Table 1: Functional components of neuropathic and chronic pain pathways, key anatomical substrates and their importance

Process and underlying mechanism	**Major neurotransmitter/s (target /tissue)**	**Time of release/ activation**	**Consequences**	**Importance/Remarks**
Pain signalling/peripheral sensitisation at primary afferent neurons				
Peripheral nociceptor sensitisation (hyperexcitability)	Substance P (receptors on peripheral terminals and NK1 receptors, plasma membrane of cell bodies, dendrites of non-stimulated neurons [19, 22, 98-100]	Early in the development of neuropathic pain	Sensitisation of peripheral terminals, increased firing rate.	This mechanism explains hyperalgesia as consequence of hypersensitisation
	Purinergic pathways[19,22]			.
Activation of purinoceptors on microglia			Induction of neuropathic pain state	
Release of excitatory amino acids (EAA)			Release of TNF-α	

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Cytokine release following tissue injury is released by macrophages and nerve cell	Cytokines (Receptors on blood monocytes)	Early, within 24 h of the onset of inflammatory response	Mediates the inflammatory state	Ectopic hyper-excitability due to increase in nerve cell interaction, resulting in a vicious cycle of inflammation
Inflammation (active macrophage infiltrate)	TNF-α		Activation and release of platelet derived growth factor (PGDF)	TNF-α is the primary inflammatory mediator involved in certain nerve injuries (e.g. lumbar disc herniation)
Activation of phospholipase A_2 (PlA_2) enzyme on cell membranes	Release of arachidonic acid from the cell membrane phospholipid		Increase in prostaglandin concentrations, which in turn increase the production of glutamate	
PlA_2 activation triggers two	Prostaglandins (Peripheral nociceptors, PGE_2		Sensitisation of peripheral nociceptors,	While IL-6 is the primary chemical mediator in pain, IL-10 is a natural

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competing pathways, i.e., cyclo-oxygenase (COX) and lipo-oxygenase (LOX)	receptors in smooth muscle)		localised pain, hypersensitivity in uninjured tissue	anti-inflammatory cytokine. The net inflammatory response is the result from these opposing effects
	Thromboxane (TXA_2 receptors on platelets)		Leukotriene-induced platelet activation and constriction of smooth muscle	
Release of interleukins	Leukotrienes (receptors on smooth muscle)	Within the first few hours of tissue injury	Increased vascular permeability and leukocyte attraction	
	IL-1β, IL-6, IL-8, IL-10 (peripheral nociceptors)[101]		Stimulation of the production of pro-inflammatory mediators such as PGE_2, COX-2, and matrix metallo-proteases (MMP)	

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Pain processing				
Central sensitisation (spinal cord)	Glutamate (Presynaptic opioid, glutamate receptors) Substance P (Calcium channels-(α_2-δ)) Protein kinase C (NMDA receptors) and purinoceptors [22, 23, 102]	Unknown	Dynamic mechanical allodynia Punctate mechanical allodynia	Spread of spinal hyper-excitability Expansion of neuronal fields [22,23,102]
Phenotypical switch Nociceptor peptides normally expressed by A δ and C fibres are expressed by large myelinated Aß fibres	Calcitonin gene-related peptide, substance P (dorsal horn receptors)	Unknown	Input from mechanoreceptor A fibres is perceived as pain (dynamic and punctuate allodynia)	Increased synaptic transmission, which is considered the most important steps in the development of chronic pain [25]

Process and underlying mechanism	**Major neurotransmitter/s** **(target /tissue)**	**Time of release/ activation**	**Consequences**	**Importance/Remarks**
Descending dysinhibition	GABA (GABA receptors) Endogenous opioids (μ receptors)	Late manifestation, months to years after neurological insult[103]	Loss of inhibitory synaptic currents	Selective apoptotic loss of GABAergic neurons in superficial dorsal horn of the spinal cord
Functional degeneration of interspinal inhibitory interneurons Decreased supraspinal descending modulation Descending facilitation	Serotonin/norepinephrine, dopamine (α-2, 5-HT receptors at the dorsal horn inhibitory interneurons) Glutamate (glutamate receptors, purinergic receptors [22, 25, 104]	Protracted several weeks after peripheral nerve injury [1,26,104]	Enhanced signal transmission in the DRG	Inhibition or prevention of apoptotic loss leading to functional degeneration could provide disease modifying effect in neuropathic pain Structures in the mesencephalic reticular formation—possibly the nucleus cuneiformis and the periaqueductal gray area are involved in central sensitisation in neuropathic pain [25] Interestingly, advanced functional MRI (fMRI) techniques show that the same brainstem structures are active in humans with allodynia

Process and underlying mechanism	**Major neurotransmitter/s (target /tissue)**	**Time of release/ activation**	**Consequences**	**Importance/Remarks**
Pain perception/plasticity in the brain				
Intense and persistent nociceptive input involving limbic circuitry. Long term down-regulation of dopamine receptors and dopamine production, enhanced glutaminergic transmission from prefrontal cortex to nucleus accumbens. [105]	Dopamine, glutamine	Plasticity onset occurs at a late stage; associated with chronicity of pain	Maintain synaptic plasticity. Develop and maintain inflammatory hyperalgesia	Similar changes occur in the brain, particularly in the cortex and can be measured experimentally and by functional magnetic resonance Imaging or PET. Dramatic alterations in cortical spatial maps can be detected after nerve injury that may contribute to phantom pain[26, 105]

Table 2: Overview of commonly used experimental models of pain in human subjects.

Model	Description	Clinical manifestation	Mechanisms	Limitations /Application
Mechanical stimulation models				
Mechanical stimulation (pinprick, pressure)	Cutaneous stimulation using von Frey filaments, cotton swab, pin-prick or pressure algometers	Allodynia, pin-prick hyperalgesia	Stimulation of nociceptors and mechanoceptors Aδ and C fibres are stimulated	• Truly noxious stimuli cannot be induced by non-specific cutaneous stimulation • Cutaneous techniques do not mimic nociception • NSAIDs, systemic ketamine, tramadol show analgesic activity [57,106]
Chemical-, heat- or cold-evoked hyperalgesia				
UVB (ultraviolet B or sunburn)	Hyperalgesia induced by exposing skin area to graded individualised doses of UV B radiation, resulting in dose related erythema	Inflammatory response, allodynia and hyperalgesia Secondary hyperalgesia in erythematous area	Central sensitisation Aδ and C fibres are stimulated	• This model is not sensitive to drugs administered systemically, applied locally or to drug combinations acting via complementary mechanisms of action • NSAID activity has been identified, but no analgesia produced by opioids[57,58]
Capsaicin-induced pain	Capsaicin is applied topically, intradermally or intramuscularly Capsaicin exposure leads to acute severe burning pain	Primary or secondary hyperalgesia up to 24 h	Activation of TRPV1 receptor Stimuli mimic the symptoms of hyperalgesia observed in neuropathic pain	• Hyperalgesia is variable as it depends on capsaicin absorption • Opioids, NMDA receptor antagonists, and calcium channel α2-δ ligands attenuate capsaicin-induced hyperalgesia • Limited activity observed with tricyclic antidepressants and cannabinoids • More C than Aδ fibres activated • Inconsistent results observed during evaluation of drugs with anti-neuropathic pain activity • Lamotrigine, desipramine showed no effects, while gabapentin suppressed hyperalgesia[57,58,106]

Mustard oil	Model of acute peripheral sensitisation Topical application for a few minutes leads to burning pain followed by an inflammatory reaction in the exposed area	Secondary hyperalgesia and allodynia in surrounding unaffected area	Activation of cation channel TRP amkyrin type I in noiciceptive neurons C fibres thought to mediate burning pain A fibres believed to mediate allodynia to light mechanical stimuli	• It has not been widely used in analgesia testing • Limitations similar to those reported with the capsaicin model[57,58,106]
Thermode burn	Hyperalgesia secondary to first degree burn by exposing healthy subjects to a heat stimulus using a contact thermode	Primary hyperalgesia at the site of exposure, secondary hyperalgesia in adjacent tissue	Central sensitisation Aδ and C fibres are stimulated along with co-activation of Aβ fibres	• NMDA receptor antagonists attenuate mechanical hyperalgesia, but effects are inconsistent with opioids • Intracellular Na channel blockers, opioid receptor antagonists, and purinergic receptor activators [57,58,106]