Supplementary Material 6

In cerebral ischemia models, neuroinflammation resulting from the action of cytokines and chemokines leads to hyperalgesia and CPSP. Precisely, ischemic stroke activates spinal dorsal horn neurons, increases the box-1 high-mobility group expression, and stimulates glial cells in the spinal cord via advanced glycation end products.[25] Ischemic stroke also causes direct damage to involved brain regions [26] including neurons that produce orexin in the lateral hypothalamus, leading to a decrease in the expression of orexin-A. The reduction in binding of orexin-A to the orexin 1 receptor in norepinephrine and the locus coeruleus serotonergic neurons, as well as the rostral ventromedial medulla, decreases neurotransmitter signaling and triggers the spinal cord glial cells' activation.[27]

Activated spinal glial cells can initiate a signaling pathway that triggers an increase in nitric oxide synthase expression. This signaling pathway, known as the nuclear factor kappa B/extracellular signal-regulated kinase/inflammatory cytokine/enzyme–$N^{(G)}$, $N^{(G)}$-dimethylarginine dimethylaminohydrolase-1/nitric oxide synthase pathway, induces a cascade of events that leads to an augmented inflammatory response and the onset of CPSP during the first 3 days. This cascade of events ultimately leads to the upregulation of inflammatory cytokines and enzymes, which contribute to the onset of CPSP 3 days later [28] (Figure 1B). These processes underscore the crucial role of neuroinflammation in CPSP, implicating altered neurotransmitter function and

potential spinal cord involvement in CPSP pathogenesis.

In thalamus hemorrhage models, glial cell activation also contributes to CPSP development: activated microglia co-localize with P_2X_4 receptors [19] and P_2X_7 receptors [17, 29] in perilesional tissues of the thalamus. When an intracerebral hemorrhage occurs in the VPL nucleus, neurons are damaged and release significant ATP. Furthermore, the P_2X_4 and P_2X_7 receptors are upregulated in activated microglia and nociceptive afferent neurons. Upon activation of the P_2X_4 receptors by ATP, microglia release BDNF and activate tyrosine kinase receptor B (TrkB) in neurons, resulting in the downregulation of potassium chloride cotransporter 2, which leads to the induction of CPSP. Additionally, upon ATP binding to the P_2X_7 receptors, microglia release interleukin-1β into the surrounding tissues and synaptic spaces, promoting glutamate release. This results in frequent neuronal firing along the thalamic cingulate pathway, leading to abnormal pain.[29] MicroRNA-133b-3p is downregulated in the ventral posterolateral nucleus (VPL). However, its overexpression can downregulate the expression of P_2X_4 receptors and reverse allodynia in CPSP rat.[30]

Following a hemorrhage in the VPL nucleus, the affected area undergoes a hypoxic microenvironment during the early stages. This induces the accumulation of hypoxia-inducible factor 1α in the cytoplasm, which triggers glial cells to express stromal cell-derived factor 1 and neurons and glial cells to express CX-C chemokine

receptor type 4 receptors. During the later stages, stromal cell-derived factor 1 binds to CX-C chemokine receptor type 4, activating glial cells and releasing proinflammatory cytokines. This leads to increased glial cell activation and neuronal excitation at the perilesional site of the thalamus, often resulting in CPSP. Furthermore, the mediator complex subunit 1/BDNF/TrkB pathway regulates microglia activity.[31] Activated microglia may alter the function of the remaining neurons, thereby contributing to the CPSP development [17, 32] (Figure 2B). Therefore, it is essential to note that the formation of a hypoxic microenvironment in the VPL following hemorrhage has significant implications for the development of CPSP. Moreover, the activated microglia and the the mediator complex subunit 1/BDNF/TrkB pathway are additional factors to consider in developing this condition.