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# 学 位 論 文 の 要 約

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## 学 位 論 文 題 名

The role of the central amygdala in integrating diverse aversive sensory information  
(多様な嫌悪感覚情報の統合における扁桃体中心核の役割)

Various aversive sensory stimuli, such as pain and predator odors, are known to induce negative emotional states, leading to avoidance behaviors and adaptive physiological responses that are essential for survival and maintaining homeostasis. The central amygdala (CeA), which receives inputs from multiple sensory systems, plays a pivotal role in processing stress and coordinating emotional responses. Exposure to aversive pain, olfactory, gustatory, and visual stimuli has been shown to upregulate c-fos mRNA expression and/or c-Fos protein production in the CeA, indicating increased neuronal activity in response to these stimuli. Previous studies have demonstrated that bilateral excitotoxic lesions of the CeA reduce conditioned place aversion induced by noxious stimuli such as intraplantar formalin injection, implicating the CeA in processing the emotional component of pain. Furthermore, optogenetic manipulation has revealed that the neuronal pathway from the parabrachial nucleus (PBN) to the CeA is critically involved in mediating pain-induced aversive states. Despite these advances, it remains unclear whether individual neurons in these brain regions integrate aversive sensory information of diverse modalities (pain, olfactory, and gustatory), or whether distinct neuronal subpopulations convey the aversive sensory information of each modality in parallel. To address this issue, the present study examines the neuronal activation by aversive sensory stimuli of diverse modalities at the single-cell level in the CeA and LPB.

### **Histological analysis of neuronal activation by two types of aversive stimuli using TRAP2;Ai9 mice**

We used TRAP2;Ai9 mice to investigate whether the individual neurons in the CeA and LPB are activated by two different aversive stimuli. In TRAP2;Ai9 mice permanent expression of a reporter gene (e.g.,tdTomato) in the activated neurons enables their long-term identification. Combination of labeling the neurons activated by the first stimulation using the TRAP2 system and labeling the neurons activated by the second stimulation using immunofluorescent staining enabled to examine whether individual neurons are activated by two different stimuli. For pain stimulation, mice were subcutaneously injected with 10% formalin solution into either hindpaw or whisper pad. For aversive olfactory stimulation, mice were exposed to 2-methyl-2-thiazoline (2MT), a predator odor. For aversive gustatory stimulation, quinine solution (bitter taste) was infused into the mice's mouth. The results suggest that TRAP labeling and immunostaining differ in sensitivity, leading to a lack of accurate quantification in the detection of neurons activated by the two types of stimuli. However, the results provide semi-quantitative data showing that some portion of CeA and LPB neurons activated by aversive sensory stimulation of two modalities.

### **Ca<sup>2+</sup> imaging to visualize the activation of CeA neurons by various aversive sensory stimuli**

In vivo Ca<sup>2+</sup> imaging with C57BL/6 J mice injected with AAVDJ-hsyn-GCaMP6m into the CeA to express GCaMP6m in the entire CeA neurons showed that some portion of CeA neurons were activated by two or more

stimuli, suggesting that the integration of information in the aversive sensory signaling pathway is only partial up to the CeA neurons.

### **Ca<sup>2+</sup> imaging to visualize the activation of PKC $\delta$ -positive CeA neurons by various aversive sensory stimuli**

In vivo calcium imaging with PKC $\delta$ -Cre mice injected with AAVDJ-Ef1a-DIO-GCaMP6m into the CeA to express GCaMP6m selectively in the PKC $\delta$ <sup>+</sup> CeA neurons showed that some portion of PKC $\delta$ <sup>+</sup> CeA neurons were activated in response to pain, olfactory, and gustatory (aversive), respectively. About a quarter of the neurons responded to two or more stimuli, suggesting that the integration of information in the aversive sensory signaling pathway is only partial up to the PKC $\delta$ <sup>+</sup> neurons in the CeA.

### **Conclusion**

The present study demonstrated that diverse aversive sensory information is only partially integrated in the CeA or its upstream brain regions such as the LPB. Future studies are needed to examine the response to aversive sensory stimuli in other neuronal populations than PKC $\delta$ <sup>+</sup> neurons within the CeA and in the neurons located in the downstream brain regions such as the VTA to clarify the neuronal mechanisms for integration of diverse aversive sensory information to mediate appropriate behavioral and autonomic responses.