論文の内容の要旨

Neural circuitry underlying backward escape behavior upon noxious light irradiation in *Drosophila* larvae

（ショウジョウバエ幼虫の後退行動を規定する神経基盤）

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Animals typically avoid dangerous situations with stereotyped escape behavior. For instance, *Drosophila* larvae perceive strong blue light as noxious and respond with stereotyped escape behavior consisting of rapid forward locomotion, head-casting, rolling, and backward locomotion. Two sensory systems mediate this larval light-induced escape behavior, the photoreceptor-containing Bolwig organ (BO), which is located on the head and the class IV dendritic arborization (C4da) neurons that tile the body wall (Fig 1). Either one of these sensory systems can evoke escape behavior, yet how the two systems are at the neural circuit level remains elusive. Thus, I asked how the two distinct sensory pathways are integrated into a neural circuitry and evoke appropriate escape behavior.

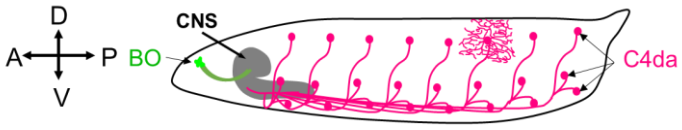

Fig1. Localization of BO and C4da which receive nociceptive light irradiation. AP and DV arrows indicate the anterior-posterior and the dorso-ventral axis, respectively.

First, to identify neurons that evoke a particular type of escape behavior, I conducted an optogenetic behavior screen. As a result, I identified two distinct sets of neurons that evoke robust backward locomotion, which is one type of escape behavior, upon optogenetic activation. The first set is reported as Moonwalker Descending Neurons (MDNs), whose activation induce backward locomotion (Bidaye et al. 2014; Carreira-Rosario et al. 2018). The other set of neurons

evoked similar behavior compared with MDN activation, yet it was composed of a different subset of novel neurons. I have named this subset of neurons as Ascending Moonwalker-like Backward neurons (AMBs).

To further characterize MDNs and AMBs, I analyzed the functional and morphological characteristics of each neuron. In order to identify the neurotransmitters, I immunostained transmitter markers and found that both neurons are choline acetyltransferase (ChAT) positive, which suggests cholinergic neurons. Next, to analyze the morphological characteristics, I expressed the dendritic and presynaptic markers in both types of neurons and found that MDNs are composed of two pairs of descending neurons whereas AMBs are consisting of one pair of ascending neurons (Fig 2).

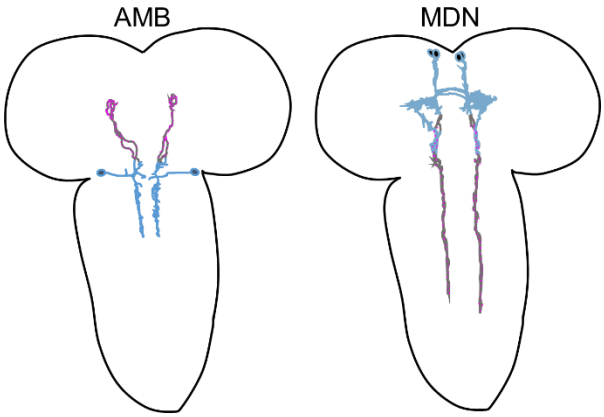

Fig2. Schematic picture of AMBs and MDNs. Light blue indicates dendritic marker, and magenta indicates presynaptic terminal marker.

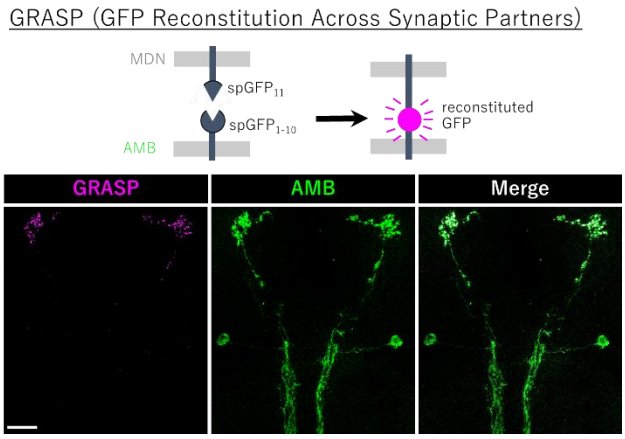

Fig 3. MDNs and AMBs connect directly. Green indicates membrane of AMBs, magenta indicates GRASP signals. Scale bar: 20μm.

Given that activation of either AMBs or MDNs triggers similar backward locomotion, I hypothesized that AMBs and MDNs might function in the same neuronal circuits. To test this possibility, I analyzed AMBs and MDNs morphologically and functionally. First, I dual-labeled AMBs and MDNs by utilizing two different binary systems: the GAL4/UAS system and the LexA/LexAop system. As a result, I found AMB axons were closely apposed to MDN dendrites, implying that AMBs are presynaptic to MDNs. To further analyze the synaptic connection, I utilized GFP Reconstitution Across Synaptic Partners (GRASP) analysis between AMBs and MDNs. As a result, I found GRASP signals locate to the presynaptic terminal of AMBs (Fig 3). In the next step, to investigate the functional connection between AMBs and MDNs, I performed calcium (Ca^{2+}) imaging of MDNs upon AMB activation. As a result, I found that optogenetic activation of AMBs significantly increased

calcium signal in MDNs (Fig 4). Finally, I asked whether MDNs might function downstream of AMBs. I optogenetically activated AMBs and observed whether MDN silencing by tetanus toxin (TNT) might affect the number of backward waves. As a result, I found that MDN silencing reduced the backward waves induced by activation of AMBs significantly (Fig 5). Taken together with my morphological and functional analyses, I conclude that AMBs function presynaptically to MDNs to evoke backward locomotion.

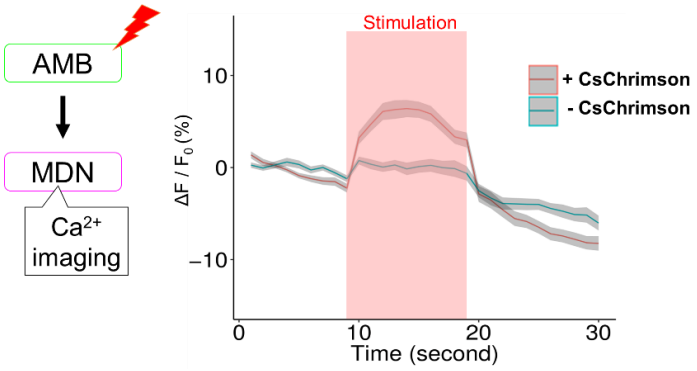

Fig 4. Optogenetic activation of AMBs increase calcium signal in MDNs.

Next, I asked which sensory pathway might contribute to the AMB-MDN pathway using optogenetic behavior assay. To this end, I activated BO or C4da neurons to induce backward locomotion while silencing AMBs or MDNs by TNT. As a result, MDN silencing inhibited backward waves induced by BO and C4da neurons, whereas AMB silencing inhibited backward waves induced by C4da neurons but not BO (Fig 6).

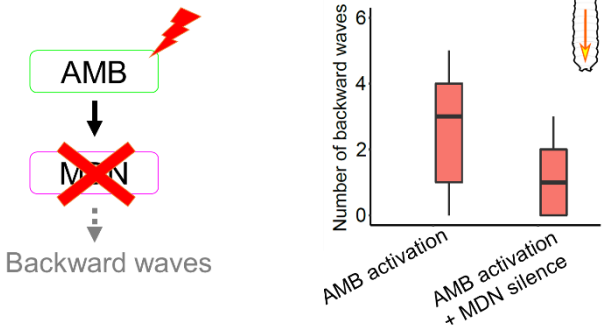

Fig 5. Backward waves induced by activation of AMBs are inhibited by silencing MDNs. I assessed statistical significance by Wilcox rank sum test. * $p < 0.05$

Finally, to confirm the functional connection between AMBs and the sensory organs, I asked whether activation of either BO or C4da neurons could trigger Ca^{2+} responses in AMBs. I expressed the calcium indicator GCaMP6m in AMBs and activated sensory neurons by CsChrimson. As a result, Optogenetic activation of C4da neurons, but not BO, resulted in a significant increase of GCaMP6m fluorescence intensity in AMBs. This result further supports the idea that AMBs mediate noxious signals from C4da neurons, but not from BO, to evoke backward locomotion. Taken together with these data, I concluded that the AMB-MDN pathway mediates noxious signals from C4da sensory neurons to evoke backward locomotion.

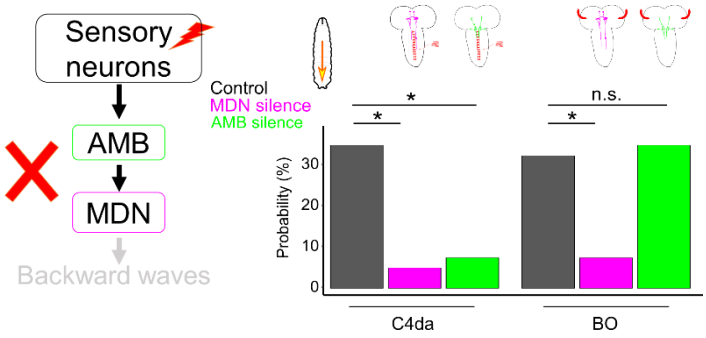

Fig 6. C4da-induced backward waves require AMB-MDN pathway while BO-induced backward waves require only MDNs, but not AMBs. I assessed statistical significance by Fisher's exact test followed by Holm method. * $p<0.05$. n.s. not significant.

In summary, my study revealed a local neural circuit that mediates backward locomotion induced by noxious light irradiation. My data indicate that AMBs, newly identified backward neurons in this study, are direct presynaptic partners of MDNs, command-like neurons for backward locomotion induced by noxious light. AMBs are essential for C4da-induced backward waves, but not required for BO-induced backward waves. Given the three points below (1) AMBs are direct presynaptic partners of MDNs; (2) AMBs do not receive BO signals; (3) MDNs are required for backward locomotion induced by noxious light, which is mediated by BO and C4da neurons redundantly, I thus propose that MDNs would work as convergence points for distinct sensory inputs to integrate signals in order to trigger backward locomotion. It would explain that the neural mechanism of the additive effect of redundant sensory pathways to induce escape behavior. My finding could further contribute to the elucidation of the circuit-based mechanism of how multiple sensory inputs are integrated to regulate specific behavior.