

博士論文（要約）

Development of Coordinated Neural Activities in the Motor

Circuits of *Drosophila* Larvae:

the Role of Sensory Feedback and Gap Junctions

（シヨウジョウバエ幼虫の運動回路における協調的活動の発

生：

感覚フィードバックとGAP結合の役割について）

47-107024

Tappei Kawasaki

川崎達平

東京大学大学院 新領域創成科学研究科

複雑理工学専攻 能瀬研究室

Abstract

In this study, I used the peristaltic locomotion of *Drosophila* embryos as a model to study the mechanism of how coordinated neural activities emerge during the development of the nervous system. Peristaltic locomotion in *Drosophila* embryos is achieved by propagation of muscle contractions from anterior to posterior or posterior to anterior of the body. The muscle movements are in turn generated by sequential activation of motor neurons in the corresponding neuromeres (segmental units of the central nervous system). Previous studies examined the development of the motor circuits in *Drosophila* larvae indirectly by observing the development of muscle activity. However, since these studies observed a global movement of muscles using muscle contraction as a measure, development of more local activities (such as activities in a single muscle or a small group of muscles) remained unknown. More importantly, activity of neurons that generates the muscle movement had not been studied. In this study, I first used calcium imaging of muscles to examine the activity of individual muscles during development and found local activity of muscles that were unnoticed in the previous studies. I then performed calcium imaging of central neurons and revealed for the first time the emergence of neuronal activity that generates larval locomotion during embryonic development. Finally, I show essential roles of gap junctions in the embryonic central circuits that autonomously (without the aid of sensory feedback) generate motor waves.

The requirement of gap junctions is transient since gap junctions are no longer required in the 3rd instar larvae. My results suggest that there are two independent and complementary circuits in the embryos that generate motor waves, one involving GJs and the other mediated by sensory feedback.

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Introduction

Mechanism of Generation of Patterned Movements

Most animals possess the ability to move. Aristotle defined and classified animals as beings that have nutritive power, self-motion and sense-perception in *De Anima II 3*. Most animals other than *Porifera* (such as sponges) and *Pracozoa* (flat animals) have the nervous system [1]. The nervous system of animals realizes self-motion and self-perception. Sensory neurons (SNs) input exterior information to interneurons (INs), INs then process information and output the appropriate activities to muscles through motor neurons (MNs) (Figure 1).

In general, a neuron has the soma, the dendrite, and the axon. The soma is spherical and contains the cell nucleus. The dendrite and the axon are fibrous; synaptic signals from other neurons are received in the dendrites and synaptic signals to other neurons are sent via the axons. Neurons are connected to each other and form complex neural circuits (Figure 2A). Synaptic signals between neurons are transmitted at the structure called the synapse. There are two types of synapses: the chemical synapse and the electrical synapse. The chemical synapse transmits synaptic signals with chemical substances called neurotransmitters. When an action potential reaches the presynaptic axon terminal, presynaptic neuron releases neurotransmitters into the synaptic cleft. Neurotransmitters activate their receptors at the post synaptic dendrite and induce various neuronal responses. The electrical synapse transmits synaptic signals via direct cell-to-cell ionic

transfer. The electrical synapse is formed with the gap junctions (GJs), which are the complex of cell adhesion molecules (Figure 2B).

Many animal movements are composition of various rhythmic patterned activities in muscles. The rhythmic pattern in muscles is the outputs of circuits in the nervous systems called CPGs (central pattern generators). CPGs are neural circuits that can generate rhythmic motor patterns in the absence of sensory feedback (SF) or descending inputs from the brain that carry specific timing information [2, 3] (Figure 3A). Many stereotyped motor outputs have been shown to be controlled by the CPGs, such as walking, breathing, and feeding [4] (Figure 3B).

Interaction between Sensory Feedback and CPGs

Although CPGs can generate rhythmic patterned activities by itself, animals should adapt to the changes in the environment to generate motor outputs appropriate for the circumstances. For this purpose, the pattern of CPGs is often modified by the inputs from SNs [5] (Figure 4A). Sensory inputs have been shown to modify the motor pattern generated by the CPGs during the flight of the *Acridida* [6] [7], swim of the *Hirudinea* [8], and locomotion of *Drosophila* [9] (Figure 4B).

Development of the Nervous Systems

How is the complex neural network constructed during development? Early work suggested that generation of neural networks can be divided into two phases: early wiring

that depends on the genetic blueprint and later rewiring that depends on the activity of neurons. Concerning the later phase of development, there exist two types of sources for the activity of neurons as describe below [10] (Figure 5).

1) Sensory inputs

Sensory inputs from the environment or those from the body (namely, SF) play important roles in the development of the nervous systems [11]. A famous example of the role of sensory inputs during the development of neural systems is the critical period in the visual system. Refinement of visual systems depends on sensory inputs from the exterior world [12]. Similarly, there is evidence that SF plays critical roles during the development of motor systems. For example, SFs modulate the development of *Drosophila* motor circuits that generate peristaltic locomotion [13]. Indeed, increase of frequency of firing in SNs during the development of motor systems brings forward the onset of coordinated activities in muscles [14] (Figure 4B). In contract, inhibition of SNs during the development of motor systems delays the onset of coordinated activities in muscles [15], and decreases the speed of larval locomotion at 2nd and 3rd instar larvae [16]

2) Spontaneous activities in the CNS

Spontaneous activities of neurons are also reported as the source of activity-dependent refinement of neural circuits. In particular, spontaneously occurring wave-like activities that propagate among a population of neurons have been reported in many sensory

systems and other brain regions including the retina [17] [18] [19] [20], cochlea [21] [22] [23], hippocampus [24] [25] and cerebellum [26] . Also in motor systems, similar sequences are observed in the spinal cord of animals such as zebrafish [27] and chick [28]. Wave-like activity seen in these different brain regions is known to develop in a similar sequence. First, spontaneous and sporadic activities emerge in some population of cells. These sporadic activities are then integrated and correlated with others gradually, and finally develop into orchestrated activities that propagate along a wide region in the CNS (Figure 6). Inhibition of the spontaneous activity during the transition from sporadic to patterned neuronal activity was reported to disturb the emergence of patterned activities in zebrafish [27]. Spontaneous neural activities during development are also reported to have function in homeostatic regulation of neural activities [29] [30]. Spontaneous neural activities regulates not only the function of neural circuit but also that of single neurons. For example, spontaneous neural activities were reported to regulate the synaptic strength in the embryonic spinal cord [31]. Thus, spontaneous activities play important roles in development of neural circuits [32]. [33].

Gap Junctions: Roles in Developing Nervous Systems

GJs are intercellular channels in animal cells that mediate direct cell-to-cell transfer of ions and small molecules. They are formed by docking of two hemichannels that are composed of hexamers of cell adhesion molecules belonging to a family of integral

membrane proteins: connexins in vertebrates and innexins in invertebrates [34]. GJs have been known to mediate the spontaneous wave-like activity in many of the systems described above [35], including the retina [36], [37], hippocampus [38], and spinal cord [27] [39] [40]. GJs-coupled networks are involved not only in wavelike activities which spread isotropically but also in rhythmic patterned neural activities [41]. These GJs-coupled networks are often transiently created at the early stage of development before the chemical synapses are formed [42]. For example, electrical coupling of lumbar MNs mediated by GJs decreases to less than half during the maturation of the developing spinal cord [43] , retina [44] [45] or cerebral cortex [46] [47] Although electrical synapses are completely replaced with the chemical synapses in some systems, they remain into later stages of development and sometimes perform different functions in other systems [48]. Thus, GJs-coupled network coordinate the neural activities especially in the early stage of development.

***Drosophila* as a model organism**

I used *Drosophila* embryos and larvae to study the development of the motor circuits. The *Drosophila* larva is an ideal model to study the mechanism of motor systems from the following reasons. First, its behavior is stereotypic and easy to quantify [49]. Second, highly sophisticated genetic tools can be used to visualize and manipulate specific component neurons in the system [50]. Third, its short life cycle (~10 days) allows

efficient genetic crosses and developmental analyses. I used these excellent features of the system to try to study the molecular and cellular mechanisms underlying the emergence of coordinated neural activities in developing neural circuits.

The Motor Systems of *Drosophila*

Drosophila larvae display several types of behaviors such as crawling, turning, bending, and rolling [51] (Figure 7A). The forward peristalsis is the most dominant behavior in *Drosophila* embryos and larvae, and is realized by the sequential muscle contraction from the posterior segment to anterior segment of the body (Figure 7B) [52]. During the backward peristalsis, which is induced when the larva receives noxious stimuli in the head, the sequential muscle contraction occurs in the opposite direction: from the anterior to posterior. These sequential muscle movements are generated by propagating activities of MNs along the segments in the CNS, called the neuromeres. The CNS of *Drosophila* is composed of the brain and ventral nerve cord (VNC) (Figure 8A). The VNC is an equivalent of the spinal cord of vertebrates and consists of three thoracic and eight abdominal neuromeres (T1 – T3, A1 – A8). Each neuromere innervates the muscles of the corresponding body segment (Figure 7C, Figure 8B). The VNC includes MNs that innervate muscles and generate various movements and INs that receive inputs from the brain, INs in the same or other segment and SNs, and process the received information.

The VNC also receives inputs from the SNs that carry sensory information from the corresponding body-wall segment.

Recent studies in this and other laboratories have identified INs that regulate larval peristaltic locomotion. These include excitatory and cholinergic INs that mediate the segmental propagation of motor activity during forward locomotion (A27h) [53] or are necessary for local muscle contraction (CLI1 and CLI2) [54], inhibitory and GABAergic INs that regulate the segmental propagation of motor activity during both forward and backward locomotion (GDL) [53], and inhibitory and glutamatergic premotor INs that regulate the speed of peristalsis (PMSIs) [55] or are implicated in the termination of motor activity during the late phase of motor cycle (GVLIs) [56]. Mathematical models of larval locomotion have also been constructed based on the roles of these INs. Crawling of *Drosophila* larvae were simulated in a virtual system, enabling one to make predictions about the effects of perturbing specific component neurons [57].

Development of the Motor Systems of *Drosophila* larvae

The motor system of *Drosophila* embryos and larvae is also an ideal model for the study of development of the neuromuscular systems. Development of motor activities of *Drosophila* embryo has previously been studied by observing muscles contraction [58, 59, 13, 60, 15, 14]. These previous studies revealed how locomotory movements emerge sequentially during embryonic development as follows. First, local muscle contractions

appear at 14h After Eggs Laying (AEL). These initial contractions are myogenic and do not require neural activity. Such myogenic movements are also reported in the embryo of a shark [61]. Then neurally-induced muscle contractions appear at 17h AEL. These activities are initially uncoordinated and span only a few segments. However, they gradually become coordinated and matured into the wavelike activities that propagate the length of the embryos at 18h AEL [15] (Figure 9).

Previous studies also showed that spontaneous activities in the CNS and SFs are necessary for the maturation of neural circuits. When the patterned neural activities in CNS were interfered during a late embryonic stage, maturation of CNS was interfered [14]. It was also reported that inhibition [16] or excitation [14] of SNs influenced the coordination of motor activities.

Outline of this Research

As described above, previous studies examined the development of the motor circuits in *Drosophila* larvae indirectly by observing the development of muscle activity. However, since these studies observed a global movement of muscles using muscle contraction as a measure, development of more local activities (such as activities in a single muscle or a small group of muscles) remained unknown. More importantly, activity of neurons that generates the muscle movement had not been studied. In this study, I first used calcium imaging of muscles to examine the activity of individual muscles during

development and found local activities of muscles that were unnoticed in the previous study. I then performed calcium imaging of central neurons and revealed for the first time the emergence of neuronal activity that generates larval locomotion during embryonic development. Finally, I show essential roles of GJs in the embryonic central circuits that autonomously (without the aid of SF) generate motor waves. The requirement of GJs is transient since GJs are no longer required in the 3rd instar larvae. My results suggest that there are two independent and complementary circuits in the embryos that generate motor waves, one involving GJs and the other mediated by sensory feedback. Based on these results, I discuss roles of GJs and sensory feedback during motor circuit development.

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Figures

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