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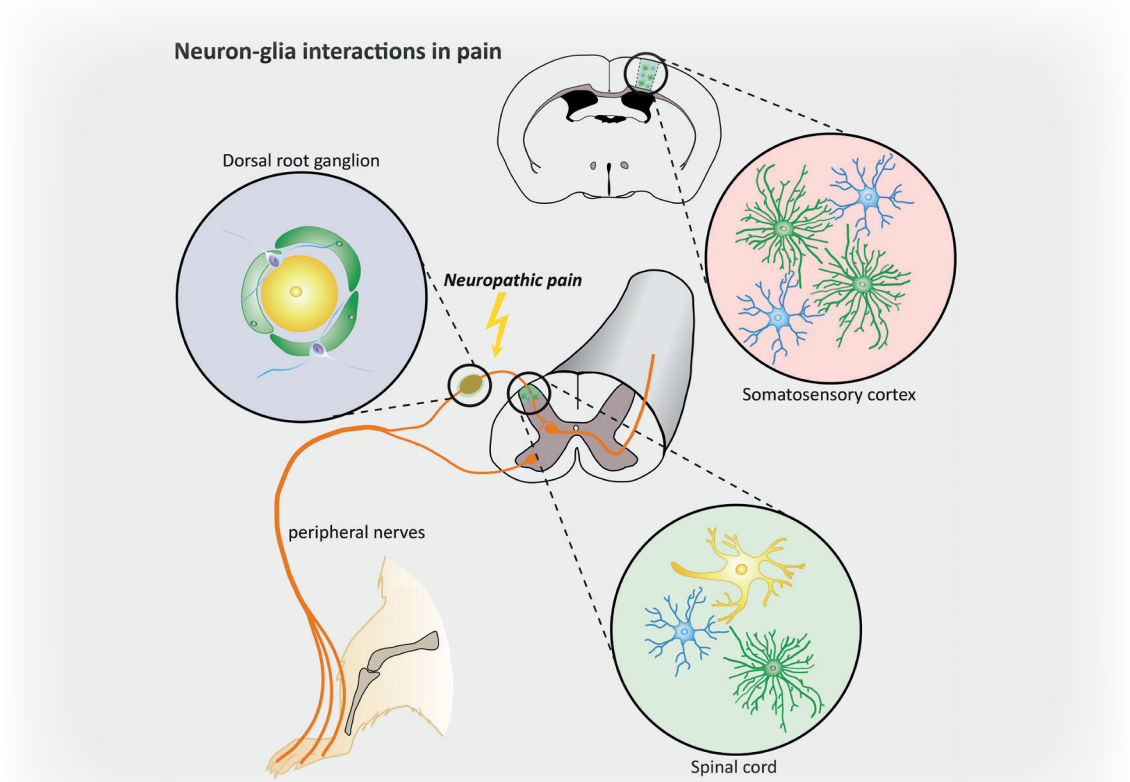

SPECIAL ISSUE:
THE CHALLENGE OF CHRONIC PAIN

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COVER ILLUSTRATION Neuroglia interactions in the central and peripheral nervous system in neuropathic pain involve proinflammatory signals leading to hyperactive sensory neurons and central pathways thereby enhancing pain transmission. Credits to: Elisa Damo (Institute of Pharmacology, Medical Faculty Heidelberg, Heidelberg University, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany) and Phillip Rieder (Molecular Physiology, Center for Integrative Physiology and Molecular Medicine (CIPMM), University of Saarland, Building 48, 66421 Homburg, Germany).

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Contents

Rohini Kuner* and Herta Flor

Editorial

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Dear reader,

Do you remember the last time you were in great physical pain? The agony coupled with a feeling of being debilitated and helpless, evoking a strong desire to escape it as soon as possible? For the sufferer, pain is a devasting and highly personal attack on one's very being. Now imagine if that pain were to persist on and on, for weeks, months or even years, long after the initial trigger is removed and no tell-tale pathology is left to show as the cause? This is the reality that millions of chronic pain patients live with world-wide. Studies suggest that every fifth European suffers from chronic pain, and that its prevalence is steadily rising, with certain types of pain, such as low back pain, showing a higher rise in prevalence than other most common disorders, such as diabetes and hypertension. The individual, emotional and socioeconomic burden of chronic pain is gigantic, and chronic pain disorders sadly continue to be under-recognized and under-treated. The opioid crisis in the United States of America has recently thrown the limitations of current pain therapy into sharp focus.

It comes therefore as no surprise that chronic pain is an area of intense investigation in the basic and clinical sciences, and represents one of the most dynamic areas in the neurosciences. The large diversity of aetiologies and types of chronic pain, the multiple anatomical avenues in the body where pain originates, is processed or modulated, its close association with the immune system, its dependence on environmental, psychosocial and contextual factors and its bilateral interactions with emotional and cognitive disorders, including fear, anxiety and depression, renders it obligatory for pain research to be multi- and interdisciplinary in order to be effective. Therefore, complementary to research ideas driven by individual laboratories, large research consortia can help to illuminate and address pain in its enormous complexity and diversity from multiple disciplinary standpoints. The Collaborative Research Center 1158 (CRC1158), funded by the German Research Foundation at Heidelberg University and its partner institutions since 2015, is one such consortium comprising a large number of preclinical and clinical researchers working collaboratively to understand structure-function relationships in neural circuits underlying pain and to elucidate how specificity and causality for pain come about. A key goal is to study how neural pathways undergo dynamic plasticity over the transition to chronic pain and to reverse this plasticity for achieving relief from chronic pain.

This special edition, comprising three articles from CRC1158 researchers and two articles from other leading pain researchers in Germany, aims to address some of the key topics of current interest in the pain field. Reflecting the broad span of chronic pain, these articles tackle very different aspects of the problem and offer ideas for designing new therapies.

Sodium channels are key to propagation of pain-related information from the periphery to the brain and represent a cornerstone of pain therapy. Yet, the full extent of their importance in pain disorders is still being unravelled, with increasing insights emerging on genetic variants of sodium channels in genetically-determined pain disorders. Jannis Körner, Natja Haag, Ingo Kurth and Angelika Lampert now describe the challenges associated with elucidating functional significance of 'variants of unclear significance' that are identified upon genetic screening of pain patients and outline a promising strategy for corelating cellular function with transcriptional changes at a single cell level.

The article by Hans-Georg Schaible, Andrea Ebersberger, Gabriel Natura and Enrique Vazquez reviews the multiple ways in which immune cells modulate pain and in turn, are subject to regulation by neuroinflammatory processes driven by the nervous system. Not only do these involve cytokines and other mediators acting in the periphery to sensitize sensory nerves sensing noxious stimuli (nociceptors), but also plasticity of microglia, the resident cells of the central nervous system.

Microglia and astrocytes, the most abundant cell type in our brain, also comprise the main focus of an article by Elisa Damo, Phillip Rieder, Ilknur Coban, Rangel Leal Silva, Frank Kirchoff, Manuela Simonetti and Amit Agarwal, which places these important cells into an entirely new spotlight, namely their potential contributions to the therapeutic effects of antidepressants in neuropathic pain. Although this may seem surprising to the non-expert, antidepressant drugs, which typically increase the synaptic availability of serotonin and noradrenaline, actually

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constitute first line therapy in neuropathic pain disorders, placed higher than opioids in terms of long-term efficacy. How does this come about? For many years, this was attributed to involvement of descending pain modulatory systems from the brainstem to the spinal cord neurons. Damo et al. now put forth strong conceptual arguments for glial cells as a key locus of site of action of antidepressant-mediated reversal of neuropathic pain.

What is to be done when the currently available arsenal of pharmacological drugs fails to relieve chronic pain? A highly encouraging line of research shows that refractory pain disorders, particularly neuropathic pain, respond favourably to different types of neuromodulation and neurostimulation therapies. In their review article, Herta Flor and Rohini Kuner discuss the rationale behind these new type of therapies as well as the problems observed in terms of variable efficacy and lack of standardization. A key hindrance has been given by major deficits in understanding the mechanistic underpinnings and nature of neural pathways involved in neurostimulation- and neuromodulation-based therapies. Both neuroimaging studies in human healthy subjects and pain patients and animal studies studying neural circuits at cellular resolution are now helping to bridge this gap and provide a basis for improving efficacy and wider applicability of these emerging therapies in chronic pain.

Is there any aspect of our lives which has not been pervaded by the Covid-19 pandemic over the last two years? Well, pain is no exception! In their article, Jonas Tesarz and Frauke Nees report that not only do certain types of pain, such as headache and musculoskeletal pain, comprise the most common symptoms and long-term manifestations of Covid 19, but also that Covid 19 critically alters brain areas involved in pain processing in form of the 'long Covid' syndrome. This is a highly topical area, and although research on this new topic is just about taking off, the repercussions for chronic pain patients and implications for pain therapy are tremendous.

We hope that these new insights will be informative to the readers and not only broaden their knowledge on the mechanisms and therapy of chronic pain, but also spur interest and active participation in multidisciplinary research on the formidable challenge of chronic pain!

On behalf of CRC1158, we would like to sincerely thank the German Neuroscience Society and the editorial team of Neuroforum for selecting this theme and providing a platform for scientific discourse with the neuroscience community. Many thanks to the authors for their valuable contributions!

Review article

Jannis Körner, Natja Haag, Ingo Kurth and Angelika Lampert*

Genetics meets function in sodium channel-related pain disorders

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Abstract: Voltage-gated sodium channels are crucial for pain perception. This is illustrated by several human genetic conditions that lead to either chronic pain or, vice versa, to congenital painlessness. The type of mutation, its impact on neuron excitability as well as the affected sodium channel subtype delineates a complex picture of the disorders. Genetic variants in sodium channels may affect the complex biophysical gating and also their trafficking, association with other proteins and more complex regulations of the channel protein and function, thus allowing us to explore the subtle but impactful effects of their dysregulation for human nociception. A detailed understanding of these pain disorders provides a unique chance to understand the detailed intricacies of nociception and pathological conditions such as neuropathic pain. With increasing awareness of the importance of sodium channel variants in neuropathic pain, more patients are genetically screened, sometimes identifying variants of unclear significance (VUS). Bioinformatic tools help to assess their potential disease causing impact, but functional studies using patch-clamp experiments in cell lines are needed to allow for reliable conclusions. Often cell lines are not sufficient to show a physiologically relevant phenotype and more complex, time intensive models, such as induced pluripotent stem cells (iPS-cells) are employed. A challenge remains to identify the role of each sodium channel VUS in the context of the detailed cellular genetic and functional context. To lay the grounds for such a detailed interpretation, we need a correlation of cellular function and genetic transcription on a single cell basis, as it is possible with the Patch-Seq technique. The more detailed our knowledge becomes on functional and genetic sensory neurons subtypes and their role in the generation of neuropathic pain, the more targeted the personal or population-based treatment can be.

Keywords: sodium channels; pain; transcriptomics; patch clamp; variant of unknown significance (VUS).

Zusammenfassung: Spannungsgesteuerte Natriumkanäle sind entscheidend für die Schmerzwahrnehmung. Dies wird durch mehrere humangenetische Erkrankungen veranschaulicht, die entweder zu chronischen Schmerzen oder umgekehrt zu angeborener Schmerzlosigkeit führen. Die Art der Mutation, ihr Einfluss auf die Erregbarkeit von Neuronen sowie der betroffene Natriumkanal-Subtyp zeichnen ein komplexes Bild der Erkrankungen. Genetische Varianten in Natriumkanälen können viel mehr als ihr biophysikalisches Schaltverhalten beeinflussen, was die Komplexität der menschlichen Nozizeption weiter unterstreicht. Ein detailliertes Verständnis dieser monogenetischen Schmerzstörungen erlaubt es in besonderer Weise, die Feinheiten der Nozizeption und ihrer Pathophysiologie, wie z. B. im Falle neuropathischer Schmerzen zu verstehen. Mit zunehmendem Bewusstsein für die Bedeutung von Natriumkanalvarianten bei neuropathischen Schmerzen werden immer mehr Patient*innen genetisch untersucht und neben klar pathogenen Varianten auch Varianten unklarer Signifikanz (VUS) identifiziert. Bioinformatische Vorhersageprogramme und Vorhersagen zur Häufigkeit einer Veränderung helfen bei der Einschätzung ihrer Krankheitsursächlichkeit, oft lässt aber nur die Untersuchung ihrer funktionellen Auswirkung, bspw. durch Patch-Clamp-Experimente in Zelllinien, zuverlässige Schlussfolgerungen zu. Oft sind für ein umfassendes Verständnis genetischer Varianten aber auch komplexere und zeitintensivere Modelle wie induzierte pluripotente Stammzellen notwendig. Es bleibt eine Herausforderung, die Rolle jeder VUS in einem Natriumkanal durch

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detaillierte zelluläre und genetische Modelle zu untersuchen. Die Korrelation zwischen Zellfunktion und Transkription auf Einzelzellbasis, wie sie mit der Patch-Seq-Technik möglich ist, trägt weiter zum Verständnis genetischer Varianten bei. Je detaillierter unser Wissen über funktionelle und genetische sensorische Neuronen-Subtypen und ihre Rolle bei der Entstehung von neuropathischen Schmerzen wird, desto zielgerichteter kann die individuelle oder allgemeingültige Schmerztherapie erfolgen.

Schlüsselwörter: Natriumkanäle; Schmerz; Transkriptom; Patch-Clamp; Variante unklarer Signifikanz (VUS).

Sodium channels and pain

The link between genetic variants and disease in certain sodium channel mutations leading to inherited chronic pain syndromes cannot be clearer: primary or inherited erythromelalgia (IEM) can be caused by clear cut mutations that often show high penetrance, which means that the person carrying the mutation will have disease symptoms (Körner and Lampert, 2020; Nau and Leipold, 2017). With this discovery not only the affected gene, SCN9A coding for Nav1.7 and its gene family members, gained center stage, also a search for pain-linked sodium channel mutations in the substantial, largely insufficiently treated patient population suffering from neuropathic pain begun (de Greef et al., 2019; Eijkenboom et al., 2019; Faber et al., 2012b; Sopacua et al., 2019). This quest revealed a plethora of candidate variants of more or less unclear significance, underlining the difficulty of a comprehensive understanding of sodium channel function in the cellular, human context.

In their seminal work Hodgkin and Huxley described in a series of papers in 1952 the role of a voltage-gated sodium ion conductance in the generation of action potentials (Raman and Ferster, 2022). By now we understand that the human genome contains nine sodium channel subtypes (Nav1.1 to Nav1.9) expressed to various extends in excitable and non-excitable tissues, fine tuning the electrical signaling (Catterall, 2012; Lampert et al., 2015, 2014). They can be associated with β-subunits (SCN1B to SCN4B) (Edokobi and Isom, 2018), other associated proteins such as intracellular growth factors (fibroblast growth factor homologous factors, FHFs) (Kanellopoulos et al., 2018; Laezza et al., 2009; Mahling et al., 2021; Nathan and Gabelli, 2021) and are set in place by well-regulated interactions with the cytoskeleton (Eshed-Eisenbach and Peles, 2021; Ghosh et al., 2018; Undrovinas et al., 1995).

Genetic variants in sodium channels may thus affect much more than their complex biophysical gating, thus allowing us to explore the subtle but impactful effects of their dysregulation for human nociception.

Genetic variants affecting sodium channel function

Three of the voltage-gated sodium channels are associated with clearly monogenic traits of altered pain perception: SCN9A (Nav1.7), SCN10A (Nav1.8), and SCN11A (Nav1.9). Individuals with loss-of-function mutations in both copies of *SCN9A* have a complete inability to experience pain (also called Congenital Insensitivity to Pain [CIP]) and lack a sense of smell (anosmia) (Cox et al., 2006; Goldberg et al., 2007; Weiss et al., 2011). The painlessness leads to biting of the tongue and fingers and non-painful bone fractures. Touch, warm and cold temperatures, proprioception, and pressure are correctly perceived in contrast to visceral pains. Sensory nerves typically show normal morphology (Cox et al., 2006), but nociceptors can also show structural alterations (McDermott et al., 2019; Yuan et al., 2013). In contrast, dominant gain-of-function point mutations in *SCN9A* lead to different phenotypes with increased pain. *SCN9A*-associated IEM is a disorder with mild-to-severe burning pain attacks affecting the hands and feet, accompanied by increased skin temperature, edema, and erythema (Yang et al., 2004). The sodium channel blocker mexiletine is sometimes helpful, as are topical lidocaine patches. However, pain relief is often only achieved with ice-cold water. Mutant Nav1.7 channels leading to IEM have mostly a lowered threshold for channel activation, thereby alleviating action potential generation and a higher frequency of repetitive firing (Bennett et al., 2019). These effects are often accompanied by an enhanced slow inactivation (Hampl et al., 2016).

Paroxysmal extreme pain disorder (PEPD) is characterized by infancy-onset episodes of severe perineal and rectal, ocular, and mandibular pain (Fertleman et al., 2006). Dominant gain-of-function mutations in *SCN9A* that cause hyperexcitability of sensory neurons are responsible for PEPD and carbamazepine is often effective. PEPD mutations lead to incomplete channel inactivation with a persistent current and resurgent current (Bennett et al., 2019; Hampl et al., 2016).

Finally, dominant gain-of-function mutations in *SCN9A* cause small fiber neuropathy (SFN), an adult-onset disorder with burning pain, typically in the feet or hands (Faber et al., 2012a). Intraepidermal nerve fiber density of small-diameter unmyelinated and thinly myelinated fibers is normally reduced. The biophysical effects of SFN linked Nav1.7 mutations is much more variable, including reduced slow inactivation, enhanced resurgent currents and alleviated

activation (Bennett et al., 2019). Heterozygous, mostly gain-of-function mutations in *SCN10A* (Faber et al., 2012b) and *SCN11A* (Huang et al., 2014; Leipold et al., 2015; Zhang et al., 2013) have also been reported in patients with SFN. Interestingly, distinct gain-of-function Nav1.9 variants also result in painlessness (King et al., 2017; Leipold et al., 2013; Phatarakijnirund et al., 2016). Patients with *SCN11A*-related pain insensitivity have additional symptoms, such as bowel motility disturbances, muscle weakness, and extreme itch. Obviously, stronger membrane depolarizations result in hypoexcitability due to depolarization block and pain insensitivity, and smaller depolarizations result in hyperexcitability and pain.

When the link between inherited pain disorders and sodium channel mutations become apparent, highly selectively Nav1.7 blockers were developed and tested as pain treatment. Unfortunately, these drugs did not show convincing efficacy in clinical trials (Kingwell, 2019). Thus, it may be the interplay of the biophysical effect of several variants on excitability, but it may also be a more complex protein regulation, including trafficking, changes in associated proteins or in the subcellular organizations, which induce the pain phenotype and would need to be addressed for more specific therapeutic approaches.

In some cases, with pain disorders, a clear and exclusive causality can be established between the clinical finding and the genetic alteration. Despite extensive exome or genome sequencing and even inheritance of the disease, a genetic cause, especially in SFN, often remains unclear. Not surprisingly, unclear genetic variants are found in sodium channel genes, which may show slight electrophysiological changes in heterologous expression systems. However, the frequency of these variants in the general population suggests that they cannot be the sole cause for the disease. Conceivable models are reduced penetrance and variable expressivity, but also other factors (diabetes mellitus, alcohol, drugs, environmental factors, etc.) that play a role in the sense of a multifactorial trait. However, in addition to such a predisposing sodium channel variant, the sum of a person's genetic variants also modulates the probability of disease.

Increasingly, complex models in terms of polygenic risk scores (PRS) need to be considered for the genetic explanation of an SFN and related pain disorders. A PRS provides an estimate of how likely a person is to have a

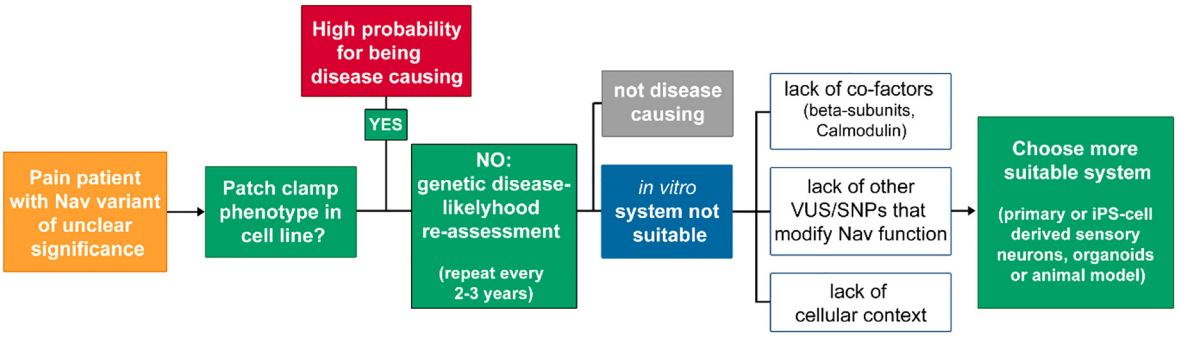

Figure 1: Decision tree to assess pathogenicity of sodium channel variants of unclear significance (VUS). The effect of the variant needs to be assessed functionally using patch-clamp, e.g., in HEK cells (green box, left). Biophysical changes in sodium channel gating increase the probability of the variant being disease causing (red box). Genetics need to be re-assessed if a first functional screen yields no or contradicting results, potentially allowing to categorize the VUS as not disease causing, or to continue using more suitable, but also more complex model systems, such as primary neurons, stem cell derived neurons, organoids, or animal models.

particular disease based solely on the many different genetic variants (Torkamani et al., 2018). Significant progress has been made in this field in recent years, particularly in the area of oncology (Lambert et al., 2019), and such models also need to be increasingly used for channelopathies. However, a good prediction also requires assessment of the variant's functional impact (Figure 1).

Ways out of the uncertainty of genetic findings

As outlined above and in Figure 1, interpretation of the pathogenicity of genetic variants identified in pain patients can be challenging. One decisive factor is whether a variant affects sodium channel gating, as small changes e.g., in the voltage-dependence of activation or fast inactivation can be sufficient to induce hyperexcitability and neuropathic pain (Bennett et al., 2019). Traditionally, variant-induced biophysical gating changes in voltage-gated sodium channels are investigated using cellular systems which can easily be studied by patch-clamp.

Heterologous expression systems and primary sensory neurons

Sodium channels can easily be expressed in HEK, CHO, or similar cell lines and studies in these systems have led to substantial improvements in the mechanistic understanding of the genotype-phenotype relations in sodium channel mutations (Meents and Lampert, 2016). Handling of these cell lines is comparably easy, and their availability is a considerable advantage. HEK cells, e.g., only express few ion channels endogenously and usually display very small sodium currents. Nevertheless, the cellular background is different from the physiological neuronal environment of peripheral sensory neurons, which can hamper e.g., post-translational protein modifications. Underlining these limitations there have been studies published on putative disease causing mutations lacking phenotypes in a HEK cell context (Le Cann et al., 2021).

Primary sensory neurons from mouse or rat dorsal root ganglia form a more physiological cellular expression system which also allows to investigate the effect of the variants on cellular excitability. The system is used as a model in the functional characterization of biophysical phenotypes both for genetic pain syndromes (with the variants overexpressed) as well as for neuropathic pain models (chronic constriction model and similar) (Körner and Lampert, 2022). However, recent drawbacks in the development of new pain therapies (Kingwell, 2019) and new insights in species differences provided by higher availability of single cell transcriptomic approaches point out substantial species differences in the nociceptive apparatus between humans and rodents (Kupari et al., 2021; Nguyen et al., 2021; Rostock et al., 2018). Therefore, growing interest in the characterization of human or human like sensory neurons from primates, pigs or humans is developing in the scientific community (Davidson et al., 2014; Körner and Lampert, 2022; Zhang et al., 2017). Specifically, pig sensory neurons are becoming more and more important, as their skin and sensory neuron subclasses seem to be more similar to humans than those of rodents.

Induced pluripotent stem cells (iPS-cells)

Due to the translational gap in pain research, there is a need for a cellular model system which resembles human

nociceptors more closely. To this end differentiation methods of induced pluripotent stem cells (iPS-cells) were developed over the past years, that allow to generate human sensory neurons and nociceptors (Lampert et al., 2020). The major advantage of this method lays in the possibility to retrieve cells of patients carrying genetic mutations and variants of unclear significance (VUS) and directly generate sensory neurons from these patients. Thus, not only the gene of interest is present, but the complete genome of the individual, including all single-nucleotide and structural variations. This allows the effects on cellular excitability to be studied in their native genetic context. Recent efforts to improve the differentiation protocol to increase reliability of results and to create specific cell types, mixtures, co-cultures of peripheral sensory neurons and associated cells are ongoing and will improve translational pain research significantly (Chambers et al., 2012; Clark et al., 2017; Eberhardt et al., 2015; McDermott et al., 2019; Namer et al., 2018; Neureiter et al., 2022).

Patch-Seq: understand ion channel function within their channelome

Patient derived neurons from iPS-cells and primary sensory neurons are heterogeneous neuronal populations with distinct functional and genetic features. The effects of sodium channel variants on cellular excitability depend on the specific cell identity as defined by its transcriptome, which includes various ion channels and associated proteins. Droplet-based single cell sequencing studies have revealed that sensory neurons in dorsal root ganglion in healthy rodents, primates and humans contain probably between 10 and 15 clearly distinct cell subtypes (Kupari et al., 2021; Nguyen et al., 2021; Tavares-Ferreira et al., 2022; Usoskin et al., 2015). Neuropathic pain is based on increased excitability of specific subclasses of sensory neurons, thus a link between electrical function and single cell gene transcription will help to understand sodium channel function and dysfunction within the cells channelome and to identify promising drug targets.

Biological systems inherently comprise a relevant fraction of variance. Thus, a separate inspection of genetic and functional features of neurons carries a certain amount of ambiguity and a deeper understanding of the link between genotype and electrical cell function often remains indirect at best. Simultaneous assessment of both, genetic and functional aspects of a single cell will help to overcome some limitations. With the emergence of a technique often referred to as Patch-Seq, this is becoming a practiced alternative (Figure 2): After functional characterization of cellular excitability by patch-clamp the cell of interest is isolated (mostly via the patch pipette) and afterwards processed by single cell RNA sequencing (Cadwell et al., 2017). Simultaneously, morphological features can be assessed by backfilling the patch pipette with biocytin or another dye and included for analysis (Lipovsek et al., 2021). Thereby, an integrated dataset with functional, transcriptomic, and morphological information in single cell resolution is generated bearing the possibility to link function and transcriptomics of neurons in an immediate manner. Those experiments have already led to profound insights on the function and identity of neurons of the central and peripheral nervous system. The results have both questioned our hierarchical concept of discrete neuronal entities (Scala et al., 2021) and our understanding of ion channels involved in different mechanically activated currents (Parpaite et al., 2021). In the future, linking these well characterized cellular subtypes to functional and morphological signatures will substantially enhance

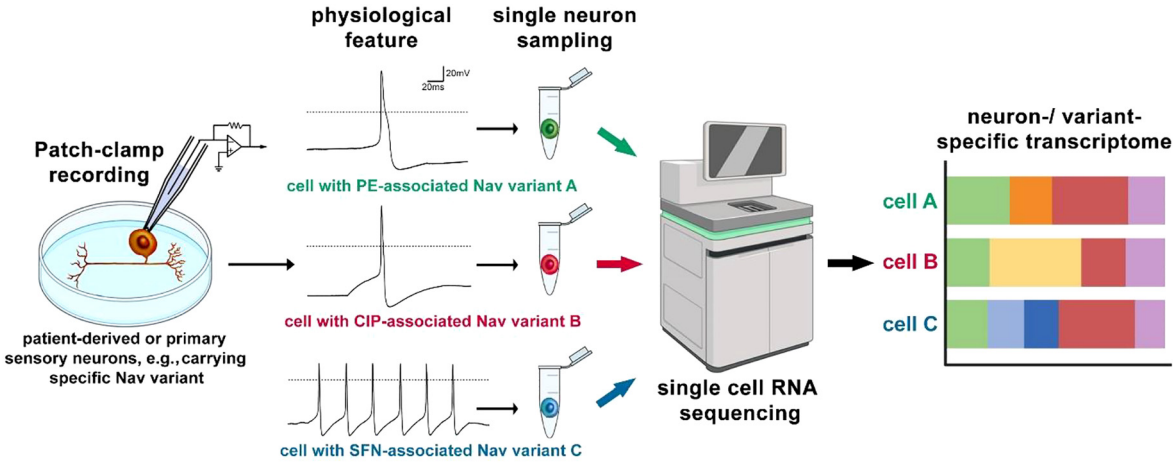

Figure 2: Patch-Seq workflow illustrating collection of functional data followed by single cell collection, their transcriptome analysis and bioinformatic workup.

our understanding of the nociceptive apparatus. Expanding these experiments to pathological pain phenotypes and genetic variants, e.g., of the sodium channel, will enlarge our comprehension of pain genesis and offer new therapeutic concepts.

Outlook translational pain research

In order to foster our understanding of sodium channel variants in human nociception, more detailed knowledge of patient phenotypes, cellular excitability and the molecular basis is needed. Therefore, a wholistic approach is needed which includes clinicians and basic scientists working hand in hand. The sodium channel network Aachen (SCN^{Aachen}, https://www.scn-aachen.rwth-aachen.de), e.g., gathers neurologists, geneticists, computer scientists, cell biologists, pain specialists, electrophysiologists, and clinician scientists, to join forces for an in-depth understanding of neuropathic pain. An increasing cohort of highly phenotyped neuropathic pain patients allows for in-depth analysis of genetic variants, characterization of iPS-cell derived sensory neuron function and a deeper understanding of each patient's individual pathophysiology.

Sodium channels are one of the few human validated pain targets, as a complete functional knock-out of Nav1.7 leads to absence of pain. Although initial attempts to develop pain medication directed towards sodium channels were not successful (Kingwell, 2019), recent efforts using new approaches (Kingwell, 2021) or targeting other sodium channel subtypes are promising (https://www.businesswire.com/news/home/20210719005192/en/Vertex-Initiates-Phase-2-Clinical-Trial-Program-for-VX-548-for-the-Treatment-of-Acute-Pain) and give hope that with the more detailed knowledge of the genetic and functional basis of nociceptive hyperexcitability sodium channels may be targeted for personalized or population-based pain treatment.

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References

Bennett, D.L., Clark, A.J., Huang, J., Waxman, S.G., and Dib-Hajj, S.D. (2019). The role of voltage-gated sodium channels in pain signaling. Physiol. Rev. *99*, 1079–1151.

Cadwell, C.R., Scala, F., Li, S., Livrizzi, G., Shen, S., Sandberg, R., Jiang, X., and Tolias, A.S. (2017). Multimodal profiling of single-cell morphology, electrophysiology, and gene expression using Patch-seq. Nat. Protoc. *12*, 2531–2553.

Catterall, W.A. (2012). Voltage-gated sodium channels at 60: Structure, function and pathophysiology. J. Physiol. *590*, 2577–2589.

Chambers, S.M., Qi, Y., Mica, Y., Lee, G., Zhang, X.-J., Niu, L., Bilsland, J., Cao, L., Stevens, E., Whiting, P., et al. (2012). Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat. Biotechnol. *30*, 715–720.

Clark, A.J., Kaller, M.S., Galino, J., Willison, H.J., Rinaldi, S., and Bennett, D.L.H. (2017). Co-cultures with stem cell-derived human sensory neurons reveal regulators of peripheral myelination. Brain J. Neurol. *140*, 898–913.

Cox, J.J., Reimann, F., Nicholas, A.K., Thornton, G., Roberts, E., Springell, K., Karbani, G., Jafri, H., Mannan, J., Raashid, Y., et al. (2006). An SCN9A channelopathy causes congenital inability to experience pain. Nature *444*, 894.

Davidson, S., Copits, B.A., Zhang, J., Page, G., Ghetti, A., and Gereau, R.W. (2014). Human sensory neurons: Membrane properties and sensitization by inflammatory mediators. Pain *155*, 1861–1870.

de Greef, B.T.A., Hoeijmakers, J.G.J., Geerts, M., Oakes, M., Church, T.J.E., Waxman, S.G., Dib-Hajj, S.D., Faber, C.G., and Merkies, I.S.J. (2019). Lacosamide in patients with Nav1.7 mutations-related small fibre neuropathy: A randomized controlled trial. Brain J. Neurol. *142*, 263–275.

Eberhardt, E., Havlicek, S., Schmidt, D., Link, A.S., Neacsu, C., Kohl, Z., Hampl, M., Kist, A.M., Klinger, A., Nau, C., et al. (2015). Pattern of functional TTX-resistant sodium channels reveals a

developmental stage of human iPSC- and ESC-derived nociceptors. Stem Cell Rep. *5*, 305–313.

Edokobi, N. and Isom, L.L. (2018). Voltage-gated sodium channel β1/β1B subunits regulate cardiac physiology and pathophysiology. Front. Physiol. *9*, 351.

Eijkenboom, I., Sopacua, M., Hoeijmakers, J.G.J., de Greef, B.T.A., Lindsey, P., Almomani, R., Marchi, M., Vanoevelen, J., Smeets, H.J.M., Waxman, S.G., et al. (2019). Yield of peripheral sodium channels gene screening in pure small fibre neuropathy. J. Neurol. Neurosurg. Psychiatry *90*, 342–352.

Eshed-Eisenbach, Y. and Peles, E. (2021). The clustering of voltage-gated sodium channels in various excitable membranes. Dev. Neurobiol. *81*, 427–437.

Faber, C.G., Hoeijmakers, J.G.J., Ahn, H.-S., Cheng, X., Han, C., Choi, J.-S., Estacion, M., Lauria, G., Vanhoutte, E.K., Gerrits, M.M., et al. (2012a). Gain of function Nav1.7 mutations in idiopathic small fiber neuropathy. Ann. Neurol. *71*, 26–39.

Faber, C.G., Lauria, G., Merkies, I.S.J., Cheng, X., Han, C., Ahn, H.-S., Persson, A.-K., Hoeijmakers, J.G.J., Gerrits, M.M., Pierro, T., et al. (2012b). Gain-of-function Nav1.8 mutations in painful neuropathy. Proc. Natl. Acad. Sci. U. S. A *109*, 19444–19449.

Fertleman, C.R., Baker, M.D., Parker, K.A., Moffatt, S., Elmslie, F.V., Abrahamsen, B., Ostman, J., Klugbauer, N., Wood, J.N., Gardiner, R.M., et al. (2006). SCN9A mutations in paroxysmal extreme pain disorder: Allelic variants underlie distinct channel defects and phenotypes. Neuron *52*, 767–774.

Ghosh, A., Sherman, D.L., and Brophy, P.J. (2018). The axonal cytoskeleton and the assembly of nodes of Ranvier. Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry *24*, 104–110.

Goldberg, Y.P., MacFarlane, J., MacDonald, M.L., Thompson, J., Dube, M.-P., Mattice, M., Fraser, R., Young, C., Hossain, S., Pape, T., et al. (2007). Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin. Genet. *71*, 311–319.

Hampl, M., Eberhardt, E., O'Reilly, A.O., and Lampert, A. (2016). Sodium channel slow inactivation interferes with open channel block. Sci. Rep. *6*, 25974.

Huang, J., Han, C., Estacion, M., Vasylyev, D., Hoeijmakers, J.G.J., Gerrits, M.M., Tyrrell, L., Lauria, G., Faber, C.G., Dib-Hajj, S.D., et al. (2014). Gain-of-function mutations in sodium channel Na(v) 1.9 in painful neuropathy. Brain J. Neurol. *137*, 1627–1642.

Kanellopoulos, A.H., Koenig, J., Huang, H., Pyrski, M., Millet, Q., Lolignier, S., Morohashi, T., Gossage, S.J., Jay, M., Linley, J.E., et al. (2018). Mapping protein interactions of sodium channel NaV1.7 using epitope-tagged gene-targeted mice. EMBO J. *37*, 427–445.

King, M.K., Leipold, E., Goehringer, J.M., Kurth, I., and Challman, T.D. (2017). Pain insensitivity: Distal S6-segment mutations in NaV1.9 emerge as critical hotspot. Neurogenetics *18*, 179–181.

Kingwell, K. (2019). Nav1.7 withholds its pain potential. Nat. Rev. Drug Discov. *18*, 321.

Kingwell, K. (2022). Navigating a new path to Nav1.7 for pain. Nat. Rev. Drug Discov. *21*, 18.

Körner, J. and Lampert, A. (2020). 5.09 - Sodium Channels. The Senses: A Comprehensive Reference. B. Fritzsch, ed., 2nd ed. (Elsevier), pp. 120–141.

Körner, J. and Lampert, A. (2022). Functional subgroups of rat and human sensory neurons: A systematic review of electrophysiological properties. Pflueg. Arch. Eur. J. Physiol., https://doi.org/10.1007/s00424-021-02656-6.

Kupari, J., Usoskin, D., Parisien, M., Lou, D., Hu, Y., Fatt, M., Lönnerberg, P., Spångberg, M., Eriksson, B., Barkas, N., et al. (2021). Single cell transcriptomics of primate sensory neurons identifies cell types associated with chronic pain. Nat. Commun. *12*, 1510.

Laezza, F., Lampert, A., Kozel, M.A., Gerber, B.R., Rush, A.M., Nerbonne, J.M., Waxman, S.G., Dib-Hajj, S.D., and Ornitz, D.M. (2009). FGF14 N-terminal splice variants differentially modulate Nav1.2 and Nav1.6-encoded sodium channels. Mol. Cell. Neurosci. *42*, 90–101.

Lambert, S.A., Abraham, G., and Inouye, M. (2019). Towards clinical utility of polygenic risk scores. Hum. Mol. Genet. *28*, R133–R142.

Lampert, A., Bennett, D.L., McDermott, L.A., Neureiter, A., Eberhardt, E., Winner, B., and Zenke, M. (2020). Human sensory neurons derived from pluripotent stem cells for disease modelling and personalized medicine. Neurobiol. Pain *8*, 100055.

Lampert, A., Eberhardt, M., and Waxman, S.G. (2014). Altered sodium channel gating as molecular basis for pain: Contribution of activation, inactivation, and resurgent currents. Voltage Gated Sodium Channels, Handbook of Experimental Pharmacology. P.C. Ruben, ed. (Springer), pp. 91–110.

Lampert, A., Stühmer, W., and Waxman, S.G. (2015). Sodium channels. eLS (encyclopedia of Life Sciences) (John Wiley & Sons), pp. 1–7.

Le Cann, K., Meents, J.E., Sudha Bhagavath Eswaran, V., Dohrn, M.F., Bott, R., Maier, A., Bialer, M., Hautvast, P., Erickson, A., Rolke, R., et al. (2021). Assessing the impact of pain-linked Nav1.7 variants: An example of two variants with no biophysical effect. Channels (Austin) *15*, 208–228.

Leipold, E., Hanson-Kahn, A., Frick, M., Gong, P., Bernstein, J.A., Voigt, M., Katona, I., Oliver Goral, R., Altmüller, J., Nürnberg, P., et al. (2015). Cold-aggravated pain in humans caused by a hyperactive NaV1.9 channel mutant. Nat. Commun. *6*, 10049.

Leipold, E., Liebmann, L., Korenke, G.C., Heinrich, T., Giesselmann, S., Baets, J., Ebbinghaus, M., Goral, R.O., Stödberg, T., Hennings, J.C., et al. (2013). A de novo gain-of-function mutation in SCN11A causes loss of pain perception. Nat. Genet. *45*, 1399–1404.

Lipovsek, M., Bardy, C., Cadwell, C.R., Hadley, K., Kobak, D., and Tripathy, S.J. (2021). Patch-seq: Past, present, and future. J. Neurosci. Off. J. Soc. Neurosci. *41*, 937–946.

Mahling, R., Rahlf, C.R., Hansen, S.C., Hayden, M.R., and Shea, M.A. (2021). Ca2+-saturated calmodulin binds tightly to the N-terminal domain of A-type fibroblast growth factor homologous factors. J. Biol. Chem. *296*, 100458.

McDermott, L.A., Weir, G.A., Themistocleous, A.C., Segerdahl, A.R., Blesneac, I., Baskozos, G., Clark, A.J., Millar, V., Peck, L.J., Ebner, D., et al. (2019). Defining the functional role of NaV1.7 in human nociception. Neuron *101*, 905–919.e8.

Meents, J. and Lampert, A. (2016). Studying sodium channel gating in heterologous expression systems. Advanced Patch-Clamp Analysis for Neuroscientists. Neuromethods, vol 113. A. Korngreen, ed. (Humana Press, New York, NY).

Namer, B., Schmidt, D., Eberhardt, E., Maroni, M., Dorfmeister, E., Kleggetveit, I.P., Kaluza, L., Meents, J., Gerlach, A., Lin, Z., et al. (2018). Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine *39*, 401–408.

Nathan, S. and Gabelli, S.B. (2021). Navigating the intricacies of cellular machinery. J. Biol. Chem. *296*, 100832.

Nau, C. and Leipold, E. (2017). Spannungsgesteuerte Natriumkanäle und Schmerz. E-Neuroforum *23*, 164–172.
Neureiter, A., Eberhardt, E., and Lampert, A. (2022). Differentiation of iPS-cells into peripheral sensory neurons. Methods Mol. Biol. 2429, Stem Cell Assays. Methods and Protocols. N. Kannan and P. Beer, eds., 1st ed. (Springer).
Nguyen, M.Q., von Buchholtz, L.J., Reker, A.N., Ryba, N.J., and Davidson, S. (2021). Single-nucleus transcriptomic analysis of human dorsal root ganglion neurons. eLife *10*, e71752.
Parpaite, T., Brosse, L., Séjourné, N., Laur, A., Mechioukhi, Y., Delmas, P., and Coste, B. (2021). Patch-seq of mouse DRG neurons reveals candidate genes for specific mechanosensory functions. Cell Rep. *37*, 109914.
Phatarakijnirund, V., Mumm, S., McAlister, W.H., Novack, D.V., Wenkert, D., Clements, K.L., and Whyte, M.P. (2016). Congenital insensitivity to pain: Fracturing without apparent skeletal pathobiology caused by an autosomal dominant, second mutation in SCN11A encoding voltage-gated sodium channel 1.9. Bone *84*, 289–298.
Raman, I.M. and Ferster, D.L. (2022). The Annotated Hodgkin and Huxley: A Reader's Guide (New Jersey, USA: Princeton University Press).
Rostock, C., Schrenk-Siemens, K., Pohle, J., and Siemens, J. (2018). Human vs. mouse nociceptors - Similarities and differences. Neuroscience *387*, 13–27.
Scala, F., Kobak, D., Bernabucci, M., Bernaerts, Y., Cadwell, C.R., Castro, J.R., Hartmanis, L., Jiang, X., Laturnus, S., Miranda, E., et al. (2021). Phenotypic variation of transcriptomic cell types in mouse motor cortex. Nature *598*, 144–150.
Sopacua, M., Hoeijmakers, J.G.J., Merkies, I.S.J., Lauria, G., Waxman, S.G., and Faber, C.G. (2019). Small-fiber neuropathy: Expanding the clinical pain universe. J. Peripher. Nerv. Syst. *24*, 19–33.
Tavares-Ferreira, D., Shiers, S., Ray, P.R., Wangzhou, A., Jeevakumar, V., Sankaranarayanan, I., Cervantes, A., Reese, J.C., Chamessian, A., Copits, B.A., et al. (2022). Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. *Sci Transl Med. 14*, eabj8186.
Torkamani, A., Wineinger, N.E., and Topol, E.J. (2018). The personal and clinical utility of polygenic risk scores. Nat. Rev. Genet. *19*, 581–590.
Undrovinas, A.I., Shander, G.S., and Makielski, J.C. (1995). Cytoskeleton modulates gating of voltage-dependent sodium channel in heart. Am. J. Physiol. *269*, H203–H214.
Usoskin, D., Furlan, A., Islam, S., Abdo, H., Lönnerberg, P., Lou, D., Hjerling-Leffler, J., Haeggström, J., Kharchenko, O., Kharchenko, P.V., et al. (2015). Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat. Neurosci. *18*, 145–153.
Weiss, J., Pyrski, M., Jacobi, E., Bufe, B., Willnecker, V., Schick, B., Zizzari, P., Gossage, S.J., Greer, C.A., Leinders-Zufall, T., et al. (2011). Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. Nature *472*, 186–190.
Yang, Y., Wang, Y., Li, S., Xu, Z., Li, H., Ma, L., Fan, J., Bu, D., Liu, B., Fan, Z., et al. (2004). Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J. Med. Genet. *41*, 171–174.
Yuan, J., Matsuura, E., Higuchi, Y., Hashiguchi, A., Nakamura, T., Nozuma, S., Sakiyama, Y., Yoshimura, A., Izumo, S., and Takashima, H. (2013). Hereditary sensory and autonomic neuropathy type IID caused by an SCN9A mutation. Neurology *80*, 1641–1649.
Zhang, X., Priest, B.T., Belfer, I., and Gold, M.S. (2017). Voltage-gated Na+ currents in human dorsal root ganglion neurons. eLife *6*, e23235.
Zhang, X.Y., Wen, J., Yang, W., Wang, C., Gao, L., Zheng, L.H., Wang, T., Ran, K., Li, Y., Li, X., et al. (2013). Gain-of-function mutations in SCN11A cause familial episodic pain. Am. J. Hum. Genet. *93*, 957–966.

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Review article

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The role of neuroimmune interactions in musculoskeletal pain

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Abstract: Interactions of the immune system and the nociceptive system play an important role in the generation and maintenance of pain in musculoskeletal diseases and in disease development. In inflamed tissue peripheral nociceptive neurons are rendered hyperexcitable by proinflammatory cytokines, antigen/antibody complexes and other immune mediators. Spinal nociceptive neurons are rendered hyperexcitable with the support of microglial cells, the immune cells of the central nervous system. The so-elicited sensitization of pain pathways has a strong impact on pain processing in the brain. On the other hand, immune processes are regulated by the nervous system. Sensory neurons, by releasing neuropeptides, and efferent neurons of the sympathetic nervous system support immune processes which promote disease development.

Keywords: central sensitization; cytokines; musculoskeletal diseases; nociceptive neuron; peripheral sensitization.

Zusammenfassung: Interaktionen zwischen dem Immunsystem und dem nozizeptiven System spielen eine wichtige Rolle in der Erzeugung und Aufrechterhaltung von Schmerzen bei muskuloskelettalen Erkrankungen und bei der Entwicklung dieser Krankheiten. Im entzündeten Gewebe erzeugen proinflammatorische Zytokine, Antigen-Antikörper-Komplexe und andere Immunmediatoren eine Übererregbarkeit peripherer nozizeptiver Neurone. Im Rückenmark entsteht durch Unterstützung der Microglia, den Immunzellen des Zentralnervensystems, eine Übererregbarkeit spinaler nozizeptiver Neurone. Die dadurch ausgelöste Sensibilisierung der nozizeptiven Bahnen hat einen starken Einfluss auf die Schmerzentstehung im Gehirn. Andererseits werden Immunprozesse durch das Nervensystem reguliert. Sensorische Neurone, die Neuropeptide freisetzen, und efferente Fasern des sympathischen Nervensystems unterstützen die Immunprozesse, die die Krankheitsentwicklung vorantreiben.

Schlüsselwörter: Nozizeptives Neuron; Periphere Sensibilisierung; Zentrale Sensibilisierung; Zytokine; muskuloskelettale Erkrankungen.

Introduction

Both the nociceptive system and the immune system are essential for body protection. By creating pain, the nociceptive system is alerting the individual about threatening stimuli from outside and disturbances in our organs and tissues. The immune system protects our body by eliminating invading organisms such as viruses, bacteria, fungi etc. These systems do not work independently of each other. Via inflammation, immune processes may activate the nociceptive system and generate pain. However, there are also direct interactions between the immune and the nociceptive system. Nociceptive neurons express receptors for immune mediators, e.g. cytokine receptors, and, *vice versa*, immune cells express receptors for neuronal mediators, e.g. adrenergic receptors and receptors for neuropeptides.

Immune processes are also important in numerous non-infectious painful musculoskeletal diseases. This is quite obvious in autoimmune diseases such as rheumatoid arthritis in which own tissue is attacked by the immune system, after the break of tolerance, involving the whole spectrum of innate and adaptive immune mechanisms (McInnes and Schett, 2011). Even musculoskeletal diseases which are considered “degenerative”, e.g. osteoarthritis, are involving immune processes. The synovium of osteoarthritic joints is often highly inflamed and contains

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macrophages and other immune cells (Eitner et al., 2017). It is likely, therefore, that immune mechanisms are widely involved in musculoskeletal pain.

In order to understand the impact of the immune system on pain generation, one has to be aware of some basic principles of pain physiology. In a healthy individual pain is only evoked by application of noxious (high intensity) stimuli to normal tissue. Such stimuli activate the nociceptive system (Figure 1) which consists of neurons specialized for the detection of noxious stimuli. This "physiological pain" causes a defense reaction and helps to avoid noxious stimuli. Clinically relevant pain, occurring during a disease, is different. "Pathophysiological pain" is evoked by normally innocuous (low intensity) stimuli, e.g. by movements in the working range of a joint, because the nociceptive system is in a hyperexcitable (sensitized) state. Both "peripheral sensitization" (hyperexcitability of primary sensory neurons) and "central sensitization" (hyperexcitability of nociceptive sensory neurons in the CNS) contribute to the sensitized state. The patient may even experience ongoing pain. Clinically relevant pain is also elicited by damage of the nociceptive system itself. This pain is called "neuropathic pain" and characterized by abnormal pain sensations such as burning, often not related to noxious stimuli.

The expression of receptors of neuronal mediators in immune cells suggests that immune mechanisms are influenced by neurons. This will be addressed in the last section.

Pain generation by immune mechanisms

Sites of interaction of the immune and the nociceptive system

Figure 1 summarizes sites of interaction of the immune and the nociceptive system relevant for pain generation.

Immune mediators such as proinflammatory cytokines act on sensory neurons at the site of the disease (e.g. the joint). There they modify the sensitivity of the sensory endings of the nociceptive neurons. Furthermore, during diseases in the musculoskeletal system immune cells such as macrophages may invade the dorsal root ganglia of the afflicted segments and influence cell bodies of the sensory neurons (Eitner et al., 2017). Another important site of neuroimmune interactions is the spinal cord. Microglial cells are resident immune cells in the central nervous system. They show many similarities to peripheral macrophages although they are derived from a separate source, the yolk sac, from where they invade the central nervous system. In the developing brain microglial cells eliminate

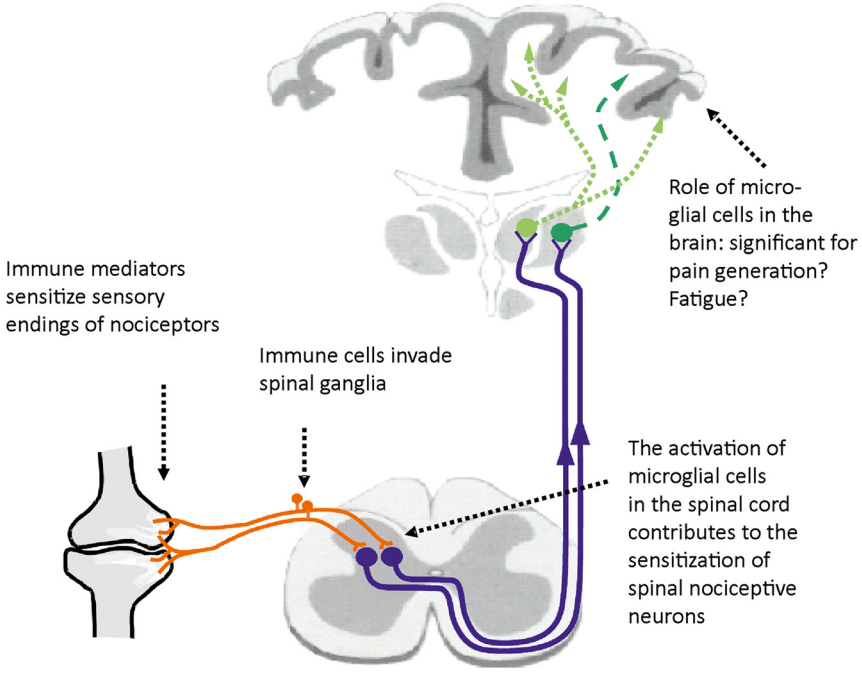

Figure 1: Schema of the nociceptive system. Sites and functions of the interaction of the immune system and the nociceptive system.

redundant synapses. During musculoskeletal diseases microglial cells can be "activated", assume a proinflammatory phenotype, and create a state of neuroinflammation. Together with astroglial cells they contribute to central sensitization in the spinal cord and thus to pain generation (McMahon and Malcangio, 2009).

The immune system of the brain is activated in neurodegenerative diseases such as Alzheimer's disease (Heppner et al., 2015). Whether the immune system of the brain and its mediators (e.g. cytokines) are important for pain generation is not clear. Cytokines are thought to cause disease symptoms such as fatigue, depression etc. (Lampa et al., 2012; Louati and Berenbaum, 2015).

The immune system and peripheral sensitization

The scheme in Figure 2A illustrates how nociceptive sensory neurons are influenced by the immune system.

It shows part of the cell membrane of a nociceptive sensory neuron. These neurons are equipped with ion channels that transduce noxious mechanical and thermal stimuli into receptor potentials (RP). The latter trigger action potentials (APs) by opening voltage-gated sodium channels which transmit the sensory information to the CNS. Under healthy conditions, ion channels of transduction are only opened by noxious (high intensity) stimuli. In addition,

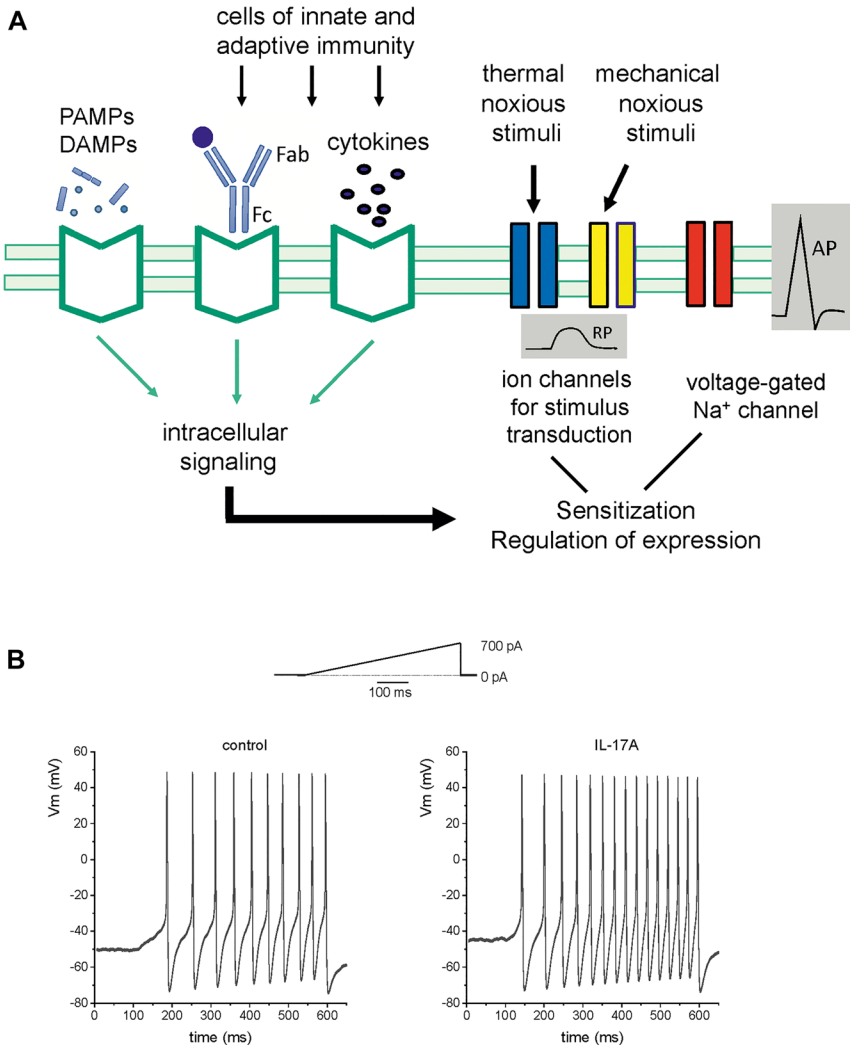

Figure 2: Membrane of a nociceptor and response to IL-17A.
A. Membrane of the sensory ending of a nociceptive sensory neuron with receptors (left side) and ion channels (right side). RP, receptor potential; AP, action potential; PAMP, pathogen associated molecular pattern; DAMP, damage associated molecular pattern. B. Effect of IL-17A on the responsiveness of an isolated cultured sensory neuron. Action potentials were elicited by a current ramp. For details see text.

sensory endings express receptors for mediators of the immune system. They activate different intracellular second messenger systems which modify ion channels of transduction and voltage-gated ion channels and lower their opening threshold. Mediators may also upregulate the channel expression. Together these processes render the sensory neuron hyperexcitable. *In vivo*, sensitized neurons exhibit a lowered excitation threshold and increased responses to suprathreshold stimuli. Thus palpation of a joint or movements in the walking range of the joint are sufficient to activate the neuron and to elicit pain.

Important sensitizing mediators are proinflammatory cytokines. A major proinflammatory cytokine in rheumatoid arthritis is tumor necrosis factor (TNF). It is released from macrophages, lymphocytes, endothelial cells, fibroblasts and others. TNF-neutralizing biologicals are key drugs in the treatment of rheumatoid arthritis. They can achieve remission of the disease although they do not cure it (McInnes and Schett, 2011). TNF is also a pain mediator. The injection of TNF into a normal knee joint induces within an hour a persistent sensitization of C-fiber nociceptors (nociceptive neurons with unmyelinated axons) of the joint (Richter et al., 2010). The nerve fibers develop pronounced responses to movements in the working range of the joint (before sensitization such stimuli evoke only few APs), and enhanced responses to noxious movements. In experimental arthritis models as well as in humans with rheumatoid arthritis the neutralization of TNF reduces pain before inflammation subsides, showing that TNF acts as a pain mediator (Ebersberger, 2018; Schaible, 2014).

Nociceptive sensory neurons are also sensitized by other proinflammatory cytokines (e.g. IL-6, IL-1β, IL-17). IL-6-neutralization causes disease remission in rheumatoid arthritis. IL-6 is also an important pain mediator. Pain sensitization by IL-6 is difficult to reverse, suggesting that IL-6 promotes chronic pain (Schaible, 2014). The role of IL-1β is unclear. Some preclinical studies showed pronociceptive effects of IL-1β (Mailhot et al., 2020), but IL-1 neutralization over weeks in clinical trials did not improve osteoarthritis pain more than placebo (Fleischmann et al., 2019). IL-17A is mainly released by Th17 cells, a specific group of T helper cells, besides the Th1 and Th2 cells. IL-17A contributes to the defence of bacteria and fungi and is involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, psoriasis arthritis, multiple sclerosis etc. (Zwicky et al., 2020). *In vivo*, IL-17A sensitizes C-fiber nociceptors of the joint for mechanical stimuli. *In vitro*, IL-17A increases the excitability of isolated and cultured sensory neurons (Figure 2B). In this neuron action potentials were elicited by a ramp of increasing current injection (starting at 100 ms, see inset). Five minutes after bath application of IL17A, the first action potential was elicited at a shorter latency (43.1 vs. 85.8 ms before IL-17A) and at less current, and during the ramp more action potentials were evoked than before IL-17A application. In cultured sensory neurons IL-17A upregulated the mechanosensitive TRPV4 ion channel, but not the TRPV1 channel, which is mainly involved in thermal hyperalgesia (Richter et al., 2019; Segond von Banchet et al., 2013).

Sensitization of nociceptive neurons can also be induced by antigen/antibody complexes. Antibodies bind antigens with their Fab-fragments, and with their Fc fragments they can bind to cells, e.g. mast cells and elicit cellular reactions. Antigen/antibody complexes can also bind to receptors for Fc fragments in nociceptive neurons and induce pronociceptive effects. This mechanism was proposed for the occurrence of pain in joints (arthralgia) before the outbreak of manifest rheumatoid arthritis. It is assumed that autoantibodies against citrullinated proteins (ACPA), play such a role (Catrina et al., 2017). Known ACPA targets in patients with rheumatoid arthritis are for example citrullinated epitopes on fibrinogen, vimentin (an ubiquitously expressed cytoskeletal protein), and collagen II (a major component of articular cartilage).

Figure 2A also displays receptors of DAMPs (damage associated molecular patterns) and PAMPs (pathogen associated molecular patterns). DAMPs consist of proteins, polysaccharides and other molecules which are released from damaged or dying cells. By activating pattern recognition receptors of immune cells, they provoke a non-infectious inflammatory response. In parallel, they can stimulate nociceptors. PAMPs are molecules of bacteria (e.g. lipopolysaccharides) which activate pattern recognition receptors (e.g. toll-like receptor 4) in cells of the innate immune system. Nociceptors express such receptors as well (Donnelly et al., 2020).

The expression of receptors for "disease mediators" on sensory neurons has important consequences. A beginning disease process may affect neurons before the disease becomes manifest. As mentioned, pain may not only occur *after* disease manifestation, it may appear *before* (Arend and Firestein, 2012; Catrina et al., 2017). Behavioral hyperalgesia *before* manifest joint pathology has also been observed in several models of immune-mediated arthritis (Ebbinghaus et al., 2019). Typically a proportion of sensory neurons shows an upregulation of ATF3, a marker of neuronal injury and stress. In glucose-6-phosphate isomerase (G6PI)-induced arthritis the ATF3-upregulation was

observed before inflammation became manifest, and it outlasted spontaneous disease remission (Ebbinghaus et al., 2019). Thus, musculoskeletal disease may exhibit a neuropathic pain component, and some musculoskeletal diseases may also be "neuronal diseases".

The immune system and central (spinal) sensitization

As shown in Figure 1, microglial cells in the spinal cord contribute to central sensitization. Hyperexcitable spinal cord neurons show lower excitation thresholds, enhanced responses to suprathreshold stimuli, and larger receptive fields. Spinal sensitization has many similarities with long term potentiation (LTP) in other areas of the CNS (Sandkühler, 2000). Sensitized ascending pain pathways will stronger activate the brain circuits involved in nociceptive processing, and they may trigger neuroplastic processes which generate a hypersensitive pain percept. Therefore, central sensitization amplifies pain intensity and often causes an expansion of painful areas beyond afflicted areas (Woolf and Salter, 2000). Many chronic pain conditions are characterized by a persistent state of central sensitization.

Spinal microglia significantly contributes to spinal sensitization (Clark et al., 2015; Ji et al., 2018). Microglial activation is induced by transmitters and neuromodulators which are released in the spinal cord from the central terminals of primary afferents. Microglial cells express receptors for such mediators including glutamate receptors (AMPA and NMDA receptors), TrkB receptors for BDNF, purinergic receptors P2X4 and P2X7 for ATP, and others (Old et al., 2015). Microglial activation is particularly intense in models of peripheral neuropathy, but can also be observed in the course of musculoskeletal diseases such as osteoarthritis (Old et al., 2015). Blocking of the activation of spinal microglial cells, e.g. by minocyclin prevents spinal sensitization (Gruber-Schoffnegger et al., 2013; König et al., 2021). Again proinflammatory cytokines play an important role in the sensitization process (Gruber-Schoffnegger et al., 2013; Old et al., 2015). There is evidence that different cytokines are not acting independently. Detailed information on interactions of TNF, IL-6, and IL-1β in the spinal cord has been published recently (König et al., 2021).

A caveat has to be added. A recent review (Mogil, 2020) pointed out qualitative sex differences in pain processing, and one area of controversy is the role of microglial activation in pain generation. It was reported that microglia is not required for mechanical pain hypersensitivity in female mice, and that female mice achieved similar levels of pain hypersensitivity using adaptive immune cells, likely T lymphocytes (Sorge et al., 2015). However, as discussed by Mogil (Mogil, 2020), some observations do not easily reconcile with the notion of the male specificity of microglial pain processing, including observations of significant effects of glial inhibitors, inhibitors of microglia-specific molecular targets or toxin-based microglial depletion in female rodents. Clearly, sex differences in the neuroimmune mediation of pain must be taken into account.

Regulation of immune processes by the nervous system

The nervous system can influence immune processes in the joint via different nerve fiber types (Levine et al., 1986). Peptidergic peripheral nociceptive sensory neurons release neuropeptides such as substance P and calcitonin gene-related peptide (CGRP, a strong vasodilator) from their sensory endings and cause "neurogenic inflammation". Efferent adrenergic postganglionic neurons of the sympathetic nervous system supply the musculoskeletal system and immune organs such as lymph nodes, spleen, and bone marrow. Many immune cells express adrenergic receptors. In addition, the organs in thorax and abdomen are supplied by efferent parasympathetic fibers (e.g. in the vagus nerve) allowing modification of immune processes by the parasympathetic nervous system. Further influences are mediated by the neuroendocrine system. The regulation of immune processes by the nervous system has also an impact on pain.

The immune system "uses" peripheral nociceptive neurons to support the induction of peripheral inflammation. Mice with selective deletion of IL-6 signaling in sensory neurons (SNS-gp130$^{-/-}$ mice which do not express the signaling unit gp130 in nociceptive sensory neurons) show significantly less release of CGRP in the joint and significantly less swelling after injection of the antigen into the knee joint (Ebbinghaus et al., 2015).

The sympathetic nervous system supports the development of inflammation (Schaible and Straub, 2014). Sympathectomized mice show a significant reduction of joint swelling and less severe pain behaviors (Ebbinghaus et al., 2012). A similar effect was observed after neutralization of TNF in the spinal cord (Boettger et al., 2010) supporting the view that spinal cord reflexes have an impact on joint inflammation (Waldburger and Firestein, 2010). Most likely the efferent effects are mediated by the sympathetic nervous system (Boettger et al., 2010).

The parasympathetic system which innervates organs in thorax and abdomen, is anti-inflammatory (Tracey, 2002). Inhibition of inflammation in the musculoskeletal system by the parasympathetic nervous system (van Maanen et al., 2010) must be indirect.

Conclusions

These data show that neuroimmune interactions are important for the understanding of musculoskeletal pain mechanisms. They further show that immune mechanisms are under neuronal control. An integrative view on both the nociceptive and the immune system may help to better understand both pain mechanisms and immune (disease) mechanisms. In the long term, neuroimmune interactions may become targets for therapeutic interventions.

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References

Arend, W.P. and Firestein, G.S. (2012). Pre-rheumatoid arthritis: Predisposition and transition to clinical synovitis. Nat. Rev. Rheumatol. *8*, 573–586.

Boettger, M.K., Weber, K., Grossmann, D., Gajda, M., Bauer, R., Bär, K.-J., Schulz, S., Voss, A., Geis, C., Bräuer, R., et al. (2010). Spinal TNF-α neutralization reduces peripheral inflammation and hyperalgesia and suppresses autonomic responses in experimental arthritis. A role for spinal TNF-α during induction and maintenance of peripheral inflammation. Arthritis Rheum. *62*, 1308–1318.

Catrina, A.I., Svensson, C.I., Malmström, V., Schett, G., and Klareskog, L. (2017). Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. Nat. Rev. Rheumatol. *13*, 79–86.

Clark, A.K., Gruber-Schoffnegger, D., Drdla-Schutting, R., Gerhold, K.J., Malcangio, M., and Sandkühler, J. (2015). Selective activation of microglia facilitates synaptic strength. J. Neurosci. *35*, 4552–4570.

Donnelly, C.R., Chen, O., and Ji, R.-R. (2020). How do sensory neurons sense danger signals? Trends Neurosci *43*, 822–838.

Ebbinghaus, M., Gajda, M., Boettger, M.K., Schaible, H.-G., and Bräuer, R. (2012). The anti-inflammatory effects of sympathectomy in murine antigen-induced arthritis are associated with a reduction of Th1 and Th17 responses. Ann. Rheum. Dis. *71*, 253–261.

Ebbinghaus, M., Müller, S., Segond von Banchet, G., Eitner, A., Wank, I., Hess, A., Hilger, I., Kamradt, T., and Schaible, H.-G. (2019). The contribution of inflammation and bone destruction to pain in arthritis – A study in murine glucose-6-phosphate isomerase (G6PI)-induced arthritis. Arthritis Rheumatol. *71*, 2016–2026.

Ebbinghaus, M., Segond von Banchet, G., Massier, J., Gajda, M., Bräuer, R., Kress, M., and Schaible, H.-G. (2015). Interleukin-6-dependent influence of nociceptive sensory neurons on antigen-induced arthritis. Arthritis Res. Ther. *17*, 334.

Ebersberger, A. (2018). The analgesic potential of cytokine neutralization with biologicals. Eur. J. Pharmacol. *835*, 19–30.

Eitner, A., Hofmann, G.O., and Schaible, H.-G. (2017). Mechanisms of osteoarthritic pain. Studies in humans and experimental models. Front. Mol. Neurosci. *10*, 349.

Fleischmann, R.M., Bliddal, H., Blanco, F.J., Schnitzer, T.J., Peterfy, C., Chen, S., Wang, L., Feng, S., Conaghan, P.G., Berenbaum, F., et al. (2019). A Phase II trial of lutikizumab, an anti-interleukin-1alpha/beta dual variable domain immunoglobulin, in knee osteoarthritis patients with synovitis. Arthritis Rheumatol. *71*, 1056–1069.

Gruber-Schoffnegger, D., Drdla-Schutting, R., Hönigsperger, C., Wunderbaldinger, G., Gassner, M., and Sandkühler, J. (2013). Induction of thermal hyperalgesia and synaptic long-term potentiation in the spinal cord lamina I by TNF-alpha and IL-1beta is mediated by glial cells. J. Neurosci. *33*, 6540–6551.

Heppner, F.L., Ransohoff, R.M., and Becher, B. (2015). Immune attack: The role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. *16*, 358–372.

Ji, R.R., Nackley, A., Huh, Y., Terrando, N., and Maixner, W. (2018). Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology *129*, 343–366.

König, C., Vazquez, E., Eß, S., Ebbinghaus, M., Vorpahl, B., Ebersberger, A., and Schaible, H.-G. (2021). Spinal interleukin-1β induces mechanical spinal hyperexcitability in rat: Interactions and redundancies with TNF and IL-6. J. Neurochem. *158*, 898–911.

Lampa, J., Westman, M., Kadetoff, D., Agreus, A.N., Le Maitre, E., Gillis-Haegerstrand, C., Andersson, M., Khademi, M., Corr, M., Christianson, C.A., et al. (2012). Peripheral inflammatory disease associated with centrally activated IL-1 system in humans and mice. Proc. Natl. Acad. Sci. U. S. A. *109*, 12728–12733.

Levine, J.D., Dardick, S.J., Roizen, M.F., Helms, C., and Basbaum, A.I. (1986). Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. J. Neurosci. *6*, 3423–3429.

Louati, K. and Berenbaum, F. (2015). Fatigue in chronic inflammation – A link to pain pathways. Arthritis Res. Ther. *254*, 17.

Mailhot, B., Christin, M., Tessandier, N., Sotoudeh, C., Bretheau, F., Turmel, R., Pellerin, E., Wang, F., Bories, C., Joly-Beauparlant, C., et al. (2020). Neuronal interleukin-1 receptors mediate pain in chronic inflammatory diseases. J. Exp. Med. *217*, e20191430.

McInnes, I.B. and Schett, G. (2011). The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. *365*, 2205–2219.

McMahon, S.B. and Malcangio, M. (2009). Current challenges in glia-pain biology. Neuron *64*, 46–54.

Mogil, J.S. (2020). Qualitative sex differences in pain processing: Emerging evidence of a biased literature. Nat. Rev. Neurosci. *21*, 353–365.
Old, E.A., Clark, A.K., and Malcangio, M. (2015). The role of glia in the spinal cord in neuropathic and inflammatory pain. Handb. Exp. Pharmacol. *227*, 145–170.
Richter, F., Natura, G., Loeser, S., Schmidt, K., Viisanen, H., and Schaible, H.-G. (2010). Tumor necrosis factor-α (TNF-α) causes persistent sensitization of joint nociceptors for mechanical stimuli. Arthritis Rheum. *62*, 3806–3814.
Richter, F., Segond von Banchet, G., and Schaible, H.-G. (2019). Transient receptor potential vanilloid 4 ion channel in C-fibres is involved in mechanonociception of the normal and inflamed joint. Sci. Rep. *9*, 10928.
Sandkühler, J. (2000). Learning and memory in pain pathways. Pain *88*, 113–118.
Schaible, H.-G. (2014). Nociceptive neurons detect cytokines in arthritis. Arthritis Res. Ther. *16*, 470.
Schaible, H.-G. and Straub, R.H. (2014). Function of the sympathetic supply in acute and chronic experimental joint inflammation. Auton. Neurosci: Basic Clin. *182*, 55–64.
Segond von Banchet, G., Boettger, M.K., König, C., Iwakura, Y., Bräuer, R., and Schaible, H.-G. (2013). Neuronal IL-17 receptor upregulates TRPV4 but not TRPV1 receptors in DRG neurons and mediates mechanical but not thermal hyperalgesia. Mol. Cell. Neurosci. *52*, 152–160.
Sorge, R.E., Mapplebeck, J.C., Rosen, S., Beggs, S., Taves, S., Alexander, J.K., Martin, L.J., Austin, J.S., Sotocinal, S.G., Chen, D., et al. (2015). Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat. Neurosci. *18*, 1081–1083.
Tracey, K.J. (2002). The inflammatory reflex. Nature *420*, 853–859.
van Maanen, M.A., Stoof, S.P., La Rosa, G.J., Vervoordeldonk, M.J., and Tak, P.P. (2010). Role of the cholinergic nervous system in rheumatoid arthritis: Aggravation of arthritis in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice. Ann. Rheum. Dis. *69*, 1717–1723.
Waldburger, J.-M. and Firestein, G.S. (2010). Regulation of peripheral inflammation by the central nervous system. Curr. Rheumatol. Rep. *12*, 370–378.
Woolf, C.J. and Salter, M.W. (2000). Neuronal plasticity: Increasing the gain in pain. Science *288*, 1765–1769.
Zwicky, P., Unger, S., and Becher, B. (2020). Targeting interleukin-17 in chronic inflammatory disease: A clinical perspective. J. Exp. Med. *217*, e20191123.

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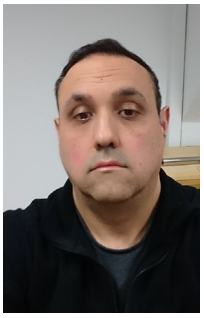

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Review article

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Glial cells as target for antidepressants in neuropathic pain

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Abstract: Several forms of chronic pain do not respond to the conventional analgesics, such as opioids, but can be treated with antidepressants, such as serotonin and noradrenalin reuptake inhibitors (SNRIs). Recent studies indicate that noradrenalin signalling is a key target for SNRI-induced analgesia in neuropathic pain. SNRIs inhibit chronic pain by blocking reuptake of noradrenalin and subsequent activation of adrenergic receptors on neurons in the dorsal horn of the spinal cord. However, in the nervous system, various subtypes of adrenergic receptors are highly expressed by astrocytes and microglial cells. Activation of these receptors on astrocytes engages complex intracellular signalling pathways and prevents inflammatory changes of microglia, which in turn can affect neuronal activity. Hence, SNRIs-induced modulations of the glial cell physiology can impact neural circuit functions and pain perception. In this review, we summarize our current knowledge on the impact of SNRIs on glial cells and in modulating chronic pain in experimental animal models.

Keywords: adrenergic receptors; astrocytes; chronic pain; microglia; serotonin and norepinephrine reuptake inhibitors (SNRI).

Zusammenfassung: Konventionelle Analgetika wie Opioide helfen häufig nicht bei chronischen Schmerzen, interessanterweise im Gegensatz zu Antidepressiva wie Serotonin- und Noradrenalin-Wiederaufnahmehemmern (SNRI). Neuere Untersuchungen zeigen nun, dass in der Tat Noradrenalin-abhängige Signalwege bei SNRI-induzierter Analgesie beteiligt sind. SNRIs induzieren erhöhte Noradrenalin-Spiegel im Dorsalhorn des Rückenmarks. Die folgende Aktivierung adrenerger Rezeptoren der Spinalneurone führt zu einer deutlichen Reduktion der neuropathischen Schmerzen. Im Nervensystem werden jedoch verschiedene Subtypen von adrenergen Rezeptoren in hohem Maße von Astrozyten und Mikrogliazellen exprimiert. Die Aktivierung dieser Rezeptoren auf Astrozyten setzt komplexe intrazelluläre Signalwege in Gang und verhindert entzündliche Veränderungen der Mikroglia, die ihrerseits die neuronale Aktivität beeinflussen können. Daher können SNRI-induzierte Modulationen der Gliazellphysiologie die Funktionen neuronaler Schaltkreise und die Schmerzwahrnehmung beeinflussen. In dieser Übersicht fassen wir unser aktuelles Wissen über die Auswirkungen von SNRIs auf Gliazellen und die Modulation chronischer Schmerzen in experimentellen Tiermodellen zusammen.

Schlüsselwörter: chronischer Schmerz; adrenerge Rezeptoren; Astrozyten; Mikrogliazellen; Noradrenalin; Serotonin- und Noradrenalin-Wiederaufnahmehemmer (SNRI).

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Introduction

Chronic pain is one of the most common global health problems, which incurs high healthcare costs and loss of productivity. In addition to impaired physical well-being, chronic pain has been linked to numerous mental comorbidities such as anxiety and depression (Bair et al., 2003), drug dependence (Salsitz, 2016), and reduced quality of life (Bair et al., 2003; Dueñas et al., 2016). At present most of the analgesic drugs used in the treatment of chronic pain including opioids and antidepressants tend to exhibit tolerance and side effects. Also, a consistent fraction of patients does not respond to commonly used analgesic drugs. For this reason, there is a global effort to find novel targets to develop more effective analgesic drugs with reduced or no side effects.

In general, analgesic drugs seem to mainly target neuronal excitability and synaptic plasticity (Carroll et al., 2007; Nakajima et al., 2012). However, very little is known about the mode of action of most of the analgesics on glial cells – a major class of cells in the nervous system. Several recent studies have demonstrated that two types of glial cells, i.e. astrocytes and microglia, play critical roles in the pathogenesis of chronic pain and its long-term maintenance (Ji et al., 2016). Indeed, activated glial cells release neuroactive factors that can be *pain-inducing* or *pain-alleviating*, which engage neurons in bidirectional communication and lead to short- and long-term changes in the neural circuit of pain. One strategy to relieve short-term pain is to block glial cell activation in response to injury, but a more promising strategy is to prompt glia to release neuroactive molecules that can avert pain induction or even lead to analgesia.

Among antidepressants, SNRIs are most frequently used to treat refractory forms of neuropathic pain (Finnerup et al., 2005, 2015; Lee and Chen, 2010). SNRIs increase the availability of two neuromodulators, serotonin (5-hydroxytryptamine, 5-HT) and noradrenalin (NA), which are known to modulate pain perception. 5-HT and NA can exert a dual effect on pain hypersensitivity (Tavares et al., 2021). 5-HT suppresses pain through the activation of 5-HT1A/B and 5-HT7 receptors in the spinal cord (Newman-Tancredi et al., 2018; Santello et al., 2017), while the activation of 5-HT3 and 5-HT2A receptors facilitates pain (Oyama et al., 1996). Although 5-HT can modulate pain, NA is the primary neuromodulator responsible for the analgesic effects of SNRIs. For NA, two mechanisms of action have been suggested (**1**) activation of noradrenergic descending pathways and (**2**) release of NA from sympathetic fibers sprouting into dorsal root ganglia (DRGs) (Kremer et al., 2016). Similar to 5-HT, while NA can reduce hyperalgesia via activation of α2 and β2 adrenergic receptors (Yalcin et al., 2010), it can evoke hyperalgesia via activation of α1 adrenergic receptor (Kohro et al., 2020). Additionally, pain conditions can induce plastic changes in specific cell-types, which can further contribute toward the pain-relieving effect of antidepressants (Kimura et al., 2013). The pain modulation by adrenergic pathways could be further shaped by immune cells and cytokines. Indeed, activation of α2a adrenergic receptors might contribute to the long-term analgesia by preventing neuroinflammatory changes such as reduced production of inflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and prostaglandins (Liu and Eisenach, 2005). Here, we want to highlight that even though we know various sites of action of SNRIs, the precise mechanism of their action on chronic pain remains elusive. It is tempting to speculate that SNRIs engage distinct pathways involving neurons and glial cells, and modulate several regions across the brain, spinal cord and DRGs (Obata, 2017). Since astrocytes and microglia express a wide variety of adrenergic receptors, these drugs could engage astrocytic and microglial signaling mechanisms to influence neuronal activity and synaptic plasticity. In this review, we will highlight some of the known effects of SNRIs on glial cells in pain modulation.

Astrocytes in pain

Astrocytes are one of the most abundant glial cells in the CNS and account for 20–40% of all glial cells (Herculano-Houzel, 2014). Astrocytes perform homeostatic functions, such as maintenance of extracellular ion (K^+) concentrations and neurotransmitter levels (glutamate and GABA), regulation of blood brain barrier, and provide energy substrates (e.g., lactate) to neurons. Several studies suggest that astrocyte activation can induce long-lasting changes in the neural circuit of pain, and play a major role in the amplification, maintenance and chronicity of pain (Ji et al., 2013). Reactive astrocytes release a variety of cytokines and chemokines, such as TNF-α and CCL2, which can potentiate chronic pain by loss of GABAergic inhibition and by strengthening pain memory traces, respectively (Gosselin et al., 2010; Kronschläger et al., 2016; Tang et al., 2021). Furthermore, astrocytes in chronic pain conditions lose their homeostatic properties such as regulation of ion and neurotransmitter levels and their receptor expression, leading to neuronal hyperexcitability and subsequently contribute to pain induction (Li et al., 2019). For example, it

has been shown that in response to peripheral nerve injury (PNI), the primary somatosensory (S1) cortex astrocytes upregulate glutamate receptors such as mGluR5, which mediates aberrant Ca^{2+} signaling and enhanced production of the synaptogenic factor thrombospondin-1 (TSP1). TSP1 promotes maintenance of chronic pain by formation of aberrant synapses that lead to the rewiring of neural circuits of pain (Kim et al., 2016a). Moreover, the optogenetic activation of spinal cord (SC) astrocytes induced mechanical allodynia and thermal hyperalgesia by disinhibiting neurons in the spinal cord dorsal horn (SDH) (Nam et al., 2016; Yamashita et al., 2014). At the same time, inhibiting astrocytic activity can reduce neuropathic pain (Meller et al., 1994).

Astrocytes and their interactions with SNRIs

Astrocytes are known to express several sub-types of α and β adrenergic receptors (Gaidin et al., 2020; Hertz et al., 2010; Salm and McCarthy, 1992), and activation of these receptors can modulate pain. A recent study identified a subgroup of astrocyte that get activated in response to a painful stimulus (intraplantar capsaicin injection). These astrocytes are located in the superficial laminae of the SDH and express transcription factor called Hes5. A direct chemogenetic activation or an activation of α1a receptors on Hes5+ astrocytes induced mechanical hyperalgesia (Kohro et al., 2020). In a PNI model, the specific deletion of α1a in Hes5+ astrocytes enhanced the analgesic action of duloxetine, a drug often prescribed for peripheral diabetic neuropathic pain (DPN) and fibromyalgia patients (Bravo et al., 2019). This finding suggests α1a adrenergic receptors on astrocytes can be a target for co-adjuvant drugs to associate with duloxetine, in order to obtain the same analgesic effect but with a lower doses and side effects (Kohro et al., 2020).

A metabolic profiling study revealed that venlafaxine affects amino acid metabolism, cellular growth, and proliferation pathways in astrocytes (Sun et al., 2017). The analgesic effect of venlafaxine was induced by alteration in the amino acid metabolism and decreased glutamate levels, which in turn impaired glutamate-dependent synaptic plasticity. In addition, venlafaxine inhibited the production of pro-inflammatory cytokines IL-6 and IL-1β, and reduced activation of two important molecular pathways of pain development, STAT3 and JNK (He et al., 2021). Another study showed that a NA and specific serotonergic antidepressant (NaSSA) mirtazapine induce the production of glial cell line-derived neurotrophic factor (GDNF) in astrocytes (Hisaoka-Nakashima et al., 2019), which reduce ectopic discharges within sensory neurons and reversed sensory hypersensitivity developed in neuropathic pain (Boucher et al., 2000).

In conclusion, astrocytes seem to play a key role in pathogenesis of neuropathic pain and might be a target of action of SNRIs (Figure 1 B, C). In future, detailed studies are essential to unravel the mechanism of action of SNRIs on astrocytes and to develop new molecules which further enhance the efficacy of SNRIs in pain treatment.

Microglia in pain

In the CNS, microglia are the resident immune cells and continuously survey the neuropil to clear up cellular debris and infectious agents (Hanisch and Kettenmann, 2007). Microglia have been shown to be active players in the pathogenesis of chronic pain (Ji and Suter, 2007). Pain differs from other neurological diseases for its rapid onset: following treatment with microglial activators and inhibitors, pain behavior will change within minutes to tens of minutes (Berta et al., 2014; Tsuda et al., 2003). Recent studies indicate that neuromodulators released by microglia can rapidly alter synaptic plasticity, a driving force for the pathogenesis of pain after tissue and nerve injury (Luo et al., 2014; Woolf and Salter, 2000).

After PNI, SDH microglia are strongly activated (Guan et al., 2016; Tsuda et al., 2005). This activation requires neuronal activity (Wen et al., 2007; Xie et al., 2009) and the release of sensory neuron-derived pro-inflammatory factors, including colony-stimulating factor 1 (CSF1), caspase-6, neuregulin-1, cytokines such as IL-1β, CCL2, CXCL1, CCL21, extracellular proteases and ATP (reviewed in Inoue and Tsuda, 2018). All these molecules have been shown to efficiently activate distinct receptors on microglia and concomitantly enhance the expression of receptors for these factors, such as P2X4, P2Y12, and CX3CR1. The activated microglia further increase production of TNF-α, IL-1β and brain-derived neurotrophic factor (BDNF), which in turn fine-tunes excitatory and inhibitory synapses, and ultimately enhance pain signal transmission to the brain. For example, TNF-α and IL-1β enhance excitatory and suppress inhibitory synaptic transmission, while BDNF disinhibits GABA-mediated inhibition in the SC (Guan et al., 2016; Kawasaki et al., 2008). Since microglial cells are engaged in neuroinflammation after nerve injury and influence synaptic connectivity and neurotransmission, they are critical for the induction of hyperalgesia and allodynia in chronic pain conditions (Figure 1 B, C).

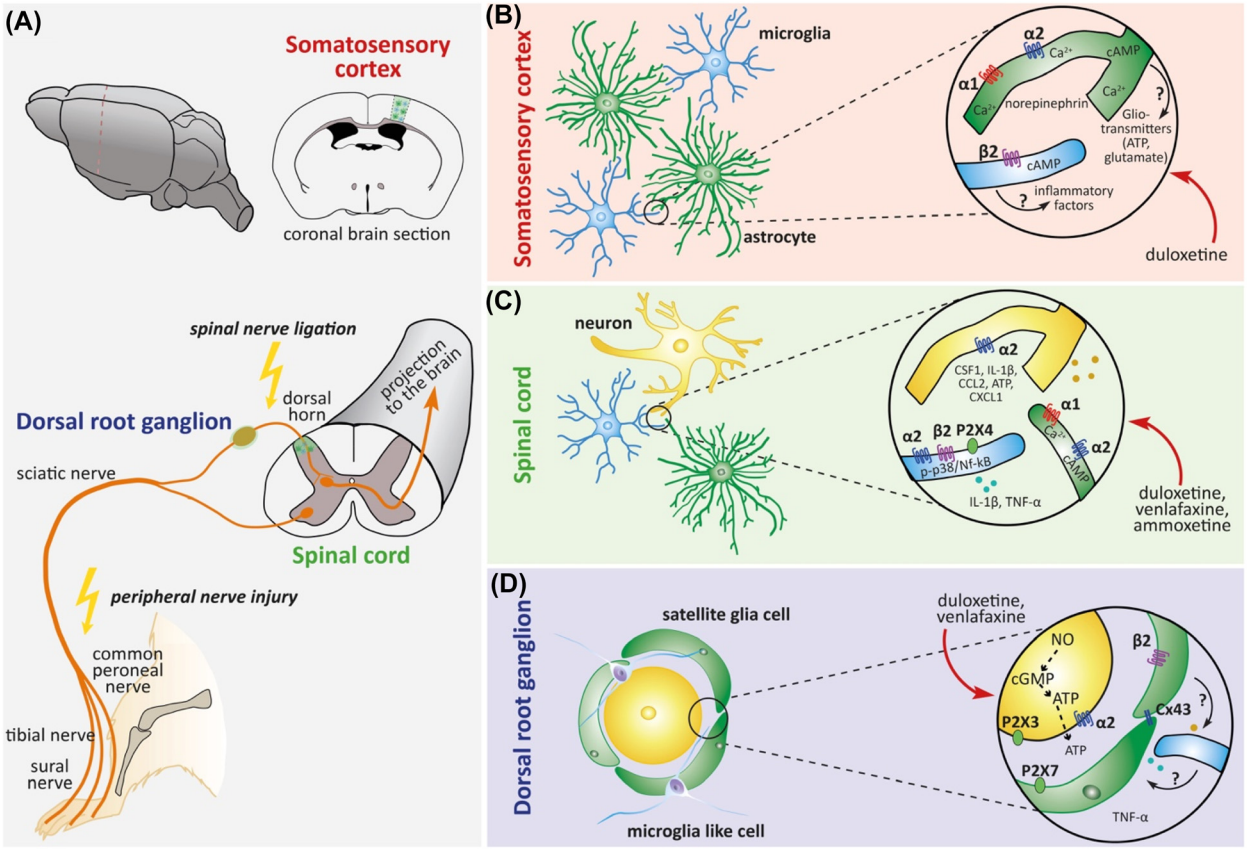

Figure 1: Action of SNRIs in peripheral and central nervous system glial cells and their involvement in chronic pain.
(A) SNRIs induce analgesia by acting in areas from the central and peripheral nervous system. **(B)** In the brain, the increase in noradrenalin concentration in the synaptic cleft induced by SNRIs can activate α1 and α2 receptors in astrocytes (green), and β2 receptors in microglia (blue). The activation of α1 adrenergic signaling in somatosensory cortex astrocytes triggers rapid astrocytic Ca^{2+} elevation (Agarwal et al., 2017), while activation of α2 receptors leads to a simultaneous increase in intracellular Ca^{2+} and reduced cAMP levels, but their consequences to the analgesic effect of SNRI is least understood. β2 receptors activation in microglia elevates intracellular levels of cAMP, which in turn can modulate neuroinflammation and impair cortical experience-dependent plasticity. The potential anti-inflammatory effect of β2 receptor activation in cortical microglia for the analgesic effect of SNRIs remains unexplored. **(C)** The action of noradrenalin in the spinal cord neural circuit seems to be a key mechanism responsible for the analgesic effect of SNRIs. Noradrenalin inhibits projections and interneurons (yellow) in the dorsal horn through activation of α2 adrenergic receptors. However, adrenergic receptors in both microglia (blue) and astrocytes (green) in the spinal cord can influence the analgesic effect of SNRIs. Activation of the α1 receptor in astrocytes of the dorsal horn induces pain, but consequences of α2 activation remains unexplored. α2 and β2 adrenergic receptors activation in spinal microglia reduces the activation p38/NF-κB pathway and the release of pro-inflammatory and neuroregulatory mediators during neuropathic pain. **(D)** The analgesic effect of SNRIs in the peripheral nervous system occurs by the activation of the β2 receptor in SGCs (green). This activation suppresses the release of ATP and TNF-α, and reduces microglia-like cells/macrophages (blue) activation in DRGs. ATP can induce mechanical sensitization by acting in P2X3 directly in nociceptors (yellow) or inducing the expression of TNF by acting in P2X7 receptors in SGSc. Given that TNF disrupts gap junctions, the SNRIs may play an indirect effect on connexins, such as connexin-4. Ca^{2+} = calcium ions; cAMP = cyclic adenosine monophosphate; ATP = adenosine triphosphate; CSF1 = colony-stimulating factor 1; IL-1β = interleukin 1 beta; TNF-α = tumor necrosis factor alpha; cGMP = cyclic guanosine monophosphate; Cx43 = connexin 43.

Microglia and their interactions with SNRIs

In particular, microglial cells express α2 and β2 adrenergic receptors, which are well known for their role in neuropathic pain (Kremer et al., 2016). Activation of these receptors was shown to attenuate microglial reactivity through reduced activation p38 MAPK pathway (Morioka et al., 2009; Zhang et al., 2016b) and can ameliorate neuropathic mechanical hypersensitivity (Choucair-Jaafar et al., 2009). In models of neuropathic pain, such as DNP or vincristine-induced neuropathic pain, duloxetine treatment reduced microglial activation (Tawfik et al., 2018). Moreover, duloxetine downregulated TNF-α and NGF levels in a rat model of intervertebral disk (IVD) degeneration (Handa et al., 2016).

Interestingly, intrathecal injection of Poly Lactic-*co*-Glycolic Acid (PLGA) nanoparticles containing a low dose of duloxetine was able to attenuate the p38/NF-κB pathway and the production of inflammatory cytokines in the rat spinal nerve ligation (SNL) model (Kim et al., 2021). Several studies highlighted an inhibitory effect of duloxetine on microglia P2X4R (Nagata et al., 2009; Yamashita et al., 2016), and the consequent analgesic effects persisted even after 5-HT and/or NA signals were inhibited by 5-HT depletion or a NA neurotoxin in a PNI induced pain hypersensitivity (Yamashita et al., 2016). Duloxetine results in a general inhibition of microglia in the spinal cord that associates with the reduction of pain hyperalgesia. In astroglia–microglia co-culture, venlafaxine prevented microglial activation, reduced pro-inflammatory cytokine secretion (IL-6 and INF-γ), and increased TGF-β release (Vollmar et al., 2008).

A potent SNRI ammoxetine was found to have analgesic effect on neuropathic pain, fibromyalgia-related pain or inflammatory pain models (Zhang et al., 2016, 2018). In a rat DNP model, which shows increased activation of microglia but not astrocytes in the SC, four weeks of ammoxetine treatment significantly reduced mechanical allodynia and improved depressive-like behavior. Similar to other SNRIs, ammoxetine reduces microglial activation, accumulation of pro-inflammatory cytokines and activation of p38 and c-Jun N-terminal kinase (JNK) in the SC (Zhang et al., 2018).

Taken together, a general mode of action of SNRIs on microglial cells is to prevent their activation and subsequent production of inflammatory cytokines, which contributes to the overall analgesic efficacy of these class of drugs (Table 1). Understanding the impact of microglia in analgesic efficacy of SNRIs can promote the development of new specific drugs that have little to no severe complications or better fit for other neuropathic conditions.

Peripheral glia in pain

For a holistic targeting of chronic pain, it is of utmost importance to not only to comprehend the changes in the SC and brain, but also the cellular and molecular changes in the DRGs (Berger et al., 2021; Berta et al., 2017; Esposito et al., 2019; Liem et al., 2016). DRGs are the part of the peripheral nervous system (PNS) recognized as targets for (neuro)-modulation to combat chronic pain (Berta et al., 2017). In the DRG, two types of glia namely satellite glial cells (SGCs) and macrophages as microglia-like cells (MLCs) are central to pain cascades (Ahimsadasan et al., 2022; Murray et al., 2021). SGCs closely attach to neurons and share many properties with astrocytes of the CNS (Hanani, 2005), including recycling the excess of extracellular glutamate (Carozzi et al., 2008), potassium buffering (Tang et al., 2010) and gap junctions (connexin 43) coupling (Spray and Hanani, 2019). SGCs become gap-junction coupled to neurons in response to neuronal injury (Kim et al., 2016b; Spray and Hanani, 2019). The macrophages in the DRGs are equivalent to the microglia of the CNS (Mori et al., 2003) and perform the task of an immune system, and get activated during acute and chronic pain (Yu et al., 2020).

When considering the sensory processing along the DRGs, Schwann cells are important cellular partners. They are the myelinating cells of the PNS and are in close contact with the neurons and immune cells. Upon peripheral injuries, Schwann cells are involved in the enhanced release of chemokines/cytokines (MCP-1, TNFα, etc.), recruitment of macrophages, gene expression changes in the sensory neurons (Martini et al., 2008; Ohtori et al., 2004; Poplawski et al., 2018; Wagner and Myers, 1996).

Interestingly, direct stimulation of DRG neurons releases ATP, activates surrounding SGCs via P2X7 receptors and leads to an increased TNF-α release from SGCs. This

Table 1: Clinically used antidepressants and their targets in CNS and PNS glial cells.

SNRI	Target	Cell type	CNS/PNS region	References
Duloxetine	GFAP, connexin 43, p38/NF-κB TNF-α, Iba1	Astrocytes, microglia SGCs,	SC, DRG	Tawfik et al. (2018), Jeanson et al. (2016), Okada et al. (2020), Sun et al. (2012), Handa et al. (2016), and Kim et al. (2021)
Ammoxetine	p-p38, p-JNK	Microglia	SC	Zhang et al. (2018)
Venlafaxine	S100B, β2-AR, iba1	Astrocytes, SGCs, microglia	PNS (DRG)	Zychowska et al. (2015) and Bohren et al. (2013)

communication between neurons and glial cells could be disrupted by blocking L-type calcium channel (Zhang et al., 2007). Furthermore, the activation of P2X7 receptors in SGCs reduced pain by downregulating P2X3 receptors in nociceptive neurons (Chen et al., 2008).

DRG glial cells and their interactions with SNRIs

Still, there is a no clear description of the exact mechanisms of action of SNRIs on the glial cells of the DRGs. Recent studies showed the involvement of various chemokines and cytokines in the induction and chronification of pain (Gosselin et al., 2010; Tang et al., 2010). Several SNRIs such as venlafaxine, duloxetine, and terbutaline have been shown to decreased TNF-α level in SGCs and attenuated neuropathic allodynia through a mechanism dependent on β2-AR (Bohren et al., 2013; Handa et al., 2016), which is exclusively expressed on SGCs, and not on neurons (Shen et al., 2022). In addition, venlafaxine was able to induce analgesic effects and weaker allodynia by reducing microglia activation as detected by Iba1-immunolabeling in the DRG (Zychowska et al., 2015).

Although the mode of action of SNRIs in DRGs is unclear, they may attenuate sensitivity to pain by reducing the recruitment of immune cells to the DRGs through inhibition of ATP and TNF-α release, which leads to decreased interaction between SGCs and DRG neurons (Figure 1D). However, an extended analysis of the signaling pathways affected by SNRIs in DRGs is needed to capture the full extent of action of these drugs across the nervous system.

Perspective

It is evident that we know very little about the role of glial cells across the nervous system in the pain modulation. In the context of the pain pathophysiology, glial cells have been mostly studied in the context of neuroinflammation with a limited number of cellular markers and the handful of chemokines and cytokines. Another caveat has been that most of the studies outlining the role of glia cells in pain have been performed in the cell culture system, which many times couldn't be directly interpolated *in vivo*. With emerging imaging technologies, cell-type specific expression of genetically encoded ion sensors, optogenetics and chemogenetics tools, mouse transgenic tools, and high-throughput single cell RNA-sequencing, metabolomics and proteomics, will enable glial-biologists and pain researchers to work closely to dissect the role of astrocytes and microglial cells in pain modulations and chronification in the PNS and CNS.

The increased understanding of the role glial cells in chronic pain will not only help us tackle already know pain conditions, but also to develop an efficient treatment strategy for the newly arising conditions. For examples, COVID-19 often causes peripheral or central neuroinflammation, it is anticipated that several chronic pain complications of COVID-19 will be neuropathic (Drożdżal et al., 2020). Although antidepressants such as SNRIs are effective in the treatment of a small class of neuropathic pain conditions, it might be worth repurposing SNRIs to treat COVID-19 related neuropathic pain. In the long-term, the key aim is to gain a precise understanding of the analgesic mechanisms of antidepressants, and to identify distinct cellular targets of these drugs. These developments will enable to further develop specific drugs or therapies to treat neuropathic pain without undesirable cognitive side effects of SNRIs.

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References

Agarwal, A., Wu, P.-H., Hughes, E.G., Fukaya, M., Tischfield, M.A., Langseth, A.J., Wirtz, D., and Bergles, D.E. (2017). Transient opening of the mitochondrial permeability transition pore induces microdomain calcium transients in astrocyte processes. Neuron *93*, 587–605.e7.

Ahimsadasan, N., Reddy, V., and Kumar, A. (2022). Neuroanatomy, gorsal root ganglion [Updated 2021 Sep 7]. StatPearls [Internet] (StatPearls Publishing: Treasure Island (FL)). https://www.ncbi.nlm.nih.gov/books/NBK532291/.

Bair, M.J., Robinson, R.L., Katon, W., and Kroenke, K. (2003). Depression and pain comorbidity: A literature review. Arch. Intern. Med. *163*, 2433–2445.

Berger, A.A., Liu, Y., Possoit, H., Rogers, A.C., Moore, W., Gress, K., Cornett, E.M., Kaye, A.D., Imani, F., Sadegi, K., et al. (2021). Dorsal root ganglion (DRG) and chronic pain. Anesthesiol. Pain Med. *11*, e113020.

Berta, T., Park, C.K., Xu, Z.Z., Xie, R.G., Liu, T., Lü, N., Liu, Y.C., and Ji, R.R. (2014). Extracellular caspase-6 drives murine inflammatory pain via microglial TNF-α secretion. J. Clin. Invest. *124*, 1173–1186.

Berta, T., Qadri, Y., Tan, P.H., and Ji, R.R. (2017). Targeting dorsal root ganglia and primary sensory neurons for the treatment of chronic pain. Expert Opin. Ther. Targets *21*, 695–703.

Bohren, Y., Tessier, L.H., Megat, S., Petitjean, H., Hugel, S., Daniel, D., Kremer, M., Fournel, S., Hein, L., Schlichter, R., et al. (2013). Antidepressants suppress neuropathic pain by a peripheral β2-adrenoceptor mediated anti-TNFα mechanism. Neurobiol. Dis. *60*, 39–50.

Boucher, T.J., Okuse, K., Bennett, D.L., Munson, J.B., Wood, J.N., and McMahon, S.B. (2000). Potent analgesic effects of GDNF in neuropathic pain states. Science *290*, 124–127.

Bravo, L., Llorca-Torralba, M., Berrocoso, E., and Micó, J.A. (2019). Monoamines as drug targets in chronic pain: Focusing on neuropathic pain. Front. Neurosci. *13*, 1268.

Carozzi, V.A., Canta, A., Oggioni, N., Ceresa, C., Marmiroli, P., Konvalinka, J., Zoia, C., Bossi, M., Ferrarese, C., Tredici, G., et al. (2008). Expression and distribution of 'high affinity' glutamate transporters GLT1, GLAST, EAAC1 and of GCPII in the rat peripheral nervous system. J. Anat. *213*, 539–546.

Carroll, I., Mackey, S., and Gaeta, R. (2007). The role of adrenergic receptors and pain: The good, the bad, and the unknown. Semin. Anesth. Perioperat. Med. Pain *26*, 17–21.

Chen, Y., Zhang, X., Wang, C., Li, G., Gu, Y., and Huang, L.Y. (2008). Activation of P2X7 receptors in glial satellite cells reduces pain through downregulation of P2X3 receptors in nociceptive neurons. Proc. Natl. Acad. Sci. U. S. A. *105*, 16773–16778.

Drożdżal, S., Rosik, J., Lechowicz, K., Machaj, F., Szostak, B., Majewski, P., Rotter, I., and Kotfis, K. (2020). COVID-19: Pain management in patients with SARS-CoV-2 infection-molecular mechanisms, challenges, and perspectives. Brain Sci. *10*, 465.

Dueñas, M., Ojeda, B., Salazar, A., Mico, J.A., and Failde, I. (2016). A review of chronic pain impact on patients, their social environment and the health care system. J. Pain Res. *9*, 457–467.

Esposito, M.F., Malayil, R., Hanes, M., and Deer, T. (2019). Unique characteristics of the dorsal root ganglion as a target for neuromodulation. Pain Med. *20*, S23–S30.

Finnerup, N.B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R.H., Gilron, I., Haanpää, M., Hansson, P., Jensen, T.S., et al. (2015). Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. Lancet Neurol. *14*, 162–173.

Finnerup, N.B., Otto, M., McQuay, H.J., Jensen, T.S., and Sindrup, S.H. (2005). Algorithm for neuropathic pain treatment: An evidence based proposal. Pain *118*, 289–305.

Gaidin, S.G., Zinchenko, V.P., Sergeev, A.I., Teplov, I.Y., Mal'tseva, V.N., and Kosenkov, A.M. (2020). Activation of alpha-2 adrenergic receptors stimulates GABA release by astrocytes. Glia *68*, 1114–1130.

Gosselin, R.D., Bebber, D., and Decosterd, I. (2010). Upregulation of the GABA transporter GAT-1 in the gracile nucleus in the spared nerve injury model of neuropathic pain. Neurosci. Lett. *480*, 132–137.

Guan, Z., Kuhn, J.A., Wang, X., Colquitt, B., Solorzano, C., Vaman, S., Guan, A.K., Evans-Reinsch, Z., Braz, J., Devor, M., et al. (2016). Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat. Neurosci. *19*, 94–101.

Hanani, M. (2005). Satellite glial cells in sensory ganglia: From form to function. Brain Res. Rev. *48*, 457–476.

Handa, J., Sekiguchi, M., Krupkova, O., and Konno, S. (2016). The effect of serotonin-noradrenaline reuptake inhibitor duloxetine on the intervertebral disk-related radiculopathy in rats. Eur. Spine J. *25*, 877–887.

Hanisch, U.K. and Kettenmann, H. (2007). Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. *10*, 1387–1394.

He, J.H., Liu, R.P., Peng, Y.M., Guo, Q., Zhu, L.B., Lian, Y.Z., Hu, B.L., Fan, H.H., Zhang, X., and Zhu, J.H. (2021). Differential and paradoxical roles of new-generation antidepressants in primary astrocytic inflammation. J. Neuroinflammation *18*, 1–14.

Herculano-Houzel, S. (2014). The glia/neuron ratio: How it varies uniformly across brain structures and species and what that means for brain physiology and evolution. Glia *62*, 1377–1391.

Hertz, L., Lovatt, D., Goldman, S.A., and Nedergaard, M. (2010). Adrenoceptors in brain: Cellular gene expression and effects on astrocytic metabolism and [Ca2+]i. Neurochem. Int. *57*, 411.

Hisaoka-Nakashima, K., Taki, S., Watanabe, S., Nakamura, Y., Nakata, Y., and Morioka, N. (2019). Mirtazapine increases glial cell line-derived neurotrophic factor production through lysophosphatidic acid 1 receptor-mediated extracellular signal-regulated kinase signaling in astrocytes. Eur. J. Pharmacol. *860*, 172539.

Inoue, K. and Tsuda, M. (2018). Microglia in neuropathic pain: Cellular and molecular mechanisms and therapeutic potential. Nat. Rev. Neurosci. *19*, 138–152.

Ji, R.R., Berta, T., and Nedergaard, M. (2013). Glia and pain: Is chronic pain a gliopathy? Pain *154*, S10–S28.

Ji, R.R., Chamessian, A., and Zhang, Y.Q. (2016). Pain regulation by non-neuronal cells and inflammation. Science *354*, 572–577.

Ji, R.R. and Suter, M.R. (2007). p38 MAPK, microglial signaling, and neuropathic pain. Mol. Pain *3*, 33.

Kawasaki, Y., Zhang, L., Cheng, J.K., and Ji, R.R. (2008). Cytokine mechanisms of central sensitization: Distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J. Neurosci. *28*, 5189–5194.

Kim, S.I., Shin, J., Tran, Q., Park, H., Kwon, H.H., Shin, N., Hwang, J.A., Shin, H.J., Lee, J., Lee, W.H., et al. (2021). Application of PLGA nanoparticles to enhance the action of duloxetine on microglia in neuropathic pain. Biomater. Sci. *9*, 6295–6307.

Kim, S.K., Hayashi, H., Ishikawa, T., Shibata, K., Shigetomi, E., Shinozaki, Y., Inada, H., Roh, S.E., Kim, S.J., Lee, G., et al. (2016a). Cortical astrocytes rewire somatosensory cortical circuits for peripheral neuropathic pain. J. Clin. Invest. *126*, 1983–1997.

Kim, Y.S., Anderson, M., Park, K., Zheng, Q., Agarwal, A., Gong, C., Saijilafu, Young, L., He, S., LaVinka, P.C., et al. (2016b). Coupled activation of primary sensory neurons contributes to chronic pain. Neuron *91*, 1085–1096.

Kimura, M., Hayashida, K., Eisenach, J.C., Saito, S., and Obata, H. (2013). Relief of hypersensitivity after nerve injury from systemic donepezil involves spinal cholinergic and γ-aminobutyric acid mechanisms. Anesthesiology *118*, 173–180.

Kohro, Y., Matsuda, T., Yoshihara, K., Kohno, K., Koga, K., Katsuragi, R., Oka, T., Tashima, R., Muneta, S., Yamane, T., et al. (2020). Spinal astrocytes in superficial laminae gate brainstem descending control of mechanosensory hypersensitivity. Nat. Neurosci. *23*, 1376–1387.

Kremer, M., Salvat, E., Muller, A., Yalcin, I., and Barrot, M. (2016). Antidepressants and gabapentinoids in neuropathic pain: Mechanistic insights. Neuroscience *338*, 183–206.

Kronschläger, M.T., Drdla-Schutting, R., Gassner, M., Honsek, S.D., Teuchmann, H.L., and Sandkühler, J. (2016). Gliogenic LTP spreads widely in nociceptive pathways. Science *354*, 1144–1148.

Lee, Y.C. and Chen, P.P. (2010). A review of SSRIs and SNRIs in neuropathic pain. Expet Opin. Pharmacother. *11*, 2813–2825.

Li, T., Chen, X., Zhang, C., Zhang, Y., and Yao, W. (2019). An update on reactive astrocytes in chronic pain. J. Neuroinflammation *16*, 140.

Liem, L., van Dongen, E., Huygen, F.J., Staats, P., and Kramer, J. (2016). The dorsal root ganglion as a therapeutic target for chronic pain. Reg. Anesth. Pain Med. *41*, 511–519.

Liu, B. and Eisenach, J.C. (2005). Hyperexcitability of axotomized and neighboring unaxotomized sensory neurons is reduced days after perineural clonidine at the site of injury. J. Neurophysiol. *94*, 3159–3167.

Luo, C., Kuner, T., and Kuner, R. (2014). Synaptic plasticity in pathological pain. Trends Neurosci. *37*, 343–355.

Martini, R., Fischer, S., López-Vales, R., and David, S. (2008). Interactions between Schwann cells and macrophages in injury and inherited demyelinating disease. Glia *56*, 1566–1577.

Meller, S.T., Dykstra, C., Grzybycki, D., Murphy, S., and Gebhart, G.F. (1994). The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropharmacology *33*, 1471–1478.

Mori, I., Goshima, F., Koshizuka, T., Imai, Y., Kohsaka, S., Koide, N., Sugiyama, T., Yoshida, T., Yokochi, T., Kimura, Y., et al. (2003). Iba1-expressing microglia respond to herpes simplex virus infection in the mouse trigeminal ganglion. Mol. Brain Res. *120*, 52–56.

Murray, I., Bhanot, G., and Bhargava, A. (2021). Neuron-glia-immune triad and cortico-limbic system in pathology of pain. Cells *10*, 1553.

Nagata, K., Imai, T., Yamashita, T., Tsuda, M., Tozaki-Saitoh, H., and Inoue, K. (2009). Antidepressants inhibit P2X4 receptor function: A possible involvement in neuropathic pain relief. Mol. Pain *5*, 20.

Nakajima, K., Obata, H., Iriuchijima, N., and Saito, S. (2012). An increase in spinal cord noradrenaline is a major contributor to the antihyperalgesic effect of antidepressants after peripheral nerve injury in the rat. Pain *153*, 990–997.

Nam, Y., Kim, J.H., Kim, J.H., Jha, M.K., Jung, J.Y., Lee, M.G., Choi, I.S., Jang, I.S., Lim, D.G., Hwang, S.H., et al. (2016). Reversible induction of pain hypersensitivity following optogenetic stimulation of spinal astrocytes. Cell Rep. *17*, 3049–3061.

Newman-Tancredi, A., Bardin, L., Auclair, A., Colpaert, F., Depoortère, R., and Varney, M.A. (2018). NLX-112, a highly selective 5-HT. Brain Res. *1688*, 1–7.

Obata, H. (2017). Analgesic mechanisms of antidepressants for neuropathic pain. Int. J. Mol. Sci. *18*, 2483.

Ohtori, S., Takahashi, K., Moriya, H., and Myers, R.R. (2004). TNF-alpha and TNF-alpha receptor type 1 upregulation in glia and neurons after peripheral nerve injury: Studies in murine DRG and spinal cord. Spine *29*, 1082–1088.

Oyama, T., Ueda, M., Kuraishi, Y., Akaike, A., and Satoh, M. (1996). Dual effect of serotonin on formalin-induced nociception in the rat spinal cord. Neurosci. Res. *25*, 129–135.

Poplawski, G., Ishikawa, T., Brifault, C., Lee-Kubli, C., Regestam, R., Henry, K.W., Shiga, Y., Kwon, H., Ohtori, S., Gonias, S.L., et al. (2018). Schwann cells regulate sensory neuron gene expression before and after peripheral nerve injury. Glia *66*, 1577–1590.

Salm, A.K. and McCarthy, K.D. (1992). The evidence for astrocytes as a target for central noradrenergic activity: Expression of adrenergic receptors. Brain Res. Bull. *29*, 265–275.

Salsitz, E.A. (2016). Chronic pain, chronic opioid addiction: A complex nexus. J. Med. Toxicol. *12*, 54–57.

Santello, M., Bisco, A., Nevian, N.E., Lacivita, E., Leopoldo, M., and Nevian, T. (2017). The brain-penetrant 5-HT. Neurobiol. Dis. *106*, 214–221.

Shen, S., Tiwari, N., Madar, J., Mehta, P., and Qiao, L.Y. (2022). Beta 2-adrenergic receptor mediates noradrenergic action to induce cyclic adenosine monophosphate response element-binding protein phosphorylation in satellite glial cells of dorsal root ganglia to regulate visceral hypersensitivity. Pain *163*, 180–192.

Spray, D.C. and Hanani, M. (2019). Gap junctions, pannexins and pain. Neurosci. Lett. *695*, 46–52.

Sun, L., Fang, L., Lian, B., Xia, J.J., Zhou, C.J., Wang, L., Mao, Q., Wang, X.F., Gong, X., Liang, Z.H., et al. (2017). Biochemical effects of venlafaxine on astrocytes as revealed by 1 H NMR-based metabolic profiling. Mol. Biosyst. *13*, 338–349.

Tang, J., Bair, M., and Descalzi, G. (2021). Reactive astrocytes: Critical players in the development of chronic pain. Front. Psychiatr. *12*, 682056.

Tang, X., Schmidt, T.M., Perez-Leighton, C.E., and Kofuji, P. (2010). Inwardly rectifying potassium channel Kir4.1 is responsible for the native inward potassium conductance of satellite glial cells in sensory ganglia. Neuroscience *166*, 397–407.

Tavares, I., Costa-Pereira, J.T., and Martins, I. (2021). Monoaminergic and opioidergic modulation of brainstem circuits: New insights into the clinical challenges of pain treatment? Front. Pain Res. *2*, 696515.

Tawfik, M.K., Helmy, S.A., Badran, D.I., and Zaitone, S.A. (2018). Neuroprotective effect of duloxetine in a mouse model of diabetic neuropathy: Role of glia suppressing mechanisms. Life Sci. *205*, 113–124.

Tsuda, M., Inoue, K., and Salter, M.W. (2005). Neuropathic pain and spinal microglia: A big problem from molecules in “small” glia. Trends Neurosci. *28*, 101–107.

Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., and Inoue, K. (2003). P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature *424*, 778–783.

Vollmar, P., Haghikia, A., Dermietzel, R., and Faustmann, P.M. (2008). Venlafaxine exhibits an anti-inflammatory effect in an inflammatory co-culture model. Int. J. Neuropsychopharmacol. *11*, 111–117.

Wagner, R. and Myers, R.R. (1996). Schwann cells produce tumor necrosis factor alpha: Expression in injured and non-injured nerves. Neuroscience *73*, 625–629.

Wen, Y.R., Suter, M.R., Kawasaki, Y., Huang, J., Pertin, M., Kohno, T., Berde, C.B., Decosterd, I., and Ji, R.R. (2007). Nerve conduction blockade in the sciatic nerve prevents but does not reverse the activation of p38 mitogen-activated protein kinase in spinal microglia in the rat spared nerve injury model. Anesthesiology *107*, 312–321.

Woolf, C.J. and Salter, M.W. (2000). Neuronal plasticity: Increasing the gain in pain. Science *288*, 1765–1769.

Xie, W., Strong, J.A., and Zhang, J.M. (2009). Early blockade of injured primary sensory afferents reduces glial cell activation in two rat neuropathic pain models. Neuroscience *160*, 847–857.

Yalcin, I., Tessier, L.H., Petit-Demoulière, N., Waltisperger, E., Hein, L., Freund-Mercier, M.J., and Barrot, M. (2010). Chronic treatment

with agonists of beta(2)-adrenergic receptors in neuropathic pain. Exp. Neurol. *221*, 115–121.
Yamashita, A., Hamada, A., Suhara, Y., Kawabe, R., Yanase, M., Kuzumaki, N., Narita, M., Matsui, R., and Okano, H. (2014). Astrocytic activation in the anterior cingulate cortex is critical for sleep disorder under neuropathic pain. Synapse *68*, 235–247.
Yamashita, T., Yamamoto, S., Zhang, J., Kometani, M., Tomiyama, D., Kohno, K., Tozaki-Saitoh, H., Inoue, K., and Tsuda, M. (2016). Duloxetine inhibits microglial P2X4 receptor function and alleviates neuropathic pain after peripheral nerve injury. PLoS One *11*, e0165189.
Yu, X., Liu, H., Hamel, K.A., Morvan, M.G., Yu, S., Leff, J., Guan, Z., Braz, J.M., and Basbaum, A.I. (2020). Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain. Nat. Commun. *11*, 264.
Zhang, T.T., Xue, R., Fan, S.Y., Fan, Q.Y., An, L., Li, J., Zhu, L., Ran, Y.H., Zhang, L.M., Zhong, B.H., et al. (2018). Ammoxetine attenuates diabetic neuropathic pain through inhibiting microglial activation and neuroinflammation in the spinal cord. J. Neuroinflammation *15*, 176.
Zhang, T.T., Xue, R., Zhu, L., Li, J., Fan, Q.Y., Zhong, B.H., Li, Y.F., Ye, C.Y., and Zhang, Y.Z. (2016). Evaluation of the analgesic effects of ammoxetine, a novel potent serotonin and norepinephrine reuptake inhibitor. Acta Pharmacol. Sin. *37*, 1154–1165.
Zhang, X., Chen, Y., Wang, C., and Huang, L.Y. (2007). Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. Proc. Natl. Acad. Sci. U. S. A. *104*, 9864–9869.
Zychowska, M., Rojewska, E., Makuch, W., Przewlocka, B., and Mika, J. (2015). The influence of microglia activation on the efficacy of amitriptyline, doxepin, milnacipran, venlafaxine and fluoxetine in a rat model of neuropathic pain. Eur. J. Pharmacol. *749*, 115–123.

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Review article

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Brain-based interventions for chronic pain

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Abstract: Brain circuits involved in pain chronicity shift from areas involved in nociceptive processing to those associated with emotional and motivational processes. They overlap with circuits relevant for anxiety, fear and depression and are characterized by deficient prefrontal control mechanisms. Noninvasive brain stimulation techniques such as repetitive transcranial magnetic stimulation, transcranial direct and alternating current stimulation directly impact on these circuits and pain. Neurofeedback and brain-computer interfaces as well as various types of cognitive and behavioral interventions also alter these circuits. The analysis of brain changes related to pain chronicity helps to mechanistically tailor interventions to patient characteristics, can increase treatment efficacy and efficiency and can identify new treatment approaches.

Keywords: magnetic resonance imaging; neurofeedback; neurostimulation; personalized treatment; psychological treatment.

Zusammenfassung: Chronische Schmerzen entstehen in Schaltkreisen des Gehirns, die weniger mit nozizeptiver Verarbeitung zu tun haben, sondern in emotionale und motivationale Verarbeitungsprozesse involviert sind. Diese überlappen mit Schaltkreisen, die für Angst, Furcht und Depression relevant sind und sind auch durch eine defiziente präfrontale Hemmung charakterisiert. Verfahren der nichtinvasiven Hirnstimulation wie die repetitive Hirnstimulation, transkranielle Gleichstromstimulation oder die transkranielle Wechselstromstimulation können diese Schaltkreise und damit Schmerz direkt beeinflussen. Neurofeedback und Gehirn-Computer Schnittstellen ebenso wie verschiedene kognitive und verhaltensbezogene Trainingsverfahren können diese Hirnregionen ebenfalls verändern. Die Analyse von Hirnveränderungen, die mit chronischem Schmerz in Zusammenhang stehen, kann Interventionen mechanistisch auf Patientencharakteristika zuschneiden, die Effektivität und Effizienz von Behandlungen verbessern und trägt dazu bei, neue Behandlungsansätze zu entwickeln.

Schlüsselwörter: Neurofeedback; kognitive Therapie und Verhaltenstherapie; Neurostimulation; funktionelle Magnetresonanztomographie; personalisierte Therapie.

Introduction and objectives

Brain-based treatments for chronic pain include treatments that directly stimulate the brain by impacting on neuronal activity by noninvasive neurostimulation, neurofeedback and brain-computer interfaces or change brain circuits indirectly by altering the psychological processing of pain or behavioral responses to pain. In recent years, more information has become available about the structural and functional brain changes that are related to chronic pain and it has been shown that these changes go beyond nociceptive circuits and involve emotional and motivational changes. Successful treatments should reverse these changes. This overview will first describe typical brain changes associated with the experience of chronic pain and then discuss how neurostimulation in animals and humans as well as neurofeedback and targeted cognitive and behavioral interventions might impact on them. Future research should focus on tailored mechanistic treatments related to specific pain-related brain mechanisms. This will also aid in the development of new and more personalized treatments.

Brain circuits involved in chronicity

Chronic pain cannot be equated with acute pain, which is characterized by brain regions involved in nociception such as the primary and secondary somatosensory cortex, the anterior cingulate cortex, the posterior insula, the periaqueductal grey and the thalamus and their respective connections (Apkarian et al., 2005; Wager et al., 2013). Evidence from longitudinal imaging studies as well

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as meta-analytic research suggest that chronic pain is characterized by a shift towards brain areas supporting emotion, motivation, and memory (Baliki et al., 2012; Hashmi et al., 2013). This involves frontal regions related to pain control but also limbic regions such as the nucleus accumbens and the hippocampus involving reward, learning and memory processes. Here multiple networks may interact and contribute to different aspects of chronic pain (Figure 1).

In addition to activation-related networks, resting state networks of the brain have been examined (Pfannmöller and Lotze, 2019). In both tonic pain models and chronic pain patient samples, Lee et al. (2021) observed that specifically resting state interconnections in the sensorimotor network and the frontoparietal network, which has been linked to attentional modulation and coping with pain, were related to ongoing pain. In addition, connectivity between the dorsomedial prefrontal cortex and the ventral striatum was also related to pain chronicity. A disrupted default mode network has also been observed in chronic pain (Alshelh et al., 2018). In resting state EEG studies increased connectivity at theta and gamma frequencies in frontal brain areas as well as global network reorganization at gamma frequencies was observed in chronic pain patients and increased high frequency oscillations in prefrontal cortex were linked to fluctuations in pain intensity (Baliki et al., 2011; May et al., 2019). In addition, widespread slowing of neural oscillations and an increase of theta, beta and alpha oscillations have been found (Pinheiro et al., 2016).

Many of these areas have been found to have structural changes, for example, altered grey matter density (cf. May, 2011). In addition, white matter changes have been examined using diffusion tensor imaging. Here specifically reduced connectivity in tracts involved in descending pain modulation has been observed in chronic pain patients and these changes have also been related to deficient cognitive pain control (for a review see Kuner and Flor, 2016). It is a matter of debate to what extent the changes seen in chronic pain are pain-specific or may reflect comorbid states auch as anxiety or depression, medication effects or if these changes are induced by the long-lasting states of pain themselves. In neuropathic pain states such as phantom limb pain or complex regional pain syndrome, alterations in the sensorimotor cortices have been prominently observed (Moseley and Flor, 2012), but areas involved in affective modulation of pain, such as the anterior insula, have also been found to be changed. Finally, descending inhibitory systems of pain control have also been found to be disturbed in chronic pain with a focus in frontal areas, the parabrachial nucleus and subcortical regions such as the hypothalamus and amygdala.

The evidence summarized above suggests that chronic pain states differ from acute pain by a stronger focus on brain circuits involved in learning and memory, affective and motivational processes that are not sufficiently targeted by classical analgesic pharmacological interventions. Similar to other neuropsychiatric diorders chronic pain states call for interventions that directly target altered brain states on a network-level (cf., Mercer Lindsay et al., 2021).

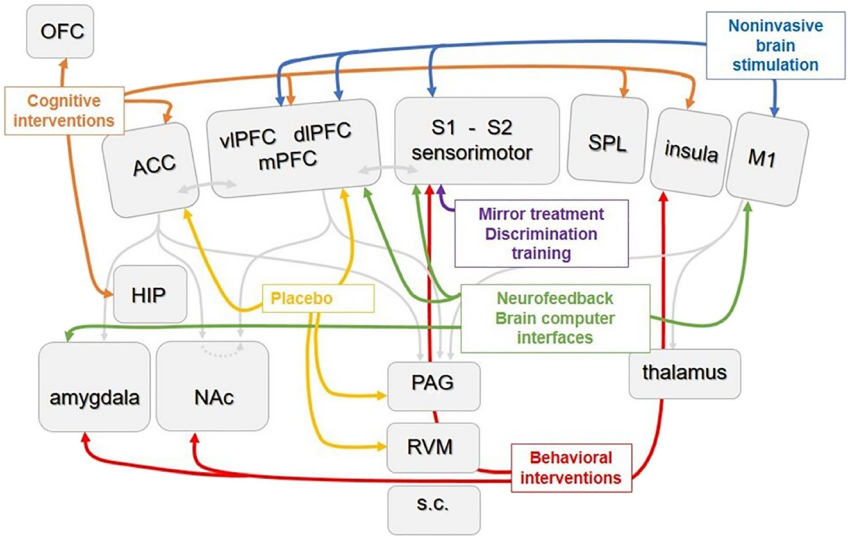

Figure 1: Brain regions that could be targeted by brain-based interventions based on neuroimaging evidence of chronic pain as well as successful interventions. The grey lines indicate brain circuits relevant for chronic pain. The colored lines refer to main targets of the brain-based interventions.

Neurostimulation

Given that network alterations in many cortical and subcortical areas have been associated with chronic pain, noninvasive stimulation of the brain by magnetic (transcranial magnetic stimulation, TMS) and electrical methods (transcranial electrical stimulation, TES) has been suggested as a means to influence circuits involved in chronic pain. TMS is based on magnetic stimulation that creates action potentials and can have inhibitory or excitatory effects, depending on the stimulation rate (Hallett, 2007). Specifically, repetitive transcranial magnetic stimulation (rTMS) with low repetition rates under or at about 1 Hz decreases and high repetition rates of ≥5 Hz increases brain excitability beyond the stimulation period, thereby promoting neuronal plasticity (Ziemann et al., 2008).

A special variant is theta burst stimulation (TBS) that has the advantage of shorter application time and can also be applied in excitatory (continuous TBS, cTBS) or inhibitory (intermittent TBS, iTBS) protocols. TES has been used as direct current stimulation (tDCS) where a weak current is introduced into the tissue by a bipolar montage, with anodal stimulation showing excitatory and cathodal stimulation inducing inhibitory effects (Paulus, 2011). In transcranial alternating current stimulation (tACS) the current alternates between the electrodes and can influence cortical rhythms. In addition, invasive brain stimulation has been employed, which has commonly used motor cortex or deep brain stimulation in areas such as the thalamus, internal capsule, and periaqueductal or periventricular grey matter. Since efficacy and long-term effects are limited, these studies will not be considered here (cf. Nguyen et al., 2011).

The most common sites for application on noninvasive brain stimulation treatments have been the motor cortex, the somatosensory cortex and the dorsolateral prefrontal cortex. High frequency rTMS of the motor cortex has produced pain relief in chronic pain patients, especially neuropathic pain patients in a number of controlled studies (for review Cruccu et al., 2016) but the quality of the studies, the duration of follow-up and the proneness to bias is often a problem. A direct comparison of excitatory repetitive TMS and excitatory (intermittent) theta burst stimulation of the motor cortex (André-Obadia et al., 2021) revealed a superiority of the 20 Hz rTMS protocol with respect to pain reduction and fatigue. The authors assumed that the optimal matching with the intrinsic oscillation might have been the decisive variable.

Choice of the dorsolateral prefrontal cortex has been motivated by the assumption that it has an important role in pain inhibition and suppression as well as cognitive control and its stimulation has been shown to have small effects on pain intensity in chronic pain (Seminowicz and Moayedi, 2017). In a chronic pain model, Seminowicz et al. (2018) applied excitatory rTMS over left DLPFC before injecting NGF in healthy subjects. Compared with sham rTMS, active rTMS significantly reduced pain severity, muscle soreness and the size of the painful body area. The authors suggested that descending modulatory circuits or effects on cognitive aspects of pain might have mediated these effects. Likewise, De Martino et al. (2019) found lower muscle pain scores and reduced pain pressure thresholds as well as decreased frontal N30 somatosensory evoked potentials and increased TMS map volume after repetitive high frequency rTMS in a nerve growth factor model of muscle pain. The authors suggested that rTMS of DLPFC modulates pain-induced sensorimotor cortical adaptations and suggest an important role of prefrontal to basal-ganglia function in sensorimotor cortical excitability and pain processing.

Small effects have also been reported for tDCS over the same regions (O'Connell et al., 2018). In patients with phantom limb pain, Kikkert et al. (2019) used anodal tDCS over the sensorimotor cortex contralateral to the amputated limb in a within-participants, double-blind, sham-controlled design. The amputees performed phantom hand movements during anodal transcranial direct current stimulation. One session of tDCS relieved phantom limb pain with reduced activity in the sensorimotor cortex. The changes in pain and sensorimotor activity were correlated with activation in the posterior insula and secondary somatosensory cortex. McPhee and Graven-Nielsen (2021) used the medial prefrontal cortex as target for excitatory tDCS in chronic back pain patients. This might be beneficial given that changes in medial prefrontal areas are characteristic of chronic pain and frontostriatal brain circuits have been found to predict chronicity (Baliki et al., 2012). The authors observed no significant effects on pain, psychological or psychophysical outcomes, however, correlational analysis suggested higher efficacy of the intervention in patients with a severely impaired descending pain inhibitory control system. Thus, the choice of the patient groups that shows deficiencies in the targeted mechanisms might be important in non-invasive brain stimulation studies.

The method of tACS has only recently be used in the control of chronic pain. Ahn et al. (2019) employed 10 Hz tACS bilaterally over the primary somatosensory cortex and showed reduction in ongoing back pain and disability in chronic low back pain patients. Electrophysiological

recordings displayed an increase in alpha oscillations in both somatosensory and frontal regions. tACS can influence brain rhythms and thus permit to make causal inferences about their significance (Hohn et al., 2019).

In terms of the diverse types of neuromodulation therapies in the human context discussed in this article, research in animal models is still scarce and mostly limited to brain stimulation approaches. Not only is it highly challenging to apply therapies based on cognitive modulation, conditioned pain modulation and neurofeedback to animal models, but also the small size of the brain presents challenges to test rTMS. Electrical brain stimulation, both invasive deep stimulation and direct current transcranial stimulation (tDCS), are being increasingly tested in animal models. Indeed, rodent models can complement and support neurostimulation-based therapies in at least two ways: (i) Broad application of neurostimulation therapies is still restricted by major concerns about their efficacy, variable duration of analgesic effects, variability of outcomes across different types of chronic pain as well as safety (Cruccu et al., 2016; Garcia-Larrea and Peyron, 2007; Lefaucheur et al., 2020; Nguyen et al., 2011). Animal studies can offer opportunities to test multiple different protocols and treatment regimens with varying strength, locus and repetitions of stimulation as well as diverse types of chronic pain at a much lower cost, level of difficulty and faster time frames, thereby playing a predictive role and helping streamline the design of clinical studies. (ii) Animal studies can provide deeper mechanistic insights into neurostimulation-induced analgesia, particularly in terms of establishing cellular resolution and causal relationships. Indeed, there is a broad consensus that the largest hindrance in implementing neurostimulation approaches in routine therapy is given by the large gaps in knowledge about the regions and identity of brain circuits that yield beneficial effects (Garcia-Larrea and Peyron, 2007; Lefaucheur et al., 2020; Nguyen et al., 2011).

As per the focus on rTMS of the primary M1 motor cortex in clinical studies, most of the animal experiments have targeted the motor cortex, with early studies employing invasive brain stimulation followed by a more recent application of tDCS in mouse brain (de Andrade et al., 2019; Franca et al., 2013; Lopes et al., 2019; Nguyen et al., 2011; Pagano et al., 2012; Souza et al., 2018). Anodal, but not cathodal, stimulation of M1 was found to suppress hypersensitivity in a number of models of acute and chronic pain. Despite this, a number of gaps remain in terms of utility of modelling motor cortex stimulation in terms of translational value, such as (i) therapeutic efficacy at chronic stages of established, typically drug therapy-refractory pain, such as is the case when a patient is likely to receive neurostimulation therapy, (ii) potential value of repeating cycles of stimulation and, (iii) potential of impacting on negative affect and affective comorbidities of chronic pain. In a recent study, we addressed these questions by longitudinally implementing two cycles of repetitive anodal M1 tDCS over five days each and observed that, as compared to single phasic stimulation, repetitive and cyclic stimulation evoked stronger analgesia, led to pain relief for longer durations (up to nine days after the last cycle), affected a larger number of modalities of pain and reversed chronically established hypersensitivity (Gan et al., 2021a). Importantly, these effects were achieved without hindering locomotion and overall motor activity, and were accompanied by suppression of pain-associated aversion and reduction in anxiety in neuropathic mice (Gan et al., 2021a). Taken together with the increasingly non-invasive and user friendly, modern designs of tDCS, these findings suggest that in the future, the patient can be put in a position to flexibly receive or self-administer pain relief on demand and choose multiple cycles of stimulation as required and as per the particular profile of pain and associated comorbidities, i.e. a significant step toward personalized medicine.

While the motor cortex is the preferred locus for brain stimulation therapies, there is much interest in targeting various areas of the prefrontal cortex (PFC) for management of chronic pain. Clinical studies in chronic pain patients yielded disappointing to mixed results (Ferreira et al., 2019; O'Connell et al., 2018), while two recent reports on healthy human subjects suggest potential beneficial effects for modulation of the DLPFC particularly in acute to subacute plasticity phenomena underlying the transition from physiological to pathological pain (De Martino et al. 2019; Seminowicz et al. 2018). Prefrontal anodal tDCS has recently also been modelled in mice (Gan et al., 2021a,b) and demonstrated efficacy of targeting the medial prefrontal and cingulate domains of the prefrontal cortex in preventing the progression of neuropathic hypersensitivity as well as reversing established neuropathic pain; interestingly, allodynia (hypersensitivity to normally innocuous stimuli) was preferentially suppressed as compared to hyperalgesia (exaggerated sensitivity to noxious stimuli). Unlike several widely used analgesic drugs, such as carbamazepine, opioids, amongst others, there was no tolerance development to repetitive cycles of prefrontal tDCS. Importantly, this study also led to key mechanistic insights into how repetitive tDCS impacts on brain function (Gan et al., 2021b). Direct recordings of cortical activity at single cell resolution in behaving mice that received anodal tDCS or sham stimulation demonstrated that while single stimulation episodes lead to an increase

in activity of neocortical neurons, spreading widely across neocortical domains, repetitive cycles of stimulation quickly leads to adaptation and remodelling of brain activity by differentially affecting the activity of specific classes of GABAergic inhibitory interneurons; this ultimately reduces the recruitment of excitatory neurons in key brain regions, which are target regions of the prefrontal cortex, upon application of stimuli that associated with allodynia, explaining thereby the analgesic effect.

Indeed, a number of preclinical studies have shed light into the nature of brain regions modulated by neocortical tDCS using c-Fos-based activity mapping (de Andrade et al., 2019; Pagano et al., 2012). While studies employing single phasic stimulation demonstrate modulation of thalamic nuclei (e.g. Pagano et al., 2012), this is not observed in studies employing repetitive stimulation regimens that yield higher efficacy and duration of analgesia (Gan et al., 2021a,b). Regions that consistently emerge as key targets across several studies include the anterior cingulate cortex, the posterior insula and diverse domains of the periaqueductal grey. For example, repetitive prefrontal tDCS specifically lowers the activity of the rostral domain of the anterior cingulate cortex, a region that is key to aversion and negative affect (Rainville et al., 1997; Tan et al., 2017). Human studies suggest that the posterior insula encodes pain intensity, serving a 'dial' function (Segerdahl et al., 2015), which is consistent with animal studies showing that the posterior insula is recruited by non-noxious stimuli in neuropathic pain and that this is suppressed by prefrontal tDCS as well as M1 tDCS. Interestingly, all of the afore-mentioned regions modulate spinal nociception; the anterior cingulate cortex via direct facilitatory cortico-spinal projections (Chen et al., 2018), the posterior insula by virtue of projecting to the periaqueductal grey (Tan et al., 2017) and the periaqueductal grey as the origin of both descending facilitatory and inhibitory systems via its diverse sub-domains (Kuner and Kuner, 2021). Accordingly, both prefrontal anodal tDCS and M1 anodal tDCS suppress the recruitment of neurons in the superficial spinal laminae that process nociceptive inputs, thus suggesting modulation of the brain-spinal cord axis in chronic pain, a mechanism that has also been implicated in analgesia via other neuromodulation-based approaches, including conditioned pain modulation and placebo analgesia (Tan and Kuner, 2021). Despite these advances, very little is known about the precise nature of the brain circuits and connectivity between specific types of neurons, which underlie long-term pain relief upon neurostimulation. Moreover, although both M1 tDCS and prefrontal tDCS suppress neuropathic pain, they show some divergent and even contradictory features, for example M1 tDCS reduces anxiety in neuropathic mice, but prefrontal tDCS does not (Gan et al., 2021a,b), and unlike prefrontal tDCS, M1 tDCS leads to a paradoxical increase in the activity of the anterior cingulate cortex (Gan et al., 2021a,b), a feature shared with opioids and placebo analgesia (Tan and Kuner, 2021). Moreover, the potential roles of structural remodelling and reorganisation in chronic pain (Kuner and Flor, 2016) by neurostimulation therapies have not been addressed at all in animal studies and represent a promising avenue for future work. The field of preclinical analysis of neurostimulation therapies is still in its infancy and we believe that it holds great promise, not only in yielding insights into neurobiological underpinnings, but also in optimizing neurostimulation-based therapies for a broader applicability.

Neurofeedback and brain-computer interfaces

Neurofeedback training measures pain-related activations in the brain, which are recorded by noninvasive brain imaging methods such as electroencephalography, magnetoencephalography or functional magnetic resonance imaging and feeds them back to the patients via acoustic or visual signals. The patients receive the instruction to increase or decrease the respective activity of certain areas, connections, or entire networks. Neurofeedback applications can be viewed as a type of brain computer interface where brain activity changes an output signal of the computer that can then be used for feedback or be employed to drive a tool such as a prosthesis.

Siniatchkin et al. (1998) investigated slow cortical potentials of the electroencephalogram in young migraineurs and found them to be elevated, indicative of cortical hyperexcitability. Feedback training, which involved lowering of the negativity of the slow cortical potential, was accompanied by significant reduction of cortical excitability as well as a significant reduction of days with migraine and other headache parameters. There were also successful attempts to alter specific EEG frequencies by feedback in patients with chronic pain, such as fibromyalgia (e.g., Barbosa-Torres and Cubo-Delgado, 2021; Caro and Winter, 2011), that have shown promising effects on pain and physiological indicators (for rewiew see Roy et al., 2020). Goldway et al. (2019) employed electrical activity that fingerprints amygdalar function and found positive short term effects on sleep and long term effects on clinical pain in fibromyalgia patients. Using functional magnetic resonance imaging, deCharms et al. (2005) gave feedback

from the rostral anterior cingulate cortex to teach chronic pain patients pain control. They reported decreases in the ongoing level of chronic pain after several sessions of training. In phantom limb pain, Yanagisawa et al. (2016) used a brain computer interface to control a robotic arm by training amputees to use phantom movement-related activity from sensorimotor cortices ipsi- or contralateral to the amputated arm. Ipsilateral activation decreased and contralateral activation increased phantom limb pain, demonstrating bidirectional control over the clinical pain. In an extended training over several days, where either actual hand movements or random activity were fed into the decoder, the extended training of real movement reduced pain for several days (Yanagisawa et al., 2020).

In sum, neurofeedback applications are promising for the control of chronic pain. They should be extended to connectivity feedback, for example to frontostriatal, fronto-amygdalar or fronto-hippocampal circuits involved in pain chronicity and need longer-term follow-ups and credible control conditions.

Cognitive and behavioral interventions

Cognitive and behavioral treatments, including exposure treatment and acceptance and commitment-based approaches, are commonly-used treatments for chronic pain and among the most effective (cf. Kundakci et al., 2021). They also have specific effects on the brain that can be used to alter brain circuits involved in chronic pain. For example, Lackner et al. (2006) used a brief cognitive-behavioral intervention that involved education, cognitive coping strategies and problem-solving training in patients with painful irritable bowel syndrome and achieved significant reductions in pain, anxiety and gastrointestinal symptoms. These improvements were accompanied by reduced activations in the parahippocampal gyrus, the amygdala and the subgenual ACC including the medial frontal cortex, all regions involved in affective and cognitive modulation of pain. Enduring cognitive changes that alter pain processing have also been observed in meditators where the anticipation and experience of pain was accompanied by altered activation of the anterior insula (Lutz et al., 2013). Yoshino et al. (2018) observed that cognitive-behavioral pain treatment resulted in normalization of intrinsic brain connectivity networks (ICN) assessed with fMRI in the orbitofrontal cortex (OFC) and inferior parietal lobule within the dorsal attention network and in the paracentral lobule within the sensorimotor network. Higher ICN connectivity strength in the OFC indicated greater improvements in pain intensity. Furthermore, ICN connectivity strength in the dorsal posterior cingulate cortex (PCC) within the dorsal attention network was negatively correlated with clinical improvements. Day et al. (2021) found substantial electroencephalography-assessed brain oscillation changes as potential mechanisms of cognitive therapy, mindfulness-meditation, and mindfulness-based cognitive therapy for chronic low back pain. A significant reduction in theta and alpha power in frontal areas and an overall reduction in beta power were found. A central power reduction was associated with reduced pain intensity in mindfulness-based treatment. Jensen et al. (2012) showed that cognitive-behavioral intervention increased specifically activity in the prefrontal cortex in response to pain in a sample of fibromyalgia patients examined with functional magnetic resonance imaging. It is likely that these interventions enhance descending pain control processes and thus reduce pain intensity, however, causal effects still need to be determined.

Cognitive and learning factors also play a role in placebo analgesia, which can be viewed as a type of psychological modulation of pain. A network ranging from dorsolateral prefrontal and orbitofrontal cortex to the rostral ACC, the periaqueductal grey, the rostroventral medulla and the spinal cord seems to mediate the effects of placebos on pain perception (Krummenacher et al., 2010). Placebo effects can be actively used to improve both psychological and pharmacological pain interventions and can contribute to the normalization of brain circuits in chronic pain.

Operant-behavioral treatments have been advocated for persons who have low activity levels, display high pain behaviors and have significant others who reinforce them for the display of pain behaviors (Thieme et al., 2007). Diers et al. (2012) showed that patients with fibromyalgia, who underwent a pain extinction retraining based on operant principles, displayed a shift from more activation in the anterior insula pre-treatment to more activation in posterior insula post-treatment. In a study that used functional magnetic resonance imaging, changes in pain-related interference were closely related to changes in activations in the posterior insula, primary somatosensory cortex, thalamus and striatum, suggesting more sensory than affective processing and better pain prediction following successful pain control. These changes differ from those shown by Jensen et al. (2012) for cognitive-behavioral treatment and thus suggest that behavioral treatments may impact on different brain circuits compared to cognitive treatments.

In phantom limb pain, sensory discrimination training compared to placebo improved tactile acuity and changed both phantom limb pain and organization of sensorimotor cortices (Flor et al., 2001). Mirror treatment to train patients with phantom limb pain to virtually move phantom re-establishes control over the phantom and reduces phantom limb pain (Chan et al., 2007). Foell et al. (2014) showed that this type of behavioral treatment leads to a reduction of both phantom limb pain and the reorganization in sensorimotor cortices and, that changes can be predicted by parietal correlates of phantom perception. In this study, persons with distorted perception of the phantom (telescoping) did not profit from mirror treatment. Recreating telescoped phantoms in augmented reality, Thogersen et al. (2020) showed that these patients can also profit from mirror training and they also showed changes in sensorimotor cortex. It can be assumed that imagined movement activates similar, but not identical, brain regions as the actual movement. MacIver et al. (2008) thus employed therapeutic mental imagery and found that it caused a significant reduction in phantom limb pain with a concomitant normalization of brain activation in the sensorimotor cortex. These studies suggest that modification of input into the affected brain region by visual feedback and imagery alone may alter pain sensation and cortical plasticity. However, it needs to be determined if imagined movement or executed movements of the phantom hand (which amputees can differentiate) are more effective.

Discussion and conclusions

In this article, pain-related alterations in the structure and function of the brain were discussed with respect to brain-based interventions. Non-invasive neurostimulation, neurofeedback, brain computer interfaces and cognitive as well as behavioral interventions can impact on brain circuits related to chronic pain. These approaches can differentially act on relevant brain circuits and thus provide specific mechanistic treatment approaches. The analysis of brain circuits related to chronic pain can thus aid in the determination of subgroups of patients that might differentially profit from certain brain-based interventions. The combination of different approaches, such as brain stimulation and cognitive interventions or neurofeedback with brain stimulation, might boost the effects of the individual treatments by acting on more than one mechanism involved in pain chronicity. It would also be interesting to add pharmacological interventions that drive brain plasticity to the training measures in order to speed up some of the desired brain changes. Animal studies are especially suited to further elucidate the mechanisms of brain-based interventions and to improve their mode of delivery. Finally, the analysis of brain mechanisms of chronic pain may also reveal new pathways that could be targeted in a specific manner. A further important issue that was not addressed here is the close interaction of central and peripheral physiological processes in chronic pain that involves site-specific disruptions in body perception and physiological regulation. These issues need to be examined in more detail in the future.

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References

Ahn, S., Prim, J.H., Alexander, M.L., McCulloch, K.L., and Frohlich, F. (2019). Identifying and engaging neuronal oscillations by transcranial alternating current stimulation in patients with chronic low back pain: A randomized, crossover, double-blind, sham-controlled pilot study. J. Pain *20*, 277 e271–277 e211.

Alshelh, Z., Marciszewski, K.K., Akhter, R., Di Pietro, F., Mills, E.P., Vickers, E.R., Peck, C.C., Murray, G.M., and Henderson, L.A. (2018). Disruption of default mode network dynamics in acute and chronic pain states. Neuroimage Clin *17*, 222–231.

Andre-Obadia, N., Magnin, M., and Garcia-Larrea, L. (2021). Theta-burst versus 20 Hz repetitive transcranial magnetic stimulation in neuropathic pain: A head-to-head comparison. Clin. Neurophysiol. *132*, 2702–2710.

Apkarian, A.V., Bushnell, M.C., Treede, R.D., and Zubieta, J.K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. Eur. J. Pain *9*, 463–484.

Baliki, M.N., Baria, A.T., and Apkarian, A.V. (2011). The cortical rhythms of chronic back pain. J. Neurosci. *31*, 13981–13990.

Baliki, M.N., Petre, B., Torbey, S., Herrmann, K.M., Huang, L., Schnitzer, T.J., Fields, H.L., and Apkarian, A.V. (2012).

Corticostriatal functional connectivity predicts transition to chronic back pain. Nat. Neurosci. *15*, 1117–1119.

Barbosa-Torres, C. and Cubo-Delgado, S. (2021). Clinical findings in SMR neurofeedback protocol training in women with fibromyalgia syndrome. Brain Sci. *11*, 1069.

Caro, X.J. and Winter, E.F. (2011). EEG biofeedback treatment improves certain attention and somatic symptoms in fibromyalgia: A pilot study. Appl. Psychophysiol. Biofeedback *36*, 193–200.

Chan, B.L., Witt, R., Charrow, A., Howard, R., Magee, A., Pasquina, P.F., Heilman, K.M., and Tsao, J.W. (2007). A randomized trial of mirror therapy for lower limb phantom pain. Ann. Neurol. *62*, S32–S33.

Chen, T., Taniguchi, W., Chen, Q.Y., Tozaki-Saitoh, H., Song, Q., Liu, R.H., Koga, K., Matsuda, T., Kaito-Sugimura, Y., Wang, J., et al. (2018). Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. Nat. Commun. *9*, 1886.

Cruccu, G., Garcia-Larrea, L., Hansson, P., Keindl, M., Lefaucheur, J.P., Paulus, W., Taylor, R., Tronnier, V., Truini, A., and Attal, N. (2016). EAN guidelines on central neurostimulation therapy in chronic pain conditions. Eur. J. Neurol. *23*, 1489–1499.

Day, M.A., Matthews, N., Mattingley, J.B., Ehde, D.M., Turner, A.P., Williams, R.M., and Jensen, M.P. (2021). Change in brain oscillations as a mechanism of mindfulness-meditation, cognitive therapy, and mindfulness-based cognitive therapy for chronic low back pain. Pain Med. *22*, 1804–1813.

de Andrade, E.M., Martinez, R.C.R., Pagano, R.L., Lopes, P.S.S., Auada, A.V.V., Gouveia, F.V., Antunes, G.F., Assis, D.V., Lebrun, I., and Fonoff, E.T. (2019). Neurochemical effects of motor cortex stimulation in the periaqueductal gray during neuropathic pain. J. Neurosurg. *132*, 239–251.

deCharms, R.C., Maeda, F., Glover, G.H., Ludlow, D., Pauly, J.M., Soneji, D., Gabrieli, J.D.E., and Mackey, S.C. (2005). Control over brain activation and pain learned by using real-time functional MRI. Proc. Natl. Acad. Sci. U. S. A. *102*, 18626–18631.

De Martino, E., Seminowicz, D.A., Schabrun, S.M., Petrini, L., and Graven-Nielsen, T. (2019). High frequency repetitive transcranial magnetic stimulation to the left dorsolateral prefrontal cortex modulates sensorimotor cortex function in the transition to sustained muscle pain. Neuroimage *186*, 93–102.

Diers, M., Yilmaz, P., Rance, M., Thieme, K., Gracely, R.H., Rolko, C., Schley, M.T., Kiessling, U., and Flor, H. (2012). Treatment-related changes in brain activation in patients with fibromyalgia syndrome. Exp. Brain Res. *218*, 619–628.

Ferreira, N.R., Junqueira, Y.N., Corrêa, N.B., Fonseca, E.O., Brito, N.B.M., Menezes, T.A., Magini, M., Fidalgo, T.K.S., Ferreira, D.M.T.P., de Lima, R.L., et al. (2019). The efficacy of transcranial direct current stimulation and transcranial magnetic stimulation for chronic orofacial pain: A systematic review. PLoS One *14*, e0221110.

Flor, H., Denke, C., Schaefer, M., and Grüsser, S. (2001). Effect of sensory discrimination training on cortical reorganisation and phantom limb pain. Lancet *357*, 1763–1764.

Foell, J., Bekrater-Bodmann, R., Diers, M., and Flor, H. (2014). Mirror therapy for phantom limb pain: Brain changes and the role of body representation. Eur. J. Pain *18*, 729–739.

Franca, N.R., Toniolo, E.F., Franciosi, A.C., Alves, A.S., de Andrade, D.C., Fonoff, E.T., Britto, L.R., and Dale, C.S. (2013). Antinociception induced by motor cortex stimulation: Somatotopy of behavioral response and profile of neuronal activation. Behav. Brain Res. *250*, 211–221.

Gan, Z., Li, H., Naser, P.V., Han, Y., Tan, L.L., Oswald, M.J., and Kuner, R. (2021a). Repetitive non-invasive prefrontal stimulation reverses neuropathic pain via neural remodelling in mice. Prog. Neurobiol. *201*, 102009.

Gan, Z., Li, H., Naser, P.V., Oswald, M.J., and Kuner, R. (2021b). Suppression of neuropathic pain and comorbidities by recurrent cycles of repetitive transcranial direct current motor cortex stimulation in mice. Sci. Rep. *11*, 9735.

Garcia-Larrea, L. and Peyron, R. (2007). Motor cortex stimulation for neuropathic pain: From phenomenology to mechanisms. Neuroimage *37*, S71–S79.

Goldway, N., Ablin, J., Lubin, O., Zamir, Y., Keynan, J.N., Or-Borichev, A., Cavazza, M., Charles, F., Intrator, N., Brill, S., et al. (2019). Volitional limbic neuromodulation exerts a beneficial clinical effect on fibromyalgia. Neuroimage *186*, 758–770.

Hallett, M. (2007). Transcranial magnetic stimulation: A primer. Neuron *55*, 187–199.

Hashmi, J.A., Baliki, M.N., Huang, L., Baria, A.T., Torbey, S., Hermann, K.M., Schnitzer, T.J., and Apkarian, A.V. (2013). Shape shifting pain: Chronification of back pain shifts brain representation from nociceptive to emotional circuits. Brain *136*, 2751–2768.

Hohn, V.D., May, E.S., and Ploner, M. (2019). From correlation towards causality: Modulating brain rhythms of pain using transcranial alternating current stimulation. Pain Rep *4*, e723.

Jensen, K.B., Kosek, E., Wicksell, R., Kemani, M., Olsson, G., Merle, J.V., Kadetoff, D., and Ingvar, M. (2012). Cognitive behavioral therapy increases pain-evoked activation of the prefrontal cortex in patients with fibromyalgia. Pain *153*, 1495–1503.

Kikkert, S., Mezue, M., O'Shea, J., Slater D.H., Johansen-Berg H., Tracey I., and Makin T.R. (2019). Neural basis of induced phantom limb pain relief. Ann. Neurol. *85*, 59–73.

Krummenacher, P., Candia, V., Folkers, G., Schedlowski, M., and Schonbachler, G. (2010). Prefrontal cortex modulates placebo analgesia. Pain *148*, 368–374.

Kundakci, B., Kaur, J., Goh, S.L., Hall, M., Doherty, M., Zhang, W., and Abhishek, A. (2021). Efficacy of nonpharmacological interventions for individual features of fibromyalgia: A systematic review and meta-analysis of randomised controlled trials. Pain, https://doi.org/10.1097/j.pain.0000000000002500.

Kuner, R. and Flor, H. (2016). Structural plasticity and reorganisation in chronic pain. Nat. Rev. Neurosci. *18*, 20–30.

Kuner, R. and Kuner, T. (2021). Cellular circuits in the brain and their modulation in acute and chronic pain. Physiol. Rev. *101*, 213–258.

Lackner, J.M., Lou Coad, M., Mertz, H.R., Wack, D.S., Katz, L.A., Krasner, S.S., Firth, R., Mahl, T.C., and Lockwood, A.H. (2006). Cognitive therapy for irritable bowel syndrome is associated with reduced limbic activity, GI symptoms, and anxiety. Behav. Res. Ther. *44*, 621–638.

Lee, J.J., Kim, H.J., Ceko, M., Park, B.Y., Lee, S.A., Park, H., Roy, M., Kim, S.G., Wager, T.D., and Woo, C.W. (2021). A neuroimaging

biomarker for sustained experimental and clinical pain. Nat. Med. *27*, 174–182.

Lefaucheur, J.P., Aleman, A., Baeken, C., Benninger, D.H., Brunelin, J., Di Lazzaro, V., Filipović, S.R., Grefkes, C., Hasan, A., Hummel, F.C., et al. (2020). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clin Neurophysiol *131*, 474–528.

Lopes, P.S.S., Campos, A.C.P., Fonoff, E.T., Britto, L.R.G., and Pagano, R.L. (2019). Motor cortex and pain control: Exploring the descending relay analgesic pathways and spinal nociceptive neurons in healthy conscious rats. Behav. Brain Funct. *15*, 5.

Lutz, A., McFarlin, D.R., Perlman, D.M., Salomons, T.V., and Davidson, R.J. (2013). Altered anterior insula activation during anticipation and experience of painful stimuli in expert meditators. Neuroimage *64*, 538–546.

MacIver, K., Lloyd, D.M., Kelly, S., Roberts, N., and Nurmikko, T. (2008). Phantom limb pain, cortical reorganization and the therapeutic effect of mental imagery. Brain *131*, 2181–2191.

May, A. (2011). Structural brain imaging: A window into chronic pain. Neuroscientist *17*, 209–220.

May, E.S., Nickel, M.M., Ta Dinh, S., Tiemann, L., Heitmann, H., Voth, I., Tolle, T.R., Gross, J., and Ploner, M. (2019). Prefrontal gamma oscillations reflect ongoing pain intensity in chronic back pain patients. Hum. Brain Mapp. *40*, 293–305.

McPhee, M.E. and Graven-Nielsen, T. (2021). Medial prefrontal transcranial direct current stimulation aimed to improve affective and attentional modulation of pain in chronic low back pain patients. J. Clin. Med. *10*, 889.

Mercer Lindsay, N., Chen, C., Gilam, G., Mackey, S., and Scherrer, G. (2021). Brain circuits for pain and its treatment. Sci. Transl. Med. *13*, eabj7360.

Moseley, G.L. and Flor, H. (2012). Targeting cortical representations in the treatment of chronic pain: A review. Neurorehabilitation Neural Repair *26*, 646–652.

Nguyen, J.P., Nizard, J., Keravel, Y., and Lefaucheur, J.P. (2011). Invasive brain stimulation for the treatment of neuropathic pain. Nat. Rev. Neurol. *7*, 699–709.

O'Connell, N.E., Marston, L., Spencer, S., DeSouza, L.H., and Wand, B.M. (2018). Non-invasive brain stimulation techniques for chronic pain. Cochrane Database Syst. Rev. *16*, CD008208. connelUpdate in: Cochrane Database Syst Rev. 2018 Apr 13;4: CD008208.

Pagano, R.L., Fonoff, E.T., Dale, C.S., Ballester, G., Teixeira, M.J., and Britto, L.R.G.D. (2012). Motor cortex stimulation inhibits thalamic sensory neurons and enhances activity of PAG neurons: Possible pathways for antinociception. Pain *153*, 2359–2369.

Paulus, W. (2011). Transcranial electrical stimulation (tES – tDCS; tRNS, tACS) methods. Neuropsychol. Rehabil. *21*, 602–617.

Pfannmöller, J. and Lotze, M. (2019). Review on biomarkers in the resting-state networks of chronic pain patients. Brain Cognit. *131*, 4–9.

Pinheiro, E.S., de Queiros, F.C., Montoya, P., Santos, C.L., do Nascimento, M.A., Ito, C.H., Silva, M., Nunes Santos, D.B., Benevides, S., Miranda, J.G., et al. (2016). Electroencephalographic patterns in chronic pain: A systematic review of the literature. PLoS One *11*, e0149085.

Rainville, P., Duncan, G.H., Price, D.D., Carrier, B., and Bushnell, M.C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science *277*, 968–971.

Roy, R., de la Vega, R., Jensen, M.P., and Miro, J. (2020). Neurofeedback for pain management: A systematic review. Front. Neurosci. *14*, 671.

Segerdahl, A.R., Mezue, M., Okell, T.W., Farrar, J.T., and Tracey, I. (2015). The dorsal posterior insula subserves a fundamental role in human pain. Nat. Neurosci. *18*, 499–500.

Seminowicz, D.A., de Martino, E., Schabrun, S.M., and Graven-Nielsen, T. (2018). Left dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation reduces the development of long-term muscle pain. Pain *159*, 2486–2492.

Seminowicz, D.A. and Moayedi, M. (2017). The dorsolateral prefrontal cortex in acute and chronic pain. J. Pain *18*, 1027–1035.

Siniatchkin, M., Gerber, W.D., Kropp, P., and Vein, A. (1998). Contingent negative variation in patients with chronic daily headache. Cephalalgia *18*, 565–569.

Souza, A., Martins, D.F., Medeiros, L.F., Nucci-Martins, C., Martins, T.C., Siteneski, A., Caumo, W., Dos Santos, A.R.S., and Torres, I.L.S. (2018). Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain. Brain Res. *1682*, 14–23.

Tan, L.L. and Kuner, R. (2021). Neocortical circuits in pain and pain relief. Nat. Rev. Neurosci. *22*, 458–471.

Tan, L.L., Pelzer, P., Heinl, C., Tang, W., Gangadharan, V., Flor, H., Sprengel, R., Kuner, T., and Kuner, R. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. Nat. Neurosci. *20*, 1591–1601.

Thieme, K., Turk, D.C., and Flor, H. (2007). Responder criteria for operant and cognitive-behavioral treatment of fibromyalgia syndrome. Arthritis Rheum. Arthritis Care Res. *57*, 830–836.

Thogersen, M., Andoh, J., Milde, C., Graven-Nielsen, T., Flor, H., and Petrini, L. (2020). Individualized augmented reality training reduces phantom pain and cortical reorganization in amputees: A proof of concept study. J. Pain *21*, 1257–1269.

Wager, T.D., Atlas, L.Y., Lindquist, M.A., Roy, M., Woo, C.W., and Kross, E. (2013). An fMRI-based neurologic signature of physical pain. N. Engl. J. Med. *368*, 1388–1397.

Yanagisawa, T., Fukuma, R., Seymour, B., Hosomi, K., Kishima, H., Shimizu, T., Yokoi, H., Hirata, M., Yoshimine, T., Kamitani, Y., et al. (2016). Induced sensorimotor brain plasticity controls pain in phantom limb patients. Nat. Commun. *7*, 13209.

Yanagisawa, T., Fukuma, R., Seymour, B., Tanaka, M., Hosomi, K., Yamashita, O., Kishima, H., Kamitani, Y., and Saitoh, Y. (2020). BCI training to move a virtual hand reduces phantom limb pain: A randomized crossover trial. Neurology *95*, e417–e426.

Yoshino, A., Okamoto, Y., Okada, G., Takamura, M., Ichikawa, N., Shibasaki, C., Yokoyama, S., Doi, M., Jinnin, R., Yamashita, H., et al. (2018). Changes in resting-state brain networks after cognitive-behavioral therapy for chronic pain. Psychol. Med. *48*, 1148–1156.

Ziemann, U., Paulus, W., Nitsche, M.A., Pascual-Leone, A., Byblow, W.D., Berardelli, A., Siebner, H.R., Classen, J., Cohen, L.G., and Rothwell, J.C. (2008). Consensus: Motor cortex plasticity protocols. Brain Stimul. *1*, 164–182.

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Review article

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Pain, the brain, and SARS-CoV-2: evidence for pain-specific alterations in brain-related structure–function properties

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Abstract: According to best current estimates, approximately 10% of those infected with SARS-CoV-2-virus experience long-term clinical and nonspecific neurological symptoms that may last for several weeks or months. This is currently referred to as "Long-COVID" or "Post-COVID-Syndrome". Based on current knowledge, the most common long-term symptoms of COVID-19 disease include fatigue and poor concentration, but particularly also headache and musculoskeletal pain. However, given the novelty of COVID-19, only a few studies have systematically evaluated the central nervous alterations in the pain processing structures of our brain. Those first insights are yet important in order to offer patients adequate therapeutic options. Based on a systematic review of the literature, we will therefore provide an overview of the central nervous alterations in the brain described in the context of SARS-CoV-2 infection, focusing on findings with brain imaging.

Keywords: brain; Coronavirus; COVID-19; imaging; pain.

Zusammenfassung: Nach aktuellen Schätzungen treten bei etwa 10% der mit dem SARS-CoV-2-Virus Infizierten klinische und unspezifische neurologische Symptome auf, die mehrere Wochen oder Monate andauern können. Dies wird aktuell als „Long-COVID" oder „Post-COVID-Syndrom" bezeichnet. Nach derzeitigem Kenntnisstand zählen Fatigue, Konzentrationsschwäche, Kopf-, und muskuloskelettale Schmerzen zu den häufigsten Spätsymptomen einer COVID-19-Erkrankung. Angesichts der Neuartigkeit von COVID-19 existieren bisher nur wenige Studien, die zentralnervöse Veränderungen in schmerzverarbeitenden Strukturen des Gehirns systematisch untersucht haben. Diese ersten Erkenntnisse sind jedoch bereits wichtig, um Betroffenen optimale Therapieoptionen bieten zu können. Ausgehend von einer systematischen Literatursuche geben wir daher einen Überblick über die im Rahmen einer SARS-CoV-2-Infektion beschriebenen zentralnervösen Veränderungen im Gehirn, wobei der Fokus auf Befunden mit bildgebender Darstellung des Gehirns liegt.

Schlüsselwörter: Gehirn; Bildgebung; Schmerz; COVID-19; Coronavirus.

Pain as a common symptom after COVID-19 infection

Persistent headache and musculoskeletal pain are both common long-term symptoms of SARS-CoV-2 infection, with a prevalence rate of ~10% each in the first year after infection (Fernández-de-Las-Peñas et al., 2021a,b). Time trend analyses indicate a biphasic course with a decrease in pain symptoms at the end of the first month after the initial COVID infection, a renewed increase after the end of the 2nd month, and with a second decrease more than six months after the acute disease phase. Moreover, a condition of critical illness requiring intensive care, like it is the case for 10–20% of all COVID-19 patients (RKI, 2021), is a strong risk factor for the development of chronic pain, and approximately 15–20% of those affected subsequently develop chronic pain conditions (Koster-Brouwer et al., 2020). In this sense, severe COVID-19 treatment trajectories are often associated with numerous non-COVID-19-specific pathologies, which in turn may promote the development of chronic pain (Friedrich et al., 2015). Furthermore, additional nonspecific neurological and psychological complains frequently accompany these pain symptoms and complicate treatment.

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This complexity and heterogeneity of the symptomatology suggest that different mechanisms and factors may contribute to Long-COVID in general and consequences of (chronic) pain in particular. Although muscle and peripheral nerve affections as well as postinfectious auto-inflammatory processes are often described, in most patients with persistent headache and musculoskeletal pain associated with COVID-19, no specific nociceptive manifestation could be identified, and central nervous processes in the sense of a nociplastic or neuropathic pain process may therefore rather be a source (Goudman et al., 2021). However, few studies examined the impact of SARS-CoV-2 infection on neuronal pain processing in the brain thus far, and the current evidence is suggesting that the SARS-CoV-2-virus influences brain neuronal networks through multiple pathways.

Using a systematic literature search in MEDLINE (10/2019 up to now) and Web of Science (10/2019 up to now), we collected all types of studies that investigated SARS-CoV2-associated central nervous system changes in humans using brain imaging techniques (Figure 1). The most commonly used techniques were computed tomography (CT), magnetic resonance imaging (MRI), derived functional magnetic resonance imaging (fMRI), and positron emission tomography (PET), as well as electroencephalography (Figure 2a). Moreover, we also included autopsy studies in our search.

Based on the identified studies, we first address the manifest structural damage that may occur in the context of acute infection and, in particular, in the context of a critical disease state with hypoxia and massive systemic inflammatory response. In this respect, we considered the findings from neuropathological studies of brains on and with COVID-19 of deceased patients and brain imaging findings from SARS-CoV-2 infected patients with an initially mild course and/or (up to) severe symptomatology. Most patients with COVID-19 develop mild to moderate disease, and only about 5–10% develop critical illness including acute respiratory distress syndrome (ARDS), septic shock, or multigrain failure (RKI, 2021).

These are the same patients who frequently show abnormalities on brain imaging and to whom much of the existing literature refers; this aspect needs to be considered both in interpreting the literature and in the diagnostic assessment of COVID-19-associated pain disorders.

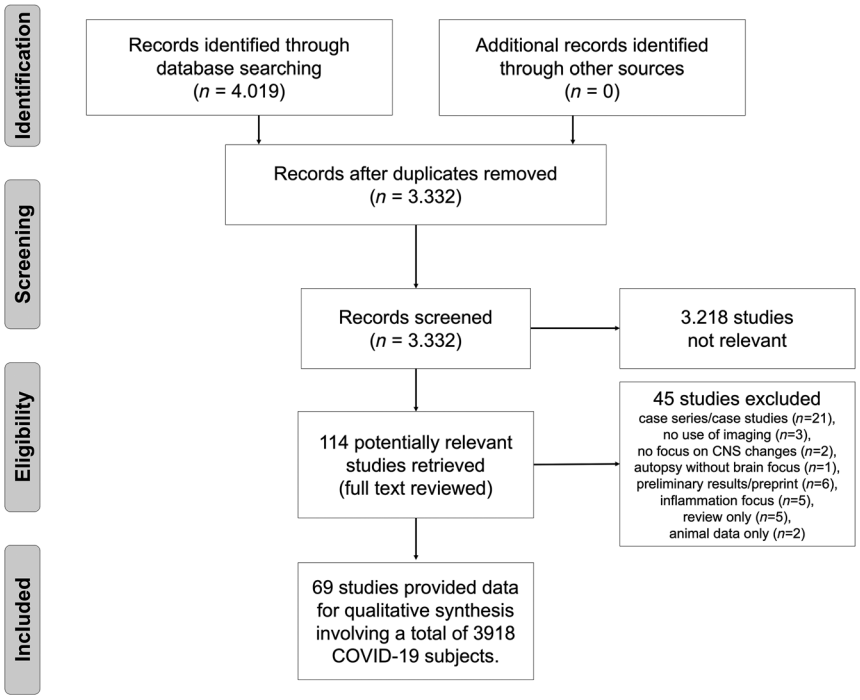

Figure 1: Using a systematic literature search of MEDLINE (10/2019 to date) and Web of Science (10/2019 to date), we collected all types of studies that investigated SARS-CoV-2-associated central nervous system changes in humans using brain imaging techniques. Studies that were limited to examining changes in the peripheral nervous system and those that did not focus on human individuals (e.g., animal studies, cell cultures, etc.…) were excluded. Because SARS-CoV-2 was not described until late 2019, only articles after that date were considered. Based on this systematic literature search, a total of 3332 articles could be classified as "potentially relevant," of which 69 ultimately met the inclusion criteria. Relevant information from each included study was extracted and summarized narratively using a specially designed data extraction form. In the context of narrative report of the results, we have focused on discussing the results of those studies that appear to be of particular relevance with respect to the question of changes in the structures involved in pain processing.

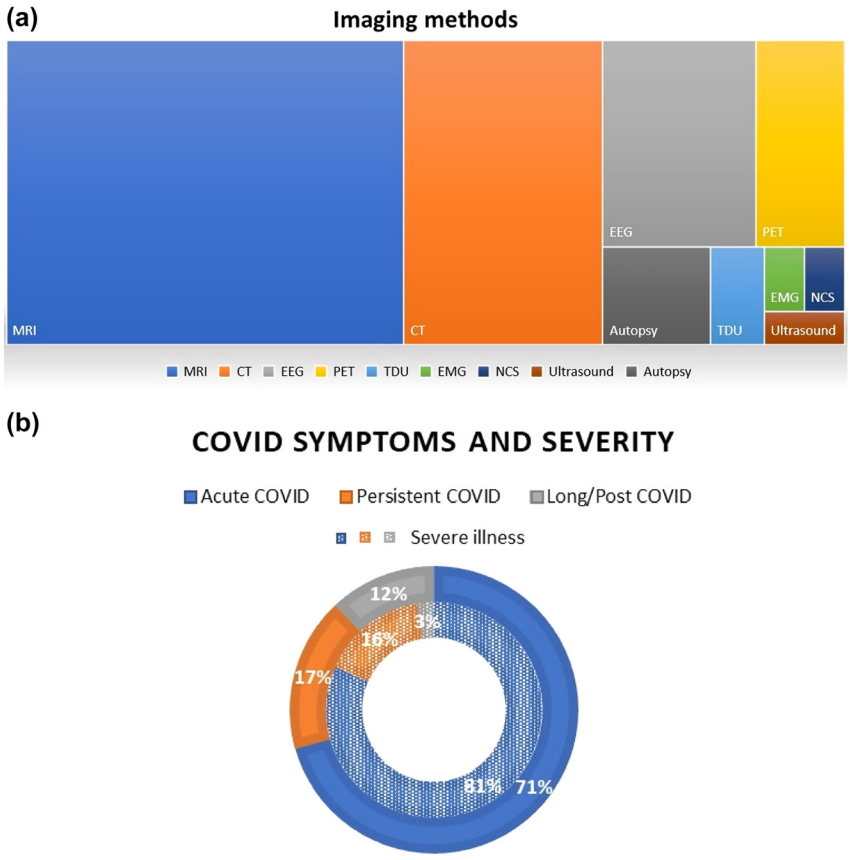

Figure 2: Methodological overview of the current evidence. The majority of studies used MRI imaging methods (a), and reported on the acute stage of COVID-19 (b).

Moreover, pathologic brain changes in the context of severe COVID-19 disease are thought to be the result of both static and dynamic processes, and there is likely a spectrum of distinct pathophysiologic processes underlying the central nervous alterations (Agarwal et al., 2021).

However, the relevant brain imaging findings on this matter strengthen the evidence that COVID-19-specific patterns of structural and functional changes can be described in addition to the many COVID-19 unspecific findings that are commonly seen in the context of "critical illness." An interesting and well described study, published in 2020, have described the detailed emerging spectrum of neurological disorders presenting 43 patients with COVID-19 (Paterson et al., 2020). Here, for example, in case of a sequential para-infectious involvement of central and peripheral nervous systems, pain-related symptoms of back pain, headache and limb weakness as well as neck and facial weakness, preserved sensation and generalized areflexia could be observed.

We link the reported brain abnormalities identified the selected studies to pain processing and discuss possible long-term consequences of COVID-19 disease and their relation to SARS-CoV-2-associated pain conditions. This approach may allow us to better understand the development of persistent pain after COVID-19 disease, identify possible "key mechanistic domains" and derive a research framework for future studies and clinical concepts.

Manifest structural damage in the context of acute infectious events

A large number of studies have described in detail the neurological complications that can be observed in the course of a COVID-19 disease. In this respect, it is striking that these complications differ in type and also in their affinity to specific brain regions known from neurological complications of other clinical pictures. Leukoencephalopathy and cerebral microbleeds are the most common MRI findings in the literature in patients with COVID-19 who have severe disease and a long duration of ventilation.

In contrast to the deep microbleeds in hypertension or the cortical microbleeds in amyloid angiopathies, the COVID-19-associated microbleeds are rather diffuse and involve the cortex, subcortex, and brainstem with a particular predilection for the corpus callosum (Agarwal et al., 2021). In addition, nonspecific white matter changes are increasingly reported in MRI studies,

preferentially localized in the frontal, parietal and temporal lobes (Kavak et al., 2021; Kremer et al., 2020), and with a clustered localization in the subcortical white matter as well as in the corpus callosum and thalami (Chougar et al., 2020; Fitsiori et al., 2020; Kelsch et al., 2021; Raman et al., 2021). Hypoxemia is the most likely explanation for this pattern, a similar phenomenon being observed in the high-altitude cerebral edema and in the severe respiratory failure. Interestingly, the corpus callosum is also the main fiber tract connecting the cerebral hemispheres and is critical in hemispheric sensory integration, attentional and inhibition processing (Banich, 1998; for a review see Zaidel and Iacoboni, 2003). Fibers of the genu and rostrum in the anterior corpus callosum bridge different prefrontal cortical regions and thus play a pivotal role in information transfer and executive processing by the frontal cortex (Clarke et al., 1989; Gazzaniga, 1995; Rueckert and Levy, 1996; Witelson, 1985). In this context, there is evidence that the prefrontal cortex serves as an inhibitory regulatory area of early sensory transmission at cortical (Alexander et al., 1976; Skinner and Yingling, 1977; Yingling and Skinner, 1977) and subcortical (Edinger et al., 1975) regions, and as a hub for sustained attention, sensory gating, or filtering of irrelevant stimuli, thereby reducing interference with higher cognitive processes (Boutros and Belger, 1999; Knight et al., 1999; Staines et al., 2002). When speaking about such cognitive inhibitions, this is where also a relation to pain perception and processing may come in, playing a critical role for analgesic processes. The analgesic effect of a clinical dose of remifentanil, an opioid agonist, can be reversed by a negative expectation about this effect (Bingel et al., 2011), whereas the expectation of pain relief can favor placebo analgesia (Benedetti et al., 2005). Similar effects could be found for distraction (Villemure and Bushnell, 2002). In the brain, beside (pre)frontal regions, also activity in afferent pain pathways and intrinsic modulatory systems in the brain, including the primary somatosensory cortex, the insula and the anterior cingulate cortex (Bantick et al., 2002; Bushnell et al., 1999; Frankenstein et al., 2001; Longe et al., 2001; Petrovic et al., 2000; Schweinhardt and Bushnell, 2010; Tracey et al., 2002; Valet et al., 2004; Villemure and Bushnell, 2002). Abnormalities of the corpus callosum as a consequence of COVID-19 may therefore also affect brain networks crucial for pain, which could, in the long run, increase the vulnerability for pain problems.

In a similar vein, the majority of studies found abnormalities in the acute phase after a COVID-19 infection in the thalamus. The thalamus is essential for nociceptive inputs, transmitted from the spinal cord to cortex (Hatanaka et al., 2003). Such plasticity is associated with all levels of pain processing in the central nervous system, where the thalamus and cortex assess nociceptive signals in context of other somatic sensory, auditory, and visual events. This can then be particularly important in pain processing also in the aftermath of COVID-19 infection, supporting cortical integration and memory formation by temporal maintenance or habituation of the response, and given (emotional) learning and memory may play a major role in the development of chronic pain, with potential associated critical structural–functional brain changes (for example, Apkarian et al., 2009; Flor, 2012; Vlaeyen and Linton, 2000). Finally, a very recent study has found changes the insula, the striatum, putamen, and the amygdala in COVID-19 patients (Qin et al., 2021).

This was specific for patients with critical illness condition and lower measured volume of the thalamus and putamen: when comparing the cerebral blood flow, the group of initially severely ill patients showed a more widespread decrease in cerebral blood flow in the subcortical nuclei, which are mainly located in the striatum and amygdala, compared with the group of initially mildly ill patients (Qin et al., 2021). There is ample evidence that all these regions the insula perform a variety of functions in humans, ranging from sensory and affective processing to high-level cognition (for example, Uddin et al., 2017), including pain processing (for example, Borsook et al., 2016; Simons et al., 2014; Wiech and Tracey, 2009). Moreover, it has been pointed out (Uddin et al., 2017) that the insular cortex lies beneath dense arterial and venous blood vessels, so it may be particularly vulnerable to hypoxia induced by pneumonia.

Together, these results suggest that the severity of COVID-19 may have different effects, including damage to critical subcortical nuclei, which may also affect pain processing, and explain the relatively high prevalence of pain problems after a COVID-19 infection (Figure 3).

Histopathological brain studies of patients dying with or from COVID-19

Numerous neuropathological autopsy studies have used histopathology to examine the brains of critical ill patients who died from COVID-19 infection, and increasingly support the morphologic correlates for the manifestation of structural cerebral damage. Primarily vascular changes, hyperemia of the meninges, and perivascular inflammation in the brain parenchyma with hypoxic neuronal damage (Colombo et al., 2021; Conklin et al., 2021; Lopez

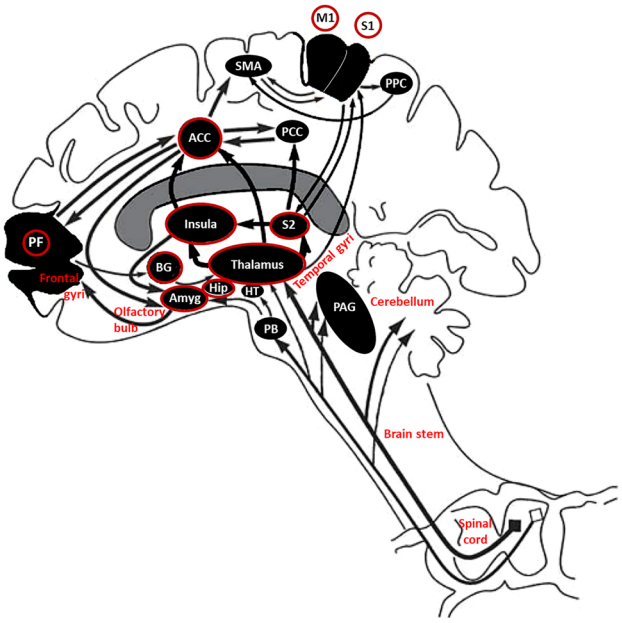

Figure 3: Overview on main brain structures involved in pain processing and perception (black and white), with those regions involved in COVID-19 symptomatology highlighted with a red circle, and those only particularly found in relation to COVID-19 in red text.

et al., 2022; Poloni et al., 2021; Wierzba-Bobrowicz et al., 2021) as well as microscopically, perivascular hemorrhage, gray matter disruption, and blood–brain barrier damage (Wierzba-Bobrowicz et al., 2021) were noted.

Brain changes caused by COVID-19 infection have therefore been termed cerebral COVID-19 angiopathy with diffuse inflammation (Wierzba-Bobrowicz et al., 2021), and such inflammatory changes in the vessel walls could be identified in particular in areas of the cerebral cortex, cerebellum, basal nuclei, and brainstem (Al-Sarraj et al., 2021; Colombo et al., 2021; Lee et al., 2021; Matschke et al., 2020). The increased vascular permeability in COVID-19 patients could also be a consequence of the cytokine storm triggering endotheliitis and general vasculopathic changes, the main factor for cerebral cortex damage being oxygen deprivation, which is also generally common in critically ill patients and thus seems to be less specific for SARS-CoV-2 infections. Only rarely viral inclusions have been detected there (Lopez et al., 2022), and especially not in the cerebrospinal fluid of patients with severe neurological sequelae (Chougar et al., 2020; Ermis et al., 2021; Helms et al., 2020; Kremer et al., 2020; Lambrecq et al., 2021; Pilotto et al., 2021). So, it remains unclear how specific the reported neuropathologic changes are to SARS-CoV-2 infection, or whether they merely represent a nonspecific consequence of severe disease progression.

EEG findings in patients with COVID-19

Based on the observed structural damage associated with COVID-19 the question now arises whether those changes might be associated with changes in neuronal excitability. The currently available EEG studies do not, however, support this assumption. The most common EEG findings were abnormal background activity (81%) and slow frontal waves (60%) (Lambrecq et al., 2021). The latter were associated with metabolic and toxic encephalopathies, for which one or more factors in most cases have been identified, or with frontal lesions. Also, in rare cases, a periodic EEG pattern was found to occur predominantly in the frontal lobe but could not be explained by MRI findings (Lambrecq et al., 2021). In addition, a different distribution of EEG bands can be detected in temporal lobe leads and hemispheric connectivity appears to be lower in COVID-19 patients. The clinical significance of these findings, however, still remains unclear (Pastor et al., 2020). Epileptiform activity as well as other pathologies in the EEG were usually associated with a history of epilepsy or seizures before SARS-CoV-2 infection (Louis et al., 2020; Pellinen et al., 2020), suggesting that SARS-CoV-2 infection does not increase neuronal excitability (Gogia et al., 2021). A specific association with persistent headache or other pain disorders has so far not been reported in the literature.

Persistent hypometabolism in positron emission tomographic imaging

Further evidence for global as well as local metabolic changes in brain activity can be reliably, with relatively good sensitivity and specificity, derived from PET studies. For example, PET studies of a longitudinal case series with repeated PET scans in seven patients with different clinical presentations of COVID-19-related encephalopathy indicated that cerebral glucose metabolism was still impaired six months after the initial onset of the disease (Kas et al., 2021). Although the clinical condition of most patients had improved after this time, various degrees of cognitive and emotional disturbances persisted. Moreover, the currently available PET analyses also show a consistent pattern of cerebral hypometabolism after SARS-CoV-2 infection. In addition, this phenomenon of cerebral hypometabolism appears to be associated with the occurrence of persistent

pain in the setting of long-COVID-syndrome (Guedj et al., 2021).

Those changes might therefore stand in direct association with above reported structural alterations following COVID-19 infection. However, the performed MRI scans in some of the PET patient populations did not reveal any specific abnormalities, while all patients still showed after six months a consistent pattern of hypometabolism in a widespread cerebral network, including the frontal cortex, anterior cingulate, insula, and caudate nucleus (Kas et al., 2021). The negative MRI findings coupled with hypometabolism patters on PET scans argue for a primarily functional rather than structural genesis. Moreover, no data on long-COVID-associated pain complaints were reported in this study, although it should be noted that the patient population of the study by Kas et al. (2021) had already in part had considerable somatic and psychic comorbidities before the infection. Given the observed brain changes in key regions for pain processing, those patients might be at a higher risk for increased or worsened pain following a COVID-19 infection.

More informative with respect to pain are PET studies in patients with persistent functional symptoms more than three weeks after initial SARS-CoV-2 infection (Guedj et al., 2021). Nearly 2/3 of these patients reported persistent pain in the sense of a long-COVID pain syndrome, with approximately 39% of the patients characterized by an initial severe course of disease, and the remaining patients by an initial mild disease pattern. Comparison of this long-COVID patient cohort with age- and sex-matched control cohorts confirmed the phenomenon of persistent cerebral hypometabolism after initial SARS-CoV-2 infection. In addition, this study identified four specific regional hypometabolic clusters: 1.) a frontal cluster (bilateral rectal/orbital gyrus, including the olfactory gyrus); 2.) a right temporal cluster (right temporal lobe, including the amygdala and hippocampus, extending to the right thalamus); 3.) a brainstem cluster (bilateral pons/medulla brainstem region); and 4.) a cerebellar cluster (bilateral cerebellum) (Guedj et al., 2021; Sollini et al., 2021) This maps findings from structural abnormalities, although, as mentioned above, a direct linkage between functional and structural changes has not been detected. Moreover, the metabolic clusters were not only highly discriminative to distinguish patients from healthy subjects (100% correct classification), but furthermore significantly correlated with pain symptomatology. Persistent pain was associated with lower metabolic values in all four clusters (Guedj et al., 2021).

This association of persistent symptoms after SARS-CoV-2 infection with hypometabolism in the frontal cortex, brainstem and thalamus, and cerebrum is noteworthy because these regions are known to be involved in the cerebral pain matrix (Borsook et al., 2010). Also, similar metabolic PET changes have been described in patients with fibromyalgia (Guedj et al., 2008). Thus, the alterations in anterior temporal perfusion suggest involvement of the limbic system. This hypoperfusion could be related to decreased affective appraisal and reactivity, which is often observed in patients with chronic pain (Gracely et al., 2002). The association of hypoperfusion in this very area with symptom severity in patients with fibromyalgia, a clinical condition similar to long-COVID-syndrome, has already been reported (Gur et al., 2002).

Cerebral hemodynamics (and endothelial dysfunction)

Of interest with regard to the high prevalence of persistent headache after COVID-19 disease are also preliminary studies of vasomotor reactivity in COVID-19 patients. These report an increase in baseline cerebral blood velocity and a decrease in vasomotor reactivity (Sonkaya et al., 2021). It is hypothesized that this may be evaluated as a consequence of endothelial dysfunction in the vascular structures of the central nervous system. This is significant because disturbances in vasomotor reactivity have also been described in relation to migraine-associated headache (Harris and Rasyid, 2020; Ornello et al., 2020; Öztürk and Karadaş, 2020). Vasomotor reactivity describes the speed with which blood vessels respond to changes in blood gases. For example, to assess this adaptability, the change in blood velocity over each stopped second is determined (breath holding index [BHI]). In a first case series, it was shown that despite an increase in baseline cerebral velocity, reduced vasodilation after breath holding can be demonstrated in patients with COVID-19, similar to migraine patients. Together with the evidence of endothelial dysfunction, increased vascular permeability, and meningeal hyperemia in COVID-19 patients, these findings may suggest a hemodynamic vascular component in COVID-19-associated headaches.

Spatial-structural remodeling over the post-acute course of the disease

To date, there has only been little research on brain changes in the post-acute course of the disease as well as related clinical manifestations. Interesting insights are

provided by a study that correlated post-COVID psychopathology with specific brain functional markers in COVID-19 survivors several weeks after the viral illness had resolved (Benedetti et al., 2021). This study demonstrated a specific correlation between the severity of depressive psychopathology and the decrease in gray matter volume in the anterior cingulate cortex (ACC), whereas posttraumatic symptoms were associated with a decrease in gray matter volume in both the ACC and bilateral insular cortex (Benedetti et al., 2021). Interestingly, no brain lesions were detected in this patient sample, and thus no association was found (Benedetti et al., 2021). Although pain symptoms were in this case not directly addressed, assumptions can be made due to the observed brain structural changes in the very same brain regions, which play an important role in pain processing. At the same time, this study also demonstrated a correlation between the severity of the initial inflammatory response in the acute stage of the disease and the subsequent white matter microstructure changes as well as functional connectivity changes weeks to months after resolution of the viral disease. A strong inflammatory response at baseline thus resulted in an increased resting-state functional connectivity, particularly within the dorsal attentional and salience networks, and between these networks and the sensorimotor network later in the post-acute course. These neuronal networks are particularly important in pain processing as well. Increased connectivity within salience and sensorimotor networks has previously been associated with persistent physical pain (Hegarty et al., 2020; Kutch et al., 2017), which is interesting in several ways. They highlight a possible common pathway for how COVID disease could lead to both the development of anhedonia and psychological pathology, as well as the development of chronic pain. In this sense, both an increase in resting-state functional connectivity within the dorsal attentional and salience networks and between these networks and the sensorimotor network, as well as a decrease in gray matter volume in both the ACC and bilateral insular cortex could be possible pathomechanisms when it comes to pain problems after COVID-19 infection.

Furthermore, studies shave also shown reduced gray matter volume in the frontal–temporal network in COVID-19 patients who received oxygen therapy or had fever compared with patients who did not receive oxygen therapy or had no fever. Also, the lower the gray matter volume in the superior, medial, and middle frontal gyri, the higher the degree of impairment, even when controlled for cerebrovascular disease. This is also corroborated by a Swedish study, which explicitly focused on patients with long-COVID symptoms persisting for more than six months and demonstrated that more than 2/3 of patients had multiple white matter lesions with a frontal and parietal distribution, particularly located in the cerebral hemispheres near the gray–white matter boundary (Hellgren et al., 2021). Here it is also important to note that MRI abnormalities were also found in patients who did not require intensive care treatment. In addition, all patients who had undergone MRI during the acute phase of COVID-19 had acquired multiple new white matter lesions at follow-up. The frontal networks may thus represent a core/hub region for brain involvement in COVID-19 beyond the presence of specific (focal) damage associated with clinical manifestations (i.e., stroke, intracranial hemorrhage, encephalitis) of COVID-19 (Duan et al., 2021), already at the acute stage (see above) and continuing into the long run even after acute and subacute viral infection.

Finally, another study has focused on the post-infection course examined COVID-19 patients with persistent nonspecific neurological symptoms (such as fatigue, loss of smell, and headache) 40–60 days after the onset of infection and since cured (Marcic et al., 2021). For this purpose, patients after mild forms of SARS-CoV-2 infection were compared with patients after moderate forms of SARS-CoV-2 infection and with healthy participants in the control group. Here, all subjects in the SARS-CoV-2 group had small, punctate, newly formed hyperintense lesions on T2 and FLAIR sequences. These lesions were distributed also primarily bilaterally frontal subcortical, bilaterally periventricular, and bilaterally juxtacortical. However, in contrast to studies in critically ill patients, participants in this study had no lesions in the corpus callosum. Of note is, yet, that the number of brain lesions correlated significantly with the intensity of the headache: Spearman correlation coefficient rho = 0.578 ($p < 0.001$). Among these, the more severe the clinical picture, the more intense and pronounced the headache. This indicates that headache is a common and important nonspecific neurological symptom, which seems to correlate with both the number of brain lesions and disease severity (Marcic et al., 2021). This is also corroborated by a Chinese study in predominantly mildly ill COVID-19 patients: even when patients recover well from pneumonia, the microstructural and functional integrity of the brain in the recovery phase of COVID-19 was still disturbed after months (Lu et al., 2020). While in this study the prevalence of headache complaints showed a decrease (25% during the acute phase vs. 10% in the follow-up survey), there was an increase in terms of muscle pain prevalence in the follow-up survey after three months (15% vs. 25%). Interestingly, COVID-19 patients from this study also showed significantly increased volumes in the bilateral olfactory cortices, hippocampus, islets, left Heschl's

gyrus, left Rolandic operculum, and right cingulate gyrus. All of these structures belonged to the central olfactory system. The olfactory cortex, also called the piriform cortex, receives axonal projections directly from the olfactory bulb and is referred to as part of the primary olfactory cortex. Interestingly, olfactory ensheathing cells (OECs) in olfactory bulb tissues have also been discussed in the context of pain. Even if not primarily in headache, but rather neuropathic pain, it has been shown that OECs, a special type of neuroglial cell that can release a variety of neurotrophic factors, inhibit the formation of glial scars and cavities, induce change in the microenvironment surrounding nerve injury, and promote nerve repair (Dai et al., 2016). Moreover, it has been shown that transplantation of microencapsulated OECs in rats can relive pain, potentially through receptors of the P2X4 subtype from the P2 purine receptor family, which play an important role in the transmission of algesia and nociception information by primary sensory neurons (Dal Ben et al., 2017).

These results indicate the presence of microstructural changes in the brain and a decrease in cerebral blood flow several months after a COVID-19 infection (Qin et al., 2021) in both patients with mild and severe COVID-19, but the changes in white matter were only smaller in the group with initial mild disease, with no significant changes in gray matter or cerebral blood flow compared to healthy controls, while the changes in patients with initial severe disease course compared to patients with initial mild COVID-19 disease course were much more profound and extensive, particularly in the frontal and limbic systems. In addition, the changes in brain microstructure and cerebral blood flow decline correlated strongly with the level of initial inflammatory markers. Although none of the patients had any specific neurological deficits, the recovered patients with initial severe disease in this study still showed gray matter atrophy in the left insula, left hippocampus, and left superior temporal gyrus, all of which are important components of the limbic system. Therefore, this study suggests a possible vulnerability of the limbic system to COVID-19 infection (Qin et al., 2021).

Conclusions and outlook

Persistent pain following SARS-CoV-2 infection is a growing clinical problem. The underlying pathophysiology is still not understood, but central nervous processes seem to be of leading importance. The current studies suggest that a SARS-CoV-2 infection may affect the central nervous pain system both via nonspecific effects in the context of severe disease courses and the associated inflammatory processes and neurological complications and via specific effects, which may also occur in mild disease courses. These lead to a hypometabolic metabolic state of the brain in specific brain regions, which include wide areas of the pain processing structures. The limbic structures, the frontal cortex, as well as thalamic and corpus callosum changes seem to be of critical importance here. Furthermore, the frontal control networks, which are also important for pain processing, as well as the dorsal attention and attentional networks, and their connectivity to the sensorimotor network appear to be affected.

Future studies should improve neuroimaging by incorporating not only DTI and volumetry, but also functional measures of task-specific and task-independent resting-state fMRI assessments and specific pain ratings and linking these not only to imaging measures but also to neuropsychological and clinical indices. While task-based fMRI allows assessment of brain responses to noxious or non-noxious stimuli as a window into understanding pain, hyperalgesia, and allodynia, resting-state fMRI can assess functional connectivity reflecting synchronous ultralow-frequency oscillations between brain areas (for example, Davis and Moayedi, 2013). Previous research has demonstrated changes in functional activity and connectivity in somatosensory, cognitive, and affective modulation of pain in various chronic pain patients (Baliki et al., 2014, 2012; Cagnie et al., 2014; Kregel et al., 2015), but such brain imaging studies are not yet consistently available in patients with COVID-19.

Information from these measurements could improve understanding of the time course of disease-related brain plasticity and capacity for reversibility and then guide future clinical practice. The correlation between individual factors and brain data could lead to individualized treatment plans, which seems necessary for potential specificities related to pain. Here, early recognition, investigation and management of neurological diseases related to COVID-19 should clearly be integrated (Paterson et al., 2020).

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References

Agarwal, S., Melmed, K., Dogra, S., Jain, R., Conway, J., Galetta, S., and Lewis, A. (2021). Increase in ventricle size and the evolution of white matter changes on serial imaging in critically ill patients with COVID-19. Neurocrit. Care *35*, 491–500.

Al-Sarraj, S., Troakes, C., Hanley, B., Osborn, M., Richardson, M.P., Hotopf, M., Bullmore, E., and Everall, I.P. (2021). Invited Review: The spectrum of neuropathology in COVID-19. Neuropathol. Appl. Neurobiol. *47*, 3–16.

Alexander, G.E., Newman, J.D., and Symmes, D. (1976). Convergence of prefrontal and acoustic inputs upon neurons in the superior temporal gyrus of the awake squirrel monkey. Brain Res. *116*, 334–338.

Apkarian, A.V., Baliki, M.N., and Geha, P.Y. (2009). Towards a theory of chronic pain. Prog. Neurobiol. *87*, 81–97.

Baliki, M.N., Mansour, A.R., Baria, A.T., and Apkarian, A.V. (2014). Functional reorganization of the default mode network across chronic pain conditions. PLoS One *9*, e106133.

Baliki, M.N., Petre, B., Torbey, S., Herrmann, K.M., Huang, L., Schnitzer, T.J., Fields, H.L., and Apkarian, A.V. (2012). Corticostriatal functional connectivity predicts transition to chronic back pain. Nat. Neurosci. *15*, 1117–1119.

Banich, M.T. (1998). The missing link: The role of interhemispheric interaction in attentional processing. Brain Cognit. *36*, 128–157.

Bantick, S.J., Wise, R.G., Ploghaus, A., Clare, S., Smith, S.M., and Tracey, I. (2002). Imaging how attention modulates pain in humans using functional MRI. Brain *125*, 310–319.

Benedetti, F., Mayberg, H.S., Stohler, C.S., and Zubieta, J.-K. (2005). Neurobiological mechanisms of the placebo effect. J. Neurosci. *25*, 10390–10402.

Benedetti, F., Palladini, M., Paolini, M., Melloni, E., Vai, B., De Lorenzo, R., Furlan, R., Rovere-Querini, P., Falini, A., and Mazza, M.G. (2021). Brain correlates of depression, post-traumatic distress, and inflammatory biomarkers in COVID-19 survivors: A multimodal magnetic resonance imaging study. Brain Behav. Immun. Health *18*, 100387.

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M.C., Ploner, M., and Tracey, I. (2011). The effect of treatment expectation on drug efficacy: Imaging the analgesic benefit of the opioid remifentanil. Sci. Transl. Med. *3*, 70ra14.

Borsook, D., Sava, S., and Becerra, L. (2010). The pain imaging revolution: Advancing pain into the 21st century. Neuroscientist *16*, 171–185.

Borsook, D., Veggeberg, R., Erpelding, N., Borra, R., Linnman, C., Burstein, R., and Becerra, L. (2016). The insula: A "hub of activity" in migraine. Neuroscientist *22*, 632–652.

Boutros, N.N. and Belger, A. (1999). Midlatency evoked potentials attenuation and augmentation reflect different aspects of sensory gating. Biol. Psychiatr. *45*, 917–922.

Bushnell, M.C., Duncan, G.H., Hofbauer, R.K., Ha, B., Chen, J.I., and Carrier, B. (1999). Pain perception: Is there a role for primary somatosensory cortex? Proc. Natl. Acad. Sci. U. S. A. *96*, 7705–7709.

Cagnie, B., Coppieters, I., Denecker, S., Six, J., Danneels, L., and Meeus, M. (2014). Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. Semin. Arthritis Rheum. *44*, 68–75.

Chougar, L., Shor, N., Weiss, N., Galanaud, D., Leclercq, D., Mathon, B., Belkacem, S., Ströer, S., Burrel, S., Boutolleau, D., et al. (2020). Retrospective observational study of brain MRI findings in patients with acute SARS-CoV-2 infection and neurologic manifestations. Radiology *297*, E313–e323.

Clarke, S., Kraftsik, R., Van der Loos, H., and Innocenti, G.M. (1989). Forms and measures of adult and developing human corpus callosum: Is there sexual dimorphism? J. Comp. Neurol. *280*, 213–230.

Colombo, D., Falasca, L., Marchioni, L., Tammaro, A., Adebanjo, G.A.R., Ippolito, G., Zumla, A., Piacentini, M., Nardacci, R., and Del Nonno, F. (2021). Neuropathology and inflammatory cell characterization in 10 autoptic COVID-19 brains. Cells *10*, 2262.

Conklin, J., Frosch, M.P., Mukerji, S.S., Rapalino, O., Maher, M.D., Schaefer, P.W., Lev, M.H., Gonzalez, R.G., Das, S., Champion, S.N., et al. (2021). Susceptibility-weighted imaging reveals cerebral microvascular injury in severe COVID-19. J. Neurol. Sci. *421*, 117308.

Dai, Q., Zhang, Z., Liu, Q., and Yu, H. (2016). The protective effect of olfactory ensheathing cells on post-injury spiral ganglion cells. Acta Otolaryngol. *136*, 1115–1120.

Dal Ben, D., Marchenkova, A., Thomas, A., Lambertucci, C., Spinaci, A., Marucci, G., Nistri, A., and Volpini, R. (2017). 2',3'-O-Substituted ATP derivatives as potent antagonists of purinergic P2X3 receptors and potential analgesic agents. Purinergic Signal. *13*, 61–74.

Davis, K.D. and Moayedi, M. (2013). Central mechanisms of pain revealed through functional and structural MRI. J. Neuroimmune Pharmacol. *8*, 518–534.

Duan, K., Premi, E., Pilotto, A., Cristillo, V., Benussi, A., Libri, I., Giunta, M., Bockholt, H.J., Liu, J., Campora, R., et al. (2021). Alterations of frontal-temporal gray matter volume associate with clinical measures of older adults with COVID-19. Neurobiol. Stress *14*, 100326.

Edinger, H.M., Siegel, A., and Troiano, R. (1975). Effect of stimulation of prefrontal cortex and amygdala on diencephalic neurons. Brain Res. *97*, 17–31.

Ermis, U., Rust, M.I., Bungenberg, J., Costa, A., Dreher, M., Balfanz, P., Marx, G., Wiesmann, M., Reetz, K., Tauber, S.C., and Schulz, J.B. (2021). Neurological symptoms in COVID-19: A cross-sectional monocentric study of hospitalized patients. Neurol. Res. Pract. *3*, 17.

Fernández-de-Las-Peñas, C., Navarro-Santana, M., Gómez-Mayordomo, V., Cuadrado, M.L., García-Azorín, D., Arendt-Nielsen, L., and Plaza-Manzano, G. (2021a). Headache as an acute and post-COVID-19 symptom in COVID-19 survivors: A meta-analysis of the current literature. Eur. J. Neurol. *28*, 3820–3825.

Fernández-de-Las-Peñas, C., Navarro-Santana, M., Plaza-Manzano, G., Palacios-Ceña, D., and Arendt-Nielsen, L. (2021b). Time course prevalence of post-COVID pain symptoms of musculoskeletal origin in patients who had survived to severe acute respiratory syndrome coronavirus 2 infection: A systematic review and meta-analysis. Pain, https://doi.org/10.1097/j.pain.0000000000002496.

Fitsiori, A., Pugin, D., Thieffry, C., Lalive, P., and Vargas, M.I. (2020). COVID-19 is associated with an unusual pattern of brain microbleeds in critically ill patients. J. Neuroimaging *30*, 593–597.

Flor, H. (2012). New developments in the understanding and management of persistent pain. Curr. Opin. Psychiatr. *25*, 109–113.

Frankenstein, U.N., Richter, W., McIntyre, M.C., and Rémy, F. (2001). Distraction modulates anterior cingulate gyrus activations during the cold pressor test. Neuroimage *14*, 827–836.

Friedrich, O., Reid, M.B., Van den Berghe, G., Vanhorebeek, I., Hermans, G., Rich, M.M., and Larsson, L. (2015). The sick and the weak: Neuropathies/myopathies in the critically ill. Physiol. Rev. *95*, 1025–1109.

Gazzaniga, M.S. (1995). Principles of human brain organization derived from split-brain studies. Neuron *14*, 217–228.

Gogia, B., Thottempudi, N., Ajam, Y., Singh, A., Ghanayem, T., Dabi, A., Fang, X., Masel, T., and Rai, P. (2021). EEG characteristics in COVID-19 survivors and non-survivors with seizures and encephalopathy. Cureus *13*, e18476.

Goudman, L., De Smedt, A., Noppen, M., and Moens, M. (2021). Is central sensitisation the missing link of persisting symptoms after COVID-19 infection? J. Clin. Med. *10*, 5594.

Gracely, R.H., Petzke, F., Wolf, J.M., and Clauw, D.J. (2002). Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. Arthritis Rheum. *46*, 1333–1343.

Guedj, E., Cammilleri, S., Niboyet, J., Dupont, P., Vidal, E., Dropinski, J.P., and Mundler, O. (2008). Clinical correlate of brain SPECT perfusion abnormalities in fibromyalgia. J. Nucl. Med. *49*, 1798–1803.

Guedj, E., Campion, J.Y., Dudouet, P., Kaphan, E., Bregeon, F., Tissot-Dupont, H., Guis, S., Barthelemy, F., Habert, P., Ceccaldi, M., et al. (2021). (18)F-FDG brain PET hypometabolism in patients with long COVID. Eur. J. Nucl. Med. Mol. Imag. *48*, 2823–2833.

Gur, A., Karakoc, M., Erdogan, S., Nas, K., Cevik, R., and Sarac, A.J. (2002). Regional cerebral blood flow and cytokines in young females with fibromyalgia. Clin. Exp. Rheumatol. *20*, 753–760.

Harris, S. and Rasyid, A. (2020). Objective diagnosis of migraine without aura with migraine vascular index: A novel formula to assess vasomotor reactivity. Ultrasound Med. Biol. *46*, 1359–1364.

Hatanaka, N., Tokuno, H., Hamada, I., Inase, M., Ito, Y., Imanishi, M., Hasegawa, N., Akazawa, T., Nambu, A., and Takada, M. (2003). Thalamocortical and intracortical connections of monkey cingulate motor areas. J. Comp. Neurol. *462*, 121–138.

Hegarty, A.K., Yani, M.S., Albishi, A., Michener, L.A., and Kutch, J.J. (2020). Salience network functional connectivity is spatially heterogeneous across sensorimotor cortex in healthy humans. Neuroimage *221*, 117177.

Hellgren, L., Birberg Thornberg, U., Samuelsson, K., Levi, R., Divanoglou, A., and Blystad, I. (2021). Brain MRI and neuropsychological findings at long-term follow-up after COVID-19 hospitalisation: An observational cohort study. BMJ Open *11*, e055164.

Helms, J., Kremer, S., Merdji, H., Schenck, M., Severac, F., Clere-Jehl, R., Studer, A., Radosavljevic, M., Kummerlen, C., Monnier, A., et al. (2020). Delirium and encephalopathy in severe COVID-19: A cohort analysis of ICU patients. Crit. Care *24*, 491.

Kas, A., Soret, M., Pyatigoskaya, N., Habert, M.O., Hesters, A., Le Guennec, L., Paccoud, O., Bombois, S., and Delorme, C. (2021). The cerebral network of COVID-19-related encephalopathy: A longitudinal voxel-based 18F-FDG-PET study. Eur. J. Nucl. Med. Mol. Imag. *48*, 2543–2557.

Kavak, S., Yildirim, M.S., Altındag, R., Mertsoy, Y., Alakus, M.F., Guleken, M.D., and Kaya, S. (2021). Correlation of neuroimaging findings with clinical presentation and laboratory data in patients with COVID-19: A single-center study. BioMed Res. Int. *2021*, 2013371.

Kelsch, R.D., Silbergleit, R., and Krishnan, A. (2021). Neuroimaging in the first 6 Weeks of the COVID-19 pandemic in an 8-hospital Campus: Observations and patterns in the brain, head and neck, and spine. J. Comput. Assist. Tomogr. *45*, 592–599.

Knight, R.T., Staines, W.R., Swick, D., and Chao, L.L. (1999). Prefrontal cortex regulates inhibition and excitation in distributed neural networks. Acta Psychol. *101*, 159–178.

Koster-Brouwer, M.E., Rijsdijk, M., van Os, W.K.M., Soliman, I.W., Slooter, A.J.C., de Lange, D.W., van Dijk, D., Bonten, M.J.M., and Cremer, O.L. (2020). Occurrence and risk factors of chronic pain after critical illness. Crit. Care Med. *48*, 680–687.

Kregel, J., Meeus, M., Malfliet, A., Dolphens, M., Danneels, L., Nijs, J., and Cagnie, B. (2015). Structural and functional brain abnormalities in chronic low back pain: A systematic review. Semin. Arthritis Rheum. *45*, 229–237.

Kremer, S., Lersy, F., de Sèze, J., Ferré, J.C., Maamar, A., Carsin-Nicol, B., Collange, O., Bonneville, F., Adam, G., Martin-Blondel, G., et al. (2020). Brain MRI findings in severe COVID-19: A retrospective observational study. Radiology *297*, E242–E251.

Kutch, J.J., Ichesco, E., Hampson, J.P., Labus, J.S., Farmer, M.A., Martucci, K.T., Ness, T.J., Deutsch, G., Apkarian, A.V., Mackey, S.C., et al. (2017). Brain signature and functional impact of centralized pain: A multidisciplinary approach to the study of chronic pelvic pain (MAPP) network study. Pain *158*, 1979–1991.

Lambrecq, V., Hanin, A., Munoz-Musat, E., Chougar, L., Gassama, S., Delorme, C., Cousyn, L., Borden, A., Damiano, M., Frazzini, V., et al. (2021). Association of clinical, biological, and brain magnetic resonance imaging findings with electroencephalographic findings for patients with COVID-19. JAMA Netw. Open *4*, e211489.

Lee, M.H., Perl, D.P., Nair, G., Li, W., Maric, D., Murray, H., Dodd, S.J., Koretsky, A.P., Watts, J.A., Cheung, V., et al. (2021). Microvascular injury in the brains of patients with covid-19. N. Engl. J. Med. *384*, 481–483.

Longe, S.E., Wise, R., Bantick, S., Lloyd, D., Johansen-Berg, H., McGlone, F., and Tracey, I. (2001). Counter-stimulatory effects on pain perception and processing are significantly altered by attention: An fMRI study. Neuroreport *12*, 2021–2025.

Lopez, G., Tonello, C., Osipova, G., Carsana, L., Biasin, M., Cappelletti, G., Pellegrinelli, A., Lauri, E., Zerbi, P., Rossi, R.S., and Nebuloni, M. (2022). Olfactory bulb SARS-CoV-2 infection is not paralleled by the presence of virus in other central nervous system areas. Neuropathol. Appl. Neurobiol. *48*, e12752.

Louis, S., Dhawan, A., Newey, C., Nair, D., Jehi, L., Hantus, S., and Punia, V. (2020). Continuous electroencephalography characteristics and acute symptomatic seizures in COVID-19 patients. Clin. Neurophysiol. *131*, 2651–2656.

Lu, Y., Li, X., Geng, D., Mei, N., Wu, P.Y., Huang, C.C., Jia, T., Zhao, Y., Wang, D., Xiao, A., and Yin, B. (2020). Cerebral micro-structural changes in COVID-19 patients - an MRI-based 3-month follow-up study. EClinicalMedicine *25*, 100484.

Marcic, L., Marcic, M., Kojundzic, S.L., Marcic, B., Capkun, V., and Vukojevic, K. (2021). Personalized approach to patient with MRI brain changes after SARS-CoV-2 infection. J. Personalized Med. *11*, 442.

Matschke, J., Lütgehetmann, M., Hagel, C., Sperhake, J.P., Schröder, A.S., Edler, C., Mushumba, H., Fitzek, A., Allweiss, L., Dandri, M., et al. (2020). Neuropathology of patients with COVID-19 in Germany: A post-mortem case series. Lancet Neurol. *19*, 919–929.

Ornello, R., Frattale, I., Caponnetto, V., Pistoia, F., and Sacco, S. (2020). Cerebral vascular reactivity and the migraine-stroke relationship: A narrative review. J. Neurol. Sci. *414*, 116887.

Öztürk, B. and Karadaş, Ö. (2020). Cerebral hemodynamic changes during migraine attacks and after triptan treatments. Noro Psikiyatr Ars. *57*, 192–196.

Pastor, J., Vega-Zelaya, L., and Martín Abad, E. (2020). Specific EEG encephalopathy pattern in SARS-CoV-2 patients. J. Clin. Med. *9*, https://doi.org/10.3390/jcm9051545.

Paterson, R.W., Paterson, R.W., Brown, R.L., Benjamin, L., Nortley, R., Wiethoff, S., Bharucha, T., Jayaseelan, D.L., Kumar, G., Raftopoulos, R.E., et al. (2020). The emerging spectrum of COVID-19 neurology: Clinical, radiological and laboratory findings. Brain *143*, 3104–3120.

Pellinen, J., Carroll, E., Friedman, D., Boffa, M., Dugan, P., Friedman, D.E., Gazzola, D., Jongeling, A., Rodriguez, A.J., and Holmes, M. (2020). Continuous EEG findings in patients with COVID-19 infection admitted to a New York academic hospital system. Epilepsia *61*, 2097–2105.

Petrovic, P., Petersson, K.M., Ghatan, P.H., Stone-Elander, S., and Ingvar, M. (2000). Pain-related cerebral activation is altered by a distracting cognitive task. Pain *85*, 19–30.

Pilotto, A., Masciocchi, S., Volonghi, I., Crabbio, M., Magni, E., De Giuli, V., Caprioli, F., Rifino, N., Sessa, M., Gennuso, M., et al. (2021). Clinical presentation and outcomes of severe acute respiratory syndrome coronavirus 2-related encephalitis: The ENCOVID multicenter study. J. Infect. Dis. *223*, 28–37.

Poloni, T.E., Medici, V., Moretti, M., Visonà, S.D., Cirrincione, A., Carlos, A.F., Davin, A., Gagliardi, S., Pansarasa, O., Cereda, C., et al. (2021). COVID-19-related neuropathology and microglial activation in elderly with and without dementia. Brain Pathol. *31*, e12997.

Qin, Y., Wu, J., Chen, T., Li, J., Zhang, G., Wu, D., Zhou, Y., Zheng, N., Cai, A., and Ning, Q., et al. (2021). Long-term microstructure and cerebral blood flow changes in patients recovered from COVID-19 without neurological manifestations. J. Clin. Invest. *131*, e147329.

Raman, B., Cassar, M.P., Tunnicliffe, E.M., Filippini, N., Griffanti, L., Alfaro-Almagro, F., Okell, T., Sheerin, F., Xie, C., Mahmod, M., et al. (2021). Medium-term effects of SARS-CoV-2 infection on multiple vital organs, exercise capacity, cognition, quality of life and mental health, post-hospital discharge. EClinicalMedicine *31*, 100683.

RKI (2021). Epidemiologischer Steckbrief zu SARS-CoV-2 und COVID-19. https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Steckbrief.html.

Rueckert, L. and Levy, J. (1996). Further evidence that the callosum is involved in sustaining attention. Neuropsychologia *34*, 927–935.

Schweinhardt, P. and Bushnell, M.C. (2010). Pain imaging in health and disease–how far have we come? J. Clin. Invest. *120*, 3788–3797.

Simons, L.E., Elman, I., and Borsook, D. (2014). Psychological processing in chronic pain: A neural systems approach. Neurosci. Biobehav. Rev. *39*, 61–78.

Skinner, J.E. and Yingling, C.D. (1977). Central gating mechanisms that regulate event-related potentials and behavior: A neural model for attention. Attention, Voluntary Contraction and Event-Related Cerebral Potentials. Progress in Clinical Neurophysiology. Desmedt, J.E., ed. (Karger), pp. 28–68.

Sollini, M., Morbelli, S., Ciccarelli, M., Cecconi, M., Aghemo, A., Morelli, P., Chiola, S., Gelardi, F., and Chiti, A. (2021). Long COVID hallmarks on [18F]FDG-PET/CT: A case-control study. Eur. J. Nucl. Med. Mol. Imag. *48*, 3187–3197.

Sonkaya, A.R., Öztrk, B., and Karadaş, Ö. (2021). Cerebral hemodynamic alterations in patients with Covid-19. Turk. J. Med. Sci. *51*, 435–439.

Staines, W.R., Graham, S.J., Black, S.E., and McIlroy, W.E. (2002). Task-relevant modulation of contralateral and ipsilateral primary somatosensory cortex and the role of a prefrontal-cortical sensory gating system. Neuroimage *15*, 190–199.

Tracey, I., Ploghaus, A., Gati, J.S., Clare, S., Smith, S., Menon, R.S., and Matthews, P.M. (2002). Imaging attentional modulation of pain in the periaqueductal gray in humans. J. Neurosci. *22*, 2748–2752.

Uddin, L.Q., Nomi, J.S., Hébert-Seropian, B., Ghaziri, J., and Boucher, O. (2017). Structure and function of the human insula. J. Clin. Neurophysiol. *34*, 300–306.

Valet, M., Sprenger, T., Boecker, H., Willoch, F., Rummeny, E., Conrad, B., Erhard, P., and Tolle, T.R. (2004). Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain–an fMRI analysis. Pain *109*, 399–408.

Villemure, C. and Bushnell, C.M. (2002). Cognitive modulation of pain: How do attention and emotion influence pain processing? Pain *95*, 195–199.

Vlaeyen, J.W.S. and Linton, S.J. (2000). Fear-avoidance and its consequences in chronic musculoskeletal pain: A state of the art. Pain *85*, 317–332.

Wiech, K. and Tracey, I. (2009). The influence of negative emotions on pain: Behavioral effects and neural mechanisms. Neuroimage *47*, 987–994.

Wierzba-Bobrowicz, T., Krajewski, P., Tarka, S., Acewicz, A., Felczak, P., Stępień, T., Golan, M.P., and Grzegorczyk, M. (2021). Neuropathological analysis of the brains of fifty-two patients with COVID-19. Folia Neuropathol. *59*, 219–231.

Witelson, S.F. (1985). The brain connection: The corpus callosum is larger in left-handers. Science *229*, 665–668.

Yingling, C.D. and Skinner, J.E. (1977). Gating of thalamic input to cerebral cortex by nucleus reticularis thalami. Attention, Voluntary Contraction and Event-Related Cerebral Potentials. Progress in Clinical Neurophysiology. Desmedt, J.E., ed. (Karger), pp. 70–96.

Zaidel, E. and Iacoboni, M. (2003). The parallel brain: The cognitive neuroscience of the corpus callosum (MIT Press: Cambridge, Massachusetts, London, England).

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Jonas Tesarz is a specialist in internal medicine and currently works as managing senior physician and associate professor at Heidelberg University Hospital, Department of General Internal Medicine and Psychosomatics. After completing his PhD at the University of Heidelberg on the neurobiology of pain processing, he initially focused on researching the role of myofascial tissue in the development and maintenance of low back pain before starting his clinical practice at the Department of General Internal Medicine and Psychosomatics (Division of Musculoskeletal Pain) at the University of Heidelberg. There he habilitated on the influence of biopsychosocial factors on chronic low back pain. In addition to researching the influence of traumatic life events on pain processing, his particular scientific interest lies in the development and scientific evaluation of novel psychological treatment approaches for the treatment of chronic pain conditions.

Obituary

Carsten Duch and Ansgar Büschges*

Hans-Joachim Pflüger – scientist, citizen, cosmopolitan

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Far too early has our colleague, Professor Hans-Joachim '*Jochen*' Pflüger, passed away on January 25, 2022, only 72 years old. This loss of our close friend and honorable companion has struck us unexpectedly, because Jochen was highly active, full of infectious energy and exciting future projects. We feel deep sorrow and grief along with his wife, Bärbel, and his family and friends. The numerous ideals, goals, and plans that we have shared with Jochen will remain alive forever.

Jochen's career and life in science

Jochen (Figure 1) was born in the post-world war period on March 7th, 1949 in Ulm, a medieval town in the southern German state of Baden-Württemberg. There, he completed his primary and high-school education and passed his qualification for general university admittance in 1968. Jochen then followed his interests in the natural and life-sciences, choosing to study biology and chemistry at the University of Stuttgart, initially with the plan to become a school teacher. In 1972 he was selected as "Aufbaustudent" (founding student) with the task of helping to establish a study program for biology at the newly founded University of Kaiserslautern in the state of Rhineland-Palatinate. In 1974 Jochen graduated with the "Staatsexamen" (state examination for teachers) in both chemistry and biology at Kaiserslautern. In the same year, Jochen started secondary school teaching, but in parallel, he engaged in post-graduate studies with Ulrich Bässler, who inspired him to undertake a doctoral thesis on the neural control of limb movements and locomotion, a topic that has fascinated him for decades thereafter. It was in Kaiserslautern where Jochen met his later spouse, Bärbel Kolodziej, a professional costume designer at the municipal theater, at that time working in "theater on the road" throughout the Rhineland-Palatinate state.

Jochen's first scientific study case was the stick insect, *Carausius morosus*. Later he added numerous other animal model species as his attention turned towards a more comparative, evolutionary approach. For his doctoral thesis, Jochen studied the neural control of body "rocking", a species-specific motor behavior expressed by phasmids for camouflage. Of debate in the field of motor control at that time was the role of neural circuits residing in the central nervous system, so called central pattern generators (CPGs), capable of generating rhythmic motor activity *versus* the role that sensory feedback signals play in producing cyclic animal movements and locomotion. In fact, deciphering the relative contributions of these two neural processes remains an issue that remains to be fully resolved for both vertebrate (Grillner and El Manira, 2020; Kiehn, 2016) and invertebrate motor control (Mantziaris et al., 2020). During his PhD, Jochen demonstrated with skillful experiments that the coordination of motor neuron activity required for stick insect rocking and walking movements can be generated without sensory feedback by a CPG network, but that the centrally generated motor activity is constantly refined by proprioceptive signals that provide feedback information about limb positions and movement velocities (Pflüger, 1977). He concluded that both CPG network activity and sensory feedback work hand in hand to control locomotion. This first peer reviewed scientific contribution typified Jochen's principle attitude: not adhering to pre-existing "dogmas", but working and reasoning openly to integrate all possible mechanisms contributing to a defined nervous system function.

Jochen completed his PhD in 1976 and then moved for approximately one year to the lab of Malcolm Burrows at the University of Cambridge for his first postdoc position studying motor control. Now working with locusts, Jochen started making full use of the 'identified neuron principle' in insects, for example, in mapping the axonal projections of mechanosensory neurons onto central motor circuitry. At a time when *in vivo* genetic labeling, identification, and

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Jochen Pflüger on a hike in the Ahrntal (South Tyrol) in 2014; (photo taken by A. Büschges).

manipulation of defined types of neurons was not yet feasible, many invertebrate nervous systems offered, with their accessible and relatively small numbers of CNS neurons, the possibility to physiologically and anatomically identify the same neurons from animal to animal. Building on this experimental advantage in insects, Jochen published a series of studies that mapped, both anatomically and functionally, mechanosensory input to identified central motor circuitry (Pflüger, 1984; Pflüger et al., 1981, 1986). During this time, he started developing a passion for the direct access to neuronal function by intracellular recordings and through the beauty of neuroanatomy, two aspects of experimental neurobiology that he considered not only to be fundamental methodological tools for scientific advancement, but also in satisfying his quest for innovative artistry. Any student in Jochen's lab learned quickly under his supervision that neurophysiology and neuroanatomy can be successfully employed according to scientific and aesthetic criteria in combination. Jochen followed his interest and fascination in improving his technical and theoretical expertise in studying insect sensorimotor circuits (e.g. Pflüger and Burrows, 1987). His relatively brief research period in Cambridge led to subsequent collaborations with his original postdoc supervisor, Malcolm Burrows, over the next four decades, starting with a study on swimming in locusts (Pflüger and Burrows, 1978; see also, Burrows and Pflüger, 1995; Pflüger and Burrows, 1987).

In 1977 Jochen returned to Germany, first to the University of Bielefeld (1977–1980) to work in the lab of Peter Görner and subsequently to the University of Konstanz (1980–1987) to the lab of Werner Rathmayer. During this period, Jochen perfected his methodological repertoire for gaining insights into the sensorimotor principles of insect motor control. In 1985 in Konstanz, Jochen passed his habilitation, entitled "*Sensorimotor interactions in the locust: Unraveling a mechanoreceptive information path*". In Germany, the habilitation, which is based on an evaluation of both research and teaching achievements, was at that time the only mandated PhD follow-up qualification to become eligible for obtaining a university professorship. During this period, Jochen contributed substantially to making the locust a so-called "model organism" in neuroethology, best exemplified by his foundation study on the organization of mechanosensory neuropiles in the locust thoracic ganglia that still remains highly relevant for studies on sensorimotor circuit structure and function in insects (Pflüger et al., 1988). While continuing to decipher mechanosensory processing in motor networks in locusts with increasingly elegant and sophisticated methods, in the Rathmayer lab Jochen also started to analyze the effects of animal toxins and neuromodulatory substances on neuromuscular systems. Today it is well accepted that in addition to synaptic connections and functional properties of sensorimotor networks, neuromodulation is an essential component for the control of movement by the nervous system (Marder, 2012).

Subsequently, in 1987 Jochen accepted the offer of a professorship in *Neurobiology and Functional Neuroanatomy* at the Free University of Berlin (FU Berlin), where he conducted his research and taught ever since. Jochen started his own lab focusing on the anatomical and functional characterization of a specific class of aminergic neurons in the locust with central and peripheral projections, so called dorsal unpaired median (DUM) neurons, named by the location of their unpaired cell bodies at the dorsal midline of the insect CNS (Kononenko et al., 2019; Stevenson et al., 1992). DUM neurons produce and release the biogenic amine, octopamine, the invertebrate analogue of norepinephrine in vertebrates (reviewed in Bräunig and Pflüger, 2001). Jochen found that, in addition to the already known functions of biogenic amines, octopamine release from DUM neurons is regulated differentially in different types of DUM cells and is temporally coupled to specific aspects of a motor program (Burrows and Pflüger, 1995; Duch et al., 1999). This finding pointed towards more precise roles of aminergic modulation for the generation of motor activity, including locomotion. On the other hand, he showed that task-specific inhibition of specific types of DUM neurons, for example during flight activity of migratory locusts, serves to switch from carbohydrate to lipid metabolism during prolonged flight (Mentel et al., 2003). Overall, Jochen's studies added important insights into

short and long-term metabolic adjustments by biogenic amines during insect locomotor behavior.

Jochen's interest in adaptive changes of neural circuit operation under the influence of neuromodulators led to him to team up with colleagues in Berlin who also worked on the functional plasticity of neuronal circuits. Particularly fruitful were his close interactions with his colleague at the Institute of Neurobiology in Berlin, Randolf Menzel, a specialist in the field of insect learning and memory research. This provided the cornerstone for multiple collaborative research consortia that have sparked invertebrate neurobiology research at the FU Berlin and beyond. Jochen and Randolf successfully applied for a Research Unit on "*Learning, Memory and Neuromodulation in Arthropods*" (1989–1995) funded by the German Research Foundation (DFG). Subsequently, Jochen's research on mechanisms of neuromodulation made an important contribution to the successful installment of the DFG-funded Collaborative Research Center 515 (CRC 515, "*Mechanisms of developmental and experience dependent nervous system plasticity*", 1996–2005). This CRC brought together neuroscience groups from multiple Berlin Universities and blue-list Research Institutions. The same inter-institutional initiative held for the DFG-funded Graduate School 837 "*Functional Insect Science*" in collaboration with the University of Potsdam. Throughout these years, Jochen's ongoing research contributed to our present understanding of the role of neuromodulation during the development and execution of motor programs and animal behavior in general (Pflüger, 1999). In 2010, Jochen finally established a new DFG-funded Research Unit on biogenic amine function in insects (FOR 1363, "*Biogenic amines in insects: coordination of physiological processes and behavior*"), which he led as the program's principal coordinator from 2010 to 2016.

Over this period, Jochen transformed his lab at the Königin-Luise-Street in Berlin Dahlem into a well-known and highly attractive location for invertebrate neuroethology colleagues, both nationally and internationally. Scientists from all over Germany and around the globe frequently visited his lab, not only because of the excellent and timely research conducted in his and other labs at the FU Berlin, but also because of the open minded, multicultural, and warm atmosphere that was present in Jochen's lab. In fact, this made his lab a virtual hub of German invertebrate neuroethology. His visiting colleagues are too many to cite, but particularly influential to Jochen's lab were longer visits by Malcom Burrows (University of Cambridge, UK), Richard B "Rick" Levine (University of Arizona, Tucson, US), and Laurence H. "Larry" Field (University of Canterbury, NZ). Viewing Jochen's list of publications provides a deeper indication to his strength in networking and collaboration. One reflection of Jochen's international standing was his appointment as adjunct professor at the ARL Division of Neurobiology at the University of Arizona, Tucson, AZ since 1991 (Pflüger et al., 1993).

Jochen – beyond the bench

Jochen was that kind of German university faculty member who was of the strong belief that universities and research institutions need people to actively "run the show" and to assume ownership in building a high-quality professional environment for all other staff associates. In doing so, Jochen contributed to the development of neurobiology and biology at the FU Berlin in serving his *alma mater* in various administrative roles, for example, as Managing Director of the Institute of Neurobiology, as Dean of the Faculty of Pharmacy, Chemistry and Biology and as an elected member in the FU Senate. At times these roles were particularly challenging and demanding, for example, during the early 2000s, when the three Berlin universities each had to restructure and reduce their study program portfolios and research positions in order to accommodate the tremendous financial cut-backs in Berlin at that time.

Jochen's activities also included supporting high school pupils, students and young researchers in graduate programs, reflected in his reviewing role for "*Jugend forscht*", a Germany-wide competition for talented pupils in the areas of science, technology, engineering and mathematics (STEM), his role in the DFG-funded Research Training Grant (RTG) "*Signalling Cascades in Living Systems*" and his activities in individually mentoring students, for example, scholars of the *Studienstiftung des deutschen Volkes (German Academic Scholarship Foundation)*.

Of similar significance was his service to the scientific community in his work for funding agencies, most prominently the DFG, for which he served as member of the neuroscience study section. His broad knowledge in neuroscience that spread into multiple related areas, together with his fair way of judging the work of colleagues, in particular when they were competing with him, made him ideally suited as a reviewer and editorial board member of scientific journals, for example, the *Journal of Comparative Physiology A*, for which Jochen served for more than two decades.

Jochen was always highly respected among his colleagues due to his undogmatic view of the different areas of neuroscience research, and commitment to promoting the discovery of new insights. This vision made him an

excellent participant on boards of national and international scientific associations, such as the *German Neuroscience Society*, the *International Society for Invertebrate Neurobiology*, the *International Society for Neuroethology*, the *Federal European Neuroscience Association* and finally the *International Brain Research Organization (IBRO)*, which organizes the *Global Federation of Neuroscience Societies*. While on all these boards, Jochen served, for example, as treasurer of FENS (2011–13), as member of the Pan-European Research Council (PERC in IBRO) and as treasurer and president of the *German Neuroscience Society* (1992–2003 and 2015–2017, respectively).

We will miss Jochen for many, many other reasons, some of which we would like to highlight here.

Jochen was a true zoologist and neuroethologist (Figure 2), who scrutinized the nervous system of an organism while always retaining and integrating perspective of the animal's specific environmental and behavioral setting. For example, when it became known that neural circuits for insect flight and walking differ with respect to their sensitivity to neuromodulators, Jochen chose to compare their systemic actions in winged locust and hawkmoth, as well as in wingless phasmids. Jochen's broad biological knowledge was known to those who had the chance to participate and observe him on one of his excursions for birdwatching. These outings were not only regularly offered in the surrounds of Berlin to students, colleagues, and friends, but he also planned and embarked on them with companion scientists throughout the globe, ranging from visits to the ornithological station at the Baltic Curonian Spit to excursions after ISN and SFN meetings. For example, in travelling by car to the ISN congress in Salamanca, Spain in 2010, Jochen had planned the trip in such a way that it essentially turned out to be a major birdwatching excursion of some 1800 km!

Jochen Pflüger, the ornithologist, in a bird watching station in the Spanish highlands on the road trip to the 2010 ISN in Salamanca, Spain; (photo taken by A. Büschges).

Working at the Free University Berlin served the cultural interest of Jochen perfectly. When living in Berlin, Jochen and Bärbel always were highly reliable and expert informants on which exhibition to visit, which concert to enjoy and which theater play to attend. Many of us had the immense pleasure of diving into the cultural life of Berlin with Jochen and Bärbel when a visit to Berlin was on the agenda. In the same way, Jochen's own collaborative visits around the globe were always accompanied by his exploration of the local cultural life and customs.

Jochen cared in a marvelous, impressive way about the history of science and society. Two examples illustrate this aspect of his personality: In 2016 Jochen was awarded the first "*Ernst Bresslau Professorship*" at the Institute of Zoology at the University of Cologne. Ernst Bresslau, the Institute's first Director in 1925, was expelled by the Nazis in 1933 based on their "Gesetz zur Wiederherstellung des Berufsbeamtentums" (law to reinstate professional officialdom) and his quarter Jewish heritage, even though he was a highly decorated military doctor during World War I. Prof. Bresslau had to leave Germany permanently with his family and took up the founder position for the Institute of Zoology at Sao Paulo in Brazil. Jochen not only honored the guest professorship by his stay at the Institute and his close collaboration with Institute colleagues, but he also looked closely into the work and life of Ernst Bresslau (Pflüger, 2017). In a fascinating way, Jochen used all possible sources of information, such as archives, media, and institutions, to collect information about the Institute's founder and his fate during the Nazi period. Jochen even visited Ernst-Bresslau's relatives in Sao Paulo to inspect the original hand-written excursion books from the latter's various field trips almost 100 years ago. These efforts led to an unexpected view on the life of the founder of the Institute of Zoology at the University of Cologne. One of Jochen's last contributions to our understanding of the history of zoology was a collaboration with Julia Heideklang and Helmut Kettenmann to translate the dissertation of Hermann Helmholtz from Latin into English and German (Heideklang et al., 2021). This early work of Helmholtz, entitled "*De fabrica systematis nervosi evertebratorum*"

attracted Jochen's passion for fine neuroanatomy, since already in 1842, it provided major insights into invertebrate neuroanatomy that still hold today.

We will always keep Jochen in our memories and hearts as an outstanding investigative scientist, an all-round biologist, a companion, close friend, and a true citizen of the world.

References

Bräunig, P. and Pflüger, H.-J. (2001). The unpaired median neurons of insects. Adv. Insect Physiol. *28*, 186–266.

Burrows, M. and Pflüger, H.-J. (1995). The action of locust neuromodulatory neurons is coupled to specific motor patterns. J. Neurophysiol. *74*, 347–357.

Duch, C., Mentel, T., and Pflüger, H.-J. (1999). Distribution and activation of different types of octopaminergic DUM neurons in the locust. J. Comp. Neurol. *403*, 119–134.

Grillner, S. and El Manira, A. (2020). Current principles of motor control, with special reference to vertebrate locomotion. Physiol. Rev. *100*, 271–320.

Heideklang, J., Pflüger, H.-J., and Kettenmann, H. (2021). De fabrica systematis nervosi evertebratorum. Die kommentierte Dissertation von/commented Thesis by Hermann Helmholtz (wbg Academic: Darmstadt).

Kiehn, O. (2016). Decoding the organization of spinal circuits that control ocomotion. Nat. Rev. Neurosci. *17*, 224–238.

Kononenko, N.L., Hartfil, S., Willer, J., Ferch, J., Wolfenberg, H., and Pflüger, H.-J. (2019). A population of descending tyraminergic/octopaminergic projection neurons of the insect deutocerebrum. J. Comp. Neurol. *527*, 1027–1038.

Mantziaris, C., Bockemühl, T., and Büschges, A. (2020). Central pattern generating networks in insect locomotion. Dev. Neurobiol. *80*, 16–30.

Marder, E. (2012). Neuromodulation of neuronal circuits: Back to the future. Neuron *76*, 1–11.

Mentel, T., Duch, C., Stypa, H., Müller, U., Wegener, G., and Pflüger, H.-J. (2003). Central modulatory neurons control fuel selection in flight muscle of migratory locust. J. Neurosci. *23*, 1109–1113.

Pflüger, H.-J. (1977). The control of the rocking movements of the phasmid Carausius morosus Br. J. Comp. Physiol. A *120*, 181–202.

Pflüger, H.-J. (1984). The large fourth abdominal intersegmental interneuron: A new type of wind-sensitive ventral cord interneuron in locusts. J. Comp. Neurol. *222*, 343–357.

Pflüger, H.-J. (1999). Neuromodulation during motor development and behavior. Curr. Opin. Neurobiol. *9*, 638–689.

Pflüger, H.-J. (2017). Professor Ernst Bresslau, founder of the zoology departments at the universities of Cologne and Sao Paulo: Lessons to learn from his life history. Zoology *122*, 1–6.

Pflüger, H., Bräunig, P., and Hustert, R. (1981). Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. Cell Tissue Res. *216*, 79–96.

Pflüger, H.-J., Bräunig, P., and Hustert, R. (1988). The organization of mechanosensory neuropiles in locust thoracic ganglia. Phil. Trans. Roy. Soc. Lond. B *321*, 1–26.

Pflüger, H.-J. and Burrows, M. (1978). Locusts use the same basic motor pattern in swimming as in jumping and kicking. J. Exp. Biol. *75*, 81–93.

Pflüger, H.-J. and Burrows, M. (1987). A strand receptor with a central cell body synapses upon spiking local interneurones in the locust. J. Comp. Physiol. A *160*, 295–304.

Pflüger, H.-J., Elson, R., Binkle, U., and Schneider, H. (1986). The central nervous organization of the motor neurones to a steering muscle in locusts. J. Exp. Biol. *120*, 403–420.

Pflüger, H.-J., Witten, J., and Levine, R.B. (1993). Fate of abdominal ventral unpaired median cells during metamorphosis of the hawkmoth, Manduca sexta. J. Comp. Neurol. *335*, 508–522.

Stevenson, P.A., Pflüger, H.-J., Eckert, M., and Rapus, J. (1992). Octopamine immunoreactive cell populations in the locust thoracic-abdominal nervous system. J. Comp. Neurol. *315*, 382–397.

Nachrichten aus der Gesellschaft

https://doi.org/10.1515/nf-2022-0010

NWG-Reisestipendien für das FENS Forum 2022 in Paris vergeben

Aus den zahlreichen Bewerbungen wurden 10 Kandidaten für ein Reisestipendium für die Teilnahme am FENS Forum 2022 in Paris, Frankreich ausgewählt.

(1) Aksan, Bahar (Heidelberg)
(2) Baldauf, Leonie (Hannover)
(3) Buchholz, Sarah (Freiburg)
(4) Burkart, Marie-Elisabeth (Leipzig)
(5) De Sa, Rafael (Berlin)
(6) Demaili, Arijana (Magdeburg)
(7) Janssen, Jan Maximilian (Linz, Österreich)
(8) Kupke, Janina (Heidelberg)
(9) Marosi, Endre (Magdeburg)
(10) Srivastava, Akash (Jena)

Herzlichen Glückwunsch!

Die Stipendiaten erhalten eine Unterstützung für die Kosten für Anreise und Unterkunft in Höhe von 500 Euro und eine Urkunde.

Einladung zur Mitgliederversammlung während des FENS Forum 2022 in Paris

Termin: Sonntag, 10. Juli 2022, 18:45–21:30 Uhr, Paris Expo Porte de Versailles
Vorläufige Tagesordnung:

(1) Begrüßung durch die Präsidentin
(2) Bestätigung des Protokolls der letzten Mitgliederversammlung
(3) Bericht des Schatzmeisters
(4) Mitteilungen
(5) Bericht Göttinger Tagung 2023
(6) Vorstandswahlen 2023
(7) Aktivitäten der Gesellschaft
(8) Verschiedenes

Vorschläge für weitere Tagesordnungspunkte müssen **bis spätestens 15. Juni 2022** bei der Geschäftsstelle eingegangen sein (gibson@mdc-berlin.de).

NEU auf dasGehirn.info

Im Februar stand wieder einmal das Thema Musik im Mittelpunkt: **Musik** ist ein Kulturgut wie kein anderes: Sie verpackt Emotion in die Mathematik der Harmonie und transportiert Information wie eine Sprache.

Das Thema **Gewalt,** eine Kooperation mit dem IRTG 2150, war im März noch einmal Schwerpunktthema, und zwar mit den folgenden Beiträgen:

Die tiefen Wurzeln der Gewalt – Aggression und Gewalt sind bis zu einem gewissen Grad Teil unserer DNA. Doch ob und wie aggressiv Menschen individuell sind, ist bekanntlich verschieden. Frühkindliche Erfahrungen und Kontrollzentren im Gehirn spielen dabei eine Rolle.

Gewalt im Gehirn – Schläge, Tritte, Gräueltaten mit Waffen – menschliche Aggression entfaltet sich trotz aller zivilisatorischen Bemühungen in fürchterlicher Vielfalt. Was passiert im Gehirn, damit es so weit kommt?

Das aggressive Geschlecht – Jack the Ripper, Ted Bundy und der Rhein-Ruhr-Ripper hatten zwei Dinge gemeinsam: Sie waren Serienmörder und sie waren Männer. Die meisten Gewaltverbrechen werden von Männern begangen. Die Forschung will klären, warum das so ist.

Der Reflex der Gewalt – Wiederholte Gewalterfahrungen schreiben sich ins Gehirn ein. Deshalb sind solche Schreckenserlebnisse bis heute ein wichtiger Risikofaktor für viele psychische Störungen.

Strategien gegen die Gewalt – Übermäßige Aggressivität ist oft ein Anzeichen für eine Verhaltensstörung oder ein psychisches Leiden. Mit unterschiedlichen Ansätzen versuchen Ärzte und Psychologen diese Störungen des Sozialverhaltens zu behandeln.

In der Mediathek sind neue Beiträge hinzugekommen:
Das Schwerpunktthema *Schlaganfall*, eine Kooperation mit den DFG-Forschungsgruppen 2879 und 2795, wurde um das Videointerview

Schlaganfall – Grundlagen und aktuelle Forschung ergänzt: Ein Schlaganfall kann verheerende Folgen haben und führt viel zu oft zum Tod. Zudem tritt er erschreckend häufig auf. Prof. Dr. Christine Rose erforscht die Effekte im Gehirn, Prof. Dr. Christoph Kleinschnitz die Behandlung; beide sprechen hier über den aktuellen Stand der Forschung

Die Summer School des TRR 265 stellt dem Portal ihren Videovortrag **Kerstin Ritter – AI in Brain Research** zur Verfügung: Im 4. Vortrag der (Covid-bedingt digitalen) Summer School des TRR 265 spricht Prof. Dr. Kerstin Ritter, Juniorprofessorin für Computational Neuroscience, Charité Campus Mitte, über den Einsatz künstlicher Intelligenz in der Hirnforschung. – Weitere Vorträge sind ebenfalls auf dasGehirn.info zu hören.

#BerlinBrains2022: Evolution des menschlichen Gehirns: Im Lauf der Evolution wurden menschliche Gehirne kognitiv immer leistungsfähiger. Welche genetischen Änderungen führten dazu? Und wie testet man, ob eine genetische Änderung kognitive Fähigkeiten ändert? Katja Nowick und Jeong-Eun Lee erläutern, wie ihre Arbeitsgruppe mit bioinformatischen Methoden menschenspezifische Gensequenzen identifiziert, die für die Evolution unseres Gehirns relevant sind. Und sie berichten, wie sie derartige Gensequenzen auf ihre Funktion hin testen.
Prof. Dr. Katja Nowick, Freie Universität Berlin
Jeong-Eun Lee, Freie Universität Berlin
Moderation: Dr. Jochen Müller

In dem Format **Frage an das Gehirn** beantworten Experten regelmäßig Fragen unserer Leser. Zuletzt ging es um die folgenden Fragen:

Musik im Kopf – Wie entsteht eigentlich ein Ohrwurm? Und wie wird man ihn wieder los?

Wie wirken sich Darmbakterien auf die Stimmung aus? – Bakterien im Darm sollen sich auf das Wohlbefinden auswirken. Wie das? Und hilft es, wenn ich jeden Tag probiotischen Joghurt löffle?

Mag das Gehirn Langeweile? – Meine Mutter behauptet, Langeweile sei gut für das Gehirn. Stimmt das und warum ist das so?

In der Rubrik **Neues aus der Wissenschaft** macht dasGehirn.info im Februar und März 2022 auf die folgenden Pressemeldungen aus den Instituten aufmerksam:

Multiple Sklerose: Zwillingsstudie entschlüsselt Einfluss von Umwelt und Genetik auf das Immunsystem | Ludwig-Maximilians-Universität München (17.02.2022)

Das kleine Einmaleins der Nervenzelle | Max-Planck-Institut für biologische Intelligenz, in Gründung (23.02.2022)

Feeling the Groove: Warum uns Samba besonders zum Tanzen bringt | Max-Planck-Institut für Kognitions- und Neurowissenschaften (28.02.2022)

Als die Synapsen-Bausteine knapp wurden: Bayreuther Biologen erklären Proteinaustausch während der Wirbeltier-Evolution | Universität Bayreuth (28.02.2022)

Milch kann MS-Symptome verstärken | Rheinische Friedrich-Wilhelms. Universität Bonn (01.03.2022)

Langfristiger Benzodiazepin-Gebrauch greift Synapsen an | Universitätsklinikum Bonn (08.03.2022)

Astrozyten-Netzwerke steuern räumliches Lernen und Gedächtnis | Universität Zürich (08.03.2022)

Wie chronischer Schmerz entsteht | Ruprecht-Karls-Universität Heidelberg (08.03.2022)

Alzheimer-Erkrankung: Schützende Immunzellen schon Jahrzehnte vor Ausbruch aktiv | Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE) (17.03.2022)

Möchten Sie eine Pressemeldung an **dasGehirn.info** weitergeben oder Ihr Institut vorstellen, wenden Sie sich bitte an Arvid Leyh (a.leyh@dasgehirn.info).

Neueintritte

Folgende Kolleginnen und Kollegen dürfen wir als Mitglieder der Neurowissenschaftlichen Gesellschaft begrüßen:

Dorukhan Acil (Leipzig)
Tuba Aktürk (Istanbul, Türkei)
Brigid Chimoita Aliero (Magdeburg)
Ayshan Aliyeva (Planegg)
Kira Andrea (Kaiserslautern)
Natalia Babushkina (Bochum)
Xianshu Bai, Dr. (Homburg)
Leonie Baldauf (Hannover)
Sanja Bauer Mikulovic, Dr. (Magdeburg)
Amina Becic (Bochum)
Despoina Binou (Jena)
Michela Borghi (Mainz)
Diba Borgmann (Köln)
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Der Mitgliedsstand zum 15. März 2022 beträgt 2.050 Mitglieder.

Ausblick

Veronica Egger
Neuromodulation of social odor discrimination in the main olfactory bulb

Ilona Grunwald-Kadow
How the body rules the nose

Markus Rothermel
Functional role of the anterior olfactory nucleus in sensory information processing

Sebastian Bitzenhofer
How the sense of smell influences cognition throughout life

Tobias Ackels
Information about space from time: The mammalian olfactory system encodes fast dynamics of the odour landscape

Ivan Manzini
Perireceptor events and peripheral modulation of odorant responses in the olfactory epithelium of vertebrates

NEUROWISSENSCHAFTLICHE GESELLSCHAFT
GERMAN NEUROSCIENCE SOCIETY

Neurowissenschaftliche Gesellschaft e.V. (NWG)

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Datum/Unterschrift des Mitglieds Datum/Unterschrift des Mitglieds

Bitte senden Sie Ihren Antrag an die Geschäftsstelle der NWG:

Stefanie Korthals
Neurowissenschaftliche Gesellschaft e.V.
MDC
Robert-Rössle-Str. 10
13092 Berlin
Email: korthals@mdc-berlin.de
Tel.: +49 30 9406 3127

Ich optiere für folgende 2 Sektionen:

- ☐ Computational Neuroscience
- ☐ Entwicklungsneurobiologie/Neurogenetik
- ☐ junge NWG (jNWG)
- ☐ Klinische Neurowissenschaften
- ☐ Kognitive Neurowissenschaften
- ☐ Molekulare Neurobiologie
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie

Ich bin Student ☐ ja ☐ nein
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