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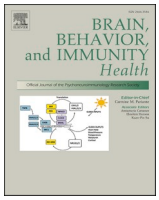

# Pain research in a petri dish? Advantages and limitations of neuro-glial primary cell cultures from structures of the nociceptive system

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A B S T R A C T

How can we learn more about pain without causing pain in humans or animals? This short review focuses on neuro-glial primary cell cultures as models to study neuro-immune interactions in the context of pain and discusses their advantages and limitations.

The field of basic pain research places scientists in an ethical dilemma. We aim to understand underlying mechanisms of pain for an improved pain therapy for humans and animals. At the same time, this regularly includes the induction of pain in model animals. Within the field of psychoneuroimmunology, the examination of the complexity of neuro-immune interactions in health and disease as well as the bi-directional communication between the brain and the periphery make animal experiments an inevitable part of pain research. To address ethical and legal considerations as well as the growing societal awareness for animal welfare, scientists push for the identification and characterization of complementary methods to implement the 3R principle of Russel and Burch. As such, methods to *replace* animal studies, *reduce* the number of animals used, and *refine* experiments are tested. Neuro-glial primary cell cultures of structures of the nociceptive system, such as dorsal root ganglia (DRG) or the spinal dorsal horn (SDH) represent useful *in vitro* tools, when research comes to a cellular and molecular level. They allow for studying mechanisms of neuronal sensitization, glial cell activation, or the role of specific inflammatory mediators and intracellular signaling cascades involved in the development of inflammatory and neuropathic pain. Moreover, DRG/SDH-cultures provide the opportunity to test novel strategies for interventions, such as pharmaceuticals or cell-based therapies targeting neuroinflammatory processes. Thereby, *in vitro* models contribute to a better understanding of neuron-glia-immune communication in the context of pain and in the advancement of pain therapies. However, this can only be one piece in a large puzzle. Our knowledge about the complexity of pain will depend on studies in humans and animals applied *in vitro* and *in vivo* and will benefit from clear and open-minded interdisciplinary communication and transparency in public outreach.

> "Pain research thus places us between horns of a troublesome ethical dilemma. We appear obligated to do something - pain research on animals - that will sometimes involve doing something else - causing pain - that we are generally obligated not to do."(Tannenbaum, 1999)
>
> Jerrold Tannenbaum: "Ethics and Pain Research in Animals", 1999

## 1. Basic pain research in the field of psychoneuroimmunology (PNI)

Pain is one of the most common reasons why people seek medical care (Goldberg and McGee, 2011). Therefore, it represents not only a significant socio-economic challenge of global outreach, but even more importantly an enormous personal physiological and psychological burden affecting patients independent of age, gender or ethnicity with an estimated 20% of the global population suffering from pain (Goldberg and McGee, 2011). In 2021, one in five U.S. citizens reported pain on most or every day during the previous 3 months, referred to as chronic pain (Rikard et al., 2023). The Global Burden of Disease (GBD) study 2017 identified low back pain and headache disorders as the top two leading Level 3 causes of years lived with disability worldwide (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). More diseases that are directly or indirectly associated with pain appear in this list and include depressive and anxiety disorders, as well as drug and alcohol use disorders. While there is a rising awareness of recognizing pain as a global health priority, adequate options to sufficiently treat pain need to be improved (Sessle, 2011). Therefore, increased efforts in pain research are warranted to uncover mechanisms of pain modulation for an advanced pain relief.

In recent decades, psychoneuroimmunology (PNI) has evolved into an outstanding interdisciplinary field of research connecting basic and clinical scientists to foster our understanding of immune-brain

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interactions in health and disease (Rummel et al., 2022; Peters et al., 2023). The bi-directional communication between the nervous and immune system is of pivotal importance in the context of pain and associated comorbidities (Zouikr and Karshikoff, 2017; Karshikoff et al., 2019). Several endogenous inflammatory mediators released by immune and glial cells modulate the neuronal excitability of primary nociceptors (peripheral sensitization) or second-order neurons in the spinal dorsal horn (central sensitization). At the same time, activation of nociceptors induces the release of inflammatory neuropeptides, such as substance P or calcitonin-gene-related peptide (CGRP) in the periphery, as well as in the dorsal horn of the spinal cord. Thereby, nociceptive stimulation promotes peripheral (neurogenic) inflammation and (neuro) inflammation in the spinal cord (Matsuda et al., 2019). Thus, pain and inflammation are directly linked to each other.

Neuroinflammatory processes in dorsal root ganglia (DRG) and the spinal dorsal horn (SDH) are important components in the development of inflammatory and neuropathic pain. Dorsal root ganglia contain cell bodies of pseudounipolar sensory neurons, which innervate the periphery and transmit the information to the SDH (Dubin and Patapoutian, 2010). These neurons express channels of the TRP-family (transient receptor potential), like TRPV1, TRPA1, TRPM3 and others that can detect noxious stimuli (Julius, 2013). Inflammation induces a rapid nociceptor sensitization via posttranslational mechanisms, like phosphorylation of TRPV1 channels (Bhave and Gereau, 2004). Cell bodies of nociceptors are surrounded by satellite glial cells, which not only have essential functions in controlling the neuronal environment, but are activated in animal models of inflammatory and neuropathic pain and contribute to peripheral sensitization by releasing inflammatory mediators (Hanani and Spray, 2020; McGinnis and Ji, 2023). Moreover, DRG contain immune cells, like macrophages and lymphocytes that are activated under inflammatory conditions and contribute to the development and resolution of neuropathic pain (Krukowski et al., 2016; Zhang et al., 2016; Laumet et al., 2020; Yu et al., 2020). Interestingly, recent studies indicate that cellular mechanisms to induce peripheral sensitization are modulated in a sex-dependent manner (Yu et al., 2020; Szabo-Pardi et al., 2021; Alexander et al., 2023; Stratton et al., 2024). Overall, the close interaction between immune and glial cells with nociceptive neurons in the DRG is essentially involved in the induction of pathological pain states and represents a particularly interesting target for novel interventions to treat pain.

Within the spinal dorsal horn, nociceptive information is transmitted to secondary afferent neurons and integrated by resident excitatory and inhibitory interneurons, which represent the majority of SDH neurons (Todd, 2017). In addition, a top-down control within the nociceptive system is mediated by descending inputs from brain regions, such as the periaqueductal gray to control the spinal synaptic transmission (Heinricher et al., 2009). Spinal mechanisms of neuroinflammation include the activation and proliferation of resident glial cells (e.g., microglia, astrocytes), infiltration of immune cells (e.g., macrophages, T-cells, neutrophils), upregulated production and release of pro-inflammatory mediators (e.g., cytokines, chemokines, prostaglandins) and alterations in neuronal excitability and synaptic plasticity (Ji et al., 2014). Similar to DRG, targeting spinal (neuro)inflammatory processes seems to be an extraordinary promising approach to achieve advanced pain relief. This does not necessarily implicate the pharmacological inhibition of pro-inflammatory signaling cascades. Interestingly, it may also involve the activation of endogenous anti-inflammatory mechanisms, such as specialized pro-resolving mediators (e.g., resolvins) (Ji, 2023) and, thereby, shifting an organism's endogenous balance from a more pro- to an anti-inflammatory direction.

While a lot of research has been performed to understand neuroinflammatory processes in DRG and SDH, our knowledge about the contribution of neuron-glia-immune interactions to modulate pain is still limited. In this context, animal models represent invaluable tools to gain novel insights into underlying mechanisms and opportunities for intervention. Nevertheless, experiments in animals can be effectively supplemented using *in vitro* approaches to acknowledge ethical considerations and the 3R principle (*see Chapter 2: Ethical considerations in pain research*).

## 2. Ethical considerations in pain research

While there is an obviously urgent need for more research to gain deeper insights into mechanisms of pain, scientists are confronted with substantial ethical and legal restrictions, which are undoubtedly for good reasons. Experimental modeling of pain in humans is hardly justifiable in terms of ethics and requires strict regulations. To this point, only a limited number of tests exist with which to study acute pain in humans, such as visceral and somatic pain (Kleine-Borgmann et al., 2022). Moreover, models of experimentally induced endotoxemia are applied in humans to study mechanisms of sickness behavior, including inflammation-induced hyperalgesia (Benson and Karshikoff, 2023). Such models are instrumental in translational science to transfer knowledge between studies in animals and humans and to investigate entities of pain, which are difficult to assess in animals, such as the role of expectation in nocebo and placebo effects or connections to comorbid psychological disorders (Benson and Karshikoff, 2023).

Compared to humans, the legislation for research on animals is less strict in most countries, but fortunately, public awareness for animal welfare is constantly rising. In Germany, the protection of animals has been elevated to a national goal of constitutional status since 2002 (Constitutional protection for animals, 2002). However, what strengthens animals welfare, confronts researchers with further restrictions. This results in an ethical dilemma between the obligation to improve pain management by studying mechanisms of pain and at the same time respecting humans and animals welfare (Tannenbaum, 1999).

In 1959, Russel and Burch published the *Principles of Humane Experimental Technique* and introduced the principle of the 3R (Replacement, Reduction, Refinement) (Russel and Burch, 1959), which was also implemented in national and international legislations, like the *European Directive 2010/63/EU on the protection of animals used for scientific purposes (Art. 4)* (European Parliament and the Council, 2010). While the directive acknowledges that 'the use of live animals continues to be necessary to protect human and animal health', 'it seeks to facilitate and promote the advancement of alternative approaches' (European Parliament and the Council, 2010). Therefore, it encourages researchers to identify and apply novel 'alternative' opportunities to realize the 3Rs in their experimental approaches.

An additional point that has been traditionally underappreciated and only recently received the attention it deserves in the context of *'Culture of Care'* is the welfare of those individuals, who care for research animals (Ferrara et al., 2022; von der Beck et al., 2024). This includes not only scientists, but moreover the caregiving staff, technicians, veterinarians and animal welfare officers as well as responsible authorities. They all have to cope with this ethical dilemma, the associated emotional stress in the workplace and public stigmatization of their work (von der Beck et al., 2024). Thus, the aim to identify innovative strategies in pain research is of eminent intrinsic interest for scientists and not only a political-societal intention.

## 3. Neuro-glial primary cultures to study cellular mechanisms of pain *in vitro*

To study the diverse facets of pain on a cellular level, scientists established an enormous variety of *in vitro* approaches. These include primary cultured tissues and dissolved cells from animals (Caterina et al., 2000; Vriens et al., 2011; Biggs et al., 2014; Leisengang et al., 2018b, 2020a) or humans (Chrysostomidou et al., 2021; Middleton et al., 2021) as well as induced pluripotent stem cell (iPSC)-derived neurons (Chrysostomidou et al., 2021; Labau et al., 2022) or immortalized DRG cell lines (Haberberger et al., 2020). Moreover, there has

been a rapid progress in the generation of three dimensional organoid models of peripheral and central nervous structures involved in pain processing (Kofman et al., 2022; Zhou et al., 2024). All of these *in vitro* methods are applied with a specific justification to answer distinct scientific questions and exert advantages or disadvantages with regard to practicability, reproducibility, complexity, transferability and, as mentioned before, ethical concerns.

Primary cell cultures of dorsal root ganglia played an essential role in the identification and characterization of receptors involved in the transduction and transmission of sensory information. These include channels of the TRP-channel family, such as TRPV1 (Caterina et al., 2000), TRPA1 (Story et al., 2003), TRPM3 (Vriens et al., 2011), or TRPM8 (McKemy et al., 2002; Peier et al., 2002) and their capability to respond to noxious, thermal, mechanical or chemical stimuli. Sensitization of TRP-channels represents a key mechanism in the development of thermal and mechanical hyperalgesia and allodynia in states of inflammatory and neuropathic pain (Huang et al., 2006; Basbaum et al., 2009). Primary cultures of DRG are regularly utilized to study modulatory effects of inflammatory mediators on nociceptor excitability. Exogenous pathogen-associated molecular patterns (PAMPs) directly activate or sensitize channels involved in transduction and transmission of nociceptive stimuli via toll-like-receptors (TLRs) (Qi et al., 2011; Boonen et al., 2018; Agalave et al., 2020). Moreover, endogenous substances released under inflammatory conditions have been applied in DRG primary cultures to investigate sensitizing capacities and underlying cellular pathways. This includes cytokines, such as interleukin (IL)-6 (Segond von Banchet et al., 2005; Obreja et al., 2005; Andratsch et al., 2009; Ebbinghaus et al., 2015) and tumor necrosis factor (TNF)-α (Nicol et al., 1997; Hensellek et al., 2007; Richter et al., 2010), as well as prostaglandin (PG)E−2 (Pitchford and Levine, 1991; Linhart et al.,

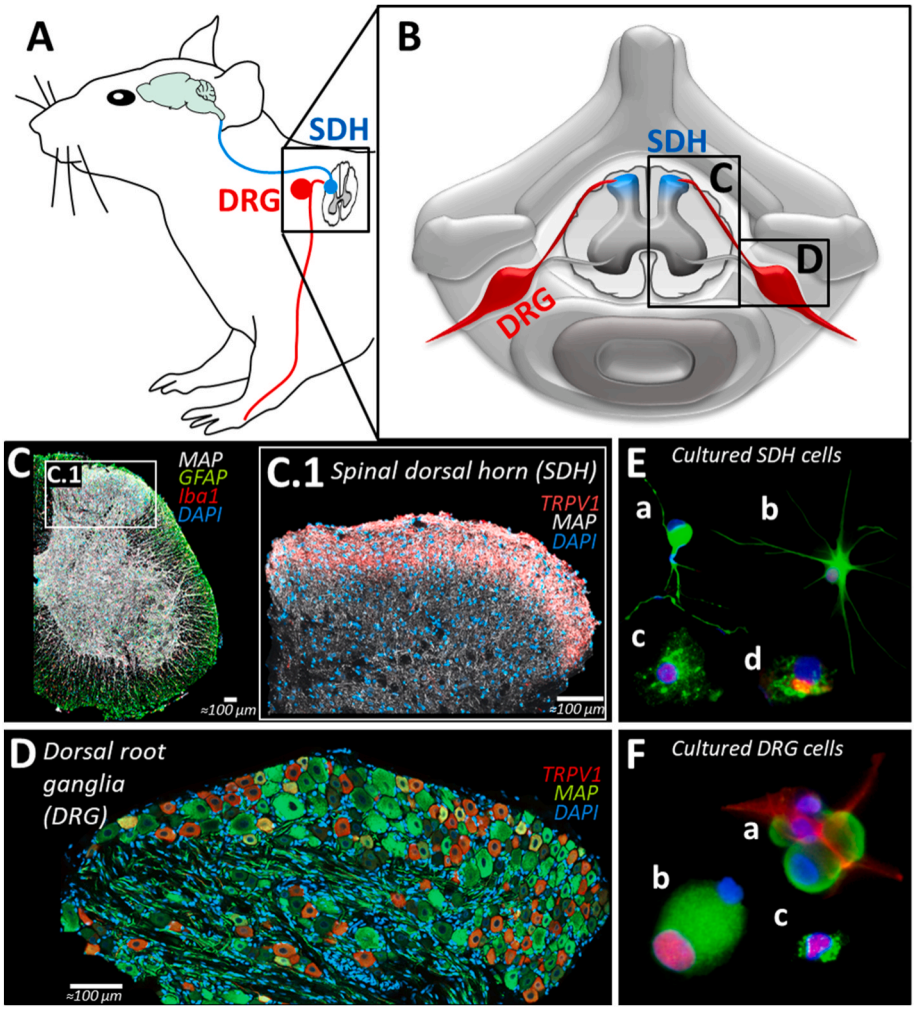


**Fig. 1.** Neuro-glial primary cell cultures of dorsal root ganglia (DRG) and the spinal dorsal horn (SDH) to study neuro-immune interactions on a cellular level
Noxious stimuli are detected by peripheral free nerve endings of nociceptive neurons (**A** + **B**; red). In the spinal dorsal horn, nociceptive stimuli are synaptically transmitted to secondary neurons (**A** + **B;** blue) that forward the information to superior brain regions. Cell bodies of sensory neurons are located in the dorsal root ganglia (DRG; **D:** MAP-positive). Nociceptors express TRP-channels (e.g., TRPV1) in peripheral nerve endings, in cell bodies in the DRG (**D**) and their central nerve endings in the SDH (**C.1**). In **E** and **F** glial and neuronal elements of DRG and SDH primary cultures as well as potential applications for studying inflammation-induced nuclear translocation of transcription factors or expression of cytokines are exemplarily illustrated with the respective markers. **E:** (a) MAP-positive neuron (green); (b) GFAP-positive astrocyte (green) and STAT3 (red); (c + d) CD68-positive microglia (green) and NFκB (c) or TNFα (d) (red); **F:** (a) MAP-positive neuron (green) and GFAP-positive satellite glial cells (red); (b) MAP positive neuron (green) and STAT3 (red); (c) CD68-positive macrophage (green) and NF-IL6 (red).
CD68: cluster of differentiation 68 (marker for activated macrophages/microglia); DAPI: 4′, 6-Diamidino-2-phenylindole dihydrochloride (nuclear staining); DRG: dorsal root ganglia; GFAP: glial fibrillary protein (marker for astrocytes and satellite glia); Iba1: ionized calcium binding adaptor molecule 1; MAP: microtubule-associated protein (neuronal marker); NF-IL6: nuclear factor interleukin 6; NFκB: nuclear factor kappa B; SDH: spinal dorsal horn; STAT3: signal transducer and activator of transcription 3; TNFα: tumor necrosis factor alpha; TRPV1: transient receptor potential vanilloid 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2003; Lin et al., 2006), or nerve growth factor (NGF) (Bonnington and McNaughton, 2003; Zhang et al., 2005) to mention only a number of examples. Besides these inflammatory mediators, DRG primary cultures are applied to study effects of substances that are administered upon medical indication and impact the sensory system, such as platinum-based chemotherapeutics in terms of peripheral neuropathy (Leisengang et al., 2018b; Leo et al., 2020, 2021; Schmitt et al., 2020). While most studies focus on sensory neurons in DRG cultures, more and more researchers also assess the role of resident glial and immune cells. Satellite glia are the predominant cellular component in naïve rat DRG cultures (Fig. 1F–a) (Leisengang et al., 2018b). Moreover, resident immune cells (e.g., macrophages) exist within these cell cultures (Fig. 1F–c). Therefore, primary DRG cultures can also be utilized to study neuron-glia-immune interactions. Inflammatory stimulation with lipopolysaccharide (LPS), a potent agonist on TLR-4 results in activation of DRG macrophages and enhanced expression and release of the pro-inflammatory cytokines TNFα and IL-6, which is attenuated in the absence of macrophages (Leisengang et al., 2018b). Sensitization of nociceptors upon stimulation with capsaicin, an agonist of the TRPV1 channel is detected after *in vitro* LPS-treatment in a dose-dependent manner (Nürnberger et al., 2022a). The elevated neuronal excitability is accompanied by an enhanced nuclear translocation of transcription factors STAT3 (signal transducer and activator of transcription 3) and NF-IL6 (nuclear factor interleukin 6) in DRG neurons (Fig. 1F–b). Interestingly, similar effects are observed, when DRG cultivation is performed after an *in vivo* LPS-challenge (Nürnberger et al., 2022b). Intraperitoneal injection of LPS results in systemic inflammation accompanied by symptoms of sickness behavior, including hyperalgesia (Harden et al., 2015). Within the DRG, the systemic LPS-challenge induces an upregulation of pro-inflammatory mediators (TNFα, IL-6, IL-1β) as well as elevated capsaicin responses in *ex vivo* cultured nociceptors (Nürnberger et al., 2022b). Moreover, LPS-induced nuclear translocation of NF-IL6 is detectable in *ex vivo* cultivated DRG macrophages and STAT3 in neurons (Nürnberger et al., 2022b). Therefore, an *in vitro* stimulation of DRG primary cultures with LPS shares important characteristics with processes that are induced by an *in vivo* LPS-challenge within the DRG.

The spinal dorsal horn represents the first main site of pain transmission and integration (Fig. 1C). Primary cultures of the SDH consist of neuronal as well as glial elements, including astrocytes, microglia and oligodendrocytes (Fig. 1E) (Leisengang et al., 2020a). Exposition of SDH cells to LPS leads to an enhanced expression and release of pro-inflammatory cytokines (e.g., TNFα, IL-6, IL-1β) as well as an activation of transcription factors (NFκB, STAT3, NF-IL6) that is predominantly observed in microglial cells or astrocytes (Fig. 1E–b + c) (Leisengang et al., 2020a). Moreover, LPS-stimulated SDH neurons show elevated $Ca^{2+}$-responses upon stimulation with glutamate, indicating a central sensitization (Leisengang et al., 2020a). Interestingly, pre-incubation with a lower LPS-dose attenuates a second LPS-induced inflammatory response in DRG and SDH cultures, as depicted by a reduced expression and release of cytokines and suppressed activation of inflammatory transcription factors, termed LPS tolerance (Nürnberger et al., 2021).

Neuro-glial primary cell cultures of the DRG and SDH are not only useful to assess pathophysiological mechanisms of neuroinflammatory processes, but can be further applied to study novel therapeutic approaches, including pharmacological substances or cell-based therapies. Gabapentin and pregabalin are drugs to treat neuropathic pain via inhibition of the

$\alpha_2\delta$-1 subunit of voltage-gated $Ca^{2+}$-channels at the presynapses resulting in a reduced release of excitatory neurotransmitters (Maneuf et al., 2003). However, treatment of DRG and SDH primary cell cultures with gabapentinoids also attenuated LPS-induced IL-6 expression and release, which most likely contributes to long-term analgesic effects (Leisengang et al., 2020b; Nürnberger et al., 2023). Interestingly, the immunomodulatory capacities of stem cells via release of exosomes and anti-inflammatory mediators represent a further promising tool to tackle neuroinflammation in the context of pain (Huh et al., 2017). Co-cultivation of SDH primary cultures with adipose tissue derived medicinal signaling cells (AdMSCs) results in a significant reduction in the inflammatory response of SDH cells by means of an attenuated LPS-induced cytokine expression and release and suppressed activation of the NFκB-pathway in SDH microglial cells (Leisengang et al., 2022).

Overall, neuro-glial primary cell cultures of the DRG and SDH represent useful tools to study neuroinflammatory processes in structures of the nociceptive system and are applicable to identify novel potential options for interventions.

## 4. Advantages and limitations of primary cell culture models

Neuro-glial primary cell cultures improve our knowledge of underlying cellular and molecular processes and are useful tools to study the impact of pro- and anti-inflammatory stimuli on specific cell types, intracellular signaling cascades, or the production of inflammatory mediators (*see Chapter 3*). Compared to other *in vivo* or *in vitro* approaches to study mechanisms of pain, there exist several advantages and limitations: The dissolved cells share characteristics with cells in the intact tissue regarding their physiological composition, like the number of nociceptive neurons in DRG (Leisengang et al., 2018b) as well as temperature responsive neurons in the SDH (Leisengang et al., 2020a) or in brain regions of the hypothalamus involved in thermoregulation (Leisengang et al., 2018a). Moreover, the existence of neuronal and glial components allows the investigation of intercellular communication via secretion of mediators, such as cytokines, and their actions on other cell types. Application of neuro-glial primary cultures is one opportunity to implement the 3R-principle (*Replacement, Reduction, Refinement*) by Russel and Burch into the field of pain research. Such *in vitro* models lead to a *Reduction* in the number of research animals because one animal can serve for several primary cultures, which are used for different treatment groups and methodological approaches. Other organs of the same animal can help to address further biomedical research questions. Moreover, tissue for cultivation can also be harvested from non-experimental animals as well as humans and thereby, represent an exciting approach in translational research (Herzberg and Bustamante, 2021). The use of cell culture models also represents a strategy of *Refinement*. It allows the investigation of cellular and molecular processes involved in pain modulation without the induction of pain in living animals in *in vivo* models. Finally, primary cell cultures allow an extraordinarily efficient workflow by means of time and costs.

However, it has to be noted that cell culture models only provide answers to a limited field of scientific questions and contribute some components toward identifying mechanisms of pain modulation. Compared to more complex three-dimensional cultivation systems, such as organotypic cultures, the physiological cell morphology and composition of intercellular connections is disrupted upon enzymatic digestion and trituration of the tissue. This cultivation process can result in alterations in the expression levels of ion channels and receptors and induce expression of inflammation-associated genes, leading to an injury-like phenotype of DRG cells (Wangzhou et al., 2020). Immune cell infiltration in DRG and SDH is one important mechanism of neuroinflammation in intact organisms, but can hardly be simulated *in vitro*. At some point, all *in vitro* models lack the presence of intact organ-organ interactions via cellular, humoral, endocrine and neuronal pathways, which are essential in the bi-directional communication between the brain and the immune system (Pflieger et al., 2018). Finally, the processing of nociceptive information on spinal cord level includes an endogenous inhibitory system that modulates transmission via descending tracts from superior brain regions (Ossipov, 2012). Therefore, *in vitro* models are not capable of replacing animal studies in terms of the 3R principle. The term of *Replacement* is regularly over-interpreted as a complete replacement and 'alternative' for *in vivo* studies. Indeed, this would overestimate the current opportunities of applicable *in vitro*

tools and could be misleading for the non-scientific community.

## 5. Conclusions and perspectives

To improve pain therapy for the billions of patients in human as well as in veterinary medicine, we depend on more research in basic and clinical sciences. A better understanding about the physiology and pathophysiology of pain is essential to identify novel targets that are of potential interest for pharmacological studies. Studying the complexity of neuroinflammatory processes in the dorsal root ganglia (DRG) and the spinal dorsal horn (SDH) represents an important component in this progress. Neuro-glial primary cell cultures represent valuable tools to study cellular mechanisms of nociception. Together with other *in vitro* tools, primary cultures promote the implementation of the 3R principle into pain research. Scientists are ethically and legally obliged to consider 'alternative' approaches when they design a project. However, it has to be acknowledged that - to date - animal experiments are not completely replaceable and still, represent an essential component of biomedical research (Domínguez-Oliva et al., 2023). Therefore, an integrated strategy combining *in vitro* and *in vivo* experiments is crucial for a comprehensive understanding of modulatory mechanisms of pain. If we aim to advance our understanding of pain, an open-minded exchange within the research community will be of tremendous importance. Furthermore, scientists need to improve public outreach and become more active members in a societal debate on animal research to communicate the advantages and limitations of 'alternative' approaches and inform about animal research (Link et al., 2024).

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## CRediT authorship contribution statement

**Stephan Leisengang:** Writing – original draft, Visualization, Supervision, Project administration, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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**Stephan Leisengang** After his graduation in Veterinary Medicine, Stephan Leisengang started his scientific career at the Institute of Veterinary Physiology and Biochemistry in Giessen under supervision of Prof. Joachim Roth and defended his thesis in 2019. In the following two years, he worked as a postdoctoral fellow and lecturer at the institute and completed the degree as Specialist in Veterinary Physiology. From 2021 to 2023 he was a post-doctoral fellow with Prof. Manfred Schedlowski at the Institute of Medical Psychology in Essen, before he took up his current position as Academic Councilor in the workgroup of Prof. Christoph Rummel in Giessen. Dr. Leisengang's work includes *in vitro* and *in vivo* models to study neuroinflammatory processes involved in the development of pathological pain. To study cellular mechanisms of neuro-immune interactions, he established neuro-glial primary cell cultures of structures of the afferent sensory system, namely, dorsal root ganglia, spinal dorsal horn, and hypothalamic nuclei. Applying these *in vitro* models, he investigates effects of pro-and anti-inflammatory stimuli on neuronal sensitization, glial activation and cytokine production. In 2022, his work on *in vitro* models in pain research was awarded with the Animal Welfare Award of the University Hospital Essen. In addition, Dr. Leisengang is particularly interested in effects of placebo analgesia. Applying an established paradigm of taste-associative learning in an animal model of inflammatory pain, he investigates effects of conditioned analgesia at behavioral and molecular levels.