Fellowship ID：BR240405

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独立行政法人日本学術振興会理事長　殿
To: President, Japan Society for the Promotion of Science

研究活動報告書
Research Report

１．受入研究者/ Host researcher

受入研究機関・部局・職 Name of Host Institution, Department and Title	東京大学・大学院理学系研究科・教授
受入研究者氏名 Host Researcher's Name	榎本 和生

２．外国人招へい研究者/ Fellow

所属研究機関・部局・職 Name of Institution, Department and Title	ワシントン大学・生物学科・教授
外国人招へい研究者氏名 Fellow's Name	PARRISH Jay Zachary

３．採用期間/ Fellowship Period

2024 年　10 月　14 日　～　2024 年　11 月　12 日

４．研究課題/ Research Theme

上皮細胞による侵害受容メカニズムの解明

５．研究活動報告/ Research Report

（1）研究活動の概要・成果/ Summary of Research Results

Work supported by this fellowship focused on defining sensory responses of epidermal cells and epidermal modulation of nociception. In a project initiated with JSPS support in 2018, we defined mechanically-evoked responses of epidermal cells in *Drosophila* larvae and human keratinocytes. In *Drosophila*, these epidermal responses drive a form of adaptive nociceptive sensitization, and we found that the molecular mediators of these epidermal mechanosensory responses are evolutionarily conserved. During the Bridge fellowship period, we revised a manuscript reporting these results, and the manuscript has since been published in eLife (Yoshino et al, 2025).

In a related line of studies supported by the Bridge Fellowship, we found that epidermal cells additionally mediate adaptive sensitization to heat. In response to noxious heat, Drosophila larvae exhibit stereotyped escape response that are initiated with nocifensive rolling. We found that exposure to prior heat sensitizes larvae to noxious thermal inputs, with the efficacy of the prior stimulus is limited by an upper threshold of < 45°C. Furthermore, we found that optogenetic stimulation of epidermal cells but not nociceptive sensory neurons induced this form of nociceptive sensitization, suggesting that epidermal responses to temperature are responsible for heat-induced nociceptive sensitization. To test this possibility directly, we characterized monitored responses to temperature change in larvae selectively expressing the

Ca^{2+} indicator GCaMP6s in epidermal cells. Indeed, we found that epidermal cells reproducibly exhibited two robust, distinctive temperature responses: responses to heat-off stimuli as well as responses to cooling from comfortable to noxious cold stimuli. These heat-off responses exhibited two notable features: first, heat-off responses required a heat threshold of ~39C prior to cooling, a temperature profile that matches thresholds for larval noxious heat-evoked behaviors. Second, heat-off calcium responses persisted for > 2 min after the temperature returned to baseline, matching the timescale of adaptive sensitization we observed in our behavioral studies. We further found that Drosophila epidermal cells are intrinisically responsive to heat-off stimuli, as acutely dissociated epidermal cells similarly responded to heat-off stimuli, and that these heat-off responses involve external calcium.

To define the molecular mediators of the epidermal heat-off responses we used a combination of pharmacological and genetic approaches. Calcium release-activated calcium channels (CRAC), which are comprised of the Ca^{2+}-selective cation channel Orai and its activator the transmembrane Ca^{2+} binding protein Stim, are required for heat-off responses in human and mouse keratinocytes. Additionally, Stim is activated by temperature paradigms comparable to those that evoke heat-off responses in *Drosophila* epidermal cells: warming beyond 37°C followed by cooling. We therefore investigated whether CRAC channels are required for the heat-off response in *Drosophila* epidermal cells. Indeed, we found that treatment with the chemical inhibitor 2-aminoethoxydiphenyl borate (2-APB) at a concentration that blocks Orai as well as several TRP channels attenuated the epidermal heat-off responses, as did epidermis-specific knockown of CRAC channel expression. Finally, we found that epidermis-specific knockown of CRAC channel expression blocked heat-induced nociceptive sensitization.

Our work defines several features of this sensitization and extends prior work defining epidermal thermosensory responses. First, the sensitization is short-lived; prior heat exposure primes larvae to future inputs that occur within seconds to minutes, potentiating escape from a harmful environment without providing indefinite sensitization to noxious inputs that would be maladaptive. Second, the sensitization is increasingly triggered by warm temperatures outside of the preferred range. Hence, as temperatures of prior stimuli approach noxious levels, so too does the state of vigilance they induce. Third, epidermal thermosensory responses that underlie the sensitization are heat-off responses, suggestive of a mechanism by which epidermal cells reinforce productive escape behavior. Prior studies demonstrated that CRAC channels mediate heat-off responses in epidermal cells and that epidermal CRAC channel inactivation affected thermal preference; our studies demonstrate that epidermal CRAC channels are required for epidermal heat-induced sensitization of nociceptors. Fourth, prior heat stimulus yields polymodal sensitization: responses to noxious thermal as well as mechanosensory inputs, suggesting that epidermal heat-evoked signals broadly sensitize nociceptors to future stimuli. The heat-evoked sensitization we describe here is remarkably similar to mechanically-evoked nociceptive sensitization seen in *Drosophila* larvae, which likewise emerges on a timescale of seconds and yields reversible, mechanical hypersensitivity to protect larvae from further insult. Heat-induced sensitization to noxious inputs has been reported in *Manduca* larvae as well, but epidermal contributions have not yet been explored. These forms of sensitization are distinct from pathological forms of nociceptive sensitization that emerge on a timescale of hours, and are long-lasting. A manuscript reporting these results is in preparation.

（2）主な研究発表（雑誌論文、学会、集会、知的財産権等）/ Main Research Publications

Yoshino J, Mali S, Williams CR, Morita T, Emerson C, Arp C, Miller S, Yin C, Motoyoshi M, Hemmi C, Ishii K, *Bautista D, *Emoto K & *Parrish JZ
Drosophila epidermal cells are intrinsically mechanosensitive and modulate nociceptive behavior outputs.
eLife 13: 95379 (2025).

Yoshino J, Chiu A, Morita T, Yin C, Tenedini FM, Sokabe T, Emoto K, and Parrish JZ. (2025) Heat-off responses of epidermal cells sensitize Drosophila larvae to noxious inputs. (in preparation)

（3）その他/ Remarks

（注）採用期間終了後 3 ヶ月以内に提出
※ (Note) Submit the form within 3 months after the expiration of fellowship.
※ 様式 1 に記載された情報を元に確認しますので、部局名等の名称含め、内容に誤りが無いか必ずご確認ください。