A13 dopamine cell group in the zona incerta is a key neuronal nucleus in nociceptive processing

Shunpei Moriya*, Tomoyuki Kuwaki

In stressful modern society, pain caused by physical and mental disorders is an increasing social health problem all over the world. Physical and mental activity depends on the state of various neurotransmitters in the central nervous system (CNS). Monoaminergic systems play important roles in regulating physiological functions including nociception. It is well known that in the CNS, there are descending pathways related to nociceptive processing known as the descending antinociceptive system (DAS). Noradrenalin and serotonin are major components of the DAS that form the locus coeruleus (LC)-spinal dorsal horn noradrenergic circuit and periaqueductal gray (PAG)-rostro ventricular medulla (RVM)-spinal dorsal horn serotonergic circuit (Jordan et al., 2008). Dopaminergic pathways also play roles in regulating nociceptive processing in the CNS.

The zona incerta (ZI) is a subthalamic nucleus located ventrolateral to the medial lemniscus and dorsomedial to the substantia nigra. It is associated with nociception, locomotor function, liquid and food intake, arousal, attention, and sexual behavior. The ZI interconnects with various brainstem nuclei. There are some reports that the ZI plays roles in regulating nociceptive processing. Some neurons located in the ZI respond to peripheral stimulation (Okada et al., 2002), and electrical stimulation of the ZI has antinociceptive effects (Prado and Roberts, 1985). These findings indicate involvement of ZI in nociceptive processing. The ZI has a heterogeneous neurochemical profile (Petronilho et al., 2012), including dopaminergic, glutamatergic, and melanin-concentrating hormone-containing neurons. The A13 dopaminergic cell group is located in the rostromedial part of the ZI (**Figure 1A**), and belongs to the medial hypothalamic system.

The A13 dopamine neurons project to the PAG, the central nucleus of the amygdala (CeA), the medial preoptic area, and the ventromedial hypothalamus (VMH). To our knowledge, little is known about how the A13 dopamine neurons are related to specific physiological conditions. Using a fiber photometry system Moriya et al. (2020) recently demonstrated that acute nociceptive stimuli increased A13 dopamine neuron activity.

In the current study, AAV-TetO-G-CaMP6 was unilaterally introduced into the ZI site in mice carrying a dopamine transporter promoter-regulated Cre recombinase transgene (**Figure 1A**). A silica fiber was used to detect and collect the green/red fluorescence of G-CaMP6/mCherry. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animals Use Committee of Kagoshima University (MD15092). After confirming G-CaMP6 expression (**Figure 1B**), G-CaMP6 florescence intensity of the A13 dopamine neurons was rapidly increased in response to the two acute nociceptive stimuli (pinch + heat (55°C)), but not by the two non-nociceptive control stimuli (touch + low heat (25°C)) (n = 6, each) (**Figure 1C** and **D**). This suggests that the A13 dopamine neurons had a higher concentration of intracellular Ca^{2+} than they did before stimulation. In this way, this calcium imaging system uses G-CaMP6 protein as a Ca^{2+} concentration detector in the intracellular space and can detect real-time specific neuronal activity with high resolution.

In the present study, the A13 dopamine neuronal somata that anatomically project to the PAG/CeA were appraised. These are key regions in nociceptive processing, which may indicate the involvement of A13-PAG/CeA dopaminergic pathways in nociceptive processing (**Figure 1E**). The peak latency value was used as the index, indicating time latency from the start of stimuli to the peak signal. The pinch/heat value was 1.50 ± 0.25/1.98 ± 0.39 seconds, thus the value was within 2 seconds. It has previously been reported that the activity of the ventral tegmental area dopamine neurons (the A10 dopaminergic cell group) was rapidly increased in response to acute pinch stimuli, and the peak latency value was approximately 3.5 seconds (Moriya et al., 2018). Because the experimental systems used differ, the A13 group and the A10 group cannot be simply compared with respect to G-CaMP6 signal response patterns. However, if the A13 group exhibits shorter peak latency than the A10 group, it can be surmised that the A13-CeA dopaminergic pathway reacts faster than the A10-CeA dopaminergic pathway. In that case the peak latency value of the activity of the A13 dopamine neuronal axons located in the CeA may be lower than that of the A10 dopamine neuronal axons located in the CeA.

We suggest that momentary (within approximately 2 seconds) fluctuation of A13 dopamine neuronal soma activity may be primarily evoked in response to nociceptive stimuli. If that is case the A10-CeA dopaminergic pathway may be partly inhibited because A13 dopaminergic input arrives at the CeA faster and occupies the dopamine 2 receptor (D2R) located in the CeA. Notably however, we have not appraised the activity of A13/A10 dopamine neuronal axons in the CeA/PAG. In a previous study the activity of B9 serotonin neuronal axons located in the LC/ventral tegmental area was rapidly increased in response to acute nociceptive stimuli (Moriya et al., 2020). In the current study immunostaining with serotonin transporter antibody confirmed co-expression of G-CaMP6/serotonin transporter located in serotonin neuronal axons in the LC/ventral tegmental area. The A13 dopamine neuronal axons located in the CeA/PAG need to be stained with DAT antibody for confirming co-expression of G-CaMP6/DAT. Because D2Rs are located in postsynaptic regions in the CeA/PAG and G-CaMP6/DAT, immunostaining with a D2R antibody may not indicate co-expression. The A11 dopamine cell groups are located in PAG, so it would be interesting to appraise the activities of A11 dopamine neurons using a fiber photometry system under optogenetic regulation of the A13-PAG dopaminergic pathway. Using the designer receptor exclusively activated by designer drugs (DREADD) system would also be interesting.

The CeA region is well known to be involved in regulating emotional behaviors including fear and panic, and the PAG/VMH regions are evidently key components of the defensive behavior system (McNaughton et al., 2004). The dopaminergic system in the CNS plays a role in regulating fear, panic, and defensive behavior, but to our knowledge few studies have attempted to assess the involvement of the A13 group in these behaviors. Some studies have reported that systemic administration of D2R agonist increases defensive reactions, and conversely D2R antagonist decreases them. D2Rs are fully expressed in the CeA/PAG/VMH. Adaptations of a D2R-acting compound or D2R-RNA interference with CeA/PAG/VMH regions can be informative. For example, when neuronal A13-CeA dopaminergic pathway activity is assessed under the influence of a D2R agonist via a fiber photometry system the fluctuation in G-CaMP6 fluorescence intensity responses to aversive stimuli may be inhibited. When the D2R in the CeA is subjected to RNA-interference, a similar phenomenon may emerge. Such studies make it possible to investigate the physiological functions of A13-PAG/CeA/VMH dopaminergic pathways in depth.

The CeA region receives input from the A10, LC noradrenalin, and dorsal raphe serotonin neurons. It has previously been reported that LC noradrenalin/dorsal raphe serotonin neuron activity was rapidly increased in response to acute nociceptive stimuli (Moriya et al., 2019). The PAG region is a key region in the DAS, and it sends neuronal output to the RVM. It has also been reported that RVM serotonin neuron activity was increased rapidly in response to acute nociceptive stimuli (Moriya et al., 2019), and the LC/RVM regions are evidently key regions in the DAS. Collectively these observations indicate that experimental systems that appraise the effects of the physiological state of the DAS under optogenetic/DREADD control of A13-CeA/PAG or of A13-CeA/PAG under optogenetic/DREADD control on the DAS are necessary. The A13-PAG dopaminergic pathway may directly affect the DAS, or the A13-CeA dopaminergic pathway may indirectly affect the DAS.

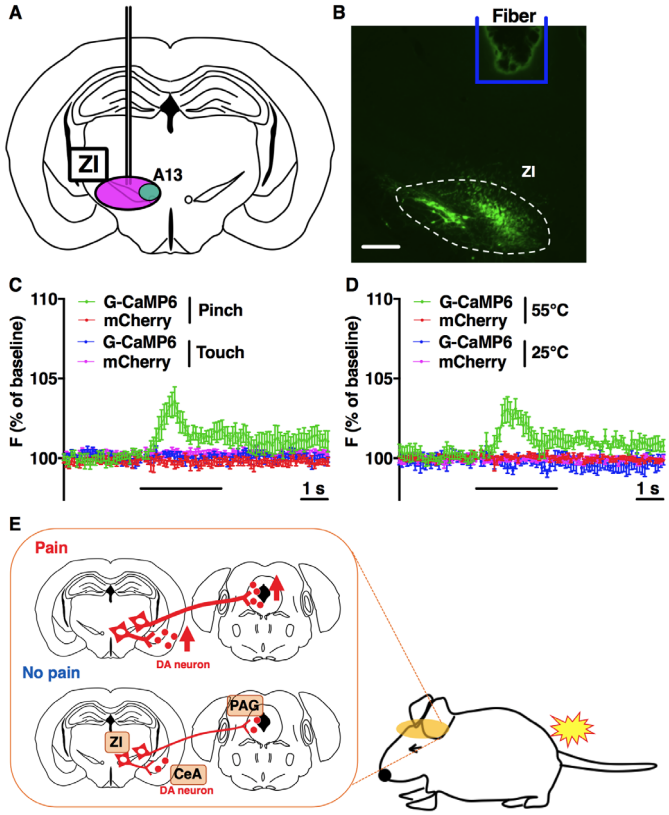

Figure 1 | **Nociceptive stimuli increased G-CaMP6 fluorescence intensity of the A13 dopaminergic cells in the zora incerta.**
(A) AAV injection into the zona incerta. A13 dopaminergic cell group is located in the rostromedial part of the zona incerta. (B) G-CaMP6 was specifically expressed in A13 dopaminergic neuronal soma. The silica fiber was inserted just above the zona incerta. (C, D) Averaged traces of fluorescence intensity of G-CaMP6 and mCherry (n = 6, each). (C) Traces in the pinch/touch groups. (D) Trace in the heat/low heat groups. (E) Involvement of A13 dopaminergic pathways in nociceptive processing. A13 dopamine neuronal soma activity is momentarily increased by pain information input.

The ZI efferents terminate at the spinal ventral horn (Shaw and Mitrofanis, 2002). Components associated with the DAS including the LC/RVM are mainly ascribed to the spinal dorsal horn, whereas the spinal ventral horn receives neuronal input from nucleus raphe obscurus serotonin neurons. To our knowledge the spinal ventral horn is not known to be involved in nociceptive processing. Thus we surmise that the descending ZI-spinal ventral horn pathway may not be involved in nociceptive processing, but this requires verification.

In clinical psychiatric medicine the main therapeutic drugs for pain are serotonin noradrenalin reuptake inhibitors, selective serotonin reuptake inhibitors, and tricyclic antidepressants. These drugs have minimal effects on the dopamine neuronal system. As mentioned above, D2Rs are expressed in postsynaptic regions of the PAG/CeA. During pain A13/A10 dopamine neuron activity is increased. In theory D2R antagonistic compounds may exert antinociceptive effects by inhibiting A13/A10-CeA/PAG pathway activity. Major tranquilizers that mainly act as D2R antagonists are prescribed to somatoform disorder patients with idiopathic pain, and they sometimes have substantial antinociceptive effects (Decoutere et al., 2011). The A10 nucleus accumbens dopaminergic pathway known as the mesolimbic system is involved in nociceptive processing (Ikemoto, 2007), and it can be a therapeutic target of major tranquilizers used to treat pain. One such target may be D2Rs located in the CeA/PAG, to which the A13 dopamine neurons project.

Major tranquilizers sometimes have harmful side effects, including high prolactin plasm which is mainly associated with the hypothalamus-pituitary dopaminergic pathway, and pharmacologic parkinsonism which is mainly associated with the substantia-striatum dopaminergic pathway. In the CNS dopamine inhibits prolactin synthesis and prolactin induces dopamine synthesis, whereas prolactin is not synthetized in the A13 dopamine neurons. An A13 targeted therapy strategy may be advantageous with regard to side effects.

Herein we have reported a study that demonstrated the involvement of A13 dopamine neurons in nociceptive processing, and discussed some related reports. Further studies focusing on A13 dopamine cell group-related signaling are required to confirm the involvement of dopaminergic signaling in nociceptive processing in the CNS.

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