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Advances in assessment of pain behaviors and mechanisms of post-operative pain models

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Each year, more than 300 million surgeries are performed worldwide, which are often accompanied by postoperative pain. To promote the healing process and prevent complications, postoperative pain should be alleviated as soon as possible. Despite intensive research efforts in this field, treatment options are still limited. This is largely due to the fact that the underlying mechanisms are poorly understood. In this review, we describe important and recent advances elucidating mechanisms of peripheral and central sensitization in preclinical models for pain after surgery. We further discuss the importance of assessing multidimensional pain-related behavior in rodents as well as tissue – and procedure specific surgical aspects to increase the translation of preclinical findings to the clinic.

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Introduction

Each year, more than 300 million surgeries are performed worldwide, and pain is one of the most common symptoms after that. Surprising is that the management of postoperative pain remains insufficient in a high number of patients, although many efforts were made to improve the situation [1–3]. Besides organizational problems related to postoperative pain management in hospitals and ambulatory surgery, the main reason for an often-unsuccessful pain management after surgery is a lack of knowledge about the mechanisms underlying postoperative pain. Current treatment options are not specific, produce side effects, and are often unsatisfactory and risky. Thus, many patients still suffer from acute pain and their consequences, including delayed recovery, increased complications, and long-term consequences like chronic postoperative pain or opioid addiction [4–6].

Preclinical pain models in rodents are essential to advance our knowledge about mechanisms inherent in pain caused by specific conditions [7]. In recent years, disease-specificity of pain models in rodents is becoming more popular and provides valuable insights into pain pathways and mechanisms that improve the translation to the clinic [8]. For postoperative pain, there is a quite strong history of preclinical rodent models imitating the surgical injury. The original and first incision model developed by Brennan *et al.* [9] used a surgical incision in the plantar aspect of the rat hind paw; this incision model is characterized by non-evoked pain behavior as well as mechanical and heat hyperalgesia, lasting for several days and corresponding with the time course of postoperative pain in patients [10,11]. Interestingly, cold hyperalgesia does not evolve after incision [12] and shows one specific difference already to some neuropathic pain models like the spinal nerve ligation and the SNI model [13,14]. In the last 20 years, many studies using the rat (and mice [15]) plantar incision model precisely demonstrated that mechanisms inherent in pain after incision injury differ significantly from those mechanisms inherent in other pain conditions like pure inflammatory or neuropathic pain [10,11]. The original rat incision model was modified concerning various parts of the body (e.g. hind paw, thigh, neck, and glabrous or hairy skin) as well as more procedural-specific postoperative pain models [16–19] and models related to pain that is more prolonged after surgery [20]. These modifications allow an investigation of different aspects of postoperative pain, including mechanisms relevant to the transition from acute to chronic pain after surgery [10,21].

Advanced assessment of postoperative pain-related behavior

Just as important as the use of disease-specific animal models seems to be the detection of relevant pain-related behaviors in animals, with rodents being especially prone to experimental limitations [22–24]. To date, assessment of reflex-based nocifensive behavior (to mechanical and thermal stimuli) represents a well-established method to characterize pain-related outcome in rodent models. However, although commonly used, their clinical relevance has been questioned recently [8].

After incision in rodents, mechanical hyperalgesia is strong and lasts for a couple of days after incision

[9,15]. In postoperative patients, punctate mechanical hyperalgesia surrounding the surgical wound has been characterized and might translate to mechanisms relevant for chronic pain after surgery [25,26]. Thus, this measure might be of importance in rodents for investigating mechanisms relevant for central sensitization and chronification of pain after incision injury. Non-evoked pain behavior is one other behavioral feature assessed after incision in rodents [9]; non-evoked guarding pain behavior might translate to pain at rest in humans after surgery. Peripheral mechanisms underlying non-evoked pain behavior after incision injury have been studied in Tim Brennan's laboratory quite extensively (some examples are Refs. [27•,28–30], for reviews compare Refs. [31,10,11]).

Interestingly, peripheral and spinal mechanisms of non-evoked pain and punctate mechanical hyperalgesia after incision differ from each other, at least in part [32,10,11]. Table 1 outlines some of the mechanisms relevant for different pain modalities, for example, non-evoked pain behavior and evoked mechanical and thermal hyperalgesia after incision injury in rats and mice after plantar incision. Molecules that regulate evoked or non-evoked pain, but not both are highlighted in red (Table 1). Thus, if studies are only testing mechanisms of one pain feature or one modality, a transfer to another modality is not possible.

Still, there are some missing links in behavioral pain research in rodents. For example, it seems crucial to establish assays that detect clinically more relevant behaviors in rodents exceeding the analysis of evoked pain [10]. Examples for such 'multidimensional' pain assays after surgery are gait analysis [33], which may represent a correlate for movement-evoked pain and its functional consequences in humans after surgery. These functional outcome parameters are not routinely assessed in humans but are currently becoming more relevant for pain-related recovery after surgery [34]. Other approaches to studying clinically relevant pain-related behaviors in rodents have been developed recently [35]; one of which is home cage monitoring [22,36] that allows detection of various social and physiological aspects related to acute and chronic pain after tissue injuries. Future studies need to

Table 1

Overview underlying peripheral and central mechanisms for evoked and non-evoked pain of plantar incision in rodents. Studies were only included if at least two of the three most common pain modalities after plantar incision were assessed. Molecules/pathways are highlighted in red if they are only specific for a pain modality after plantar incision

	Pain modalities	Periphery	Central
Non evoked pain behavior	Pain at rest Guarding pain Spontaneous pain Weight-bearing	ASIC3 [73] Nav1.7 [50] NGF [70] TRPV1 [71] TRPA1 [72] Tryptase [45]	DAGLβ [36] GLT, GAT [32] iNOS [74] MOR [75] NPY [76] p38 [32] SSTR2 [51]
Evoked pain behavior	Mechanical hyperalgesia	ASIC3 [73] Caspase 1 [77] CGRPα [47] Nav1.7 [50] PI3-Kinase [78] SARM/NF-κB signaling [33] TLR4/NF-κB signaling [39•] TRPA1 [72] Tryptase [45]	$GABA_A$, $GABA_B$ [57] iNOS [74] MOR [75] NPY [76] pERK 1/2 [79] PI3-Kinase [78] SSTR2 [80] TLR4/NF-κB signaling [39•]
	Heat hyperalgesia	ASIC3 [73] Caspase 1 [77] CGRPα [47] Nav1.7 [50] NGF [70] PI3-Kinase [78] SARM/NF-κB signaling [33] TLR4/NF-κB signaling [39•] TRPV1 [71]	iNOS [74] NPY [76] PI3-Kinase [78] TLR4/NF-κB signaling [39•]

Abbreviations: **ASIC3**, Acid-sensing ion channel 3; **CGRPα**, Calcitonin gene-related peptide-α; **DAGLβ**, Diacylglycerol lipase; **$GABA_A$**, $GABA_A$ receptor; **$GABA_B$**, $GABA_B$ receptor; **GLAST**, Glutamate aspartate transporter; **GLT-1**, Glutamate transporter 1; **iNOS**, Inducible nitric oxide synthases; **MOR**, μ-opioid receptor; **Nav1.7**, Voltage-gated sodium channel 1.7; **NF-κB**, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; **NGF**, Nerve growth factor; **NPY,** Neuropeptide Y; **p38,** p38 mitogen-activated protein kinases; **pERK1/2**, Phospho-Extracellular signal–regulated kinases1/2; **PI3-kinase**, Phosphoinositide 3-kinases; **SARM**, sterile alpha-motif-containing and armadillo-motif-containing protein; **SSTR2**, Somatostatin receptor type 2; **TRPA1**, Transient receptor potential cation channel 1; **TRPV1**, transient receptor potential cation channel subfamily V member 1; **TLR4**, Toll-like receptor 4.

determine the most relevant and best-suited assays that allow translation of preclinical rodent models back to patients after surgery.

Tissue damage caused by surgical intervention – peripheral sensitization in postoperative pain

Every surgical intervention leads to procedure-specific tissue trauma, which is accompanied by activation of immuno-competent skin-resident cells [37], axon loss [16], release of inflammatory mediators [38] via multiple signaling pathways, for example, TLR4/NF-κB pathway [39•], and the induction of an ischemic-like condition around the injury [21,40]. Under these conditions, the type/severity of tissue damage, retraction time, and the associated sensitization of nociceptors determine which of these processes are activated and which pain modalities emerge [21]. Therefore, it is essential to evaluate and interpret findings from preclinical studies (pain-related behavioral changes, the release of inflammatory mediators, etc.) in the light of procedure-specific tissue traumas. This may, in fact, translate well to the procedure – specific treatment approach in humans after surgery (www.postoppain.org).

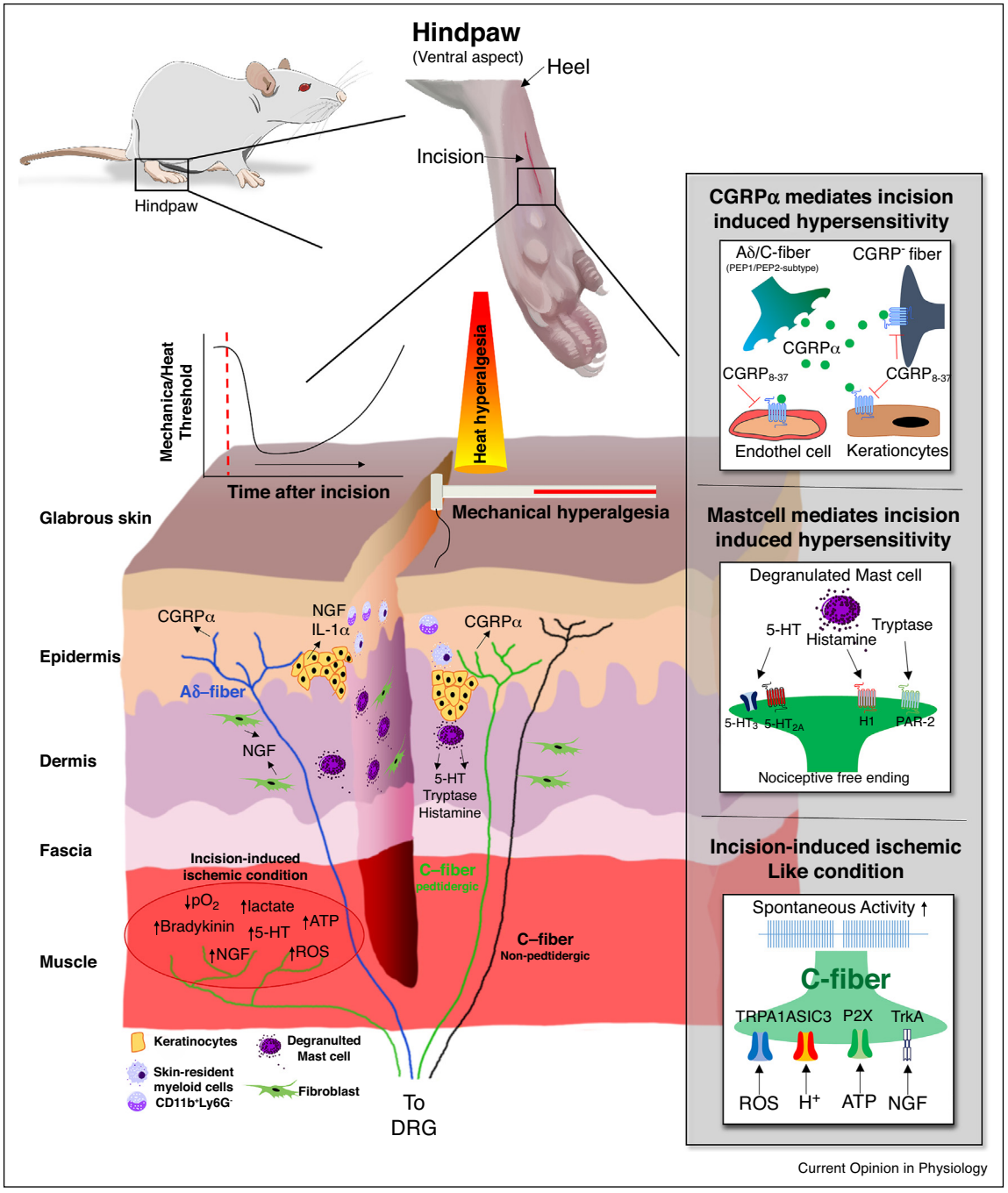
Incision of the muscle layer is part of many surgeries; in rodents, there is an associated formation of hypoxia (↓pO_2) and H_2O_2-enriched environment [41•], as well as the release of H^+ (↓pH), lactate, ATP, bradykinin, NGF and other mediators, that leads to the spontaneous activation of C-fibers which mainly contributes to guarding pain behavior after incision in rodents [40] (Figure 1). In this context, it has been shown recently that the presence of reactive oxygen species (ROS) is responsible for the initiation and maintenance of post-incisional non-evoked guarding behavior by activating Transient Receptor Potential Ankyrin 1 (TRPA1, predominantly expressed on C-fibers) after (glabrous) skin and muscle incision [27•]. In contrast, a skin incision only leads to decreased mechanical/heat thresholds and sensitization of mechano-sensitive Aδ-fibers [42] and does not result in spontaneous activation of C-fibers or non-evoked pain behavior [28,43]. Many rodent studies analyzed incision-induced processes in dermal and epidermal layers, such as the local release of various types of cytokines (IL-1β, IL-6, TNFα), chemokines and neuro-immune modulators (histamine, tryptase, 5-HT, NGF, CGRPα) from recruited fibroblasts [44], mast cells [45,46], sensitized keratinocytes [44], Schwann cells [44], peptidergic nerve fibers [47] or skin-resident myeloid cells (CD11b + Ly6G) [37]. Time-related changes in the tissue environment (tissue pH, lactate concentration, oxygen tension, activation of skin-resident cells, recruitment of immune cells, etc.) could be observed over periods up to 10 days after plantar incision [21]. The local release of inflammatory mediators after incision directly sensitizes peripheral nociceptors in multiple tissue layers [43,48] (peripheral sensitization). This sensitization leads to local neuroinflammation and neuroimmune responses in dorsal root ganglia neurons (DRG) and the superficial layer of the spinal cord. Glial cells (Satellite glia cells (SGC) and microglia), in DRG, synthesize and release cytokines as well as chemokines and neurotrophins (NT) via multiple pathways, for example, TLR4/NF-κB signaling [39•] that modulate the neuronal activity and thereby immediately contribute to mechanical hypersensitivity [49••] following plantar incision (Figure 2). Nerve Growth Factor (NGF) might play a special role as it is transported from the site of injury in peptidergic $TrkA^+$ fibers to the somata in the DRG [44]. Here it may modulate downstream signaling and neuronal excitability, for example, through increasing expression of $Na_v1.7$ channels [50].

Local pharmacological modulation of peripheral sensitization might prove an effective method to relieve acute pain following surgery. However, this is a difficult undertaking and includes some pitfalls, such as application route (intrawound infiltration versus systemic application), timing (before, during or after surgical intervention) and other parameters [39•]. Many studies recently demonstrated a positive effect on pain-related behavior after incision by targeting TLR4/NF-κB signaling [39•], activating somatostatin receptor 2 [51] or inhibition of several sodium channels [49••]. However, local and systemic interventions may lead to unintended outcomes, such as delayed wound healing [52] or increased evoked pain-related behavior [53]. Thus, many factors need to be considered before translating findings to the clinic. A further important aspect, which is not yet addressed in the literature, is the immune status of laboratory animals that live under sterile housing conditions, which differs significantly from that of free-living rodents [54] and also very likely from that of patients. Future studies could, therefore, explore the role of the animals' immune status on different pain-related behaviors to increase further translatability of rodent pain research (for further details see Refs. [54,23]).

The way of postoperative pain into the central nervous system – central sensitization

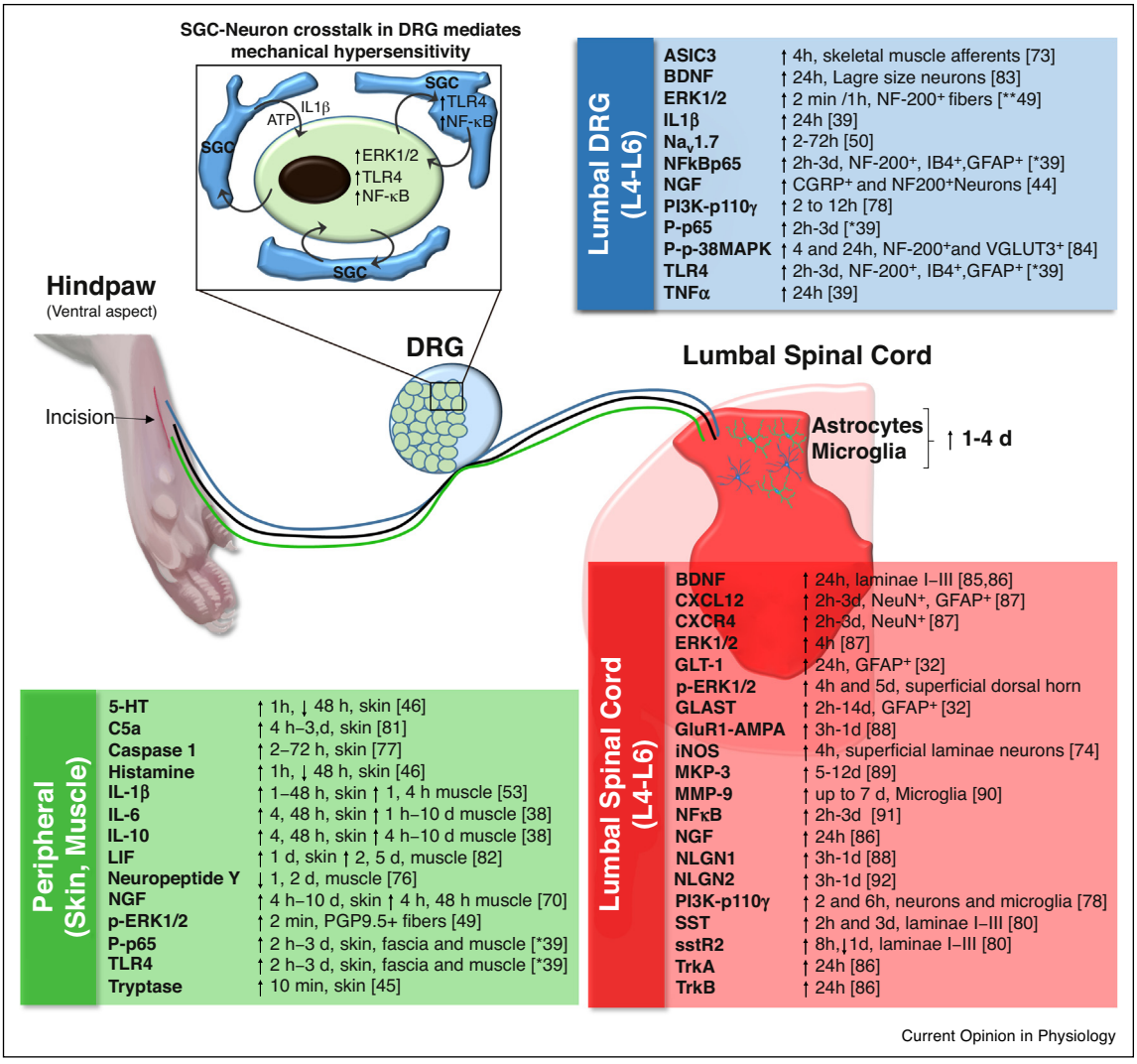
It has been reported that central sensitization/synaptic plasticity in the spinal and supraspinal nervous system is critical for the induction and maintenance of acute and chronic postoperative pain [55]. Main findings from neurophysiological and behavioral assessments in the plantar incision model were an expansion of receptive fields of dorsal horn neurons (DHN) and secondary mechanical hyperalgesia [56] after incision injury, respectively. Pharmacological studies elucidated the role of AMPA receptors, especially those containing the Ca^{2+} permeable AMPA receptor subunit (GluR1), $GABA_A$ and $GABA_B$-receptors [57], and glutamate transporters [32] (Figure 2) in the DHN. Other pharmacological spinal mechanisms inherent in pain behavior after surgery are

Figure 1



Peripheral sensitization is associated with layer-dependent processes in the plantar incision model. Incision of the deep muscle layer initiates an ischemic environment associated with the release of algesic substances that increase the spontaneous activity of multiple C-fiber population, which results in non-evoked pain behavior. Activation of skin-resident immune cells and sensitization of keratinocytes/Schwann cells contribute to decreased thresholds for external mechanical and heat stimuli and increased receptive fields through the release of pain mediators.
Abbreviations: **5-HT**, Serotonin; **ASIC3**, Acid-sensing ion channel 3; **ATP**, Adensonine Triphosphate; **CGRP**, Calcitonin Gene-Related Peptide; **CGRP8-37**, Calcitonin Gene-Related Peptide Fragment 8–37; **H^+**, Hygrogen/proton; **H1**, Histamine receptor; **H_2O_2**, Hydrogen peroxide; **IL-1β**, Interleukin-1β; **Na_v**, voltage-gated sodium channel; **NGF**, Nerve growth factor; **PAR-2**, Protease activated receptor 2; **P2X**, P2X purinoreceptor; **ROS**, Reactive Oxygen Species; **TrkA**, Tropomyosin receptor kinase A; PEP1/PEP2, CGRP-postive peptidergic neurons [47]; **TRPA1**, Transient receptor potential cation channel 1; ↑ increased, ↓ decreased, inhibition.

Figure 2



Plantar incision activates pro-inflammatory pathways that are associated with regulation and recruitment of glia cells, receptor trafficking and mediator release of dorsal root ganglion neurons (DRG) and dorsal horn neurons (DHN) of lumbal spinal cord [81–92].
Abbreviations: **ASIC3**, Acid-sensing ion channel 3; **ATP**, Adenosine triphosphate; **BDNF**, Brain-derived neurotrophic factor; **CXCL12**, C-X-C motif chemokine 12; **CXCR4**, C-X-C chemokine receptor type 4; **ERK1/2**, Extracellular signal–regulated kinases1/2; **GFAP**, Glial fibrillary acidic protein; **GLAST**, Glutamate aspartate transporter, **GluR1-AMPA**, R1-subunit of AMPA receptor; **GLT-1**, Glutamate transporter 1; **IB4**, Isolectin IB4 (marker for Non-peptidergic C fibers); **IL-1β**, Interleukin-1β; **iNOS**, Inducible nitric oxide synthases; **MKP-3**, Mitogen-activated protein kinase phosphatase 3; **MMP-9**, Matrix metallopeptidase 9; **NeuN**, Neuronal Nuclei (neuron marker); **NF-200**, Neurofilaments 200 kD (marker for myelinated Aδ fibers, Aβ fibers, and proprioceptors), **NF-κB**, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; **NGF**, Nerve growth factor, **Na$_v$1.7**, Voltage-gated sodium channel 1.7; **P-p65**, Phospho-NF-κB p65; **P-p38MAPK**, Phospho-p38 mitogen-activated protein kinases; **PI3K-p110γ**, Phosphoinositide 3-kinases p110 catalytic subunit; **SGC**, Satellite glia cells, **SST**, Somatostatin; **sstR2**, Somatostatin receptor type 2; **TLR4**, Toll-like receptor 4; **TNFα**, Tumor necrosis factor α; **TrkA**, Tropomyosin receptor kinase A; **TrkB**, Tropomyosin receptor kinase B; **VGLUT3**, Vesicular glutamate transporter type 3 (marker for Myelinated Aδ fibers, Aβ fibers, and proprioceptors); ↑ increased ↓ decreased expression, h = hours, d = days.

outlined in Table 1. The use of novel molecular methods may help discover previously unidentified spinal or supraspinal signaling pathways relevant to developing central sensitization after surgery [58••]. These studies might detect novel drug targets and drive pain-modality and entity-specific research for postoperative pain [59,32,10]. Recent studies revealed for the first time cerebral processing relevant to mechanical hyperalgesia after incision by using functional magnetic resonance imaging (fMRI) and fMR-spectroscopy (fMRS) in the thalamus [60] helping understand central processing and sensitization under conditions of postoperative pain.

Postoperative pain and lifestyle – influence of sleep, psychological factors, and diet

Effects of modern civilization, such as sleep disruption, psychological disorders, excessive alcohol drinking, and obesity are risk factors for the development of more serve acute pain and facilitate the transition to chronic pain after surgery [61–63]. Consequently, depression-like behavior [64••], perioperative stress [65], sleep disruption [66•], ethanol consumption [67] and high-fat diet [68••] all prolonged mechanical hyperalgesia and subsequent wound healing in the plantar incision model. These findings are associated with increased/prolonged neuroimmune activation evidenced by increased density and activation of spinal microglia/astrocytes [68••,64••], increased phosphorylation of spinal GluA1 receptor subunits [67], high levels of pro-inflammatory cytokines in the periphery and central structures or disturbance in the wound healing and recovery time [68••,64••]. Interestingly, caloric restriction before the incision was associated with reduced mechanical and heat hyperalgesia and pain at rest after incision and reinforced analgesic efficiency of parecoxib and morphine, presumably due to decreased levels of pro-inflammatory cytokines [69•]. Such studies are relevant to uncover underlying mechanisms associated with an increased risk of severe and prolonged acute pain and the transition to chronic pain after surgery.

Together, the exciting advances in postoperative pain research illustrate the importance of procedural-specific postoperative pain models in combination with a more versatile examination of species-specific behavioral changes. To further increase translatability of preclinical research, a sound understanding of immune-modulatory factors (stress, sleep, diet, exposure to pathogens, gender, etc.) on pain development seems critical.

Conflict of interest statement

Nothing declared.

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