(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	LI, Kai
Thesis Title	Belly roll: an Ly6 protein regulating nociceptive escape behaviors by modulating peptidergic interneuron excitability in *Drosophila melanogaster*		

(Thesis Summary)

Ensuring an appropriate response to threatening stimuli is essential for the survival of organisms. Nociception, the sensory mechanism that enables animals to perceive and avoid potentially harmful stimuli, is a critical component of this process. Although considerable research has been conducted on nociceptive circuitry, how genetic factors impact relevant escape responses is still largely unknown.

In this study, the applicant investigated the natural variation in nociceptive escape behavior among *Drosophila melanogaster* larvae and conducted a comprehensive genome-wide association analysis to identify genetic variations underlying the behaviors. Through this analysis, the applicant identified Belly roll (Bero), a member of the Ly6/α-neurotoxin family, as a potential regulator of nociceptive escape behavior in *Drosophila*. Using pan-neuronal knockdown screens and *bero* null mutant animal, the applicant discovered the inhibitory role of Bero in escape behavior. Further investigations revealed that Bero is expressed in multiple subgroups of peptidergic neurons, including abdominal leucokinin-producing neurons (ABLK neurons). Knockdown of *bero* in ABLK neurons resulted in an enhanced escape behavior, while overexpression of *bero* led to suppression of the behavior. Additionally, the applicant demonstrated that ABLK neurons respond to nociceptor activation, and optogenetic activation of ABLK neuron elicits bending behavior, which represents the initial phase within the overall sequence of escape behavior. Intriguingly, the knockdown of *bero* in ABLK neurons resulted in a reduction of persistent neuronal activity and an increase in evoked nociceptive responses. These results suggest that Bero in ABLK neurons acts as an inhibitory factor for neuronal activity.

Bero belongs to the Ly6/α-neurotoxin protein superfamily, encoding a diverse range of membrane-tethered and secreted polypeptides. Previous studies reported critical roles of cis-interactions between Ly6 family members and their target proteins. Ly6 proteins in mammals demonstrate diverse effects on the functional activity of distinct subtypes of nicotinic acetylcholine receptors (nAChRs), thereby effectively modulating neuronal excitability. In *Drosophila*, the Ly6-like protein known as Quiver /Sleepless exerts a dual effect on sleep regulation, as it not only diminishes cholinergic synaptic transmission through the antagonistic modulation of nAChRs but also diminishes neuronal excitability by amplifying the expression levels and open probability of Shaker potassium channels. Based on these previous findings, the applicant discussed how Bero interacts with its hypothetical target proteins in ABLK neurons and controls persistent neuronal activity and evoked nociceptive responses.

In summary, this study unveils the genetic regulation of nociceptive escape behavior in *Drosophila* larvae, highlighting the significance of ABLK neurons and the inhibitory influence of Bero. These findings contribute to further understanding of modulation of escape responses in *Drosophila melanogaster* larvae.

(Form 2)

(Thesis Evaluation Summary)

The applicant reported the genetic regulation of nociceptive escape behavior in *Drosophila* larvae, emphasizing the pivotal role of ABLK neurons and the inhibitory effect exerted by Bero. Since the Bero protein in ABLK neurons was shown to be essential for the regulation of escape behavior, future work should address how Bero controls the two types of activities of ABLK neurons: the persistent fluctuating activity and the evoked nociceptive response. The applicant proposed a hypothesis that *bero* expression in ABLK neurons promotes the generation of persistent fluctuating activities, which then inhibits evoked nociceptive responses in ABLK neurons, thereby downregulating the nociceptive escape behavior. In this scenario, it is likely that the elevated intracellular concentration of calcium ions inhibits the opening of voltage-gated cation channels, thereby suppressing nociceptive responses in ABLK neurons. This possibility can be tested and validated by inhibiting calcium-dependent inactivation (CDI) of the related channels in ABLK neurons. In addition, the identification of Bero target proteins in ABLK neurons is a key issue for future studies. Further studies using affinity purification mass spectrometry would identify the underlying protein-protein interactions in a high-throughput and unbiased manner. Another point of discussion was concerning the *trans*-interactions of Bero protein, as opposed to the hypothesized *cis*-interaction. It should be investigated in the future whether Bero protein interacts with cell surface proteins in neurons other than ABLK neurons and thereby influences escape behavior. The applicant also hypothesized that the persistent activities of ABLK neurons may reflect some internal stress and that ABLK neurons may serve as an integrating hub to modulate the nociceptive responses based on these internal inputs. It should be validated in the future whether the stressed animals show robust persistent activities in ABLK neurons and whether the stressed animals are less sensitive to the heat stimulus in the heat probe assay.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of neuroscience, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On July 4th, 2023, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published. (Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.) Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) :