Unilateral Odor Input Activates Bilateral Premotor Areas in the Moth Brain

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Abstract: Bilateral integration of olfactory inputs from the left and right olfactory organs facilitates odor source localization in humans, rodents, fish, and insects. We investigated representation of bilateral odor input by a population of neurons in the lateral accessory lobe, a premotor area in the brain of the moth. The circuit generates the locomotor command for pheromone-source orientation behavior. We analyzed the physiological changes in response to stimulation on the left or right antennae by using intracellular recording and staining techniques with sharp glass microelectrode. Most of the interneurons showed similar patterns of excitatory activity in response to both left and right side stimulations. These results suggest that the LAL integrates bilateral odor information. We will discuss the command signal for moth walking behavior.

Keywords: command signal, insect, premotor area, central pattern generation, reticulospinal cell

1. INTRODUCTION

Animals have a pair of olfactory organs. The bilateral integration of olfactory inputs from the left and right olfactory organs facilitates odor source localization. Analysis of neural mechanisms for bilateral odor integration is important for understanding odor localization strategy of animals. In this context, moths are one of the model systems used for odor source localization.

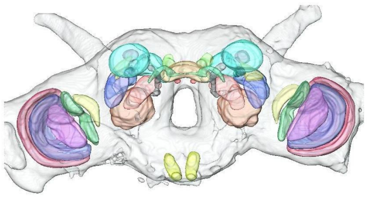

Fig. 1. Neuronal structures in the moth brain.

Male moths show mating behavior in response to the sex pheromones emitted by conspecific females. Silkmoths *Bombyx mori* show sustained walking behavior called the mating dance in response to transient pheromone exposure, and the walking direction is dependent on the stimulation: moths show straight walking to the right side to pheromone stimulation on the right antenna, and vice versa [1]. A series of electrophysiological, anatomical, and behavioral studies in *B. mori* has identified a neuronal circuit called the lateral accessory lobe (LAL) as the brain center of walking behavior [2,3]. A group of descending neurons that commands walking behavior has dendrites in the LAL.

Herein, we investigate the neural representation of pheromone input in the LAL. We recorded the physiology of the LAL interneurons in response to stimulation on either side of antennae and systematically analyzed their response properties as the first step to understanding the neural mechanisms underlying bilateral odor integration.

2. METHODS

Bombyx mori (Lepidoptera: Bombycidae) were reared under a long-day photoperiod regime (16/8-h L/D). Electrodes prepared from thin-walled glass capillaries by using a puller. The electrodes were filled with 5% Lucifer yellow solution in order to stain the neurons. The electrodes were inserted using a micromanipulator. The acquired signals were stored in a computer by using an A/D converter. Spike analysis was done by custom made programs written in MATLAB.

The odorant was applied to a piece of filter paper and inserted into a glass stimulant cartridge. We applied 10 ng of bombykol (the major pheromone component of *B. mori*) to the filter paper. Stimulus was applied to either side of the antenna. Compressed pure air was passed through a charcoal filter, and each stimulus was applied at approximately 35 cm/s. The moths were exposed to the odor for 200 ms, after which an exhaust tube was placed on the opposite side and the odor was removed (exit cartridge, ø~4.5 cm; 15 cm from the antennae; ~55 cm/s).

We stained each neuron by iontophoreticd dye injection with constant hyperpolarizing current. The brain was fixed, dehydrated and cleared. Each stained neuron was frontally imaged using a confocal imaging system. Serial optical sections were acquired throughout the entire depth of the neuron, and 3-D reconstructions of the labeled neurons were generated from these sections. Neuron and neuropil tracing was carried out using a image processing software AVIZO 6.0.

3. RESULTS

The LAL consists of 2 major interneuron types: unilateral neurons (UNs) and bilateral interneruons (BNs). Both UNs and BNs had dendrtic branches in single LAL. BNs also had axonal processes in the contralateral LAL.

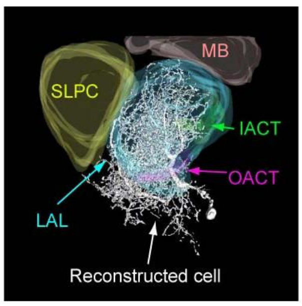

Fig. 2. Morphology of the LAL interneuron.

In this study, we examined the odor response properties of the LAL interneurons to unilateral pheromone inputs. Our results are based on physiologically and morphologically identified neurons (n = 26).

We characterized the morphology and physiology of 10 LAL UNs that innervated the LAL on one side. We performed pheromone stimulation on left or right antenna and arranged the data by ipsi- and contralateral stimulations, according to the soma position. All UNs showed firing rate change in response to pheromone stimulation (Fig. 3). One UN showed an inhibitory response and the other 9 UNs showed an excitatory response. Hence in most cases, unilateral pheromone stimulation triggers the excitatory activity of the neurons in bilateral LALs.

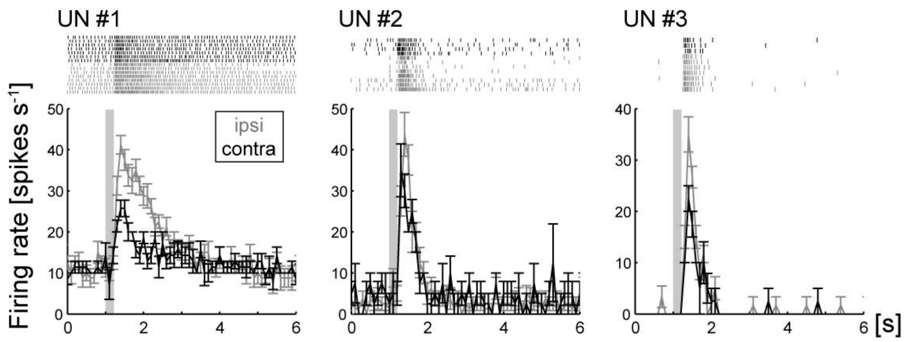

Fig. 3. Three examples of the odor response of the LAL unilateral interneurons. Response to ipsilateral antenna (ipsi, gray) evoked stronger activity than that to contralateral one (contra, black) in each case.

We characterized the morphology and physiology of 16 LAL BNs that innervated LALs in both the left and right hemispheres. All BNs had smooth processes in the LAL, which is the same side of the soma and the blebby processes in contralateral LAL. Ten BNs had branches in the ventral protocerebrum. In most cases, responses to the unilateral and contralateral stimulations were similar but a difference was observed in the response intensity in some cases. Three BNs showed persistent firing activity that lasted over 30 s, but did not show flip-flop activity, which was observed in several types of descending neurons [2]. One BN showed an inhibitory response to unilateral pheromone stimulation and an excitatory response to contralateral stimulation.

We compared neuronal responses to the ipsi- and contralateral stimulations. We calculated the firing rate change of UNs and BNs (Fig. 4). In both types, the time course of the population responses were similar, but the response to ipsilateral stimulation showed slightly higher amplitude than that to contralateral stimulation.

Finally, we examined the cell-type specific feature. We compared 3 parameters: response intensity, response duration, and response latency. When we compared the firing rate for each time window, there was a difference during the early phase of the response (Fig.4A, B). There were no significant differences in response duration and latency between the populations, suggesting that the importance of the total neuronal activity for command generation of the waling behavior.

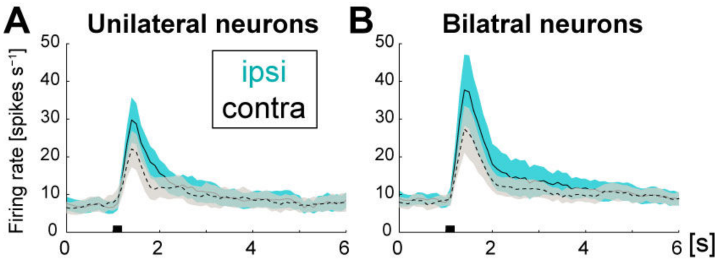

Fig. 4. Population activity in response to the odor stimulation of the LAL unilateral interneurons (A) and bilatreral interneurons (B).

4. DISCUSSION

We revealed bilateral activation of the LAL for unilateral odor input in moth, and also reported the reliability of response, which have not been examined [3]. When moths receive pheromone input on one side, they show initial surge activity (unidirectional turn) toward the input side [1]. The present study suggests that both sides of the premotor areas are active during the initial phase of behavior. There is a possibility that the slight difference among the activities of LALs on both sides might be important for determining the walking direction. We will discuss the process how the neural activity is transmitted to the pattern generating circuit.

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