氏　　名	大橋（オオハシ）　ひろ乃（ヒロノ）
所　　属	理工学研究科　生命科学専攻
学位の種類	博士（理学）
学位記番号	理工博　第277号
学位授与の日付	平成30年9月30日
課程・論文の別	学位規則第4条第1項該当
学位論文題名	Genetic studies of hunger-driven reduction of behavioral responses to noxious heat in *Drosophila* ショウジョウバエにおける空腹による侵害熱反応低下の遺伝学的研究（英文）
論文審査委員	主査　准教授　坂井　貴臣 委員　教　授　相垣　敏郎 委員　准教授　髙橋　文

【論文の内容の要旨】

Feeding is essential for animal survival in nature and influences internal satiety-hunger state. The hunger can modify not only feeding behavior but also other behaviors or physiology. The powerful genetic tools in the fruitfly *Drosophila melanogaster* have been used to clarify the cellular and molecular mechanisms of modification of innate and learned behavior induced by hunger. Food deprivation promotes the activity of olfactory and sweet taste neurons, and consequently induces active food-search behavior and gustatory response to sugar. In contrast, sensitivity to bitter taste and optomotor reflex based on the neural processing of visual motion is reduced in food-deprived flies. In addition, food deprivation is known to influence context-dependent CO_2 avoidance, sleep, and memory in *Drosophila*. Thus, hunger has a significant impact on modification of sensory systems or brain functions in *Drosophila*.

Nociception, the neuronal process for reception and/or perception of acute pain, is essential for recognition of injury and survival in nature. In vertebrates and invertebrates, animals show a variety of escape responses to noxious stimuli, and several types of stimuli (e.g., noxious heat, noxious chemicals and harsh mechanical stimulation) can activate the specific nociceptors. In rats, acute food deprivation reduces formalin-induced nociceptive behaviors, indicating that hunger also affects nociception in rats. However, it remains unknown whether hunger-driven reduction of responses to noxious stimuli is prevalent among other animal species. *Drosophila melanogaster*, fruits fly, reacts to variety of noxious stimulus and many studies focused on noxious heat. Many genes associated

with heat nociception have been reported in *Drosophila*. Thus, *Drosophila* can be used to identify genes and molecular mechanisms regulating the hunger-driven reduction of responses to noxious heat. In this study, we examined whether adult flies also show the hunger-driven reduction of responses to noxious heat stimuli and how the responses are controlled by the nervous system.

When wild-type adults are placed on a hot plate (≧ 44°C), they show robust jumping behavior as a response to noxious heat. First, I examined whether food deprivation affects responses to noxious heat. When wild-type flies were food-deprived for 12h (referred to as 12h FD), responses to noxious heat were reduced. However, 12h FD slightly increased locomotor activity, and did not affect climbing acativity. In contrast to 12h FD, when wild-type flies were food-deprived for 6 and 18h (referred to as 6h and 18h FD), responses to noxious heat were not impaired. In addition, I confirmed that food intake in food-deprived flies for 12h increased in comparison with the control flies. These results indicate that 12h FD induces hunger.

To investigate whether brain regulates hunger-driven reduction of responses to noxious heat, I used decapitated flies in the experiments. Decapitated flies are known to retain a variety of functional sensory inputs, although it is unknown whether they can respond to noxious heat. Decapitated wild-type flies showed tumbling at 44 °C. In *Drosophila*, the Painless (Pain) Transient Receptor Potential (TRP) channel is thought to be a heat nociceptor because *pain* mutations inhibit the responses to noxious heat. Frequency of tumbling was significantly reduced by *pain* mutations, indicating that the tumbling is a response to noxious heat in decapitated flies. Next, I examined whether food deprivation also affects responses to noxious heat in decapitated flies. When flies were decapitated after 12h FD, the responses to noxious heat did not change, suggesting that hunger-driven reduction of responses to noxious heat results from the modification of physiological states of the brain.

In *Drosophila*, complex signaling networks using neuropeptides regulates hunger-satiety state. A neuropeptide, Leucokinin (Lk) and its receptor [Lk receptor (Lkr)] are known to regulate meal size. However, it still remains unclear whether food deprivation modifies fly behaviors through Lk signaling. First, I confirmed that temporal electrical silencing of adult Lk neurons impairs the reduction of responses to noxious heat. Next, I generated Lkr^{KO} using CRISPR/Cas9 system. Heterozygous and homozygous flies for Lkr^{KO} also did not show the hunger-driven reduction of responses to noxious heat, indicating that that Lk/Lk receptor (Lkr) signaling pathway induces the hunger-driven reduction of responses to noxious heat. Using anti-Lk antibody, we examined whether 12h FD affects Lk levels in the brain neurons. Compared with the control, Lk signal in food-deprived flies significantly decreased. However, qRT-PCR analysis revealed that no significantly differences are detected in Lk mRNA level between food-deprived flies and the control flies. Taken these results together, it is possible that hunger induces Lk release in the brain neurons and consequently responses to noxious heat are inhibited.

In this study, I found that hunger induces reduction of responses to noxious heat through Lk/Lkr signaling in *Drosophila*. This system may improve a possibility for flies to find available feeding site under blazing heat in summer.