Genetic Studies on Hypothalamus Functions in Zebrafish

Ailani, Deepak

Doctor of Philosophy

Department of Genetics
School of Life Science
SOKENDAI (The Graduate University for Advanced Studies)

The ability to move is crucial for the survival of an animal. Although there are many forms of locomotion such as walking, running, flying and swimming, all these forms employ a rhythmic and alternating motor activity that allows an animal to move from one place to another. Basic tasks such as food-seeking or escape from predators require precise modulation of locomotor activity. In vertebrates, a brain region called the hypothalamus has been shown to play an important role in regulation of locomotor activity. A study in decorticate cats showed that electrical activation of the hypothalamus evoked locomotor activity proportional to the strength of the activation, whereas ablation of the hypothalamus abolished all spontaneous locomotion. A study in freely moving rats, which used movable electrodes to electrically stimulate various hypothalamic sites, showed that activation of the lateral hypothalamus induced exploratory locomotion, while activation of the medial hypothalamus induced escape jumps. A study in anesthetized rats showed that electrical and chemical activations of the perifornical and lateral hypothalamus evoked locomotor stepping. Although these studies demonstrated the involvement of the hypothalamus in regulating locomotion, the functional circuits and cell populations mediating locomotor activity were still unclear.

Zebrafish is a freshwater teleost fish and has been widely used as a model system to study the development and function of brain circuits. As a vertebrate, zebrafish has a brain structure similar to mammals. Several reports have suggested a homology between the mammalian and the fish hypothalamus. Functional homologs of the hypothalamic nuclei that mediate neuroendocrine response in mammals have been identified in zebrafish. Zebrafish also offers several powerful genetic tools such as the Gal4-UAS system. Our lab has developed *Tol2* transposon-mediated gene trap and enhancer trap methods and we have generated transgenic fish lines that express Gal4 in specific tissues and organs, including specific neural populations in the brain. Also the Gal4-UAS system can be used to study the

function of Gal4 expressing neurons by inhibiting their activity using genetically encoded neurotoxins such as tetanus toxin and botulinum toxin. Furthermore, transparency of zebrafish at embryonic stages make them ideal for visualizing brain activities in live and behaving animals. Using genetically encoded calcium indicators, the Gal4-UAS system can be used to monitor the neural activity of Gal4 expressing neurons in intact and behaving zebrafish larvae.

In this study, I aimed to investigate hypothalamic neuronal circuits and cell populations mediating locomotor activity using the model vertebrate zebrafish. I performed a genetic screen using Tol2 transposon mediated gene trap and enhancer trap methods and generated transgenic fish that expressed Gal4 in specific brain regions in larval zebrafish. Then, I isolated a transgenic line named 116A that labeled a subpopulation of neurons in the caudal hypothalamus (cH), the intermediate hypothalamus (iH) and the paraventricular organ (PVO), and another line named 1121A that labeled a subpopulation of neurons in the cH, iH, PVO and the telencephalon. I performed calcium imaging using 116A-gal4;UAS:GCaMP6s and 1121A-gal4;UAS:GCaMP6s double transgenic fish, and found that the hypothalamic neurons labeled in these lines were activated when larval zebrafish performed spontaneous tail-flips. I hypothesized that those neuronal populations may regulate locomotor activity.

To test this hypothesis, I analyzed the activity of the neurons labeled in 116A in freely swimming larvae after transfer to an imaging chamber. The locomotor activity of the larvae decreased gradually in the chamber, due to a process called habituation. I found that the activity of 116A-neurons also decreased during the habituation. To further investigate their functions, I inhibited the neural activities by crossing 116A fish with the UAS-effector fish carrying the botulinum neurotoxin gene. The double transgenic fish showed reduced locomotor activity, and specifically reduced swimming bout frequency. This indicated that

the genetically tagged cH, iH, and PVO cells in the hypothalamus play a crucial role in regulating locomotor activity in zebrafish larvae.

To further explore the function of hypothalamic neurons in locomotion, I analyzed the activity of neurons at single-cell resolution by using the 1121A line. I set up a system for optomotor response (OMR) in which swimming behaviors are evoked in head restrained larvae upon onset of visual stimuli, namely moving black-and-red gratings, under a spinning-disk confocal microscope. I found that the cH and iH neurons in the 1121A line were activated synchronously upon onset of the OMR stimuli. Furthermore, when the speed of moving stimulus was increased, the locomotor activity of the head restrained larvae and the activity of those cH and iH neurons also increased accordingly. I also showed that the activities of the telencephalic 1121A-neurons increased upon onset of the moving visual stimuli and some of these activities were correlated with those observed in the hypothalamus, suggesting a functional connection between these two areas.

To characterize the neuronal types in 116A and 1121A lines, I performed immunohistochemistry. I found that the neuronal population labelled by 116A was mostly serotonergic. Analysis of neuronal population labelled in 1121A showed that it included most of the serotonergic neurons in cH, iH and PVO and several non-serotonergic cells as well. Serotonergic neurons have been found in the hypothalamus of several non-placental vertebrate species, including zebrafish. In the placental mammals however, serotonergic neurons are absent from the hypothalamus and are found exclusively in the raphe nuclei, indicating that the mammalian serotonergic system is an exception.

Previous studies that employed c-fos and pERK staining to detect neuronal activation reported that aversive stimuli activated the caudal hypothalamic area in larval zebrafish. To determine if the hypothalamic neurons labeled in the 116A and 1121A lines are involved in this activity, I applied aversive stimuli, such as mustard oil and heat, to the transgenic larvae,

and analyzed them by calcium imaging. I found that the neurons labeled in 116A and 1121A lines were strongly activated by exposure to these aversive stimuli, suggesting that these neurons may also be involved in aversive responses.

Thus, my present study provided clues to understanding functions of a genetically identified neuronal population located in the caudal hypothalamus, the intermediate hypothalamus and the paraventricular organ of the larval zebrafish.