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研究課題名（和文）Genetic and circuit control of decision-making and action-planning in associative learning

研究課題名（英文）Genetic and circuit control of decision-making and action-planning in associative learning

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交付決定額（研究期間全体）：（直接経費）　　2,000,000円

研究成果の概要（和文）：動物は行動目標や経験に基づいて、様々な行動や環境の選択肢の中からどのように選択するのだろうか。等温追跡行動を維持できない変異体のスクリーニングから、Gタンパク質共役型受容体キナーゼ遺伝子のヌル変異を持つ変異体は、培養温度での等温追跡を維持できないことを発見した。 grk-1は神経系に広く発現している。その後の解析により、グルタミン酸作動性介在ニューロンがgrk-1シグナルを通じて等温性トラッキングの維持に部分的に関与していることが判明した。本研究は、動的環境下における目標達成のための精密な感覚運動計算に関する遺伝学的知見を提供するものである。

研究成果の学術的意義や社会的意義
We identify a GPCR kinase involved in the decision-making process to keep tracking along a preferred temperature with food experience. A similar mechanism may share with the reward system in the human brain; therefore, it could serve as a potential medical target for psychiatric disorders.

研究成果の概要（英文）：How do animals select among various behavioral and environmental alternatives based on behavioral goals and experiences? From a screen for mutants defective in maintaining isothermal tracking behavior, we found mutants carrying null mutations in the G-protein coupled receptor kinase gene failed to maintain isothermal tracks at the cultivation temperature. grk-1 expresses broadly in the nervous systems, including the thermosensory neurons AFD, AWC, the interneurons AIY, and RIA. However, simultaneously expressing grk-1 in AFD, AWC, AIY, and RIA was insufficient to restore the isothermal tracking defect in the grk-1 null mutant. Subsequent analyses identify a glutamatergic interneuron is partially involved in maintaining isothermal tracking through grk-1 signaling. Our study provides genetic insights into the precise sensorimotor computation of goal-driven behaviors in a dynamic environment.

研究分野：Neuroscience

キーワード：Decision-making　Associative learning　C. elegans　GPCR kinase　Neural circuit

１．研究開始当初の背景

How do animals select among various behavioral and environmental alternatives and plan sensory-motor coordination based on behavioral goals and experiences? In the quest to uncover genetic and circuit regulation of the decision-making process in learning behaviors, we used a simple decision-making model in an organism with both genome and connectome fully revealed: *C. elegans* isothermal tracking behavior. On a temperature gradient under its physiological range, the nematode *C. elegans* can track isothermally at the temperatures associated with their cultivation experiences as a result of active coordination between sensory inputs and motor outputs. Previously, we have identified key circuit neurons involved in the isothermal tracking behavior (Ikeda et al., 2020; Mori & Ohshima, 1995). Part of the circuit neurons includes the thermosensory neurons AFD, AWC, and the interneurons AIY and RIA. **However, the genetic and circuit mechanisms of the decision-making and sensorimotor computation to maintain isothermal tracking precisely at the cultivation temperature remain unexplored.**

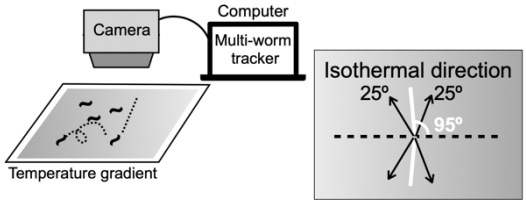
２．研究の目的

This research aims to elucidate the genetic and circuit control of *C. elegans* isothermal tracking behavior (IT behavior). Specifically, we focus on the decision-making and sensorimotor integration processes required for executing IT behavior at the cultivation temperature instead of other behaviors or temperatures on a given temperature gradient. To precisely keep tracking the cultivation temperature on a temperature gradient, animals need to process the time series of concomitant thermal stimuli while moving to accurately revise their tracking direction toward the right temperature with a behavioral goal of searching for food. The genetic and neural computation behind the behavioral regulation at the cultivation temperature is an elegant model of efficient information processing from setting behavioral goals and making decisions to actions in a dynamic environment such as a temperature gradient.

３．研究の方法

Analysis of isothermal tracking behavior

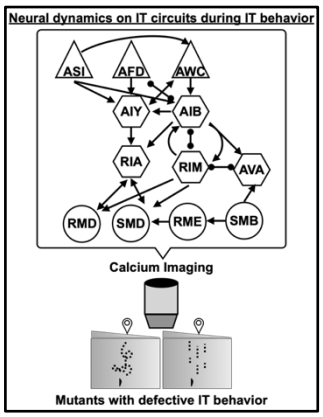
C. elegans isothermal tracking behavior represents a precise sensory-motor coordination of animals as in tracking specifically at a preferred temperature with food association. To quantitatively analyze the temporal transitions of animals' behavioral strategies to initiate, maintain and terminate IT behaviors, we employed a worm-tracking system, the Multi-Worm Tracker (MWT), to simultaneously record and analyze a population of up to 150 worms freely navigating on a temperature gradient (Ikeda et al., 2020 Swierczek et al., 2011). Under this setup, we define isothermal tracking as when animals keep tracking along the same temperature (IT direction) for more than 20 seconds.



$$\text{Fraction of IT} = \frac{\text{Total Isothermal tracking time (sec)}}{\text{Total forward running time (sec)}} \times 100\%$$

Imaging of neural circuit dynamics

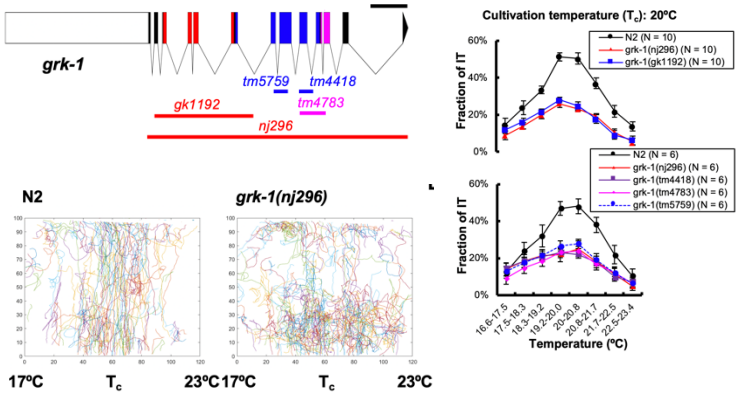
To understand circuit encoding of isothermal tracking behavior, we express a FRET-based calcium indicator Yellow Cameleon in specific neurons to analyze temperature-evoked neural activation under different temperature programs. We express the calcium indicator specifically in some of the key IT circuit neurons and analyze the neural activity in some mutants to get insight into how the mutations contribute to the temperature-evoked circuit dynamics.



４．研究成果

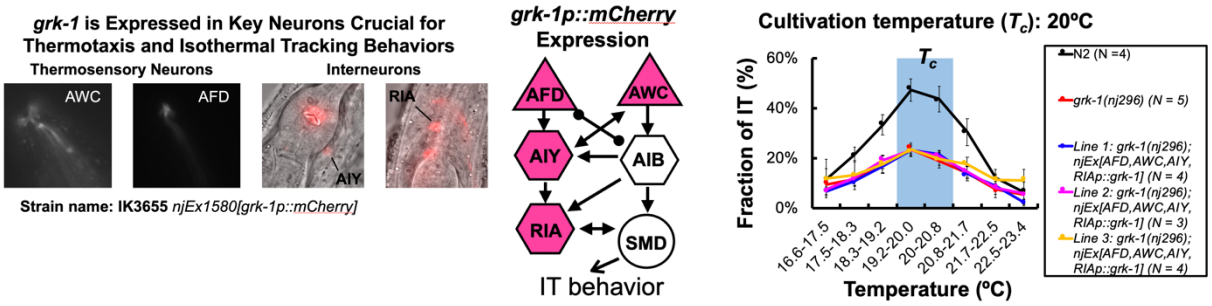
A GPCR kinase gene controls *C. elegans* isothermal tracking behavior

We selected candidate genes that might specify information-processing but not sensation or locomotion based on the gene expression profile in the IT circuit neurons. Among the 28 candidate mutants tested, we identified a null mutation in gene locus encoding a human GPCR kinase homolog that contributes to the decision-making process involved in maintaining IT behavior.



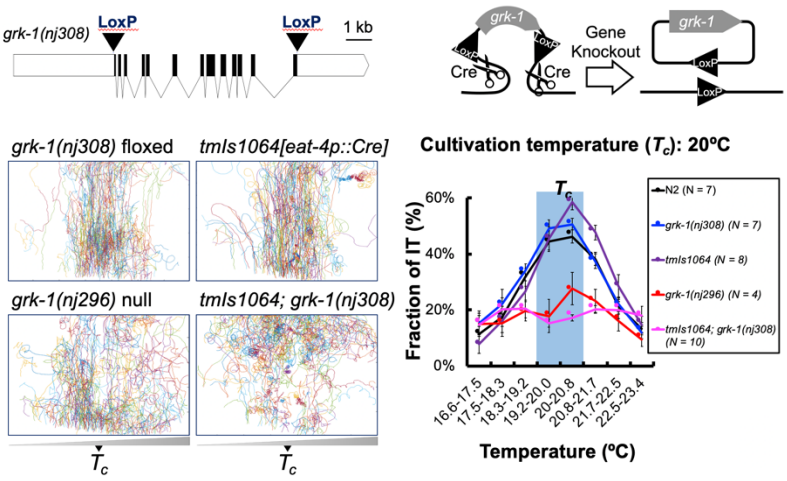
Expression analysis showed expression in major neural circuits of IT behavior

Expressing a red fluorescent protein by a 3.8-kb promoter region upstream of the gene start codon, we identified reporter expression in many neurons in the IT circuit. However, re-introducing the GPCR-encoding DNA specifically in the thermosensory neurons AFD, AWC, and interneurons AIY and RIA failed to restore the mutant defect in maintaining IT behavior, suggesting the involvement of other neurons in the AFD-AWC-AIT-RIA circuit or the site-of-action lies in a different set of neurons.



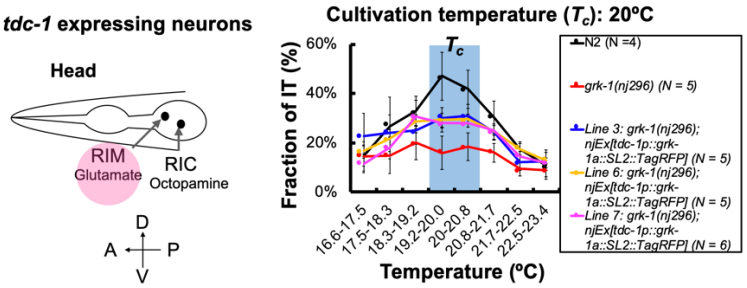
The GRK-1/GPCR kinase is required in glutamatergic neurons to maintain IT behaviors

Using a Cre-recombination system to knock down the GPCR kinase gene specifically in the glutamatergic neurons phenocopied the GPCR kinase null mutant IT phenotype. This suggests that GRK-1/GPCR kinase is required in the glutamatergic neurons to maintain IT behavior.



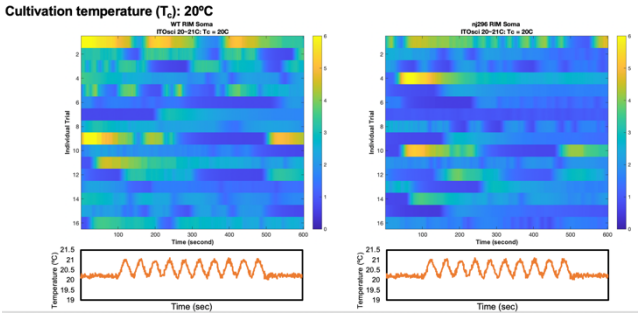
Expressing GRK-1 in a glutamatergic neuron partially rescued the IT defect of the null mutant

To identify the site-of-action of *grk-1* in maintaining IT behavior, we used a series of promoters targeting different subsets of neurons and examined their behaviors under the Multi-worm Tracker. We tested 20 different promoters in our initial rescue experiment and narrowed down the expression in a glutamatergic interneuron RIM showed a partial rescue effect in promoting IT behavior. Our mutant analysis identifies an uncharacterized regulatory role of RIM in maintaining *C. elegans* IT behavior through *grk-1* signaling.



Neural activity of RIM showed comparable responses between the wild-type and *grk-1* mutant

How may *grk-1* affect temperature-evoked neural activity in the RIM interneuron? To address this question, we recorded the calcium dynamics in RIM under different temperature programs. To better capture the temperature dynamics during IT behavior, we stimulated the interneuron RIM with a sinusoidal temperature program oscillating near the cultivation temperature (20°C). Under the conditions tested, we did not find compelling differences in the evoked neural activation of RIM in the wild type versus the *grk-1* mutant. This result suggests that *grk-1* may function downstream of the calcium dynamics in the interneuron RIM to maintain IT behavior.



５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Tzu-Ting Huang*, Shunji Nakano and Ikue Mori
２．発表標題 Genetic Mechanisms of Isothermal Tracking Behavior in Caenorhabditis elegans
３．学会等名 23rd International C. elegans conference（国際学会）
４．発表年 2021年

１．発表者名 Tzu-Ting Huang
２．発表標題 Genetic mechanisms of Caenorhabditis elegans isothermal tracking behavior
３．学会等名 線虫研究の未来を創る会2021
４．発表年 2021年

１．発表者名 Tzu-Ting Huang*, Shunji Nakano and Ikue Mori
２．発表標題 Genetic mechanisms of Caenorhabditis elegans isothermal tracking behavior
３．学会等名 Neuro2022（国際学会）
４．発表年 2022年

１．発表者名 Tzu-Ting Huang
２．発表標題 Genetic and circuit mechanisms of C. elegans isothermal tracking behavior
３．学会等名 線虫研究の未来を創る会2022
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計1件

国際研究集会	開催年
The 64th Annual Drosophila Research Conference	2023年～2023年

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関