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RESEARCH HIGHLIGHT

Exploring Neural Substrates Underlying the Execution of Behavior Across the Whole Brain

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Among various types of behaviors, locomotion is the foundation for exploration, navigation, foraging, and approaching. Proper locomotion enables animals to move from one place to another *via* a sequence of actions and is essential for their survival. An excess, paucity, or imbalance of movements is considered to be a movement disorder which can impair the quality of or threaten survival [1]. Movement disorders that appear in neurodegenerative diseases are becoming an increasing menace to human health [2]. Studying the neural mechanism underlying each action during locomotion is the key to understand and explore therapy for such disorders.

Each action during locomotion is tightly controlled by neural substrates in the brain. In mammals, the basal ganglia are crucial for controlling voluntary movements and their dysfunction has been implicated in neurodegenerative diseases in which movement disorder is the main sign, like Parkinson's disease [3]. In the basal ganglia, the direct and indirect pathways, which consist of D1 and D2 dopamine receptor-expressing medial spiny neurons and distinct downstream nuclei, promote and inhibit initiation of movements, respectively [3]. In the lamprey, the locomotor regions in both the mesencephalon and the diencephalon have been reported to coordinate movements [4, 5]. These studies have uncovered the tip of the iceberg of how locomotion is executed or controlled in the brain. However, in most cases even a single action recruits many brain areas, let alone locomotion that involves sequential actions. Moreover, given that the correlation between neural activity and spontaneous action during locomotion is difficult to study, compared to cue-triggered and trial-based behaviors it is even harder to identify the neural substrates of spontaneous behavior.

To approach an in-depth understanding of the execution and control of locomotion in the brain, the first step is to identify neural substrates whose activities correlate with motor behavior across whole brain. After identifying these substrates, recording and perturbation of their activities may reveal how they function in the behavior. Immediate-early genes (e.g. c-fos and arc) have been widely used as a tool to facilitate neuronal activity mapping on the brain-wide scale at cellular resolution [6], but mapping such activity during behavior by real-time *in vivo* rather than post-hoc *in situ* assessment is being developed. Functional MRI (fMRI) allows monitoring whole-brain activities during behavior execution and provides evidence of correlations between neural activities in specific brain areas and specific behavioral tasks [7], but the causal link is hard to dissect using fMRI. Moreover, the spatiotemporal resolution of fMRI is relatively low, preventing the further exploration of synaptic and circuit mechanisms. The development of optogenetic methods has provided a brand-new opportunity for manipulating the activity of neurons at single-neuron resolution [8]. Unfortunately, such methods have not yet been applied to non-human primates and humans due to technical limitations. On the other hand, although optogenetic methods have been used in rodents, zebrafish, *Drosophila*, and *Caenorhabditis elegans*, it is still hard to characterize the neural mechanisms of locomotion across the whole brain due to technical challenges for monitoring neuronal activities across the whole brain during the execution of behavior.

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In a recent study [9], using larval zebrafish as a model, Dunn and Mu *et al*. have dissected the neural substrates of turning behavior (Fig. 1A) during locomotion in the absence of sensory cues across the whole brain by developing a fictive behavioral paradigm which simulates free-swimming of zebrafish larvae. In this paradigm, fish were paralyzed or weakly-paralyzed and two electrodes were located near peripheral motor nerves on the left and right sides (Fig. 1B). Fictive actions were then decoded from the recorded electrophysiological signals. After verifying the decoding algorithm in weakly-paralyzed fish in which decoded fictive directions accurately matched residual tail movements, Dunn and Mu *et al*. found similar behavioral sequences in fictively-behaving and freely-swimming zebrafish larvae. The turning direction of spontaneous swimming is not random but has a biased spatiotemporal pattern (Fig. 1A). Fish prefer to turn in the same direction for $\sim$6 s, on average.

This fictive behavioral system in fish provides a platform to monitor neural activity across the whole brain during the execution of behavior using light-sheet microscopy (Fig. 1B). By expressing the genetically coded calcium indicator GCaMP6 in almost all neurons, the authors mapped brain-wide neural activity during fictive locomotion. The analysis of the behavioral correlation of each neuron's activity in the whole brain revealed that neurons in the anterior rhombencephalic turning region (ARTR), rhombomeres 4–6, the inferior olive, and in the vicinity of and overlapping with the reticulospinal system are prominently direction-tuned. Among these, ARTR neurons showed the strongest behavioral correlation (Fig. 1C). Unilateral two-photon laser lesions of ARTR neurons led to biased turning toward the direction of the intact half of the ARTR, while optogenetic activation of ARTR neurons resulted in an increase in the number of turns in the direction ipsilateral to the stimulated side. These findings reveal an uncharacterized neural cluster contributing to the control of turning direction during spontaneous locomotion. The technology used in this study is rather cutting-edge. Integration of light-sheet imaging, two-photon manipulation, and a fictive behavioral paradigm in transgenic fish expressing GCaMP and/or ReaChR provides a new solution to dissect the neural circuits underlying behavior execution. In previous studies, identification of the neural circuits underlying behaviors was through recording, imaging, or manipulating specific neurons during behavior [10–13] and the specificity largely relied on the specific location, promoter expression, or labeling of recorded, imaged, or manipulated neurons. However, in many cases, the specific location or molecular markers were not identified for particular neurons. More importantly, neurons in the same location or expressing the same molecular marker may play different roles in the execution of a particular behavior [14], presenting major problems in dissecting the neural circuits underlying behaviors with the widely-applied spatial or genetic targeting strategies. The technology used in this study has established a new paradigm, which allows the identification of specific neurons based on functional properties correlated with the behavior. More importantly, after functional identification in the same fish, Dunn and Mu *et al*. applied loss/gain-of-function manipulations in these neurons' activities to suggest a causal link between the ARTR neurons and patterned directional motor output.

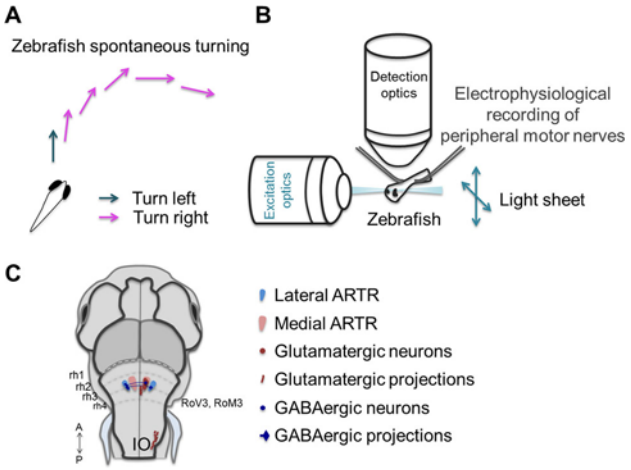

Fig. 1 ARTR neurons contribute to the control of spontaneous turning direction in zebrafish. **A** Spontaneous turning shows a biased spatiotemporal pattern. **B** Apparatus for monitoring whole-brain activity during fictive swimming by light-sheet imaging. **C** Possible neural circuit contributing to the control of turning direction. A, anterior; P, posterior; ARTR, anterior rhombencephalic turning region; IO, inferior olive; RoV3 and RoM3, two types of reticulospinal neurons; rth1–4, rhombomeres 1–4. Adapted from the reference [9] with permission.

A possible limitation of functional identification using *in vivo* whole-brain light sheet imaging is the difficulty of figuring out the identity and the morphology of neurons. In this study, the authors partly solved these problems. First, they combined whole-brain imaging with genetic methods to dissect the identity of ARTR neurons. By using transgenic lines with specific molecular markers expressed in neurons, they found that the ARTR neurons in the medial clusters are glutamatergic while the lateral clusters are primarily GABAergic. Furthermore, they combined whole-brain imaging and photoactivatable GFP to trace the projections of ARTR neurons and found that the lateral GABAergic neurons may form a mutually inhibitory circuit with both contralateral GABAergic and glutamatergic ARTR clusters, and medial glutamatergic ARTR neurons project to areas where premotor neurons are located (Fig. 1C). Such connections might lead to the formation of antiphasic activity of ARTR neurons that further activates

premotor neurons and enables patterned directional motor output.

An important question regarding the function of ARTR neurons in turning behavior is the ethological significance of biased successive turning. In this study, Dunn and Mu *et al*. used a two-state Markov model to simulate turning trajectories through virtual space and found that biased turning is more efficient in covering a restricted area than random turning. It is speculated that ARTR neurons may be recruited in foraging and exploration of the local environment. The application of computational simulation also contributes much to the understanding of neural functions in behavior.

Taken together, Dunn and Mu *et al*. set up a fictive behavioral paradigm and combined it with whole-brain imaging, two-photon manipulation, optogenetics, genetics, and photoactivatable GFP to identify a neural cluster called ARTR underlying the control of turning direction during spontaneous locomotion. This suggests that each action of locomotion is controlled and executed by different neural substrates. To reveal the whole picture of one behavior, the information flow across the whole brain during behavior needs to be monitored and tracked. This study provides a new platform for studying the neural substrates underlying behavior.

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