Neuroimmunomodulation

Review Article

Neuroimmunomodulation 2023;30:250–267
DOI: 10.1159/000534467

Received: May 30, 2023
Accepted: October 2, 2023
Published online: October 5, 2023

How Can Experimental Endotoxemia Contribute to Our Understanding of Pain? A Narrative Review

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Keywords
Inflammation · Lipopolysaccharide · Cytokines · Sickness behavior · Pain

Abstract
The immune system and the central nervous system exchange information continuously. This communication is a prerequisite for adaptive responses to physiological and psychological stressors. While the implicate relationship between inflammation and pain is increasingly recognized in clinical cohorts, the underlying mechanisms and the possibilities for pharmacological and psychological approaches aimed at neuro-immune communication in pain are not fully understood yet. This calls for preclinical models which build a bridge from clinical research to laboratory research. Experimental models of systemic inflammation (experimental endotoxemia) in humans have been increasingly recognized as an approach to study the direct and causal effects of inflammation on pain perception. This narrative review provides an overview of what experimental endotoxemia studies on pain have been able to clarify so far. We report that experimental endotoxemia results in a reproducible increase in pain sensitivity, particularly for pressure and visceral pain (deep pain), which is reflected in responses of brain areas involved in pain processing. Increased levels of blood inflammatory cytokines are required for this effect, but cytokine levels do not always predict pain intensity. We address sex-dependent differences in immunological responses to endotoxin and discuss why these differences do not necessarily translate to differences in behavioral measures. We summarize psychological and cognitive factors that may moderate pain sensitization driven by immune activation. Together, studying the immune-driven changes in pain during endotoxemia offers a deeper mechanistic understanding of the role of inflammation in chronic pain. Experimental endotoxemia models can specifically help to tease out inflammatory mechanisms underlying individual differences, vulnerabilities, and comorbid psychological problems in pain syndromes. The model offers the opportunity to test the efficacy of interventions, increasing their translational applicability for personalized medical approaches.

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Introduction

Human experimental models of systemic immune activation give scientists an opportunity to study the direct and causal effects of inflammation on behavior and underlying brain mechanisms. In the last decade, these models have been increasingly utilized to disentangle the unique and distinct relationships between systemic immunological responses and changes in the central nervous system (CNS). Several recent reviews have addressed different aspects of experimental inflammatory models in humans [1–3], but predominantly referred to mood changes and psychiatric disorders. In this narrative review, we focus on what the model of experimental endotoxemia can teach us about pain, a transdiagnostic burden observed in various medical conditions. We give an overview of what has been learned about inflammation and pain by studying sickness behavior in humans. We discuss how experimental inflammatory models can help us understand complex, clinical pathologies, such as chronic pain. We also highlight aspects of inflammation and pain that need attention in future pain research.

Crosstalk between the Immune System and the Brain

The immune system and the CNS are in close and continuous exchange of information. This allows adaptive responses of the immune system to environmental conditions as well as to physiological and psychological stressors. At the same time, the brain receives information about the status of the immune system via afferent communication pathways [4, 5]. This is a prerequisite to adapt our behavior to a disease situation, e.g., in case of an acute infection or inflammatory condition. To this end, immune messengers such as pro-inflammatory cytokines can, in addition to local and systemic immune responses, also influence CNS activity via humoral and neural pathways. This induces changes in behavior and well-being, which are summarized as "sickness behavior" or "sickness syndrome" [4]. Sickness behavior is characterized by nonspecific physical and psychological symptoms, including increased pain sensitivity, dysthymia and anxiety, fatigue, changes in sleep and appetite, and mild cognitive impairment [2, 4, 5]. During an acute inflammatory response, elicited, e.g., by tissue damage from injury or surgery, acute infections, or vaccinations, sickness symptoms are an unpleasant but adaptive response which supports energy allocation and convalescence. A healthy immune response is dependent on the responsiveness of the immune system and paralleled regulatory functions. However, when the immune system is not able to fight the infection or physiological trauma, when the inflammation arises from faulty internal signals, or when the regulatory functions are disturbed, the inflammation may become chronic, with detrimental consequences for health-related quality of life [2, 6, 7], but also for morbidity and mortality [8].

Inflammation in Mental and Somatic Health

Many of the noncommunicable disorders that are the main cause of death worldwide [9] involve a chronic low-grade systemic inflammation. Inflammatory activity has also been linked to psychiatric disorders [3] and to chronic pain (see section 3). Chronic pain is among the most important predictors of years lived with disability, and pain is the leading cause for seeking medical care [10, 11]. Chronic pain afflicts about 20% of the population and is profoundly costly for society and often devastating for the individual [11]. Only 30–40% of patients report at least 50% reductions in pain when using the most potent drugs [12], and similar response variation is seen for behavioral treatment. This condition is a great challenge for current science, both in terms of finding better treatment and understanding who is at risk of developing chronic pain.

Individuals with chronic pain commonly present with psychological comorbidity, including, for example, anxiety, depression, fatigue, and disturbed sleep [13, 14], which are problems similar to the components of sickness behavior. These comorbidities can often affect the person's quality of life and functioning as much as the pain intensity itself and may negatively influence the effects of treatment. Inflammation has been suggested as a core mechanism driving both pain and psychological comorbidities [15]. Understanding how the effect of inflammatory activity differs between individuals is therefore fundamental for personalized medicine for noncommunicable disorders such as chronic pain. However, teasing out the causal effect of inflammation in clinical populations is very difficult, given the large individual differences in individual differences in symptoms, comorbidities, and related medication, but also age-related diseases and biological changes, as well as lifestyle factors. Lifestyle factors can include nutrition, physical activity, alcohol, etc., and lately, environmental aspects are considered well [16]. Moreover, the studied processes change and adapt over time [14, 17–19]. Against this background, experimental models of inflammation are invaluable to analyze causal effects of systemic inflammation on the CNS in humans.

Modeling Sickness Behavior: Experimental Endotoxemia

Experimental endotoxemia has emerged as a translational model to assess neuro-immune communication in pain [2, 6, 20]. The experimental application of endotoxins such as lipopolysaccharide (LPS) allows the reliable induction of an acute inflammatory state under controlled laboratory conditions in nonclinical cohorts without risk of infection or long-term side effects. As a prototypical pathogen-associated molecular pattern, LPS induces the synthesis and release of pro-inflammatory cytokines by immune cells via toll-like receptor-4 (TLR-4)-dependent pathways [2, 5]. The intravenous (i.v.) injection or infusion of LPS leads to transient increases in circulating immune mediators including pro- and anti-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, IL-8, and IL-10, as well as neuroendocrine mediators such as cortisol [21, 22]. Depending on the dose, LPS administration induces various physical and psychological symptoms of sickness behavior, including changes in pain sensitivity and mood. Effects on immune mediators can be observed about 60–90 min after LPS injection, and immune parameters return to baseline about 4–5 h post-injection (p.i.) depending on LPS dose. C-reactive protein (CRP) rises about 6 h p.i., with a peak at about 24 h p.i. (see also Engler and colleagues [23] in this article collection). In contrast to animal studies (for review, see [24]), human experimental endotoxemia utilizes comparatively low doses of LPS due to a higher responsiveness of the human immune system to endotoxin. In pain research, the majority of studies were conducted using doses of 0.4–0.8 ng/kg body weight; in human research, these were termed low-to-moderate doses. Low LPS doses are well tolerated and induce rather mild but noticeable symptoms of sickness. Few studies have used high doses up to 2 ng/kg body weight LPS [25–27], which are typically used in sepsis research and can result in more pronounced symptoms [28]. In general, experimental endotoxemia studies require elaborate safety measures, including repeated laboratory assessments (i.e., assessment of blood cell counts, liver enzymes, renal parameters, electrolytes, coagulation factors, CRP) and physical examinations, as well as continuous monitoring of volunteers by a physician. Minimal monitoring should include repeated assessment of body temperature, heart rate, blood pressure, and general well-being. In addition, rapid access to an emergency unit is required in the unlikely case of adverse reactions. The resource-intensive study procedures are however outweighed by some key strength including the long-standing experience with the model which has been used for more than 100 years in human research, and the possibility to conduct pain experiments under highly standardized conditions [2, 20, 28]. It should be exceptionally useful in filling some of the remaining gaps on immune-to-CNS mechanisms in humans, as outlined below [2, 24].

Inflammation and Pain

Acute pain is one of the strongest survival signals available to us. It activates the stress-response system and changes behavior to protect an individual in the case of an injury or acute health condition [29]. Increased pain during immune activation can be seen as one such adaptive regulatory mechanism. An increased sensitivity will make the individual more attentive to the body and to potential tissue damage derived from the infection or trauma/wound. Pain also motivates an individual to be still and to reduce locomotion and energy expenditure while increasing protective and healing behaviors. Chronic pain, however, is not believed to have any evolutionary advantages but rather an unwanted consequence and/or maladaptation of our flexible nervous and pain system. Chronic pain is a type of pain that continues or recurs for more than 3 months, even when the initial painful insult has subsided or healed. The International Association for the Study of Pain (IASP) recently published new guidelines on chronic pain classifications based on the assumed underlying mechanisms [30, 31]. The pain can arise from nerve damage (like neuropathic pain), localized inflammation (like rheumatoid arthritis), or an unknown origin (like fibromyalgia) (International Association for the Study of Pain [IASP], terminology available online: https://www.iasp-pain.org/terminology?navItemNumber=576#Centralsensitization [accessed on September 14, 2023]). However, symptoms and mechanisms involved in chronic pain are often transdiagnostic. Chronic pain can involve pain from deep within the body or superficially from the skin [11]. Chronic pain mechanisms, characteristics, risk factors, and treatment options have been reviewed extensively elsewhere [11, 32–35] and are not the focus of this review. Here, we will focus on some of the challenges clinical pain research faces and how the LPS model can help overcome some of them.

Increased inflammation has been shown repeatedly in several pain populations, e.g., [36–41] in the past decade, and many inflammatory mechanisms involved in the chronification of pain have emerged [6, 7, 11, 41, 43]. For rheumatoid arthritis, immunological treatments are useful for many patients [35]. For other types of pain, novel anti-inflammatory/immunomodulatory drugs are lacking [34], but the literature on

inflammation and chronic pain suggests they may be needed to increase treatment efficacy. Some novel approaches, for example, target DNA repair mechanism and offer a promising approach to reverse neuronal dysfunction induced by inflammatory mediator [44]. Moreover, pain intensity, the primary treatment target, is not easily detangled from other aspects of a pain syndrome: disrupted sleep, lack of physical activity and potential weight gain, life stress, and mood deterioration – all of which will affect inflammatory levels in the blood or vice versa.

Challenges when Studying Inflammation in Clinical Pain Populations

Many studies have aimed at assessing the role of inflammation and inflammatory markers in patients with chronic pain, with yet often inconclusive and contradictory findings. Furthermore, the biomarkers studied are predominantly unspecific. For example, higher central and systemic IL-8 levels were found in fibromyalgia repeatedly, e.g., [45–47]. On the other hand, higher IL-8 levels were related to less widespread pain in chronic pelvic pain [48] and fewer depressive symptoms [49] in treatment-resistant depression in women but not in men. Moreover, in exploratory proteomics analyses, distinct networks of blood markers appear related to defined aspects of the disorder, such as pain intensity, pain sensitivity, or psychological comorbidity [50, 51].

An important distinction that is often overlooked in clinical pain research is that between ongoing inflammation versus immunoreactivity. Recent literature suggests priming effects on the immune system [52], even the innate immune system [53]. This means that two individuals may have similar levels of biomarkers in an unprovoked state (i.e., in the absence of an immune-activating stressor) but may show different biomarker expressions (i.e., activation patterns) when a stressor is present. Ongoing inflammation, as measured in the blood, for example, represents what the immune system is doing at the time of the study/blood draw and could be seen as a state-like aspect of immune function. Inflammatory reactivity, on the other hand, represents the immune system's capacity to react to adversity and could thus be seen as a trait-like aspect of immune function. The distinction may be of importance for pain, as recent pain studies suggest that immunoreactivity (as assessed by in vitro LPS stimulations of white blood cells) is a better predictor of noninflammatory pain progression than the cytokine levels in the blood [54–56].

To understand the complex interactions between systemic inflammation and pain, we may need to expand the measures used to assess inflammation. In vitro stimulations may be promising for clinical studies, but they do not capture the full spectra of the immune response [57], nor do they say anything about how an immune response translates into pain sensitivity and emotions for an individual [57]. Experimental endotoxemia can be seen as a model that mimics a real-life stressor (infection) very well and gives us an understanding of a systemic and behavioral response pattern during a "natural" immune challenge. Below, we outline in detail how immune activation affects the pain system and changes pain perception in human experimental endotoxemia.

Pain Perception during Experimental Endotoxemia

To assess pain sensitivity during experimental endotoxemia, various experimental methods have been used. A variety of pain models and outcome measures was implemented for different pain modalities (see Table 1 for an overview). As outlined in detail in the next sections, pain models comprised models of musculoskeletal pain (e.g., algometry, pressure pain thresholds [PPTs]), cutaneous pain (e.g., pinprick, electrical stimuli), heat/cold pain (e.g., thermode, cold pressor task), and visceral pain (rectal distensions). Deep pain stimuli are described as "dull" or "pressing" (e.g., pressure pain stimuli/algometry) and primarily mediated by unmyelinated C-fibers, while cutaneous pain stimuli, described as "pricking," are mediated primarily by small myelinated Aδ-fibers [58]. As an outcome measure, pain sensitivity has been quantified as pain threshold (at what intensity a stimulus is perceived as painful), pain tolerance (how long a painful stimulus is tolerated), or pain ratings on Visual Analogue Scales (VAS) or Numeric Rating Scales (NRS) (how painful or unpleasant a stimulus is). To assess the neural correlates of endotoxin effects on pain, few studies have implemented functional magnetic resonance imaging (fMRI) to measure blood oxygen level-dependent responses to painful stimuli and pain-associated signals. One recent study used magnetic resonance spectroscopy that allows analyzing biochemical changes in pain-related brain networks [59], reviewed in [60]. Complementing findings from studies using pain-provoking tests such as algometry or pinprick, spontaneous changes in pain sensitivity were assessed using self-report on pain-related symptoms, e.g., headaches, muscles, or joint pain.

Unprovoked/Spontaneous Pain Symptoms

Unprovoked, spontaneous symptoms during endotoxemia are typically assessed with symptom checklists such as the SicknessQ [69] or the Generic Assessment of Side Effects [21, 70]. Spontaneous pain symptoms are

Table 1. Summary of endotoxemia studies on pain

Reference	Dose	Sample	Design	Time points	Pain model	Pain measures and main findings	Predictors/ correlates	Comments/ explanation
Deep pain stimuli								
Benson et al. [61], 2020	0.4 ng 0.8 ng		Pooled data set*	B/ C, +3 h	Pressure algometry	PPT ↓	HADS anxiety, TNF-α	*Pooled from Benson et al. [62], 2012, Wegner et al. [63], 2014, Wegner et al. [64], 2015
De Goeij et al. [25], 2013	2.0 ng	47m	Between* (27 LPS/12 PLA)	B, +2	Pressure algometry	PPT ↓	IL-6, TNF-α, IL-10, IL-1RA: all n.s.	*Not randomized
Hijma et al. [27], 2020	1.0 ng 2.0 ng	12m 12m	Crossover + no treatment	B, +2, +4, +8, +10	Tourniquet cuff*	PDT (1 ng: ↓ up to +6) PTT (2 ng: ↓ at +2) AUC ↔	Not assessed	*More typically used as an ischemic pain model
Janum et al. [26], 2016	2.0 ng	17m	Repeated LPS exposure on 2 days*	B, +2, +6	Pressure algometry	PPT ↓ at +2 and +6 h on both study days	TNF-α, IL-6: n.s.	*Nicotine patch application on 1 day, w/o effect
Karshikoff et al. [65], 2015	0.6 ng 0.8 ng	29f/ 23m 1f/7m	Between (31 LPS/21 PLA) Crossover	B, +1.5	Pressure algometry	PPT ↓* PPT ↓	IL-8 Mood: n.s.	*Similar effects in men and women
Karshikoff et al. [66], 2016	0.6 ng	29f/ 22m	Between, fMRI (31 LPS/ 21 PLA)	B, +1.5	Pressure algometry (thumbnail)	PPT ↓ BOLD: vlPFC ↓, rACC ↓, aINS ↑	Not assessed	
Wegner et al. [63], 2014	0.4 ng 0.8 ng	57m	Between (19, 0.8 ng LPS/20, 0.4 ng LPS/ 18 PLA)	B, +1, +3, +6	Pressure algometry	0.4 ng: PPT ↓/↔* 0.8 ng: PPT ↓ only at +3 h	IL-6, mood (TNF-α, IL-8, IL-10, state anxiety: all n.s.)	*Reduced PPT only for erector spinae
Wegner et al. [64], 2015	0.4 ng	20f/ 20m	Crossover	+3	Pressure algometry	PPT ↓*	TNF-α (in men)	*Similar effects in men and women
Visceral pain stimuli								
Benson et al. [62], 2012	0.4 ng	11m	Crossover	+2	Rectal distensions	RPT ↓ RST ↓ VAS (↑)	RPT: IL-6, IL-10	
Benson et al. [67], 2015	0.4 ng	27m	Between, fMRI (14 LPS/ 12 PLA)	B, +2	Rectal distensions	RPT ↓ RST ↔ VAS ↔ BOLD: dlPFC ↑, pINS ↑, aMCC↑, somatosensory cortices ↑	RPT: IL-6	
Wegner et al. [64], 2015	0.4 ng	20f 20m	Crossover	+2	Rectal distensions	RPT ↓* RST ↓*	RPT: TNF-α (in men)	*Similar effects in men and women
Mechanical pain stimuli								
Benson et al. [67], 2015	0.4 ng	27m	Between, fMRI (14 LPS/12 P)	B, +3	Pinprick	VAS ↔	n.s.	
De Goeij et al. [25], 2013	2.0 ng	47m	Between* (27 LPS/12 PLA)	B, +2	Electrical pain	EPT ↓	IL-6, TNF-α, IL-10, IL-1RA: all n.s.	
Hijma et al. [27], 2020	1.0 ng 2.0 ng	12m 12m	Crossover + no treatment	B, +2, +4, +8, +10	Electrical pain (burst and stair)	Electrical stair PTT ↓ at +2 for 1 ng and 2 ng Electrical burst PDT ↓ for 1 ng	Not assessed	

Table 1 (continued)

Reference	Dose	Sample	Design	Time points	Pain model	Pain measures and main findings	Predictors/ correlates	Comments/ explanation
Hutchinson et al. [68], 2013	0.4 ng	12m	Crossover	B, 3.5 h	Von Frey hairs, brush + intradermal capsaicin	Capsaicin-induced allodynia and hyperalgesia ↑	IL-6	
Wegner et al. [63], 2014	0.4 ng 0.8 ng	57m	Between (19 0.8 ng LPS/20 0.4 ng LPS/ 18 PLA)	B, +1, +3, +6	Pinprick, brush	NRS ↔	TNF-α, IL-6, IL-8, IL-10, state anxiety, mood: all n.s.	
Heat/cold pain stimuli								
de Goeij et al. [25], 2013	2.0 ng	47m	Between* (27 LPS/12 PLA)	B, +2	Cold pressor task	CPT ↓ NRS ↑	IL-6, TNF-α, IL-10, IL-1RA: all n.s.	
Hijma et al. [27], 2020	1.0 ng 2.0 ng	12m 12m	Crossover + no treatment	B, +2, +4, +8, +10	Thermode Cold pressor task	Heat PDT ↓ at +2 for 1 ng and 2 ng Cold pressor PTT ↓ (1 ng) Cold pressor AAC ↓ (1 ng)	Not assessed	
Janum et al. [26], 2016	2.0 ng	17m	Repeated LPS exposure on 2 days*	B, +2, +6	Thermode: tonic heat stimuli	VAS ↑ at 2 h on both study days	TNF-α, IL-6: n.s.	
Karshikoff et al. [65], 2015	0.6 ng 0.8 ng	29f/ 23m 1f/7m	Between (31 LPS/21 PLA) Crossover	B, +1.5	Thermode Cold pressor task	HPT/CPT ↔ Heat/cold detection thresholds ↔ VAS for suprathreshold noxious heat stimuli ↑ in women, not men	VAS heat pain: IL-6, IL-8	
Wegner et al. [63], 2014	0.4 ng 0.8 ng	57m	Between (19 0.8 ng LPS/20 0.4 ng LPS/ 18 PLA)	B, +6*	Cold pressor task	CPT ↔ NRS ↔	TNF-α, IL-6, IL-8, IL-10, state anxiety, mood: all n.s.	*Not assessed at +1 h, +3 h

Dose, LPS dose in ng/kg body weight; sample, number of participants (m = male/*f* = female); design, study design (PLA = placebo); time points, B, baseline assessment; +1, 1 hour post injection (etc.). Pain measures and main findings: PPT, pressure pain thresholds; PDT, pain detection threshold; PTT, pain tolerance threshold; AUC, areas under curve; RPT, rectal pain threshold; RST, rectal sensory threshold; VAS, Visual Analogue Scale; EPT, electrical pain threshold; NRS, Numerical Rating Scale; AAC, area above curve; HPT, heat pain threshold; CPT, cold pain threshold; dlPFC, dorsolateral prefrontal cortex; vlPFC, ventrolateral prefrontal cortex; rACC, rostral anterior cingulate cortex; aINS, anterior insula; pINS, posterior insula; aMCC, anterior midcingulate cortex. ↔ no change/no effect observed; ↑ increase; ↓ decrease. Predictors/correlates: HADS, Hospital Anxiety and Depression Scale; n.s., not significant. *Explanations/comments to be found in the last column.

highly prevalent after endotoxin application. For example, of $N = 128$ healthy volunteers who received either 0.4–0.8 ng/kg body weight LPS or placebo, up to 75% of the LPS-treated volunteers reported headaches (vs. 15% in the placebo group), and approximately 25–40% reported back, joint, and/or muscle pain (placebo: 2–6%) during the first 6 h after injection [21]. Interestingly, the prevalence of headache (up to 52%) and muscle pain (up to 23%) increased up to 24 h after LPS application, i.e., when the acute immune activation was already resolved [21]. Muscle pain and headache are also the most common symptoms in response to higher doses of 1–2 ng LPS [26].

One resting-state fMRI study offers insights regarding the neural networks involved in LPS-induced spontaneous pain symptoms. In this randomized, placebo-controlled trial, self-reported back pain, headache, and global sickness symptoms (assessed with the SicknessQ) were increased after application of 0.6 ng/kg LPS, along with an increase in the resting state connectivity between

the left anterior insula and the left midcingulate cortex. Resting state connectivity was correlated with the increase in self-reported back pain and tended to correlate with increased global sickness scores, but was not related to increased headache [71].

Musculoskeletal Pain Sensitivity

PPTs are the most frequently used outcome measure in experimental endotoxemia studies as they represent an established and easy-to-handle measure of primarily C-fiber-mediated deep pain sensitivity [72]. PPTs are reportedly decreased in patients with inflammatory and chronic musculoskeletal pain conditions [73] and have a predictive value for therapy responsiveness [74]. In response to doses of 0.4–2 ng/kg LPS, significant decreases of PPTs have been consistently reported for different muscles and body regions [25, 26, 61, 63–66]. Body regions sensitive to LPS include the lower back, the shoulder region (m. infraspinatus, m. supraspinatus, m. trapezius, m. deltoideus), and the legs (m. quadriceps, m. gluteus maximus, m. gastrocnemius), supporting that endotoxin effects on pressure pain sensitivity are broadly distributed and not restricted to certain body areas or body parts. The effects seem to be dependent on the timing of pain testing and LPS dose (see section 4).

The neural correlates of LPS-induced changes in pressure pain sensitivity have been assessed in an fMRI study by Karshikoff et al. [66]. Individually calibrated pressure pain stimuli were applied at the thumbnail 1.5 h after the injection of endotoxin (0.6 ng/kg body weight LPS) or placebo. Although all pain stimuli were calibrated to a pressure corresponding to VAS = 50 mm in all volunteers, painful stimulation in the LPS group when compared to the placebo group led to greater activity in the anterior insular cortex, which plays a major role in the processing and integration of interoceptive signals with affective-emotional components of pain. Interestingly, activity in the ventrolateral prefrontal cortex and the rostral anterior cingulate cortex, i.e., in brain regions involved in descending pain inhibition, was decreased in the LPS group. Additional analysis on sex differences revealed that pressure pain-induced activity of the rostral anterior cingulate cortex was lower in women when compared to men, which may contribute to sex differences in LPS-induced pain sensitization [66].

While all previous studies used algometers or comparable devices to induce pressure pain, Hijma et al. [27] were the only ones to use a tourniquet cuff placed over the gastrocnemius muscle, a procedure that is also known as "ischemic pain test." Pressure pain detection thresholds (assessed with VAS) and pain tolerance thresholds were repeatedly tested up to 10 h after the injection of either 1 or 2 ng/kg LPS versus placebo and no treatment in a crossover study design. Decreased pressure pain detection thresholds were observed in volunteers who received 1 ng/kg but not 2 ng/kg LPS [27]. Similar to a study by Wegner et al. [63], pain-detection thresholds were decreased during the early phase of inflammation, i.e., when concentrations of circulating cytokines were increased, but not at later time points when acute inflammation had subsided. Use of the tourniquet model allowed testing pressure pain tolerance, which was decreased only at 2 h in response to the 2 ng/kg dose [27]. In summary, existing studies have consistently documented reduced PPT during the acute inflammatory response to LPS, i.e., 1.5–4 h post-injection.

Visceral Pain Sensitivity

Visceral pain, i.e., pain arising from the inner organs, is in various aspects different to somatic pain. Painful visceral stimuli are conveyed via a distinct innervation, and visceral pain is perceived as more diffuse, unpleasant, and burdening when compared to somatic pain [75]. The pressure-controlled rectal distension model offers a valid, clinically relevant method to experimentally assess visceral pain thresholds and pain ratings in patients with functional and inflammatory bowel conditions and in nonclinical cohorts. In a series of studies [62, 64, 67], low-dose LPS (i.e., 0.4 ng/kg) was implemented to test effects on rectal sensory and pain thresholds. Consistent across studies, significant decreases in visceral pain thresholds were found during LPS-induced acute systemic inflammation, in line with visceral hyperalgesia. LPS-induced decreases in pain thresholds were similar in men and women [64]. Results for visceral sensory thresholds (i.e., the lowest distension pressure detectable) were less conclusive. Two behavioral studies with a crossover design showed significant decreases in sensory thresholds [62, 64], which were not found in a between-group fMRI study [67]. Two of the studies additionally assessed VAS ratings of rectal distension stimuli [62, 67], showing that stimuli were rated as more unpleasant and inducing greater urge to defecate, while no effect was found for pain intensity. In one study implementing brain imaging [67], LPS administration led to more pronounced rectal distension-induced blood oxygen level-dependent responses within the posterior insula, dorsolateral prefrontal, anterior midcingulate, and somatosensory cortices. Within the LPS group, more pronounced cytokine responses correlated significantly with enhanced rectal

pain-induced neural activation in dorsolateral prefrontal cortex and anterior midcingulate. No significant LPS effects were observed on neural responses to non-painful rectal distensions. In summary, endotoxin-induced systemic inflammation leads to reliable and reproducible decreases in visceral pain thresholds. In line with findings on PPT (see above), this indicates that experimental endotoxemia increases deep pain sensitivity.

Mechanical Pain Sensitivity

Sensitivity to pricking or sharp stimuli was assessed in yet few studies by using pinprick or electrical stimuli. While pain ratings for pinprick stimuli of different intensities did not differ between LPS and placebo groups in response to 0.4–0.8 ng/kg LPS [63, 67], a higher dose of 2 ng/kg LPS led to a higher sensitivity to pinprick [25]. Interestingly, Hutchinson et al. [68] documented a significant increase in allodynia and hyperalgesia to mechanical stimuli following intradermal capsaicin injection 3.5 h after i.v. low-dose (0.4 ng/kg) LPS, but not placebo administration. The intradermal injection of capsaicin induces flare, pain, allodynia, and hyperalgesia via activation of transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors [68]. Since another study did not observe allodynia after application of a similar LPS dose [63], this may indicate that LPS-induced systemic inflammation increases the susceptibility of peripheral nerve fibers to the pain-sensitizing effects of capsaicin.

Sensitivity to electrical pain stimuli has only been tested in the context of higher LPS doses of 1–2 ng/kg. Reduced pain tolerance thresholds for electrical stair and burst stimuli were reported for the early phase of acute inflammation (i.e., 2 h p.i.) in response to 1 and 2 ng/kg, respectively [25, 27], in line with findings on sensitivity changes to pinprick. In summary, existing evidence supports that low-dose LPS does not change sensitivity to Aδ-fiber-mediated pinprick stimuli, contrasting the findings on C-fiber-mediated deep pain sensitivity (e.g., pressure pain). Higher LPS doses seem, however, to induce hyperalgesia in response to mechanical pain stimuli.

Heat/Cold Pain Sensitivity

Three studies implemented thermodes to test LPS effects on heat pain thresholds as well as on ratings of suprathreshold stimuli (i.e., intense pain stimuli). For heat pain thresholds, no effect of low-dose LPS (0.6 ng/kg) was detected at 3.5 h p.i. [65]. When suprathreshold noxious heat stimuli were applied in the same experiment, significantly increased pain ratings were present in women but not in men after LPS when compared to placebo application [65]. Two other studies were conducted in men only. Janum et al. [26] showed that tonic heat pain stimuli at temperatures between 45°C and 48°C were rated as more painful 2 h but not 6 h after LPS administration. This finding is complemented by the crossover study by Hijma et al. [27], who reported decreased pain detection thresholds 2 h after injection of 1 and 2 ng/kg LPS but not at latter time points (i.e., 4, 6, and 10 h p.i.). Together, LPS effects for heat pain sensitivity seem greater in response to high-dose LPS and for suprathreshold stimuli, while effects to low-dose LPS are less consistent.

Cold pain thresholds were assessed in a study by Karshikoff et al. [65] using a thermode. Cold pain thresholds 3.5 h after application of 0.6 ng/kg LPS did not differ from a placebo condition [65]. In the remaining studies, the cold pressor task was utilized to test cold pain sensitivity. In this task, volunteers are instructed to hold their hands in iced water as long as possible to assess cold pain tolerance, and repeated pain ratings can be collected. The existing findings for cold pressor tasks are conflicting and do not offer a clear picture: A study by Wegner et al. [63] reported no effects of 0.4 or 0.8 ng/kg LPS on cold pain tolerance or ratings when compared to placebo. However, this negative finding can only be interpreted in a careful way since cold pain sensitivity was measured 6 h after LPS application, i.e., when the acute inflammatory response had already subsided. Two studies used higher doses of LPS. In a between-group study by de Goeij and colleagues [25], volunteers treated with 2 ng/kg LPS reported more pain during the cold pressor test, and a lower number of participants were able to complete the cold pressor task when compared to the placebo control group. In the crossover study by Hijma et al. [27], cold pain ratings and cold pain tolerance were changed in a group that received 1 ng/kg when compared to the placebo condition, but not in another group of this study that received 2 ng/kg.

Conclusion Regarding Pain Modalities

In summary, immune activation results in a stable and reproducible increase in pain sensitivity for deep and visceral pain, but not as clearly for pain perceived with the skin. PPTs and visceral pain sensitivity are most strongly linked to widespread pain, such as fibromyalgia, and gut pain, such as irritable bowel syndrome. This type of pain is defined as nociplastic pain, i.e., pain of unknown origin that depends on central sensitization [11]. The reproducible inflammation-driven increase in pain sensitivity is reflected in changes in brain function during

experimental endotoxemia, which suggests that the effect is not merely limited to peripheral nerve endings. In the following sections, we will elucidate how levels of inflammation and sex, as well as how emotions and cognition relate to pain perception during endotoxemia.

Inflammatory Hits, Timing, and Sex Affect Pain

Biological systems involved in pain are not static but adapt and change over time [14, 17–19, 76]. A major challenge in clinical pain research is that measured inflammation may be a pathological part of the condition but also a physiological part of a healing process [76], depending on the timing of measurements, the progression of the disease in an individual, and the intensity and type of the inflammation. Research also shows that repeated hits of immunological challenges may cause a wide range of problems over time [52, 77, 78], such as an increased risk for psychiatric disorders later in life and heightened immunological and neurological sensitivity. While acute models of inflammation are limited to modeling long-term effects, they may contribute to our knowledge regarding moderating factors which contribute to increased pain during inflammation, especially if novel settings and study approaches are utilized (see below).

Female sex is repeatedly identified as a risk factor for developing pain, maintaining pain, having the pain spread to multiple sites, and having psychological comorbidity [11, 13, 14, 79]. Women have almost double the risk for chronic pain following several types of surgery [80]. Men and women exhibit fundamental differences in immune responses and neuroimmune interactions that may drive pain chronicity [79]. Genetic and cellular human and animal studies show that in males, macrophage- and microglia-dependent mechanisms dominate in pain conditions [13], while pain-related immune mechanisms specific for women seem to involve T-cells. In females, pain sensitivity seems to change with cycle phase and sex hormone concentration, but findings are inconsistent [81, 82]. The menstrual cycle may also influence TLR-4 responsivity with implications for pain symptoms [83], with a lower production and release of IL-1β, IL-6, and TNF-α in response to ex vivo LPS stimulation during the follicular phase [84]. These findings highlight the complexity of sex differences [13]. Also, divergent immune responses, or the well-documented higher pain sensitivity in women, do not simply translate into sex differences in pain occurrence or intensity or cognitive and affective pain components [13, 79].

Timing and Dose Can Affect Pain during Experimental Endotoxemia

If LPS effects on pain occur in a time- and dose-dependent manner, this raises an important question from a scientific perspective, as outlined above, but also from a methodological perspective: knowledge regarding the time phase with increased pain sensitivity after LPS injection will help to develop valid study designs and to avoid measurements at too early or too late time points, which will result in false negative findings. It is also an important prerequisite to choose a LPS dose, which reliably increases pain sensitivity without unnecessary symptom burden for volunteers. Women should be included in the same period of the menstrual cycle, and the use of hormonal contraceptives should be investigated and controlled for.

Existing evidence supports that changes in pain sensitivity occur most reliably 1.5–4 h after LPS injection. No effects have been observed at earlier time points, e.g., 1 h p.i., or later than 5–6 h after application of low LPS doses (i.e., 0.4–0.8 ng/kg). For higher doses, Janum et al. [26] showed decreased PPT up 6 h after injection of 2 ng/kg LPS. Hijma et al. [27] assessed LPS effects on various pain measures at 8 h and 10 h p.i. of 1 and 2 ng/kg LPS, with no evidence of changes. However, this is not surprising given that the acute inflammatory response has largely abated at that time. Together, findings support that LPS-induced changes in pain sensitivity should be tested 2–4 h p.i., but not at earlier or later time points.

Since this phase of inflammation is characterized by both pain sensitization and increased plasma concentrations of pro-inflammatory cytokine including TNF-α, IL-6, and IL-8, a number of studies have explored the associations between changes in pain measures and peak cytokine increases. Herein, contrary to what one would expect, no clear picture from correlation and regression analyses emerged across studies (see Table 1). While some studies reported significant associations between LPS-induced changes in pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, and pain outcomes [61, 65], others did not find such associations [25, 26] (Table 1).

Another way to experimentally approach associations between cytokine concentrations and pain is to implement different LPS doses within one study. This is based on observations that higher LPS doses induce a more pronounced increase in plasma cytokines. Two studies have systematically tested dose-dependent effects of LPS on pain sensitivity thus far. Wegner et al. [63] compared the effects of two low LPS doses (0.4 ng/kg vs. 0.8 ng/kg). A study by Hijma et al. [27] implemented two groups that received 1 ng/kg versus 2 ng/kg LPS. For the comparison

of low LPS doses, Wegner et al. [63] found a dose-dependent effect for deep pain sensitivity, with lower PPT when the higher LPS dose was applied. For high LPS doses, the study by Hijma et al. [27] did not support a clear pattern of dose-dependent effects for high LPS doses. However, given the substantial differences in the design and methods of the two studies, it remains elusive if dose-dependent effects occur in lower dose ranges but not in response to high doses of LPS. A study by Janum et al. [26] tested the replicability of LPS effects on pain sensitivity and applied a dose of 2 ng/kg on two consecutive study days. The second LPS application resulted in lower cytokine responses but comparable effects on PPT as well as on tonic heat pain VAS ratings.

Taking all findings together, the inflammation-induced pain sensitivity does seem limited to when the immune system is most active and subsides when blood cytokine levels decrease in nonclinical individuals. However, the association between LPS-induced increases in cytokine concentrations and changes in pain sensitivity is not linear, and more sophisticated statistical approaches than linear regression analyses are needed to address this point.

Effects of Sex on Pain during Experimental Endotoxemia

In human endotoxemia models, we can evaluate the differences between men and women in terms of immune responsiveness, but also if and how increasing levels of immune activation translate into the behavioral changes. Building on well-documented sex differences in immune responses to inflammatory challenges as well as in pain sensitivity, sex-dependent differences in LPS-induced pain have been hypothesized. However, despite significantly higher circulating cytokine concentrations in female compared to male volunteers, no difference was found in LPS-induced changes in deep pain sensitivity, i.e., visceral and PPTs [64, 65], as well as in heat pain sensitivity [65]. As both existing studies implemented low-dose LPS, it remains open if these findings also apply to high LPS doses. Interestingly, Karshikoff et al. [65] reported sex-dependent differences in responses to suprathreshold heat pain stimuli, which represent an intense pain stimulus, as well as differences in the central modulation of pain. In women, a less effective central pain modulation emerged in diffuse noxious inhibitory control, which was complemented by altered brain responses to pressure pain [66] after low-dose LPS (0.6 ng). This may reflect a mechanism that puts women at an increased risk for pain chronification during states of inflammation [66].

To summarize, the differences in immune function between men and women do not simply translate to differences in pain perception, nor do levels of pro-inflammatory blood cytokines. Clinically, the pressing questions are how pre-existing vulnerabilities, dysfunctional immune regulation, or repeated challenges tap into and modulate these systems.

Mood and Cognition Interact with Inflammation and Pain

It is well known that anxiety and depression are risk factors for developing pain [11]. Recent research suggests bidirectional effects and common physiological pathways [7, 85, 86]. As mentioned above, chronic pain commonly appears with psychological comorbidities, and these aspects are not easily disentangled in the clinical population. Pain comorbidity could represent a double-hit challenge for the CNS, as discussed above. Moreover, some studies suggest that patients experiencing both pain and mood disturbances may in fact show unique inflammatory characteristics [50, 51, 87]. For example, treatment-resistant depressed patients who experience pain comorbidity have higher blood IL-6 and granulocyte-macrophage colony-stimulating factor levels compared to patients without pain [88]. The group who had pain also responded with a more pronounced decrease of several inflammatory cytokines in the blood in response to ketamine treatment compared to patients without pain comorbidity. Similarly, individuals in a community sample who reported both negative effect and pain showed higher blood CRP levels, but not those reporting just one of the symptoms [89]. Reflecting the close interrelationships between inflammation and mood changes, the term affective immunology has been implemented for this emerging field of research [90].

As with emotions, cognition is also involved in pain perceptions. One prominent example is the well-described impact of positive (or negative) expectations on pain perception in the context of placebo analgesia and nocebo hyperalgesia [91, 92]. If maladaptive, cognitions can increase the risk of developing pathological pain [85, 86]. In fact, cognitive behavioral therapy is one of the most common treatments in modern chronic pain treatment [93] and targets cognitive aspects primarily. There are not many studies investigating if inflammation affect behavioral pain treatment, but three studies suggest that lower baseline inflammation predicts better behavioral treatment outcomes [94–96]. These studies are however correlational, and potential mechanisms remain unclear.

Most neuroimaging studies in the field of psychoneuroimmunology have departed from depression research, and thus areas that have been identified as inflammation-sensitive in humans are predominantly areas belonging to the limbic and reward systems, as well as insular cortices [3, 97]. Interestingly, recent studies suggest that brain changes related to inflammation may be distinct from brain changes related to depressive symptoms [98] or another common comorbid problem in pain syndromes, namely stress [99]. Beckmann et al. [98] show that blood levels of inflammation were associated to the strength of interaction between the default mode network and the limbic system in depressed patients, while depressive symptoms were more related to the connectivity to the executive and attention networks. A study by Kim et al. [99] describes different connectivity patterns for inflammation versus repeated stress in healthy adults and argues for a double-hit effect of inflammation and stress on the brain. These findings are intriguing, as they suggest that inflammation has distinct and measurable effects on the brain that may enforce, interact with, or modulate several pathways known to affect pain.

Using experimental endotoxemia as a model, scientists strive to understand if inflammation-induced mood and cognitive changes act as parallel or convergent processes with pain perception during inflammation and if mood and cognition moderate or mediate inflammation-induced pain sensitivity. These studies shed light on effects of individual differences, pre-existing vulnerabilities, and expectations on pain perception in the context of inflammation.

Mood and Pain Sensitization in Experimental Endotoxemia

Negative mood is a core symptom during systemic inflammation. Experimental endotoxemia reportedly induces mood changes, with transient states of negative, depression-like mood [100, 101] and anxiety [102, 103], as well as an altered cognitive processing of emotional information [100] (for review, see [2]). This is of high clinical relevance since pain and negative emotions show complex and reciprocal interconnections, and depressive symptoms may exacerbate and maintain pain symptoms as outlined above. In line with studies in clinical populations, several endotoxin studies revealed significant associations between measures of mood changes and pain sensitivity (see Table 1). However, these findings were not unequivocal, and reported correlations were rather moderate to low. Complementing analyses on mood changes during endotoxemia, which can resemble comorbid mood symptoms during an inflammatory state, recent studies have focused on the putative impact of psychological states and traits measured before endotoxin administration, i.e., before the onset of the inflammatory response to endotoxin. This offers the opportunity to identify putative vulnerability factors that contribute to more pronounced symptoms of sickness behavior. Existing data indicate that negative affectivity and psychological distress before endotoxin administration are associated with greater physical and psychological sickness symptoms [21, 102]. These findings were demonstrated in nonclinical cohorts that were carefully screened for psychiatric conditions and thus support the notion that even subclinical alterations in mood and negative affectivity may affect sickness behavior. This appears to be true for pain sensitivity as well, that is part of the induced sickness behavior during endotoxemia. Recently, a merged dataset with $N = 98$ healthy volunteers who received either low-dose LPS or placebo was used to analyze psychological and immunological predictors of LPS effects on deep pain sensitivity [61]. Herein, anxiety scores assessed before LPS administration emerged as a significant, independent predictor of increased pressure pain sensitivity in multiple regression models [61], supporting that pre-existing affective symptoms may act as a vulnerability factor for increased pain sensitization during inflammation.

Extending the correlational findings outlined above, few studies have used experimental paradigms to actively manipulate mood during endotoxemia. The close relationship of emotional and pain-related brain responses during systemic inflammation was first shown in an experiment implementing a social exclusion paradigm [104]. In this fMRI study, volunteers received an i.v. injection of 0.8 ng/kg LPS or placebo. Two hours after injection, volunteers participated in a virtual ball game with other "teammates". De facto, a computer generated the "teammates" responses in an inclusion control condition and an exclusion condition, in which volunteers were "excluded" from the ball game. Brain activity during the virtual ball game was assessed using one-way ANOVA contrasting neural activity during exclusion compared to inclusion for the endotoxin group compared to the control group. To this end, inclusion and exclusion periods were modeled as epochs individually for each participant. At the behavioral level, the exclusion condition led to negative mood and feelings of social isolation. Neural activation patterns during social exclusion, when compared to inclusion, did not differ between endotoxin and placebo groups (except one region of the occipital cortex). Exploratory whole-brain regression

analyses conducted within the LPS group indicated that IL-6 increases (from baseline to fMRI scanning) were associated with greater activity of the bilateral anterior and posterior insula, i.e., in brain regions involved in the processing of pain and interoceptive information. IL-6 increases were also associated with greater activation in brain regions that have been involved in mentalizing processes and the understanding of the minds or behaviors of other persons. The interrelationship between inflammation, negative mood, and brain activation shown in this experiment may be sex-/gender-specific, as only females but not males showed an association between IL-6 increases and depressed mood that was mediated by neural activity in dorsal anterior cingulate cortex and the anterior insula [104].

A recent fMRI study [105] documented increased visceral unpleasantness ratings as well as greater neural responses to pain-predictive cues within the caudate nucleus and hippocampus when negative (sad) mood was experimentally induced during endotoxemia. Based on a crossover study design, all volunteers underwent pain testing in experimentally induced sad and neutral mood states during experimental endotoxemia as well as in a placebo control condition. The observed interaction of inflammation and sad mood suggests that a context that induces negative mood (e.g., a negative treatment context) may amplify the effects of systemic inflammation on the experience of pain. This may be explained by a greater susceptibility to negative mood stimuli during inflammation, which in turn increases the effects of inflammation on pain. This finding underlines the importance to avoid a negative and to provide a positive treatment context in inflammatory conditions. Such a treatment context could be achieved, e.g., by avoiding negative suggestions and ambiguous information in patient-provider communication and reducing stress- and anxiety-provoking stimuli in the treatment surrounding (e.g., noises and alarms from medical equipment), while adapting an authentic, positive, and empathic communication style and providing a safe and comfy treatment setting (for further information, see e.g. [92, 106–108]).

In summary, the existing findings support that negative mood contributes to increased pain sensitivity during endotoxemia. This appears to happen on two levels. Pain during inflammation is influenced by pre-existing psychological traits and/or negative affectivity and distress, which represent vulnerabilities for pain. Moreover, systemic inflammation may increase the susceptibility to contexts that induce negative mood stimuli as well as to negative social cues during endotoxemia, with implications for pain sensitivity and pain amplification, representing an inflammation-associated pathway for comorbidity.

Cognitive Pain Modulation: Learning and Expectations during Experimental Endotoxemia

Learning to predict pain and aversive symptoms is an important prerequisite to avoid future harm. However, maladaptive pain-related learning and memory processes may also contribute to the chronification of pain [75]. Against the background that inflammation can interfere with learning and memory processes, two studies have addressed LPS effects on pain-related learning and memory in the visceral pain model [22, 109]. Implementing a classical fear-conditioning paradigm with painful rectal distensions as unconditioned stimuli and visual cues as conditioned stimuli, the impact of endotoxemia on the acquisition and extinction of pain-related fear was assessed in a series of complementary experiments. Despite significant inflammatory responses to endotoxin, the studies consistently showed that inflammation did not affect the acquisition or extinction of pain-associated fear memories at the behavioral level, as indicated by comparable valence ratings for the pain-predictive cues in endotoxin and placebo control conditions [22, 109]. However, if pain-related fear conditioning was conducted during endotoxemia, the neural activation in fear circuitry during extinction was enhanced in response to pain-predictive cues, and negative valence ratings for visceral stimuli were increased during unexpected re-exposure to distensions [109]. This suggests that pain-related fear learning during inflammation may promote the establishment of a more robust neural fear memory trace, which contributes to a more unpleasant experience of visceral pain [109]. A further understanding of how inflammation shapes the acquisition and extinction of pain may inform the development of behavioral treatment approaches.

Recent advances in the field of placebo and nocebo research have offered insights on how expectations influence various symptoms including pain and hyperalgesia [91, 92]. The clinical and societal relevance of understanding the impact of expectations on inflammation-induced symptoms and sickness behavior became particularly evident during the pandemic, e.g., when facing the high number of nocebo responses in the context of COVID-19 vaccination trials (e.g. [110, 111]). While experimental research specifically aimed at placebo effects on inflammation-induced pain is currently underway (e.g. [108]), two studies have investigated expectation effects on the broader range of sickness

behavior symptoms in the context of experimental endotoxemia. In a study by Lasselin et al. [112], healthy volunteers were asked about their expectations regarding psychological and physical sickness symptoms induced by LPS administration prior to injection. Expected symptoms were significantly associated with negative mood experienced after LPS administration but not with physical sickness symptoms. Interestingly, volunteers who expected particularly low symptom intensity showed stronger mood-related symptoms after LPS administration. This suggests that a mismatch between expected and experienced symptoms may contribute to more pronounced symptoms [112]. The second study analyzed data from control groups of previously conducted endotoxin trials [113]. Volunteers who falsely stated that they had received endotoxin (even though in fact they were randomized to the control group and received a saline solution) were classified as "nocebo responders." Nocebo responders reported significantly more pronounced sickness behavior than those who correctly assigned themselves to the control group. The number and intensity of sickness symptoms were associated with higher state anxiety among the "nocebo responders," which potentially contributed to increased symptom awareness [113].

In summary, current evidence, though yet scarce, supports that inflammation may influence pain-related learning and memory processes and contribute to maladaptive pain behavior. Studies aimed at placebo and nocebo effects in the context of sickness behavior indicate that expectation likely modulates inflammation-induced symptoms. Collectively, these findings will support the further development of behavioral strategies to prevent and ameliorate sickness behavior symptoms including pain in the context of inflammatory conditions.

Methodological Considerations

When interpreting the findings from endotoxin studies, differences in methodology, pain models, pain measures, and study design must be taken into account. To take models of pressure pain sensitivity as an example, with the exception of one study, all studies used algometry as a widely accepted and clinically relevant measure of deep pain sensitivity, with consistent findings across studies. One study conducted by Hijma et al. [27] utilized a pressure-controlled tourniquet to test pressure pain sensitivity, with partially negative results, suggesting that the tourniquet test might be a method that is not as sensitive as pressure algometry. Moreover, the pain outcomes used across studies were not consistent, as some studies defined, e.g., pain detection thresholds, while other studies defined pain tolerance or pain ratings as the primary outcome. Thus, contradictory findings may at least be in part explained by the use of different outcome measures across studies, making it more difficult to conclude if endotoxin has an impact within a certain pain modality or model. Thus, the interpretation of findings should take into account if physical measures like pressure or temperature or psychological measures like VAS or NRS are reported. Future studies should utilize refined and standardized pain tests that comprise both physical and psychological outcome measures within one pain modality. When assessing psychological components of pain, affective-emotional facets should also be included. Yet, most studies have aimed at sensory-discriminative measures of pain such as pain thresholds or pain intensity ratings. The affective-emotional and motivational components of pain have rarely been addressed yet, although they are more closely related to patient suffering and have high clinical relevance.

When designing and interpreting endotoxin studies, the pros and cons of between group versus crossover study designs are of relevance: in crossover studies, the same participant reports two (or more) times at the laboratory to receive LPS or placebo in a randomized order (i.e., LPS – placebo or placebo – LPS), with a washout period of several days. This has the advantage to control for inter-individual variance and trait characteristics; however, it bears the risk of unblinding participants and study personnel, especially if higher LPS doses are applied. While the problem of potential order effects is not given in between-subject studies, a higher inter-individual variance requires a larger sample size to guarantee sufficient statistical power. Yet, only one study has implemented a no treatment control group [27], which can be informative given that negative treatment expectations may affect pain outcomes in the placebo control group when a (in fact) non-active substance is injected (see below). When interpreting negative findings, the sample size should be critically evaluated. Many existing studies report on samples that are – due to the complexity of the model – rather small, with as few as 10–20 participants per group or condition. This may reduce statistical power and lead to false-negative findings. Finally, the timing of pain testing may also result in negative findings. Some studies have tested pain sensitivity very early (i.e., earlier than 1.5 h p.i.) or at late time points (i.e., later than 5 h p.i.). For example, Hijma et al. [27] conducted repeated pain tests up to 10 h after LPS injection and included results from all time points into one analysis, resulting in "negative findings" and the

interpretation that the LPS model is not suitable for pain research. However, secondary analyses within the same article revealed significant changes in pain sensitivity during the acute phase of inflammation (i.e., 2–4 h p.i.), which were consistent with other reports. Thus, it seems that the "negative findings" could be explained by the analysis strategy and the inclusion of several time points which were outside the acute phase of inflammation. A harmonization and standardization of protocols and procedures is required in future studies to increase the comparability and demonstrate the robustness of the findings.

Future Directions

As outlined in this review, the endotoxemia model has been used extensively by now. The model offers a promising approach for proof-of-concept studies to test the feasibility, efficacy, as well as the underlying mechanism of pharmacological and behavioral interventions aimed at pain-resolving and/or anti-inflammatory interventions [2, 20]. Interestingly, one previous trial [114] indicated beneficial effects of meditation and relaxation techniques on cytokine profiles during endotoxemia; however, effects on pain and other sickness symptoms remain elusive yet.

Due to the acute response, the similarity to a natural infection, and no known long-term complications (see Engler et al. [23] in this article collection), the model of experimental endotoxemia should be safe for individuals with some vulnerability as well. To the best of our knowledge, only one study has used the model in a chronic pain population so far. This study in a small sample of women with fibromyalgia suggested that low-dose LPS may induce different changes in magnetic resonance spectroscopy-derived brain temperature and brain metabolites during systemic inflammation when compared to healthy controls [59]. One further study has investigated prior vulnerabilities [115] by injecting healthy obese individuals with LPS. The higher baseline inflammatory levels associated with obesity did affect cognitive aspects of sickness behavior. To closer mimic long-term effects and vulnerabilities for clinical disorders, individuals who have been under stress or who have mild pain, fatigue, or allergies could be investigated with low LPS doses. In clinical populations, where endotoxemia is inappropriate, in vitro measures complementary to blood samples may reveal important immunoreactive functions. Moreover, the relationship between in vivo and ex vivo biomarkers, and blood and cerebrospinal fluid markers, need further investigation.

In clinical and neuroimaging studies of complex disorders, inflammatory activity needs to be taken into account to a greater extent, for disease-specific symptoms but also for comorbid symptoms. The research community needs to be aware of the direct effects of systemic inflammation on brain function. Nonspecific and transdiagnostic sickness symptoms may hold important information in itself, and several surveys have been developed to assess sickness behavior, e.g. [21, 69]. Finally, the classical pro-inflammatory cytokines and blood markers that are usually studied (such as IL-6 and CRP) point to relationships between the immune system and central and behavioral changes, but these markers are not useful from a treatment perspective. We hope that the psychoneuroimmunology community will come to use more exploratory biomarker analyses [116, 117] and multi-omics techniques [16] to investigate further biomarkers involved in immune-to-brain communication.

Conclusion

In the past 10 years, human experimental endotoxemia has increasingly been recognized in pain research as an externally valid, translational model allowing to analyze effects of inflammation on different facets of pain. A major advantage of the model is that a transient acute inflammatory state can be reliably induced in nonclinical cohorts, which offers the unique opportunity to assess direct and causal effects of inflammation on behavior and underlying brain mechanisms under well-controlled laboratory conditions.

Experimental endotoxemia induces reproducible changes in pain sensitivity for C-fiber-mediated deep pain and visceral pain stimuli during acute inflammation. Within the brain, these effects are mirrored by altered responses within insular, cingulate, somatosensory, and prefrontal regions involved in pain processing, supporting that endotoxin-induced changes are not limited to mere effects on peripheral nerve fibers. These findings provide a close link to clinical pain in different inflammatory and functional pain conditions, which involve central sensitization as a key mechanism of hyperalgesia. Different from deep pain, endotoxin effects on cutaneous and thermal pain are less well-studied and less clear.

Endotoxin-induced changes in pain sensitivity are restricted to the phase of acute inflammation, which is characterized by increased levels of pro-inflammatory mediators such as pro-inflammatory cytokines. Despite this timely overlap of systemic inflammation and pain sensitization, no clear picture emerged for the question if

pain sensitization increases with a higher inflammatory burden, i.e., with higher circulating cytokine concentrations or a greater endotoxin dose. Existing findings rather support that immune changes do not linearly translate into pain. It is more likely that a certain inflammatory threshold must be exceeded for the inflammatory signal to lead to sensitization and/or symptom development. This notion is also in line with endotoxin studies showing that changes in pain sensitization were independent of sex, despite a substantially stronger immune response in women.

For a valid interpretation of existing findings and for designing future endotoxin studies, some methodological considerations need to be taken into consideration. Besides potential influences of the timing of pain testing and endotoxin dosage, the outcome measures for pain assessment should be critically evaluated. Existing studies used different indicators of pain sensitization, ranging from pain threshold and pain tolerance to pain ratings. The use of different pain outcomes across studies may not only explain contradicting findings within one pain modality. It is also conceivable that "positive" findings are overrepresented, while "negative" findings are less commonly reported. Future studies should strive to assess physiological and psychological measures of pain and to capture also facets of the pain experience that closely resemble patient suffering such as pain unpleasantness in addition to measuring pain intensity or pain thresholds.

Recent advances have aimed to disentangle the close interrelationship between inflammation, mood, (maladaptive) cognitions, and pain. Negative affectivity and distress existing before immune activation seem to act as vulnerability factors for increased pain sensitization. Endotoxemia may increase the susceptibility to negative mood stimuli and negative social cues and may affect pain-related learning and memory processes, contributing to pain amplification. Together with evidence that sickness behavior symptoms including pain are likely modifiable by expectations (i.e., placebo and nocebo effects), these findings support the further development of behavioral strategies to prevent and ameliorate pain and pain-associated symptoms in the context of inflammatory conditions.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation: project ID 422744262–TRR 289). The funding organization was not involved in the writing of the manuscript or in the decision to submit the article for publication.

Author Contributions

S.B. and B.K. contributed equally to the conceptualization of the work, research of the literature, drafting and revising the manuscript, and approval of the final version.

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