashi, H. Saitoh, M. Takagi, K. Shin-ya, K. Honda, Y. Kondoh, T. Saito, Y. Nakao, H. Osada, K. Y. J. Zhang, M. Yoshida, A. Ito, Spectomycin B1 as a novel SUMOylation inhibitor that directly binds to SUMO E2. *ACS Chem. Biol.* **8**, 2635–2642 (2013).
45. F. Heblich, A. Tran Van Minh, J. Hendrich, K. Watschinger, A. C. Dolphin, Time course and specificity of the pharmacological disruption of the trafficking of voltage-gated calcium channels by gabapentin. *Channels* **2**, 4–9 (2008).
46. J. Hendrich, A. T. van Minh, F. Heblich, M. Nieto-Rostro, K. Watschinger, J. Striessnig, J. Wratten, A. Davies, A. C. Dolphin, Pharmacological disruption of calcium channel trafficking by the $\alpha 2\delta$ ligand gabapentin. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3628–3633 (2008).
47. S. R. A. Alles, M. V. Bandet, K. Eppler, M. C. Noh, I. R. Winship, G. Baker, K. Ballanyi, P. A. Smith, Acute anti-allodynic action of gabapentin in dorsal horn and primary somatosensory cortex: Correlation of behavioural and physiological data. *Neuropharmacology* **113**, 576–590 (2017).
48. S. R. A. Alles, P. A. Smith, Comparison of ex vivo and in vitro actions of gabapentin in superficial dorsal horn and the role of extra-spinal sites of drug action. *Neurosci. Lett.* **694**, 148–153 (2019).
49. T. Fukuoka, K. Miyoshi, K. Noguchi, De novo expression of Nav1.7 in injured putative proprioceptive afferents: Multiple tetrodotoxin-sensitive sodium channels are retained in the rat dorsal root after spinal nerve ligation. *Neuroscience* **284**, 693–706 (2015).
50. X. Qiao, T. R. Werkman, J. A. Gorter, W. J. Wadman, E. A. van Vliet, Expression of sodium channel α subunits 1.1, 1.2 and 1.6 in rat hippocampus after kainic acid-induced epilepsy. *Epilepsy Res.* **106**, 17–28 (2013).
51. M. Gupta, H. J. Lee, C. J. Barden, D. F. Weaver, The blood-brain barrier (BBB) score. *J. Med. Chem.* **62**, 9824–9836 (2019).
52. D. I. MacDonald, S. Sikandar, J. Weiss, M. Pyrski, A. P. Luiz, Q. Millet, E. C. Emery, F. Mancini, G. D. Iannetti, S. R. A. Alles, M. Arcangeletti, J. Zhao, J. J. Cox, R. M. Brownstone, F. Zufall, J. N. Wood, A central mechanism of analgesia in mice and humans lacking the sodium channel $Na_V1.7$. *Neuron* **109**, 1497–1512.e6 (2021).
53. J. Sun, L. Li, L. Yang, G. Duan, T. Ma, N. Li, Y. Liu, J. Yao, J. Y. Liu, X. Zhang, Novel SCN9 Amissense mutations contribute to congenital insensitivity to pain: Unexpected correlation between electrophysiological characterization and clinical phenotype. *Mol. Pain* **16**, 174480692092388 (2020).
54. E. C. Emery, A. M. Habib, J. J. Cox, A. K. Nicholas, F. M. Gribble, C. G. Woods, F. Reimann, Novel SCN9A mutations underlying extreme pain phenotypes: Unexpected electrophysiological and clinical phenotype correlations. *J. Neurosci.* **35**, 7674–7681 (2015).
55. A. Mueller, H. Starobova, M. Morgan, Z. Dekan, O. Cheneval, C. I. Schroeder, P. F. Alewood, J. R. Deuis, I. Vetter, Antiallodynic effects of the selective NaV1.7 inhibitor Pn3a in a mouse model of acute postsurgical pain: Evidence for analgesic synergy with opioids and baclofen. *Pain* **160**, 1766–1780 (2019).
56. V. Pereira, Q. Millet, J. Aramburu, C. Lopez-Rodriguez, C. Gaveriaux-Ruff, J. N. Wood, Analgesia linked to Nav1.7 loss of function requires μ- and δ-opioid receptors. *Wellcome Open Res* **3**, 101 (2018).

57. C. B. PERT, S. H. SNYDER, Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.* **10**, 868–879 (1974).
58. D. A. Brase, Is intracellular sodium involved in the mechanism of tolerance to opioid drugs? *Med. Hypotheses* **32**, 161–167 (1990).
59. P. Puttfarcken, L. L. Werling, S. R. Brown, T. E. Cote, B. M. Cox, Sodium regulation of agonist binding at opioid receptors. I. Effects of sodium replacement on binding at mu- and delta-type receptors in 7315c and NG108-15 cells and cell membranes. *Mol. Pharmacol.* **30**, 81–89 (1986).
60. L. L. Werling, S. R. Brown, P. Puttfarcken, B. M. Cox, Sodium regulation of agonist binding at opioid receptors. II. Effects of sodium replacement on opioid binding in guinea pig cortical membranes. *Mol. Pharmacol.* **30**, 90–95 (1986).
61. C. Chen, B. Xu, X. Shi, M. Zhang, Q. Zhang, T. Zhang, W. Zhao, R. Zhang, Z. Wang, N. Li, Q. Fang, GpTx-1 and [Ala^5, Phe^6, Leu^{26}, Arg^{28}]GpTx-1, two peptide $Na_V1.7$ inhibitors: Analgesic and tolerance properties at the spinal level. *Br. J. Pharmacol.* **175**, 3911–3927 (2018).
62. L. A. McDermott, G. A. Weir, A. C. Themistocleous, A. R. Segerdahl, I. Blesneac, G. Baskozos, A. J. Clark, V. Millar, L. J. Peck, D. Ebner, I. Tracey, J. Serra, D. L. Bennett, Defining the Functional Role of $Na_V1.7$ in human nociception. *Neuron* **101**, 905–919.e8 (2019).
63. L. Chen, P. R. Effraim, J. Carrara, P. Zhao, F. B. Dib-Hajj, S. D. Dib-Hajj, S. G. Waxman, Pharmacological characterization of a rat $Na_V1.7$ loss-of-function model with insensitivity to pain. *Pain* **161**, 1350–1360 (2020).
64. K. J. Sufka, Conditioned place preference paradigm: A novel approach for analgesic drug assessment against chronic pain. *Pain* **58**, 355–366 (1994).
65. R. Baron, C. Maier, N. Attal, A. Binder, D. Bouhassira, G. Cruccu, N. B. Finnerup, M. Haanpää, P. Hansson, P. Hüllemann, T. S. Jensen, R. Freynhagen, J. D. Kennedy, W. Magerl, T. Mainka, M. Reimer, A. S. C. Rice, M. Segerdahl, J. Serra, S. Sindrup, C. Sommer, T. Tölle, J. Vollert, R. D. Treede; German Neuropathic Pain Research Network (DFNS), and the EUROPAIN, and NEUROPAIN consortia, Peripheral neuropathic pain: A mechanism-related organizing principle based on sensory profiles. *Pain* **158**, 261–272 (2017).
66. N. B. Finnerup, N. Attal, S. Haroutounian, E. McNicol, R. Baron, R. H. Dworkin, I. Gilron, M. Haanpää, P. Hansson, T. S. Jensen, P. R. Kamerman, K. Lund, A. Moore, S. N. Raja, A. S. C. Rice, M. Rowbotham, E. Sena, P. Siddall, B. H. Smith, M. Wallace, Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. *Lancet Neurol.* **14**, 162–173 (2015).
67. R. L. Kraus, F. Zhao, P. S. Pall, D. Zhou, J. D. Vardigan, A. Danziger, Y. Li, C. Daley, J. E. Ballard, M. K. Clements, R. M. Klein, M. A. Holahan, T. J. Greshock, R. M. Kim, M. E. Layton, C. S. Burgey, J. Serra, D. A. Henze, A. K. Houghton, $Na_V1.7$ target modulation and efficacy can be measured in nonhuman primate assays. *Sci. Transl. Med.* **13**, eaay1050 (2021).
68. A. Dickenson, Why are sodium channel modulators not yet pharmacotherapeutic trailblazers for neuropathic pain? *Expert Opin. Pharmacother.* **22**, 1635–1637 (2021).
69. R. D. C. Team, *R: A Language and Environment for Statistical Computing*. R. F. f. S. Computing, Ed., (2011).
70. Y. Zhou, S. Cai, A. Moutal, J. Yu, K. Gómez, C. L. Madura, Z. Shan, N. Y. N. Pham, M. J. Serafini, A. Dorame, D. D. Scott, L. François-Moutal, S. Perez-Miller, M. Patek, M. Khanna, R. Khanna, The natural flavonoid naringenin elicits analgesia through inhibition of NaV1.8 voltage-gated sodium channels. *ACS Chem. Neurosci.* **10**, 4834–4846 (2019).
71. L. François-Moutal, Y. Wang, A. Moutal, K. E. Cottier, O. K. Melemedjian, X. Yang, Y. Wang, W. Ju, T. M. Largent-Milnes, M. Khanna, T. W. Vanderah, R. Khanna, A membrane-delimited N-myristoylated CRMP2 peptide aptamer inhibits CaV2.2 trafficking and reverses inflammatory and postoperative pain behaviors. *Pain* **156**, 1247–1264 (2015).
72. F. Golebiowski, A. Szulc, M. Sakowicz, A. Szutowicz, T. Pawelczyk, Expression level of Ubc9 protein in rat tissues. *Acta Biochim. Pol.* **50**, 1065–1073 (2003).
73. A. Moutal, L. François-Moutal, S. Perez-Miller, K. Cottier, L. A. Chew, S. K. Yeon, J. Dai, K. D. Park, M. Khanna, R. Khanna, (S)-Lacosamide binding to collapsin response mediator protein 2 (CRMP2) regulates CaV2.2 activity by subverting its phosphorylation by Cdk5. *Mol. Neurobiol.* **53**, 1959–1976 (2016).
74. L. François-Moutal, R. Felemban, D. D. Scott, M. R. Sayegh, V. G. Miranda, S. Perez-Miller, R. Khanna, V. Gokhale, D. C. Zarnescu, M. Khanna, Small molecule targeting TDP-43's RNA recognition motifs reduces locomotor defects in a *Drosophila* model of amyotrophic lateral sclerosis (ALS). *ACS Chem. Biol.* **14**, 2006–2013 (2019).
75. S. Davidson, B. A. Copits, J. Zhang, G. Page, A. Ghetti, R. W. Gereau, Human sensory neurons: Membrane properties and sensitization by inflammatory mediators. *Pain* **155**, 1861–1870 (2014).
76. M. M. Ibrahim, A. Patwardhan, K. B. Gilbraith, A. Moutal, X. Yang, L. A. Chew, T. Largent-Milnes, T. P. Malan, T. W. Vanderah, F. Porreca, R. Khanna, Long-lasting antinociceptive effects of green light in acute and chronic pain in rats. *Pain* **158**, 347–360 (2017).
77. A. Moutal, W. Li, Y. Wang, W. Ju, S. Luo, S. Cai, L. François-Moutal, S. Perez-Miller, J. Hu, E. T. Dustrude, T. W. Vanderah, V. Gokhale, M. Khanna, R. Khanna, Homology-guided mutational analysis reveals the functional requirements for antinociceptive specificity of collapsin response mediator protein 2-derived peptides. *Br. J. Pharmacol.* **175**, 2244–2260 (2018).
78. Z. Shan, S. Cai, J. Yu, Z. Zhang, T. G. M. Vallecillo, M. J. Serafini, A. M. Thomas, N. Y. N. Pham, S. S. Bellampalli, A. Moutal, Y. Zhou, G.-B. Xu, Y.-M. Xu, S. Luo, M. Patek, J. M. Streicher, A. A. L. Gunatilaka, R. Khanna, Reversal of peripheral neuropathic pain by the small-molecule natural product physalin F via block of CaV2.3 (R-type) and CaV2.2 (N-type) voltage-gated calcium channels. *ACS Chem. Neurosci.* **10**, 2939–2955 (2019).
79. Z. Zhou, Q. Gong, B. Ye, Z. Fan, J. C. Makielski, G. A. Robertson, C. T. January, Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys. J.* **74**, 230–241 (1998).
80. P. Zhang, F. Ye, A. C. Bastidas, A. P. Kornev, J. Wu, M. H. Ginsberg, S. S. Taylor, An isoform-specific myristylation switch targets type II PKA holoenzymes to membranes. *Structure* **23**, 1563–1572 (2015).
81. P. Zhang, E. V. Smith-Nguyen, M. M. Keshwani, M. S. Deal, A. P. Kornev, S. S. Taylor, Structure and allostery of the PKA RIIβ tetrameric holoenzyme. *Science* **335**, 712–716 (2012).
82. J. Isensee, M. Kaufholz, M. J. Knape, J. Hasenauer, H. Hammerich, H. Gonczarowska-Jorge, R. P. Zahedi, F. Schwede, F. W. Herberg, T. Hucho, PKA-RII subunit phosphorylation precedes activation by cAMP and regulates activity termination. *J. Cell Biol.* **217**, 2167–2184 (2018).
83. M. Roederer, Compensation in flow cytometry. *Curr. Protoc. Cytom.* **Chapter 1**, Unit 1.14 (2002).
84. Y. Wang, K. D. Park, C. Salomé, S. M. Wilson, J. P. Stables, R. Liu, R. Khanna, H. Kohn, Development and characterization of novel derivatives of the antiepileptic drug lacosamide that exhibit far greater enhancement in slow inactivation of voltage-gated sodium channels. *ACS Chem. Nerosci.* **2**, 90–106 (2011).
85. B. Grubinska, L. Chen, M. Alsaloum, N. Rampal, D. J. Matson, C. Yang, K. Taborn, M. Zhang, B. Youngblood, D. Liu, E. Galbreath, S. Allred, M. Lepherd, R. Ferrando, T. J. Kornecook, S. G. Lehto, S. G. Waxman, B. D. Moyer, S. Dib-Hajj, J. Gingras, Rat Na(V)1.7 loss-of-function genetic model: Deficient nociceptive and neuropathic pain behavior with retained olfactory function and intra-epidermal nerve fibers. *Mol. Pain* **15**, 1744806919881846 (2019).
86. C. J. Laedermann, N. Syam, M. Pertin, I. Decosterd, H. Abriel, β1- and β3- voltage-gated sodium channel subunits modulate cell surface expression and glycosylation of Nav1.7 in HEK293 cells. *Front Cell Neurosci* **7**, 137 (2013).
87. I. Decosterd, C. J. Woolf, Spared nerve injury: An animal model of persistent peripheral neuropathic pain. *Pain* **87**, 149–158 (2000).
88. L. Liu, J. K. Coller, L. R. Watkins, A. A. Somogyi, M. R. Hutchinson, Naloxone-precipitated morphine withdrawal behavior and brain IL-1β expression: Comparison of different mouse strains. *Brain Behav. Immun.* **25**, 1223–1232 (2011).
89. R. D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: A primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **229**, 327–336 (1977).
90. J. Yu, Q. Fang, G.-D. Lou, W.-T. Shou, J.-X. Yue, Y.-Y. Tang, W.-W. Hou, T.-L. Xu, H. Ohtsu, S.-H. Zhang, Z. Chen, Histamine modulation of acute nociception involves regulation of Nav1.8 in primary afferent neurons in mice. *CNS Neurosci. Ther.* **19**, 649–658 (2013).

Acknowledgments: We thank K. B. Gonzalez for performing the chronic dosing experiment, H. Ellingson for performing naïve CPP, and F. Porreca and A. Patwardhan for helpful discussions on this project. **Funding:** This study was supported by the NIH awards NINDS (NS098772 and NS120663 to R.K.), NIDA (DA042852 to R.K.), and NCI (CA200417 to A.G.H.). T.W. was supported by T32DA024628. **Author contributions:** R.K. developed the concept, designed the experiments, and supervised all aspects of this project. A.M., A.D., D.D.S., S.S.B., L.F.-M., S.P.-M., and T.W. collected and analyzed the data. R.C. and V.G. synthesized the compounds. A.C., S.L., T.W., and C.M. performed animal behavior studies. S.C., K.G., J.Y., L.A.C., Y.Z., and Z.S. performed electrophysiology recordings. J.I. and T.H. performed HCS and FISH. V.G., M.K., M.P., and S.P.-M. performed docking studies. R.K. and A.M. wrote the manuscript. A.G.H., T.W.V., and R.K. supervised all aspects of this project. All authors had the opportunity to discuss the results and comment on the manuscript. **Competing interests:** R.K., M.K., and V.G. are cofounders of Regulonix LLC, a company developing nonopioid drugs for chronic pain. In addition, R.K., M.K., R.C., and V.G. have patents US10287334 (non-narcotic CRMP2 peptides targeting sodium channels for chronic pain) and US10441586 (SUMOylation inhibitors and uses thereof) issued to Regulonix LLC. The other authors declare that they have no competing interests. R.K. and A.M. are cofounders of ElutheriaTx Inc., a company developing gene therapy approaches for chronic pain. **Data and materials availability:** All data associated with this study are present in the paper or the Supplementary Materials.

Submitted 17 February 2021
Resubmitted 14 July 2021
Accepted 17 August 2021
Published 10 November 2021
10.1126/scitranslmed.abh1314

PAIN

Oncostatin M can sensitize sensory neurons in inflammatory pruritus

Pang-Yen Tseng and Mark A. Hoon*

Chronic itch is a major symptom of many inflammatory skin diseases. This type of pruritus is thought to be facilitated by cytokines that activate cutaneous nerve fibers; however, the molecular components and mechanisms involved are poorly understood. We found that the cytokine oncostatin M (OSM) is highly up-regulated in psoriasis, atopic dermatitis, and cutaneous T cell lymphoma, diseases associated with chronic itch. OSM receptor (OSMR) is expressed by itch-selective natriuretic polypeptide B (Nppb) neurons, and single-cell sequencing showed that OSM is mainly produced by dermal T cells and monocytes. Unlike canonical pruritogens, OSM does not activate sensory neurons. Instead, it sensitizes neurons by potentiating neural responses to pruritogens and by enhancing neural excitability. Knockout of OSMR in sensory neurons attenuated OSM-sensitized itch and inflammatory itch in mice, and pharmacological antagonism of the OSMR complex effectively alleviated pruritus in experimental inflammatory dermatitis in a rodent model. Together, our results uncover OSM as an itch neuromodulator and reveal OSM signal transduction as a potential target for antipruritic therapy.

INTRODUCTION

Itch associated with cutaneous diseases is thought to arise from the activation of sensory neurons that innervate the skin (*1*). This activation often takes place in the context of skin diseases where the immune system is also activated frequently as a result of barrier disruption (*2*). For many persistent skin diseases, chronic itch is not only a minor side effect but also severely reduces the quality of life of patients (*3*–*6*). Because most chronic itch is difficult to treat effectively, it is, globally, a major unmet health concern (*7*–*9*).

Pruritic agents, both produced endogenously and those encountered via contact with exogenous sources, stimulate itch neurons that, in turn, via connections to the central nervous system, produce the percept of itch and evoke the urge to scratch (*6*, *10*–*12*). In mice, three classes of peripheral itch neurons have been characterized, those that express the itch receptor Mrgpra3, Mrgprd neurons, and those that express natriuretic polypeptide B (Nppb) (*13*–*16*). Itch-inducing agents such as monoamines (*17*), lipids (*18*, *19*), proteases (*20*–*22*), peptides, and some cytokines including interleukin-31 (IL-31), IL-4, and IL-13 (*4*, *23*–*25*) can directly depolarize sensory neurons that evoke pruriception (*26*, *27*). However, the data for cytokines serving as direct pruritogens are limited and remain uncertain. Emphasizing the importance of cytokine reception in itch, agents targeting cytokine receptors have shown clinical value in treating atopic dermatitis (*24*, *28*). During chronic itch, the processes that drive the continuous urge to scratch are not well understood but may not be the same as those that elicit acute itch. An example of this is histamine, which is known to be a key mediator of acute itch but, except in urticaria, is not thought to be a major mediator for chronic itch. The pruritogens involved in chronic itch are still poorly understood and may include underappreciated substances such as leukotrienes (*29*).

In this study, we examined the transcriptomic profile and single-cell sequencing data of skin from human subjects with skin diseases associated with chronic itch and uncovered that, in inflammatory skin biopsies, oncostatin M (OSM) is overwhelmingly expressed by several dermal immune cells including T cells, monocytes, dendritic cells, and mast cells. Although OSM has previously been implicated as a general agent found during inflammation (*30*–*32*), a role for this cytokine in itch has not been established. Physiological studies showed that OSM directly modulated Nppb neurons by enhancing neural activity instead of depolarizing neurons, a mechanism very different from canonical pruritogens. This OSM-mediated sensitization resulted in exaggerated itch in inflammatory skin. Genetic studies and single-cell sequencing data suggested that OSM also indirectly evoked itch by stimulating stromal cells in skin. Last, we show that inhibiting OSM receptor (OSMR) complex, in a mouse model, can alleviate itch and skin inflammation in dermatitis. Together, we showed that OSM is the predominant cytokine in skin inflammation that multidimensionally modulates sensory neurons to induce skin pruritus.

RESULTS

Nppb neurons express multiple cytokine receptors

Given the reported involvement of cytokines in chronic itch, we were interested in profiling the expression of their receptors in sensory neurons to more fully describe these interactions (*23*, *27*). Initially, we examined the cytokine receptors expressed by Nppb neurons because these neurons have been shown to be involved in itch; the expression of the neuropeptide Nppb (released by these cells) is increased in chronic itch (*33*); and IL-31 receptor (IL31ra), a receptor associated with chronic itch (*34*, *35*), is expressed by these cells. Whereas IL31ra is expressed only in Nppb neurons (*36*–*39*), the expression of other cytokine receptors has not been defined in vivo (*40*). To determine the receptor transcriptome of Nppb neurons, we collected dissociated Nppb neurons (genetically labeled) (Fig. 1A) and performed pooled-cell RNA sequencing (RNA-seq). Sequencing data from pooled Trpm8 and Trpv1 cells (Fig. 1B) were used for comparison to identify signaling molecules enriched in Nppb neurons. As anticipated, this analysis revealed the selective enrichment of the previously reported receptor for Il31ra, as well as the OSMR and an important downstream signaling molecule, Janus

Molecular Genetics Section, National Institute of Dental and Craniofacial Research/NIH, 35 Convent Drive, Bethesda, MD 20892, USA.
*Corresponding author. Email: mark.hoon@nih.gov

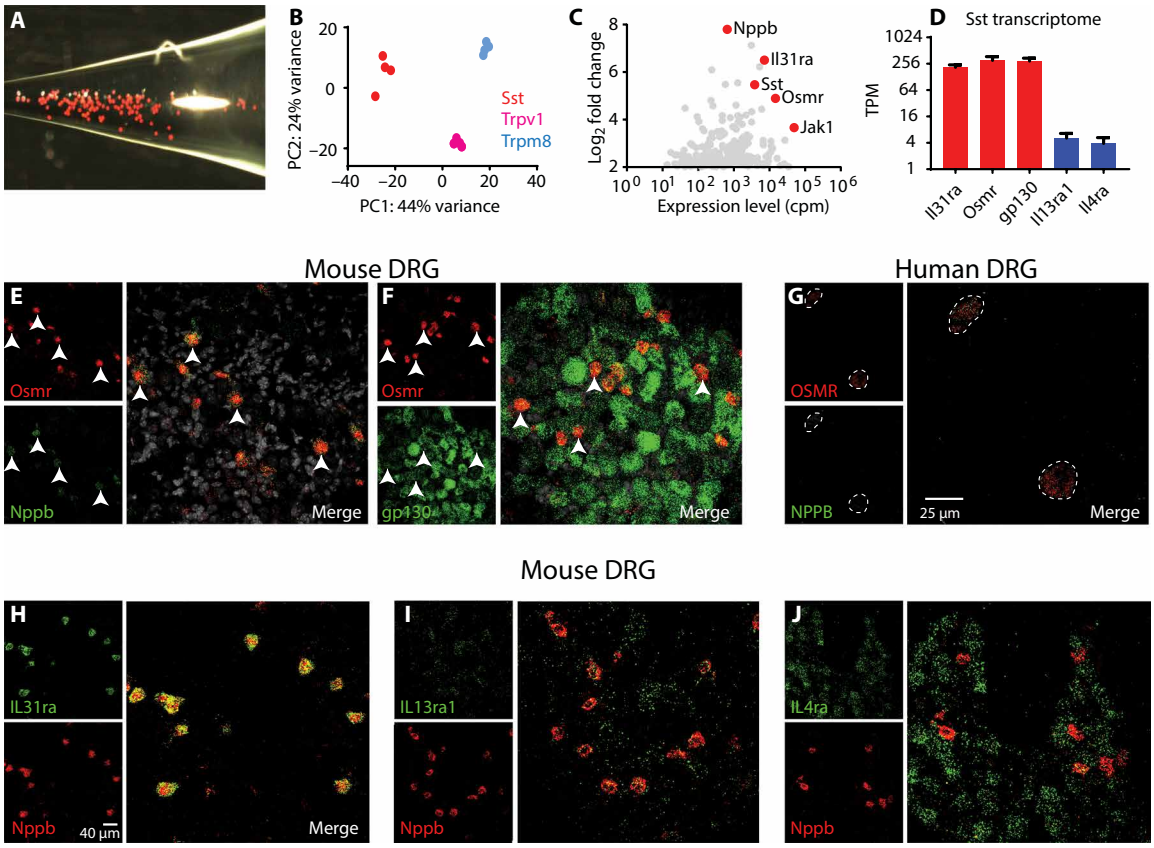


Fig. 1. Nppb neurons express multiple cytokine receptor complex. (**A**) Representative image of dissociated Nppb neurons from Sst-cre:: AAV9-CAG-Flex-tdTomato mice. (**B**) Principal components analysis of clustered RNA-seq data from Sst, Trpm8, and Trpv1 neurons ($n = 4$ RNA libraries for each class of neuron) (GSE125626). (**C**) Analysis of differential gene expression between Sst and Trpm8 neurons with estimated gene abundance, counts per million (cpm). Genes enriched in Sst neurons are indicated in red. (**D**) Quantification of transcript abundances, transcripts per million (TPM), of indicated genes; data are presented as means ± SEM. (**E** and **F**) Representative images of multilabel in situ hybridization (ISH) of mouse DRG with indicated genes. (**G**) Images of ISH of human DRG hybridized with probes for OSMR and NPPB. (**H** to **J**) Images of sections of mouse DRG hybridized with probes to the indicated genes.

kinase 1 (Jak1) (Fig. 1C). OSMR is thought to form heterodimers with IL31ra to transduce IL-31 signals (*31*, *41*). In addition, along with being a co-receptor for IL-31 signaling, OSMR acts as a receptor for OSM together with the co-receptor gp130 (*30*–*32*). Analysis of sequencing data showed that Nppb neurons also express high amounts of gp130 (Il6st) (Fig. 1D), indicating that Nppb neurons express components necessary for the detection of OSM. In addition, we found that Nppb neurons express Il13ra1 and Il4ra, which can form receptor complexes to transduce IL-13 and IL-4 signals, respectively (*42*). The expression amounts of Il13ra1 and Il4ra were about 50-fold lower than that of OSMR, gp130, and Il31ra (Fig. 1D and Table 1). To validate these RNA-seq results and to get a better insight into which cells express cytokine receptors, we performed multilabel in situ hybridization (ISH) on both mouse and human dorsal root ganglion (DRG). In agreement with our sequencing results, in mouse DRG, about 90% of OSMR-expressing neurons also coexpressed Nppb ($Osmr^+Nppb^+/Osmr^+ = 548/613 = 89\%$, $Osmr^+Nppb^+/Nppb^+ = 548/567 = 92\%$, $n = 5$ mice; Fig. 1E), although there is a fraction of neurons that express low amounts of OSMR that are Nppb negative and Trpv1 positive (fig. S1). The OSMR neurons also coexpressed the co-receptor gp130 that is broadly expressed in many DRG neurons ($Osmr^+gp130^+/Osmr^+ = 164/166$, $n = 3$ mice; Fig. 1F). In human sensory neurons, similar to what we found in mouse DRG, OSMR and NPPB are highly coexpressed (219/223 cells, $n = 5$ donors; Fig. 1G and fig. S1A). For other cytokine receptor subunits, we found that IL31ra, similar to OSMR, is selectively expressed in Nppb neurons (Fig. 1H). Although Nppb neurons express Il13ra1 and Il4ra, both receptors are found in many other cell types, and their expressions are much lower than that of OSMR or IL31ra (Fig. 1, I and J). Together, these results show that Nppb neurons, in both mouse and human DRG, express receptor complexes for transducing multiple cytokine signals including those for OSM.

Expression of OSM is prominently increased in skin disorders associated with chronic itch

Elevated expression of IL-31, IL-4, and IL-13 has been proposed to be causative for some types of itch, but it is unclear whether these are the only cytokines involved in modulating pruritic responses (*23*, *24*, *43*). To better define cytokine expression in diseased skin associated with inflammatory itch and to use physiologically relevant results for comparison, we examined several RNA-seq datasets from human subjects with different pruritic skin diseases. We analyzed RNA-seq data from skin biopsies of patients with psoriasis

Table 1. Transcript abundance of cytokine receptors in Nppb neurons. The transcript abundance [transcripts per million (TPM) ± SEM] of cytokine receptors in Nppb neurons (averaged from four samples). * indicates gene expression levels are statistically larger than zero ($P < 0.0001$).

Interleukin receptors			gp130 (IL6ST) co-receptors						
Il31ra*	**Il13ra1**	**gp130***	**Osmr***	**Il4ra**	**Il6ra**	**Cntfr**	**Il11ra1**	**Il11ra2**	**Il27ra**
213 ± 29	5.1 ± 1.4	295 ± 48	306 ± 65	3.9 ± 1.4	0.52 ± 0.28	31 ± 9.3	9.3 ± 5.7	0	0.14 ± 0.06

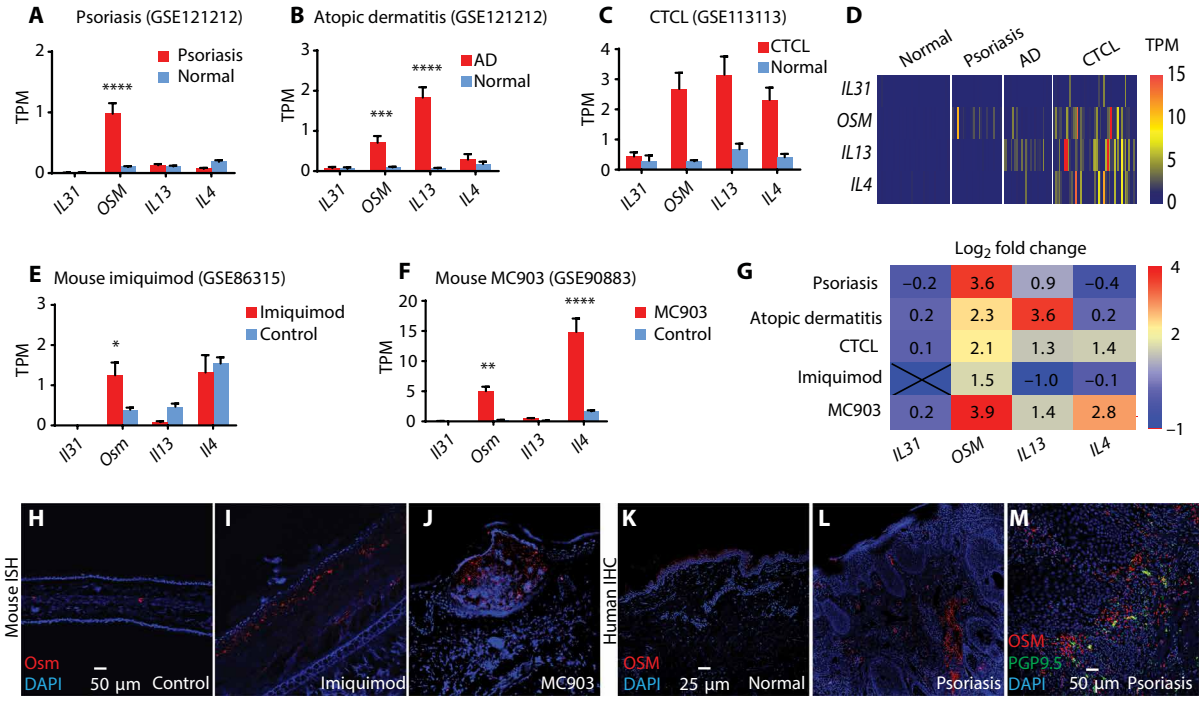


Fig. 2. OSM is up-regulated in various inflammatory skin diseases. (**A** to **D**) Quantification of transcript abundance (TPM) of IL-31, OSM, IL-13, and IL-4 in healthy, psoriasis (A), atopic dermatitis (AD) (B), and CTCL (C) skin. Accession numbers of data are indicated. (A) Twenty-eight patients and 38 healthy controls (for OSM, ****$P < 0.0001$). (B) Twenty-seven patients and 38 healthy controls (for OSM, ***$P = 0.0002$ and for IL-13, ****$P < 0.0001$). (C) Forty-nine patients and three healthy controls. (D) The heatmaps display the estimated transcript abundances (TPM) of IL-31, OSM, IL-13, and IL-4 (rows) in each patient (columns) from these datasets. (**E** and **F**) Quantification of transcript abundance in the imiquimod-induced psoriasiform dermatitis mouse model (E) and the MC903-induced atopic dermatitis–like mouse model (F). (E) Four imiquimod-treated and four control mice (**$P = 0.023$). (F) $n = 4$ MC903-treated and $n = 4$ control mice (for OSM, **$P = 0.0016$ and for IL-4, ****$P < 0.0001$). Data presented in (A) to (C), (E), and (F) are means ± SEM (two-way ANOVA with Sidak post hoc test). (**G**) Using DESeq2, the differential gene expression of IL-31, OSM, IL-13, and IL-4 was determined between lesioned and healthy skin samples. Fold changes are presented on a $\log_2$ scale. (**H** to **J**) Representative images of ISH of mouse skin hybridized with probes to OSM (red) counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue), (H) normal mouse ear, (I) 2 weeks after daily imiquimod treatment, and (J) 12 days after daily MC903 treatment. (**K** to **M**) Immunohistochemistry (IHC) of OSM (red) in formalin-fixed paraffin-embedded skin biopsies from a healthy donor (K) and from a patient with psoriasis (L). (M) Representative image of OSM immunoreactivity (red) and anti–PGP9.5-positive peripheral nerve fibers (green).

(GSE54456 and GSE121212) (*44*), atopic dermatitis (GSE121212) (*45*), and cutaneous T cell lymphoma (CTCL) (GSE113113) (*46*), all conditions that, in most cases, result in considerable pruritus (*43*, *47*, *48*). We found that amounts of OSM transcripts were significantly up-regulated in the skin from human patients with psoriasis, $P < 0.0001$, and atopic dermatitis, $P = 0.0002$, and were higher compared to controls in CTCL (Fig. 2, A to C, and Table 2). For the human samples, not all subjects had elevated OSM (Fig. 2D), suggesting that there is heterogeneity in these disease groups and there may be several factors that produce itch. In addition, we analyzed RNA-seq datasets from mouse models of atopic dermatitis, MC903 (GSE90883) (*27*), and an imiquimod-induced psoriasiform mouse model (GSE86315) (*49*). In the imiquimod mouse model, OSM was the only significantly up-regulated cytokine, $P = 0.023$, and in the MC903 mouse model, OSM expression was significantly increased together with IL-4 ($P = 0.0016$ and $P < 0.0001$, respectively) (see Fig. 2, E and F, and Table 2). Differential gene expression analyses also revealed that IL-6 is up-regulated in human psoriasis, atopic dermatitis, and imiquimod mouse model ($\log_2$ fold changes are 2.2, 2.1, and 1.4, respectively; Table 2); however, the expression of the IL-6 receptor, IL6Ra, is extremely low in Nppb neurons and is not significantly higher than zero ($P = 0.16$; Table 1). OSM is the cytokine that is commonly up-regulated in these inflammatory skin conditions (Fig. 2G). To validate our results, we performed ISH on

Table 2. Differential gene expression analyses of interleukins in skin diseases. Differential gene expression analyses of interleukins in inflammatory skin conditions (inflamed skin versus normal skin). The numbers indicate the expression of genes that are up- or down-regulated for inflamed skin samples versus normal skin (log_2 scale). N.d., not detected. The analyses were performed with R package DESeq2. LIF, Leukemia inhibitory factor; CNTF, Ciliary neurotrophic factor; CTF1, Cardiotrophin-1.

Disease	Interleukins			Interleukins for gp130 (IL6ST) complex receptors						
	IL-31	IL-4	IL-13	OSM	IL-6	LIF	CNTF	CTF1	IL-11	IL-27
Psoriasis	−0.1	−0.9	0.4	3.5	2.2	−1.6	−0.9	−0.6	0.6	0.4
AD	0.2	0.2	3.6	2.3	2.1	−1.7	0.3	0.04	0.4	−0.4
CTCL	0.09	1.4	1.3	2.1	−2.2	−0.8	0.4	0.4	0.5	1.3
Imiquimod	N.d.	0.05	−1.4	1.8	1.4	0.8	−0.3	−0.5	0.4	−0.06

skin from imiquimod- or MC903-treated mice. Corroborating our analysis of sequencing results, we found a marked increase in OSM expression in lesioned skin from both models of dermatitis (Fig. 2, I and J). We also examined OSM expression in skin biopsy samples from patients with psoriasis and found high OSM immunoreactivity in four of nine specimens we examined (Fig. 2L). Furthermore, the OSM-positive staining in these samples often accumulated around peripheral nerve fibers, consistent with potential neuroimmune cross-talk (Fig. 2M).

OSM selectively sensitizes Nppb neurons

Our findings that OSM is elevated in skin samples of diseased skin associated with chronic itch and OSMR is expressed in itch sensory neurons suggest that OSM may contribute to skin pruritus. Therefore, we hypothesized that OSM might act as a classic pruritogen that directly activates sensory neurons. To test this hypothesis, we first examined in vitro whether OSM can cause an increase in intracellular calcium by imaging dissociated DRG neurons expressing the calcium reporter GCaMP6. We expected that if OSM activates Nppb neurons, then intracellular calcium would rise in a subset of the GCaMP6-expressing neurons. We imaged 236 capsaicin-responsive DRG neurons and observed 68 neurons that were activated by leukotriene C4 (LTC4; 100 nM), histamine (100 μM), and capsaicin (10 μM), cells we previously showed that express Nppb (*19*). However, neither these cells nor any of the other neurons labeled with GCaMP6 responded to OSM [1 μg/ml; ~100-fold above the half maximal effective concentration (EC50)] (Fig. 3, A and B). To further confirm this observation, we performed whole-cell electrophysiological recording on Sst-cre:: AAV9-CAG-Flex-tdTomato neurons (Nppb neurons) (*19*, *50*). Nppb neurons are small-diameter neurons with an averaged diameter of 14.2 ± 2.2 μm (Fig. 3C). Applying OSM did not evoke noticeable currents on these neurons (Fig. 3D). By contrast, histamine (100 μM) evoked sizable transient inward currents (Fig. 3, D and E). Therefore, OSM does not act like a classic pruritogen because it cannot directly depolarize itch neurons. Although OSM does not elicit a rapid depolarization of Nppb neurons, it could nonetheless stimulate other cellular signaling pathways, such as activation of kinases, which might modulate neuronal activity in other ways. Therefore, next, to examine whether OSM can modulate neuronal responses to pruritogens, we recorded histamine-evoked whole-cell currents before and after short-term exposure to OSM. Although antihistamines are not useful for the treatment of psoriasis, atopic dermatitis, and CTCL, the pruritogens that underlie these chronic itch conditions are not well characterized. Therefore, for these mechanistic experiments, we used histamine in lieu of the itch-inducing agents active in chronic itch. In naïve Nppb neurons, repetitive histamine challenge rapidly desensitizes histamine-evoked currents by 62.5 ± 4.6% (Fig. 3, F and H). Pretreatment with OSM (1 μg/ml) for 2 min increased histamine-induced currents by 31.8 ± 17.1% (Fig. 3, G and H). Because Nppb neurons also express receptor complexes for IL-31 and IL-13, we next tested whether these two cytokines have similar effects on the histamine-elicited responses. We found that pretreatment with IL-31, similar to OSM treatment, potentiated histamine-induced currents (Fig. 3H and fig. S2A). However, IL-13 pretreatment failed to produce potentiation of histamine responses (Fig. 3H and fig. S2B). The OSM-dependent potentiation of histamine-induced activation was selective to Nppb neurons because administration of OSM on Mrgpra3-cre-GFP neurons failed to potentiate histamine currents (fig. S2, C and D). Given that OSM treatment potentiates histamine responses, we wondered whether OSM might also enhance the baseline excitability of Nppb neurons. For these studies, to probe for changes in excitability, we examined neuronal activity before and after OSM treatment using a current injection protocol. Naïve Nppb neurons, under the conditions we used in our studies, are almost resistant to current injection-induced action potential firing, on average, firing only a single action potential with an injection of 300 pA (Fig. 3I). Short-term OSM treatments (1 to 2 min) did not change the detectable excitability of Nppb neurons (fig. S2, E to G). However, although short-term exposure did not alter the activity of Nppb neurons, we wondered whether longer exposure might. When we incubated neurons with OSM for longer times, most Nppb neurons displayed tonic firing in response to small injections of current (Fig. 3, J and K). Similar to OSM, IL-31 exerted the same effect on neuronal excitability, whereas treatment with IL-13 had no detectable effects (Fig. 3K and fig. S2, H and I).

OSM is secreted by various dermal immune cells

OSM is a pleiotropic cytokine that is expressed in activated T cells, macrophages, dendritic cells, and neutrophils (*51*–*54*). To identify the cellular source of OSM in chronic skin pruritus, we examined published single-cell RNA-seq data of whole-skin biopsies collected from patients with CTCL (GSE128531) (*55*). In healthy skin biopsies, we detected very few cells expressing OSM, and these cells co-expressed pan-leukocyte marker CD45 (Fig. 4, A to C). The fraction of OSM-expressing cells profoundly increased in CTCL skin biopsies (from 0.37 ± 0.07% to 6.08 ± 2.6%, $P = 0.016$) (Fig. 4, D to F). Because OSM is mainly expressed by CD45 cells, we further characterized these cells and found that most T cells ($CD4^+$ or $CD3^+$) and monocytes ($CD14^+$) coexpressed OSM (Fig. 4, G to I). A small

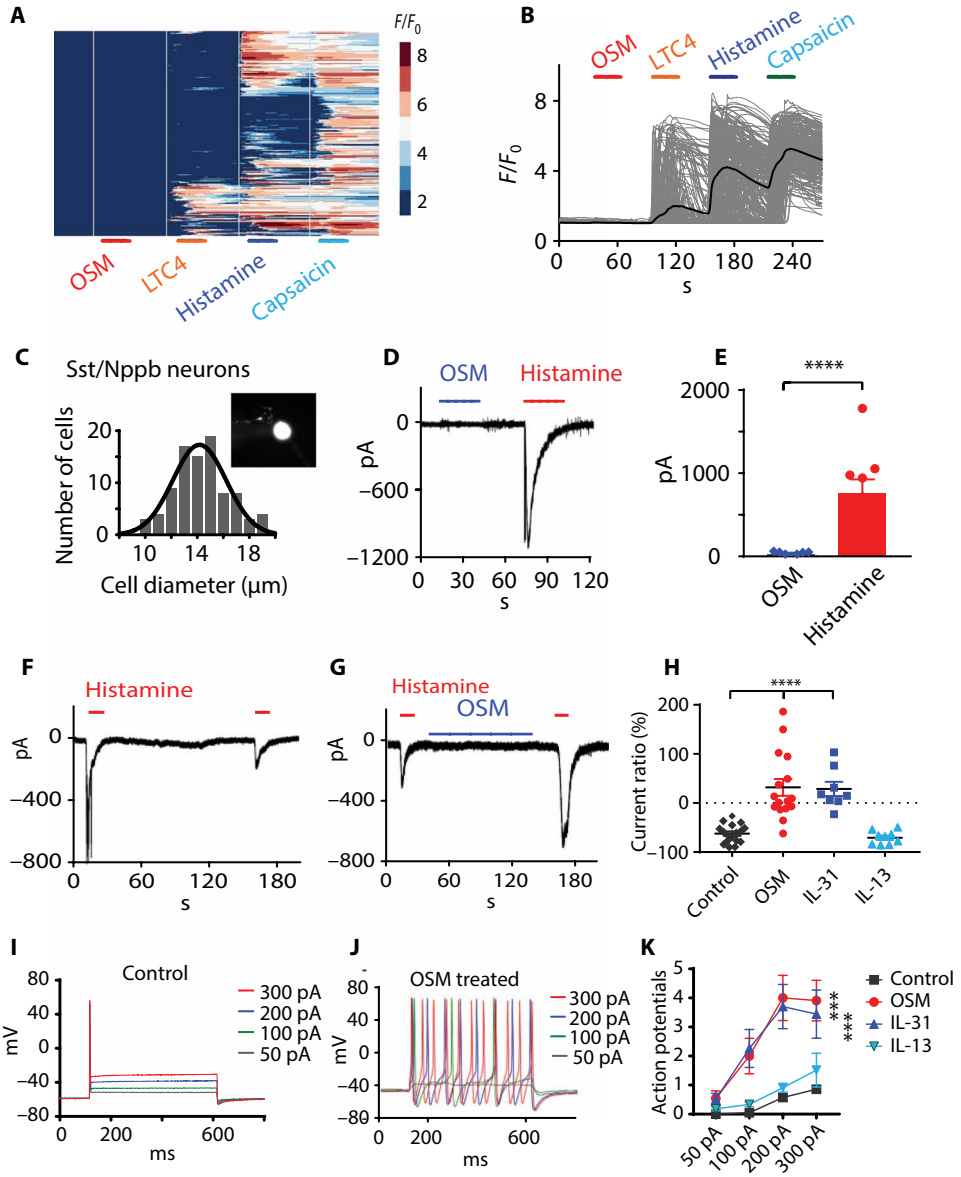


Fig. 3. OSM sensitizes Nppb neurons. (**A** and **B**) Calcium imaging of dissociated Trpv1-GCaMP6 DRG neurons challenged (30 s; indicated with bars) with OSM (1 μg/ml), LTC4 (100 nM), histamine (10 μM), and capsaicin (10 μM) (326 neurons, $n = 3$ mice). The colored scale bar represents normalized changes of fluorescence signals. The solid line in (B) indicates the averaged response of all the capsaicin-responsive neurons. (**C**) Quantification of cell diameter of Nppb neurons (inset) ($n = 90$). (**D** and **E**) Whole-cell voltage-clamp recordings (held at −60 mV) of dissociated Nppb-tdTomato neurons exposed to OSM alone (blue line; 1 μg/ml) and histamine (red line; 100 μM). (E) $n = 9$ neurons from two mice, $^{****}P < 0.0001$, two-tailed Student's t test (F and G). (**F** to **H**) Electrophysiological traces of Nppb neuron responses. (F) Responses to histamine challenge without OSM pretreatment and (G) after pretreatment with OSM (1 μg/ml) for 2 min. (H) Summary of the cytokine-mediated effects on histamine-evoked currents. Repetitive histamine ($n = 17$), pretreatment with OSM ($n = 16$), or IL-31 ($n = 8$) (for OSM, $^{****}P < 0.0001$ and for IL-31, $P < 0.0001$), one-way ANOVA with Dunnett's post hoc test. Pretreatment with IL-13 ($n = 9$) ($P = 0.95$). (**I** to **K**) Electrophysiological traces of Nppb neurons under current clamp conditions. (I) Control conditions and (J) after incubation with OSM (100 ng/ml) for 2 to 3 days. (K) Quantification of effects of cytokine. Prolonged incubation with OSM ($n = 22$) or IL-31 ($n = 27$), IL-13 ($n = 33$), and controls ($n = 21$); $^{***}P = 0.0003$, $^{***}P = 0.0003$, and $P = 0.7950$, respectively, two-way ANOVA with Dunnett's post hoc test. Data are presented as means ± SEM.

fraction of OSM-expressing immune cells express CD1C, a marker for dermal dendritic cells, and a minor number of OSM-expressing cells are marked by tryptase, a marker of mast cells (Fig. 4, J and K). When comparing OSM expression with other cytokines including IL-31, IL-13, and IL-4, we found that OSM-expressing cells outnumbered the cells expressing other cytokines ($P = 0.016$, 0.04, and 0.02, respectively), suggesting a predominant role of OSM in skin inflammation (Fig. 4, L to N). To further support the involvement of OSM in inflammatory itch, we analyzed single-cell sequencing data from psoriatic skin biopsies (EGAS00001002927) (*56*). In agreement with our analysis of CTCL data, OSM was coexpressed with CD4, CD3, CD14, and CD45 cells (Fig. 4, O to Q). To validate these sequencing data, we performed immunohistochemistry on psoriatic skin biopsies. We found that, in healthy human skin biopsies, the infiltration of $CD4^+$- and OSM^+-expressing cells was very low (Fig. 4R). However, in three of five psoriatic skin biopsies, we observed massive $CD4^+$ cell infiltration and strong OSM immunoreactivity in skin samples from patients with psoriasis (Fig. 4S). Together, these analyses uncovered a pivotal role of OSM in skin inflammation. Furthermore, OSM could be the major cytokine in neuroimmune interactions between sensory neurons and multiple dermal immune cells.

OSM can induce and exaggerate itch

Our physiological studies suggest that OSM can sensitize itch-selective sensory neurons by increasing neuronal activity and excitability. To examine the functional consequences of this neuromodulation in vivo, we assayed the effects of OSM on behavioral responses in mice. For these studies, we injected recombinant mouse OSM (1 μg/μl, 10 μl) alone or together with histamine intradermally into the nape of the neck and recorded scratching responses. As expected, from our calcium imaging and electrophysiological experiments, OSM evoked only minimal itch responses shortly after administration (Fig. 5A), which was not statistically different from saline injection

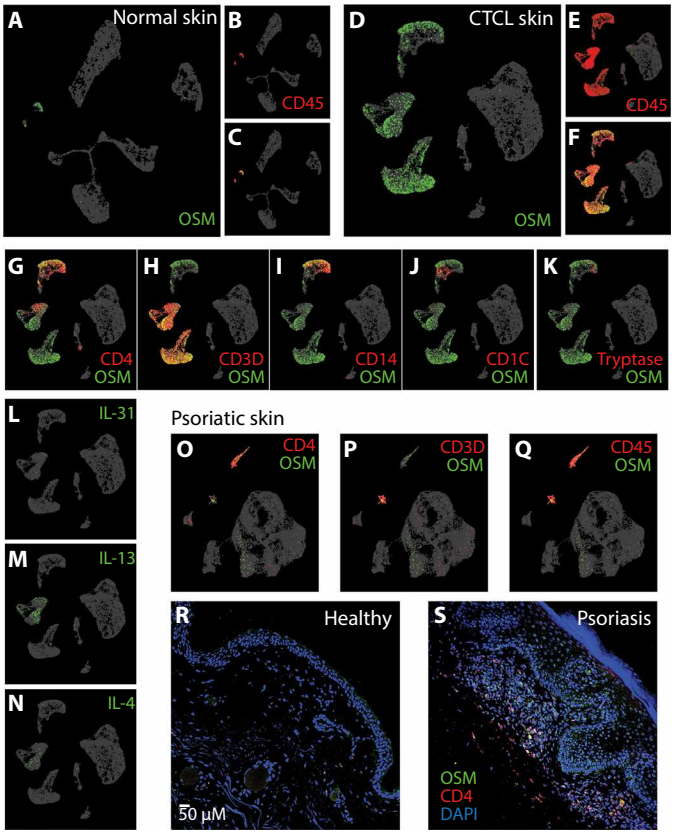


Fig. 4. OSM is predominantly expressed in skin T cells. (**A** to **C**) Uniform manifold approximation and projection (UMAP) plot of single-cell RNA-seq data (GSE128531) pooled from four normal skin biopsies (14,179 cells) showing expression pattern of OSM (green) and pan-leukocyte marker CD45 (PTPRC) (red). (**D** to **F**) UMAP plot of GSE128531 dataset, which included five CTCL skin biopsies (30,663 cells) showing expression of OSM (green) and CD45 (red). (**G** to **K**) Coexpression of OSM (green) with markers for immune cell genes $CD4^+$ and $CD3^+$ (T cells) (G and H), $CD14^+$ (monocytes) (I), CD1C (dendritic cells) (J), and tryptase (mast cell) (K). (**L** to **N**) Expression of IL-31, IL-13, and IL-4 as indicated. (**O** to **S**) UMAP plots of single-cell sequencing data (EGAS00001002927) of psoriatic skin biopsies (21,025 cells pooled from three patients). Coexpression of OSM with CD4 (O), CD3 (P), and CD45 (Q). (R and S) Representative images of parafilm-embedded human skin sections immunostained against OSM (green) and CD4 (red) in healthy controls (R) and in psoriatic skin samples (S).

(in the first 30 min after administration). In contrast to its lack of effect on its own, OSM profoundly potentiated histamine- and leukotriene-evoked scratching bouts (Fig. 5, B and C). Although OSM alone did not evoke increases in scratching (over saline) in the first 30 min after injection, when we extended our observation time and recorded scratch responses up to 90 min after treatment, we found that mice slowly developed intense itch behavior about 30 to 40 min after OSM injection (Fig. 5D). During the 30 to 60 second period, OSM challenge significantly increased scratching bouts compared to saline-injected controls (51.6 ± 15.5, $P = 0.033$) (Fig. 5E). It is known that OSMR, similar to Il31Ra (*35*), is expressed by many stromal cells such as keratinocytes, and analysis of single-cell sequencing of skin samples showed that OSMR is expressed by keratinocytes, fibroblasts, and endothelial cells (fig. S3E), and potentially, activation of these cells could be responsible for the late-onset scratching induced by OSM administration. Therefore, OSM might be acting on cells in the skin and sensory neurons. To investigate whether OSM potentiates scratching and whether OSM-evoked slow-onset scratching responses were caused by peripheral sensitization of sensory neurons, we generated DRG-specific Osmr conditional knockout mice (Osmr cKO). These cKO mice were made by crossing $Osmr^{fl/fl}$ mice with Trpv1-Cre animals to permit all Osmr expression to be eliminated including Nppb-negative, Trpv1-positive neurons (fig. S1). Congruent with OSM causing itch by sensitization of sensory neurons, the loss of Osmr in DRG neurons significantly reduced the scratching bouts evoked by coadministration of OSM and histamine (34.3 ± 5.6 scratching bouts in cKO mice versus 60.6 ± 6.5 bouts in littermate controls, $P = 0.007$) (Fig. 5F). OSM-evoked delayed scratching responses in Osmr cKO mice were also significantly reduced (27.8 ± 8.9 scratching bouts in cKO mice versus 55.7 ± 10 bouts in control littermates, $P = 0.049$) (Fig. 5G). Analysis of the phenotype of knockout mice in the imiquimod model of chronic itch also showed that Osmr in sensory neurons is important for the development of increased scratching and skin hypertrophy (Fig. 5, H to K). Together, these results suggest that OSM can induce and exaggerate itch by sensitizing sensory neurons.

Pharmacological inhibition of OSM signaling reduces inflammatory itch

Findings from our bioinformatic analyses and physiological and behavioral studies suggest that OSM evokes inflammatory skin pruritus through multiple pathways. We next tested whether systemic disruption of the OSM signaling cascade alleviates itch behavior in a model of chronic skin inflammation. Because a selective OSMR inhibitor is not commercially available, we used a gp130 inhibitor, SC144 (*57*), to inhibit the OSMR/gp130 receptor complex. Initially, we tested whether SC144 would inhibit potentiation of histamine responses in vitro. We performed whole-cell recordings on Nppb neurons and examined the effects of SC144 (10 µM) treatment upon OSM-mediated sensitization. Inhibition of OSM signaling effectively prevented sensitization of Nppb neurons (Fig. 6, A and B). At the behavioral level, SC-144 treatment (10 mg/kg) almost completely inhibited OSM-evoked delayed scratching responses (Fig. 6C). Showing the selectivity of this reagent, histamine-evoked immediate scratching was not affected by SC-144 administration (Fig. 6D). Although SC-144 could inhibit the activation of gp130 receptor complexes other than gp130-OSMR, other potential co-receptors for gp130 are not expressed at amounts expected to be functional in Nppb neurons (Table 1). In addition, apart from IL-6, the expression of cytokines that can activate other gp130 receptor complexes is not up-regulated in the imiquimod model of itch and in patients' samples of skin diseases associated with chronic itch (Table 2). These results indicate that, at a behavioral level, disrupting OSMR/gp130 receptor signaling can inhibit OSM-evoked itch through Nppb neurons. To test whether this strategy is also effective for inflammatory skin pruritus, we used a mouse model of psoriasis (*58*, *59*) and tested whether SC-144 can reduce the severity of dermatitis and its associated itch phenotypes. In this model, mice received daily imiquimod treatment (TARO; 0.05 g per mouse per day) to induce psoriasiform dermatitis (Fig. 6E), and at day 7, these animals display intense scratching behavior directed to the site of imiquimod administration. We found that SC-144 treatment greatly reduced spontaneous scratching behavior (Fig. 6, F and G). In addition, this treatment also mitigated skin inflammation, with reduced ear

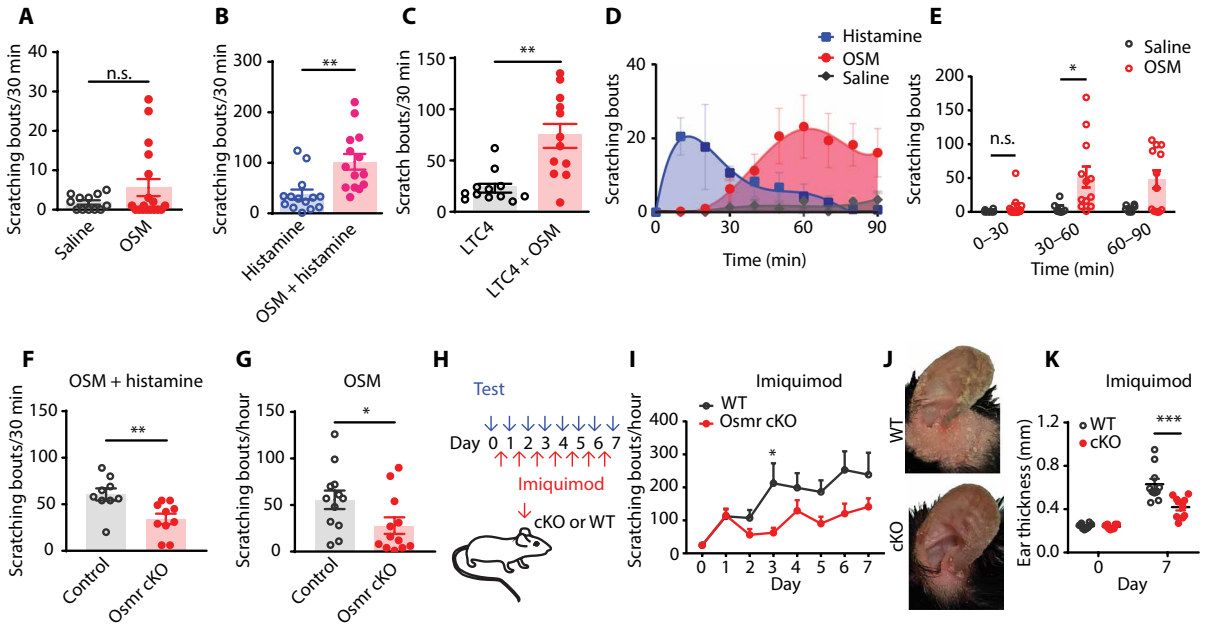


Fig. 5. OSM can induce and exaggerate itch. (**A**) Scratching bouts elicited by intradermal injection of recombinant mouse OSM (1 μg/μl, 10 μl; $n = 18$) in the first 30 min after injection, compared to saline injection [$n = 12$; n.s. (not significant), $P = 0.1$], (**B**) by injection of histamine alone (10 μg; $n = 18$) and by co-injection of histamine with OSM (10 μg; $n = 14$), $^{**}P = 0.002$, and (**C**) by LTC4 alone ($n = 12$) and co-injection of LTC4 with OSM ($n = 12$), $^{**}P = 0.0005$. (A to C) Data are presented as means ± SEM, two-tailed Student's *t* test. (**D**) Examination of time course of OSM ($n = 12$)– and histamine ($n = 8$)–induced scratching responses and (**E**) quantification of scratching bouts, $^{*}P = 0.033$, ANOVA Sidak's multiple comparison test. (**F** and **G**) Examination of the effects of sensory neuron–specific knockout of Osmr on OSM-induced itch behavior. (F) Coadministration of OSM and histamine (10 μg in 10 μl) into cKO mice ($n = 10$) during 30-min trials using automated recording of scratch bouts compared to littermate controls ($n = 9$), $^{**}P = 0.007$. (G) OSM injection in cKO mice ($n = 12$) compared to control littermates ($n = 12$), $^{*}P = 0.049$. (F and G) Data are presented as means ± SEM, two-tailed Student's *t* test. (**H** to **K**) Examination of the effect of sensory neuron–specific elimination of Osmr expression in the imiquimod model of chronic itch. (H) Schematic depicting the time course for the development of imiquimod-induced chronic itch. Imiquimod was topically applied to mouse ears for seven consecutive days. Scratching bouts were measured daily before imiquimod treatment, and the severity of skin inflammation was assessed by measurements of ear thickness. (I) Osmr Trpv1-cre cKO mice ($n = 10$) compared to wild-type control mice ($n = 10$) at day 3, $^{*}P = 0.023$ (ANOVA Sidak's multiple comparison test). (J) Representative images of ears of control and Osmr cKO mice treated with imiquimod at day 7. (K) Quantification at day 7 of ear thickness in cKO mice ($n = 10$) compared to control littermates ($n = 10$) (ANOVA Sidak's multiple comparison test, $^{***}P = 0.0001$); data are presented as means ± SEM. WT, wild type.

thickness in SC-144–treated mice (Fig. 6H). Together, these results are consistent with OSM being a mediator promoting persistent itch during chronic skin inflammation (fig. S4).

DISCUSSION

OSM is a pleiotropic cytokine that belongs to the IL-6 family of cytokines (*31*, *60*). Its receptor, OSMR, forms heterodimers with either IL31RA or gp130 (IL6ST) to transduce IL-31 or OSM, respectively (*30*, *31*, *61*). It is well known that OSM contributes to many physiological processes, including hematopoiesis, bone remodeling, and liver development. However, OSM can also cause many deleterious conditions including T helper 1 (T_H1)– and T_H2-mediated skin and lung inflammation, arthritis, colitis, and some cancers (*30*, *32*, *61*). Although the role of OSM or OSMR in itch was not explored, it was reported that missense mutations in OSMR in familiar primary localized cutaneous amyloidosis are associated with severe pruritus (*62*–*64*), suggesting that OSM/OSMR axis may play roles in skin pruritus, which may include perturbations of sensory neuron function and/or an imbalance of cytokine receptor signaling in keratinocytes. Here, we showed that OSMR is preferentially expressed by itch-selective sensory neurons. OSM expression amounts are elevated in various skin diseases linked with itch. These data strongly suggested that OSM can directly modulate itch-selective sensory neurons during chronic skin inflammation. Our functional studies showed that the mode of action of OSM is different from classic pruritogens and cytokines IL-4, IL-13, or IL31 (*26*, *27*, *33*); OSM acts to augment pruritogen-induced activity. First, OSM causes potentiation of neural responses to pruritogens. The latter studies were performed with histamine as a pruritogen because the active pruritogens in these types of itch are not well characterized. Therefore, although we used histamine in our mechanistic studies, we cannot exclude that other pruritogens might produce different effects on responses in itch neurons. Second, OSM has the longer-term effect of converting action potential firing of sensory neurons from phasic to tonic. The process of cytokine-mediated sensitization of sensory neurons caused a relatively fast increase in activity and a much slower modification of neural activity. The latter effect might be due to changes in gene expression (*65*). The rapid effect of OSM was similar to that reported to be elicited by inflammatory mediators to heat sensitization (*66*, *67*). The long-term modulation of OSM on neuronal excitability also has precedence in that IL-1 was reported to enhance excitability causing allodynia (*68*, *69*). Our observation that OSM also evoked late-onset scratching responses parallels results

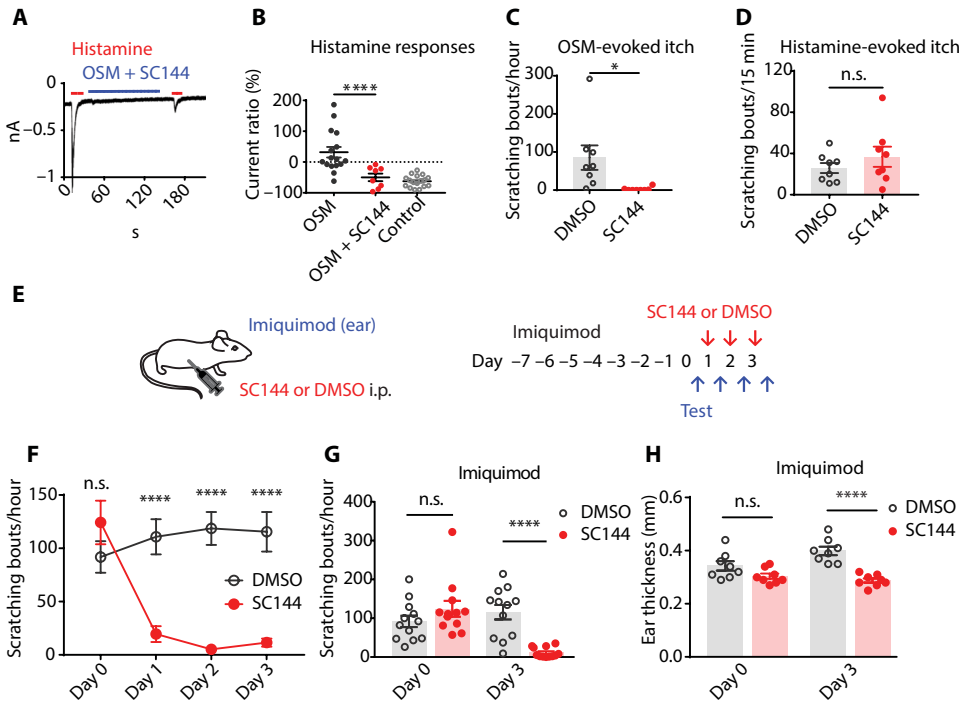


Fig. 6. Disruption of OSM signaling mitigates cutaneous inflammatory scratching. (**A** and **B**) Representative traces of voltage-clamp recording of Nppb neurons. (A) Administration of histamine (red line) or histamine after treatment with SC144 (10 μM) and OSM (1 μg/ml) (blue line). (B) Quantification of repetitive histamine-evoked currents after OSM treatment ($n = 16$ neurons) compared with OSM + SC-144 treatment ($n = 8$), ****$P = 0.0008$. (**C**) Effects of SC144 on OSM-induced delayed scratching. Thirty to 60 min after treatment, mice administered SC144 ($n = 8$) were compared with control mice ($n = 8$) injected with vehicle [dimethyl sulfoxide (DMSO)], *$P = 0.02$; data are presented as means ± SEM, two-tailed Student's t test. (**D**) Effects of SC144 on histamine scratching. During the first 15 min after histamine injection, scratching bouts in the presence of SC144 ($n = 8$) were compared with control DMSO ($n = 8$); n.s., $P = 0.3$, two-tailed Student's t test; data are presented as means ± SEM. (**E**) Experimental design of tests on the efficacy of SC144 for itch in the imiquimod-induced psoriasiform dermatitis model. Mouse ears were topically treated with imiquimod for seven consecutive days to establish dermatitis. Immediately before SC144 or vehicle treatment, the numbers of scratching bouts were measured (day 0). At days 1, 2, and 3, SC144 or vehicle was intraperitoneally injected into mice 30 min before behavioral tests. (**F** to **H**) Examination of the effects of SC144 on imiquimod-induced itch and skin inflammation. (F) At day 0, before treatment, scratching bouts in SC144 were compared to DMSO treatment group; n.s., $P = 0.36$. In the DMSO group, scratching bouts were compared with SC144 treatment, at days 1, 2, and 3, ****$P < 0.001$ (ANOVA Sidak's multiple comparison test). (G) Quantification of scratching bouts for control DMSO group ($n = 12$) compared to the SC144-treated group ($n = 12$) at day 0 (n.s., $P = 0.15$) and at day 3 (****$P < 0.0001$) (ANOVA Sidak's multiple comparison test). (H) Quantification of ear thickness in the SC-144 treatment group compared to the control DMSO group at day 3, ****$P < 0.0001$ (ANOVA Sidak's multiple comparison test); data are presented as means ± SEM.

from human subjects where it was reported that cutaneous challenge with IL-31 induces late-onset itch (*70*).

Our bulk and single-cell sequencing analyses revealed the overwhelming expression of OSM compared to other well-known cytokines, including IL-31, IL-13, or IL-4. Therefore, it is tempting to speculate that OSM might play a major role on neuronal activity in many cutaneous diseases where barrier disruption leads to inflammation (*2*, *4*) and chronic itch. However, there was also heterogeneity in increased cytokine expression with elevated expression of OSM, IL-31, IL-13, and IL-4 in different samples. Because the quantification of these cytokines was made at fixed times, the differences in expression of cytokines may reflect difference in stages of disease, or they could reflect differences in types of inflammation. In addition, we have no information on the itch phenotype of these subjects.

Single-cell sequencing data also revealed that OSM is expressed by many dermal immune cells including T cells, monocytes, dendritic cells, and mast cells. This suggests that OSM might be a universal cytokine for neuroimmune communications between sensory neurons and dermal immune cells, which might explain its prevalence in many types of pruritus. In a mouse model of psoriasis chronic itch, we showed that either the elimination of OSMR from sensory neurons or the interference of OSMR signaling (with SC-144) reduced scratching. Although these results are predictive of OSMR signaling in psoriasis, future studies will be needed to determine whether it is similarly effective for other types of inflammatory itch. We also note that OSMR is expressed in keratinocytes and that the expression of IL-6 is increased and at least some of the effects of SC-144 treatment may be on nonsensory neurons and non-Nppb neurons (which express IL6Ra). This broader effect may account for the larger reduction of scratch elicited by SC-144 compared to elimination of OSMR from sensory neurons and may also be a benefit for the treatment of inflammatory skin conditions that may have a component of pain. In the future, it will be important to use Osmr-specific antagonist to determine whether the effects of SC-144 are selective for Osmr or occur via inhibition of other gp130 receptor–mediated processes. In addition, further preclinical and clinical studies are required to determine the effectiveness of OSMR antagonism and to examine whether adverse effects and toxicity are associated with these agents. Regardless of the exact mechanisms, SC-144 shows potential for treatment of inflammatory skin pruritus.

Chronic itch is a common symptom and a major complaint in dermatology clinics (*71*, *72*). From clinical perspectives, itch is classified into several categories including dermatologic, systemic, neurologic, psychogenic, mixed, and others (*73*). Recently, it has been suggested that neurologic itch can be further divided into neuropathic itch and neurogenic itch (*3*, *5*, *72*, *74*). Neuropathic itch, a term borrowed from neuropathic pain (*75*, *76*), conceptually involves induction of neuronal hyperexcitability. However, the molecular features of neuropathic itch are not well defined. Pruritogens, such as histamine, serotonin, chloroquine, and leukotriene, activate G protein–coupled receptors that subsequently depolarize sensory neurons evoking relatively short-term onset scratching responses (*19*, *77*). Our electrophysiological recordings showed that histamine quickly desensitized Nppb neurons, and therefore, this type of

pruritogen probably does not, on their own, cause long-term hyperexcitability. Here, we found that OSM modulates sensory neurons and evokes itch by slowly increasing neuronal sensitivity and excitability. Therefore, this cytokine-mediated neuronal modulation seems to fit the mechanistic definition of neuropathic itch suggested by others (*3*, *5*, *72*, *74*). Furthermore, because OSMR is a common subunit for transducing OSM and IL-31 signaling, antagonizing OSMR may be a promising strategy to control chronic inflammatory itch for conditions where expression of cytokines is elevated.

MATERIALS AND METHODS

Study design

The primary research objective was to determine the cytokines involved in the production of inflammatory pruritus and whether itch could be reduced by antagonism of OSMR. All other hypotheses were related to these objectives. The research subjects and units of investigation were primary-cultured DRG cells, DRG tissue from mice and human donors, and mice in controlled laboratory experiments. Sample sizes for in vitro and cell-based electrophysiological assays were those used by other laboratories in the field. For animal experiments, sample sizes were based on experience and were of a size generally used in the itch field. Data were not excluded in any studies.

Statistical analysis

Prism 7.0 (GraphPad Software) was used for statistical analyses. Differences between mean values were analyzed using unpaired two-tailed Student's *t* test or analysis of variance (ANOVA) for multiple comparisons as indicated in the figure legends. Differences were considered significant for $*P < 0.05$. Exact *P* values, definition, and number of replicates as well as definitions of center and dispersion are given in the respective figure legend. No statistical method was used to predetermine sample sizes.

SUPPLEMENTARY MATERIALS

www.science.org/doi/10.1126/scitranslmed.abe3037
Materials and Methods
Figs. S1 to S4
Data file S1
References (*78*–*90*)

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. G. Yosipovitch, M. W. Greaves, M. Schmelz, Itch. *Lancet* **361**, 690–694 (2003).
2. G. Yosipovitch, L. Misery, E. Proksch, M. Metz, S. Ständer, M. Schmelz, Skin barrier damage and itch: Review of mechanisms, topical management and future directions. *Acta Derm. Venereol.* **99**, 1201–1209 (2019).
3. L. Misery, E. Brenaut, R. le Garrec, C. Abasq, S. Genestet, P. Marcorelles, F. Zagnoli, Neuropathic pruritus. *Nat. Rev. Neurol.* **10**, 408–416 (2014).
4. E. A. Lerner, Pathophysiology of itch. *Dermatol. Clin.* **36**, 175–177 (2018).
5. J. Hachisuka, M. C. Chiang, S. E. Ross, Itch and neuropathic itch. *Pain* **159**, 603–609 (2018).
6. A. Ikoma, M. Steinhoff, S. Stander, G. Yosipovitch, M. Schmelz, The neurobiology of itch. *Nat. Rev. Neurosci.* **7**, 535–547 (2006).
7. S. P. Kini, L. DeLong, E. Veledar, A. McKenzie-Brown, M. Schaufele, S. C. Chen, The impact of pruritus on quality of life: The skin equivalent of pain. *Arch. Dermatol.* **147**, 1153–1156 (2011).
8. R. J. Hay, N. E. Johns, H. C. Williams, I. W. Bolliger, R. P. Dellavalle, D. J. Margolis, R. Marks, L. Naldi, M. A. Weinstock, S. K. Wulf, C. Michaud, C. J. L. Murray, M. Naghavi, The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions. *J. Invest. Dermatol.* **134**, 1527–1534 (2014).
9. B. Leader, C. W. Carr, S. C. Chen, Pruritus epidemiology and quality of life. *Handb. Exp. Pharmacol.* **226**, 15–38 (2015).
10. D. M. Bautista, S. R. Wilson, M. A. Hoon, Why we scratch an itch: The molecules, cells and circuits of itch. *Nat. Neurosci.* **17**, 175–182 (2014).
11. M. A. Hoon, Molecular dissection of itch. *Curr. Opin. Neurobiol.* **34**, 61–66 (2015).
12. B. McNeil, X. Dong, Peripheral mechanisms of itch. *Neurosci. Bull.* **28**, 100–110 (2012).
13. L. Han, C. Ma, Q. Liu, H. J. Weng, Y. Cui, Z. Tang, Y. Kim, H. Nie, L. Qu, K. N. Patel, Z. Li, B. McNeil, S. He, Y. Guan, B. Xiao, R. H. LaMotte, X. Dong, A subpopulation of nociceptors specifically linked to itch. *Nat. Neurosci.* **16**, 174–182 (2013).
14. S. K. Mishra, M. A. Hoon, The cells and circuitry for itch responses in mice. *Science* **340**, 968–971 (2013).
15. G. Gatto, K. M. Smith, S. E. Ross, M. Goulding, Neuronal diversity in the somatosensory system: Bridging the gap between cell type and function. *Curr. Opin. Neurobiol.* **56**, 167–174 (2019).
16. L. Qu, N. Fan, C. Ma, T. Wang, L. Han, K. Fu, Y. Wang, S. G. Shimada, X. Dong, R. H. LaMotte, Enhanced excitability of MRGPRA3- and MRGPRD-positive nociceptors in a model of inflammatory itch and pain. *Brain* **137**, 1039–1050 (2014).
17. J. Meixiong, M. Anderson, N. Limjunyawong, M. F. Sabbagh, E. Hu, M. R. Mack, L. K. Oetjen, F. Wang, B. S. Kim, X. Dong, Activation of mast-cell-expressed Mas-related G-protein-coupled receptors drives non-histaminergic itch. *Immunity* **50**, 1163–1171.e5 (2019).
18. R. Z. Hill, T. Morita, R. B. Brem, D. M. Bautista, S1PR3 mediates itch and pain via distinct TRP channel-dependent pathways. *J. Neurosci.* **38**, 7833–7843 (2018).
19. H. J. Solinski, M. C. Kriegbaum, P.-Y. Tseng, T. W. Earnest, X. Gu, A. Barik, A. T. Chesler, M. A. Hoon, Nppb neurons are sensors of mast cell-induced itch. *Cell Rep.* **26**, 3561–3573. e4 (2019).
20. T. Akiyama, E. A. Lerner, E. Carstens, Protease-activated receptors and itch. *Handb. Exp. Pharmacol.* **226**, 219–235 (2015).
21. V. B. Reddy, A. O. Iuga, S. G. Shimada, R. H. LaMotte, E. A. Lerner, Cowhage-evoked itch is mediated by a novel cysteine protease: A ligand of protease-activated receptors. *J. Neurosci.* **28**, 4331–4335 (2008).
22. V. B. Reddy, S. Sun, E. Azimi, S. B. Elmariah, X. Dong, E. A. Lerner, Redefining the concept of protease-activated receptors: Cathepsin S evokes itch via activation of Mrgprs. *Nat. Commun.* **6**, 7864 (2015).
23. E. R. Storan, S. M. O'Gorman, I. D. McDonald, M. Steinhoff, Role of cytokines and chemokines in itch. *Handb. Exp. Pharmacol.* **226**, 163–176 (2015).
24. A. M. Trier, B. S. Kim, Cytokine modulation of atopic itch. *Curr. Opin. Immunol.* **54**, 7–12 (2018).
25. M. Campion, L. Smith, S. Gatault, C. Métais, J. Buddenkotte, M. Steinhoff, Interleukin-4 and interleukin-13 evoke scratching behaviour in mice. *Exp. Dermatol.* **28**, 1501–1504 (2019).
26. F. Cevikbas, X. Wang, T. Akiyama, C. Kempkes, T. Savinko, A. Antal, G. Kukova, T. Buhl, A. Ikoma, J. Buddenkotte, V. Soumelis, M. Feld, H. Alenius, S. R. Dillon, E. Carstens, B. Homey, A. Basbaum, M. Steinhoff, A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J. Allergy Clin. Immunol.* **133**, 448–460.e7 (2014).
27. L. K. Oetjen, M. R. Mack, J. Feng, T. M. Whelan, H. Niu, C. J. Guo, S. Chen, A. M. Trier, A. Z. Xu, S. V. Tripathi, J. Luo, X. Gao, L. Yang, S. L. Hamilton, P. L. Wang, J. R. Brestoff, M. L. Council, R. Brasington, A. Schaffer, F. Brombacher, C.-S. Hsieh, R. W. Gereau IV, M. J. Miller, Z.-F. Chen, H. Hu, S. Davidson, Q. Liu, B. S. Kim, Sensory neurons co-opt classical immune signaling pathways to mediate chronic itch. *Cell* **171**, 217–228.e13 (2017).
28. M. R. Mack, B. S. Kim, The itch-scratch cycle: A neuroimmune perspective. *Trends Immunol.* **39**, 980–991 (2018).
29. F. Wang, A. M. Trier, F. Li, S. Kim, Z. Chen, J. N. Chai, M. R. Mack, S. A. Morrison, J. D. Hamilton, J. Baek, T.-L. B. Yang, A. M. Ver Heul, A. Z. Xu, Z. Xie, X. Dong, M. Kubo, H. Hu, C.-S. Hsieh, X. Dong, Q. Liu, D. J. Margolis, M. Ardeleanu, M. J. Miller, B. S. Kim, A basophil-neuronal axis promotes itch. *Cell* **184**, 422–440.e17 (2021).
30. C. D. Richards, The enigmatic cytokine oncostatin m and roles in disease. *ISRN Inflamm.* **2013**, 1–23 (2013).
31. H. M. Hermanns, Oncostatin M and interleukin-31: Cytokines, receptors, signal transduction and physiology. *Cytokine Growth Factor Rev.* **26**, 545–558 (2015).
32. N. R. West, B. M. J. Owens, A. N. Hegazy, The oncostatin M-stromal cell axis in health and disease. *Scand. J. Immunol.* **88**, e12694 (2018).
33. J. Meng, M. Moriyama, M. Feld, J. Buddenkotte, T. Buhl, A. Szöllösi, J. Zhang, P. Miller, A. Ghetti, M. Fischer, P. W. Reeh, C. Shan, J. Wang, M. Steinhoff, New mechanism underlying IL-31-induced atopic dermatitis. *J. Allergy Clin. Immunol.* **141**, 1677–1689.e8 (2018).
34. I. S. Bagci, T. Ruzicka, IL-31: A new key player in dermatology and beyond. *J. Allergy Clin. Immunol.* **141**, 858–866 (2018).
35. L. A. Nattkemper, M. E. Martinez-Escala, A. B. Gelman, E. M. Singer, A. H. Rook, J. Guitart, G. Yosipovitch, Cutaneous T-cell lymphoma and pruritus: The expression of IL-31 and its receptors in the skin. *Acta Derm. Venereol.* **96**, 894–898 (2016).
36. I. M. Chiu, L. B. Barrett, E. K. Williams, D. E. Strochlic, S. Lee, A. D. Weyer, S. Lou, G. S. Bryman, D. P. Roberson, N. Ghasemlou, C. Piccoli, E. Ahat, V. Wang, E. J. Cobos,

C. L. Stucky, Q. Ma, S. D. Liberles, C. J. Woolf, Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. *eLife* **3**, (2014).
37. D. Usoskin, A. Furlan, S. Islam, H. Abdo, P. Lönnerberg, D. Lou, J. Hjerling-Leffler, J. Haeggström, O. Kharchenko, P. V. Kharchenko, S. Linnarsson, P. Ernfors, Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat. Neurosci.* **18**, 145–153 (2015).
38. C. L. Li, K. C. Li, D. Wu, Y. Chen, H. Luo, J. R. Zhao, S. S. Wang, M. M. Sun, Y. J. Lu, Y. Q. Zhong, X. Y. Hu, R. Hou, B. B. Zhou, L. Bao, H. S. Xiao, X. Zhang, Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell Res.* **26**, 967 (2016).
39. M. Q. Nguyen, Y. Wu, L. S. Bonilla, L. J. von Buchholtz, N. J. P. Ryba, Diversity amongst trigeminal neurons revealed by high throughput single cell sequencing. *PLOS ONE* **12**, e0185543 (2017).
40. H. Ma, T. Gao, J. E. T. Jakobsson, H. M. Weman, B. Xu, D. Larhammar, M. C. Lagerström, The neuropeptide Y Y_2 receptor is coexpressed with Nppb in primary afferent neurons and Y_2 activation reduces histaminergic and IL-31-induced itch. *J. Pharmacol. Exp. Ther.* **372**, 73–82 (2020).
41. S. R. Dillon, C. Sprecher, A. Hammond, J. Bilsborough, M. Rosenfeld-Franklin, S. R. Presnell, H. S. Haugen, M. Maurer, B. Harder, J. Johnston, S. Bort, S. Mudri, J. L. Kuijper, T. Bukowski, P. Shea, D. L. Dong, M. Dasovich, F. J. Grant, L. Lockwood, S. D. Levin, C. LeCiel, K. Waggie, H. Day, S. Topouzis, J. Kramer, R. Kuestner, Z. Chen, D. Foster, J. Parrish-Novak, J. A. Gross, Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat. Immunol.* **5**, 752–760 (2004).
42. S. L. LaPorte, Z. S. Juo, J. Vaclavikova, L. A. Colf, X. Qi, N. M. Heller, A. D. Keegan, K. C. Garcia, Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259–272 (2008).
43. N. K. Mollanazar, P. K. Smith, G. Yosipovitch, Mediators of chronic pruritus in atopic dermatitis: Getting the itch out? *Clin Rev Allergy Immunol* **51**, 263–292 (2016).
44. B. Li, L. C. Tsoi, W. R. Swindell, J. E. Gudjonsson, T. Tejasvi, A. Johnston, J. Ding, P. E. Stuart, X. Xing, J. J. Kochkodan, J. J. Voorhees, H. M. Kang, R. P. Nair, G. R. Abecasis, J. T. Elder, Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms. *J. Invest. Dermatol.* **134**, 1828–1838 (2014).
45. L. C. Tsoi, E. Rodriguez, F. Degenhardt, H. Baurecht, U. Wehkamp, N. Volks, S. Szymczak, W. R. Swindell, M. K. Sarkar, K. Raja, S. Shao, M. Patrick, Y. Gao, R. Uppala, B. E. Perez White, S. Getsios, P. W. Harms, E. Maverakis, J. T. Elder, A. Franke, J. E. Gudjonsson, S. Weidinger, Atopic dermatitis is an IL-13-dominant disease with greater molecular heterogeneity compared to psoriasis. *J. Invest. Dermatol.* **139**, 1480–1489 (2019).
46. C. Querfeld, S. Leung, P. L. Myskowski, S. A. Curran, D. A. Goldman, G. Heller, X. Wu, S. H. Kil, S. Sharma, K. J. Finn, S. Horwitz, A. Moskowitz, B. Mehrara, S. T. Rosen, A. C. Halpern, J. W. Young, Primary T cells from cutaneous T-cell lymphoma skin explants display an exhausted immune checkpoint profile. *Cancer Immunol. Res.* **6**, 900–909 (2018).
47. K. Ahern, E. S. Gilmore, B. Poligone, Pruritus in cutaneous T-cell lymphoma: A review. *J. Am. Acad. Dermatol.* **67**, 760–768 (2012).
48. G. Yosipovitch, J. D. Bernhard, Clinical practice. Chronic pruritus. *N. Engl. J. Med.* **368**, 1625–1634 (2013).
49. W. R. Swindell, K. A. Michaels, A. J. Sutter, D. Diaconu, Y. Fritz, X. Xing, M. K. Sarkar, Y. Liang, A. Tsoi, J. E. Gudjonsson, N. L. Ward, Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis. *Genome Med.* **9**, 24 (2017).
50. J. Huang, E. Polgár, H. J. Solinski, S. K. Mishra, P. Y. Tseng, N. Iwagaki, K. A. Boyle, A. C. Dickie, M. C. Kriegbaum, H. Wildner, H. U. Zeilhofer, M. Watanabe, J. S. Riddell, A. J. Todd, M. A. Hoon, Circuit dissection of the role of somatostatin in itch and pain. *Nat. Neurosci.* **21**, 707–716 (2018).
51. P. Repovic, E. N. Benveniste, Prostaglandin E2 is a novel inducer of oncostatin-M expression in macrophages and microglia. *J. Neurosci.* **22**, 5334–5343 (2002).
52. A. Cross, S. W. Edwards, R. C. Bucknall, R. J. Moots, Secretion of oncostatin M by neutrophils in rheumatoid arthritis. *Arthritis Rheum.* **50**, 1430–1436 (2004).
53. M. Tanaka, A. Miyajima, Oncostatin M, a multifunctional cytokine. *Rev. Physiol. Biochem. Pharmacol.* **149**, 39–52 (2003).
54. K. Boniface, C. Diveu, F. Morel, N. Pedretti, J. Froger, E. Ravon, M. Garcia, E. Venereau, L. Preisser, E. Guignouard, G. Guillet, G. Dagregorio, J. Pène, J. P. Moles, H. Yssel, S. Chevalier, F. X. Bernard, H. Gascan, J. C. Lecron, Oncostatin M secreted by skin infiltrating T lymphocytes is a potent keratinocyte activator involved in skin inflammation. *J. Immunol.* **178**, 4615–4622 (2007).
55. A. M. Gaydosik, T. Tabib, L. J. Geskin, C. A. Bayan, J. F. Conway, R. Lafyatis, P. Fuschiotti, Single-cell lymphocyte heterogeneity in advanced cutaneous T-cell lymphoma skin tumors. *Clin. Cancer Res.* **25**, 4443–4454 (2019).
56. J. B. Cheng, A. J. Sedgewick, A. I. Finnegan, P. Harirchian, J. Lee, S. Kwon, M. S. Fassett, J. Golovato, M. Gray, R. Ghadially, W. Liao, B. E. Perez White, T. M. Mauro, T. Mully, E. A. Kim, H. Sbitany, I. M. Neuhaus, R. C. Grekin, S. S. Yu, J. W. Gray, E. Purdom, R. Paus, C. J. Vaske, S. C. Benz, J. S. Song, R. J. Cho, Transcriptional programming of normal and inflamed human epidermis at single-cell resolution. *Cell Rep.* **25**, 871–883 (2018).
57. S. Xu, F. Grande, A. Garofalo, N. Neamati, Discovery of a novel orally active small-molecule gp130 inhibitor for the treatment of ovarian cancer. *Mol. Cancer Ther.* **12**, 937–949 (2013).
58. L. van der Fits, S. Mourits, J. S. A. Voerman, M. Kant, L. Boon, J. D. Laman, F. Cornelissen, A. M. Mus, E. Florencia, E. P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* **182**, 5836–5845 (2009).
59. K. Sakai, K. M. Sanders, M. R. Youssef, K. M. Yanushefski, L. Jensen, G. Yosipovitch, T. Akiyama, Mouse model of imiquimod-induced psoriatic itch. *Pain* **157**, 2536–2543 (2016).
60. S. Rose-John, Interleukin-6 family cytokines. *Cold Spring Harb. Perspect. Biol.* **10**, (2018).
61. N. R. West, A. N. Hegazy, B. M. J. Owens, S. J. Bullers, B. Linggi, S. Buonocore, M. Coccia, D. Görtz, S. This, K. Stockenhuber, J. Pott, M. Friedrich, G. Ryzhakov, F. Baribaud, C. Brodmerkel, C. Cieluch, N. Rahman, G. Müller-Newen, R. J. Owens, A. A. Kühl, K. J. Maloy, S. E. Plevy; Oxford IBD Cohort Investigators, S. Keshav, S. P. L. Travis, F. Powrie, Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nat. Med.* **23**, 579–589 (2017).
62. K. Arita, A. P. South, G. Hans-Filho, T. H. Sakuma, J. Lai-Cheong, S. Clements, M. Odashiro, D. N. Odashiro, G. Hans-Neto, N. R. Hans, M. V. Holder, B. S. Bhogal, S. T. Hartshorne, M. Akiyama, H. Shimizu, J. A. McGrath, Oncostatin M receptor-beta mutations underlie familial primary localized cutaneous amyloidosis. *Am. J. Hum. Genet.* **82**, 73–80 (2008).
63. M. W. Lin, D. D. Lee, T. T. Liu, Y. F. Lin, S. Y. Chen, C. C. Huang, H. Y. Weng, Y. F. Liu, A. Tanaka, K. Arita, J. Lai-Cheong, F. Palisson, Y. T. Chang, C. K. Wong, I. Matsuura, J. A. McGrath, S. F. Tsai, Novel IL31RA gene mutation and ancestral OSMR mutant allele in familial primary cutaneous amyloidosis. *Eur. J. Hum. Genet.* **18**, 26–32 (2010).
64. M. Saeedi, A. Ebrahim-Habibi, A. Haghighi, F. Zarrabi, M. M. Amoli, R. M. Robati, A novel missense mutation in oncostatin M receptor beta causing primary localized cutaneous amyloidosis. *Biomed. Res. Int.* **2014**, 1–6 (2014).
65. J. Xu, P. Zanvit, L. Hu, P.-Y. Tseng, N. Liu, F. Wang, O. Liu, D. Zhang, W. Jin, N. Guo, Y. Han, J. Yin, A. Cain, M. A. Hoon, S. Wang, W. JunChen, The cytokine TGF-β induces interleukin-31 expression from dermal dendritic cells to activate sensory neurons and stimulate wound itching. *Immunity* **53**, 371–383.e5 (2020).
66. M. Langeslag, C. E. Constantin, M. Andratsch, S. Quarta, N. Mair, M. Kress, Oncostatin M induces heat hypersensitivity by gp130-dependent sensitization of TRPV1 in sensory neurons. *Mol. Pain* **7**, 1744-8069-7-102 (2011).
67. O. Obreja, W. Biasio, M. Andratsch, K. S. Lips, P. K. Rathee, A. Ludwig, S. Rose-John, M. Kress, Fast modulation of heat-activated ionic current by proinflammatory interleukin 6 in rat sensory neurons. *Brain* **128**, 1634–1641 (2005).
68. A. M. Binshtok, H. Wang, K. Zimmermann, F. Amaya, D. Vardeh, L. Shi, G. J. Brenner, R. R. Ji, B. P. Bean, C. J. Woolf, T. A. Samad, Nociceptors are interleukin-1beta sensors. *J. Neurosci.* **28**, 14062–14073 (2008).
69. P. L. Stemkowski, M. C. Noh, Y. Chen, P. A. Smith, Increased excitability of medium-sized dorsal root ganglion neurons by prolonged interleukin-1β exposure is K^+ channel dependent and reversible. *J. Physiol.* **593**, 3739–3755 (2015).
70. T. Hawro, R. Saluja, K. Weller, S. Altrichter, M. Metz, M. Maurer, Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. *Allergy* **69**, 113–117 (2014).
71. L. S. Wong, T. Wu, C. H. Lee, Inflammatory and noninflammatory itch: Implications in pathophysiology-directed treatments. *Int. J. Mol. Sci.* **18**, (2017).
72. M. Steinhoff, M. Schmelz, I. L. Szabo, A. L. Oaklander, Clinical presentation, management, and pathophysiology of neuropathic itch. *Lancet Neurol.* **17**, 709–720 (2018).
73. S. Stander, E. Weisshaar, T. Mettang, J. C. Szepietowski, E. Carstens, A. Ikoma, N. V. Bergasa, U. Gieler, L. Misery, J. Wallengren, U. Darsow, M. Streit, D. Metze, T. A. Luger, M. W. Greaves, M. Schmelz, G. Yosipovitch, J. D. Bernhard, Clinical classification of itch: A position paper of the International Forum for the Study of Itch. *Acta Derm. Venereol.* **87**, 291–294 (2007).
74. M. Steinhoff, A. L. Oaklander, I. L. Szabo, S. Stander, M. Schmelz, Neuropathic itch. *Pain* **160** (Suppl 1), S11–S16 (2019).
75. T. S. Jensen, R. Baron, M. Haanpää, E. Kalso, J. D. Loeser, A. S. C. Rice, R. D. Treede, A new definition of neuropathic pain. *Pain* **152**, 2204–2205 (2011).
76. R. D. Treede, T. S. Jensen, J. N. Campbell, G. Cruccu, J. O. Dostrovsky, J. W. Griffin, P. Hansson, R. Hughes, T. Nurmikko, J. Serra, Neuropathic pain: Redefinition and a grading system for clinical and research purposes. *Neurology* **70**, 1630–1635 (2008).
77. Q. Liu, Z. Tang, L. Surdenikova, S. Kim, K. N. Patel, A. Kim, F. Ru, Y. Guan, H. J. Weng, Y. Geng, B. J. Undem, M. Kollarik, Z. F. Chen, D. J. Anderson, X. Dong, Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* **139**, 1353–1365 (2009).
78. H. Taniguchi, M. He, P. Wu, S. Kim, R. Paik, K. Sugino, D. Kvitsani, Y. Fu, J. Lu, Y. Lin, G. Miyoshi, Y. Shima, G. Fishell, S. B. Nelson, Z. J. Huang, A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* **71**, 995–1013 (2011).
79. D. J. Cavanaugh, A. T. Chesler, J. M. Braz, N. M. Shah, D. Julius, A. I. Basbaum, Restriction of transient receptor potential vanilloid-1 to the peptidergic subset of primary afferent

neurons follows its developmental downregulation in nonpeptidergic neurons. *J. Neurosci.* **31**, 10119–10127 (2011).
80. D. A. Yarmolinsky, Y. Peng, L. A. Pogorzala, M. Rutlin, M. A. Hoon, C. S. Zuker, Coding and plasticity in the mammalian thermosensory system. *Neuron* **92**, 1079–1092 (2016).
81. R. Leinonen, H. Sugawara, M. Shumway; International Nucleotide Sequence Database Collaboration, The sequence read archive. *Nucleic Acids Res.* **39**, D19–D21 (2011).
82. N. L. Bray, H. Pimentel, P. Melsted, L. Pachter, Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.* **34**, 525–527 (2016).
83. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
84. R. Satija, J. A. Farrell, D. Gennert, A. F. Schier, A. Regev, Spatial reconstruction of single-cell gene expression data. *Nat. Biotechnol.* **33**, 495–502 (2015).
85. Z. Li, P. Y. Tseng, V. Tiwari, Q. Xu, S. Q. He, Y. Wang, Q. Zheng, L. Han, Z. Wu, A. L. Blobaum, Y. Cui, V. Tiwari, S. Sun, Y. Cheng, J. H. Y. Huang-Lionnet, Y. Geng, B. Xiao, J. Peng, C. Hopkins, S. N. Raja, Y. Guan, X. Dong, Targeting human Mas-related G protein-coupled receptor X1 to inhibit persistent pain. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E1996–E2005 (2017).
86. P.-Y. Z. Tseng, Q. Zheng, Z. Li, X. Dong, MrgprX1 mediates neuronal excitability and itch through tetrodotoxin-resistant sodium channels. *Itch* **4**, e28 (2019).
87. T. W. Chen, T. J. Wardill, Y. Sun, S. R. Pulver, S. L. Renninger, A. Baohan, E. R. Schreiter, R. A. Kerr, M. B. Orger, V. Jayaraman, L. L. Looger, K. Svoboda, D. S. Kim, Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295–300 (2013).
88. N. Inagaki, K. Igeta, J. F. Kim, M. Nagao, N. Shiraishi, N. Nakamura, H. Nagai, Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80. *Eur. J. Pharmacol.* **448**, 175–183 (2002).
89. M. Q. Nguyen, C. E. Le Pichon, N. Ryba, Stereotyped transcriptomic transformation of somatosensory neurons in response to injury. *eLife* **8**, (2019).
90. W. Renthal, I. Tochitsky, L. Yang, Y.-C. Cheng, E. Li, R. Kawaguchi, D. H. Geschwind, C. J. Woolf, Transcriptional reprogramming of distinct peripheral sensory neuron subtypes after axonal injury. *Neuron* **108**, 128–144.e9 (2020).

Funding: This work was supported by the intramural research program of the National Institute of Dental and Craniofacial Research, NIH, project ZIADE000721-19 (to M.A.H.). Human tissue was obtained from the NIH NeuroBioBank at the University of Maryland, Baltimore, MD. This work used the computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov), and DNA sequencing was carried out by the NIDCR Genomics and Computational Biology Core. **Author contributions:** P.-Y.T. and M.A.H. designed the experiments. P.-Y.T. performed experiments and analyzed data. P.-Y.T. and M.A.H. wrote and edited the paper. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study are present in the paper or the Supplementary Materials.

Submitted 12 August 2020
Resubmitted 29 January 2021
Accepted 13 August 2021
Published 10 November 2021
10.1126/scitranslmed.abe3037