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Neural Basis of Odor-source Searching Behavior in Insect Brain Systems Evaluated with a Mobile Robot

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Introduction

More than 3 million species of insects live around the world in a variety of environments, and display a diversity of sophisticated behaviors adapted to these environments. Our research is aimed at understanding how the brain systems of insects process constantly changing environmental information and generate adaptive behaviors. Specifically, we are investigating how odor information is processed and modified by other sensory modalities and experience (i.e. learning and memory). It is well known that males of many moth species can detect their species-specific pheromones at low concentrations and orient successfully toward the odor source (e.g. females) even though the odor-source is far away. This may depend not only on high sensitivity to olfactory information by insect olfactory receptors, but also on superior behavioral strategies or algorithms based on processing by neural networks in the insect brain (Arbas *et al.*, 1993; Kanzaki, 1998). Insects have become an excellent model for understanding adaptive control in biological systems which has inspired research and development of control and communication in engineered systems.

It is our long-term goal to understand the behavioral and neural basis of behavior of insects. To this end we have investigated the algorithms used to search for and locate a pheromone source and its underlying control mechanisms in the brain of the male silk moth, *Bombyx mori*. To evaluate the behavioral model we have implemented it in an insect-size mobile robot as a controller for the robot behavior.

Strategies of the odor-source searching

Male *B. mori* exhibit a characteristic zigzagging pattern as they walk upwind to pheromones released by females of the same species (Kanzaki *et al.*, 1992; Kanzaki, 1998). Upwind walking towards the pheromone source is controlled by a largely internally generated program of steering which is triggered by the detection of an intermittent distribution of pheromone by the antennae. Once initiated by a single puff of pheromone, this program consists of brief bout of straight-line walking, zigzag turns and subsequent looping behavior (turns of >360°). Upon stimulation male moths exhibit straight-line walking in the direction of the antenna to which the stimulation was applied. Upon the loss of pheromone stimulation, males exhibit zigzagging walking with the time interval between turns increasing significantly after each turn, followed by looping behavior. This preprogrammed sequence of movements is 'reset' and 'restarted' from the beginning in response to pulsed pheromonal stimulation.

It is known that odor is typically distributed by wind and therefore exists not as a continuous concentration gradient but as a patchy intermittent plume (Murlis *et al.*, 1992). As a result, the male moths often show a variety of walking patterns depending on the distribution of the odor filaments in the air. The pheromone-triggered preprogrammed zigzag turns and reset mechanism together with the intermittent structure of the pheromone plume are the underlying behavioral basis for the odor-searching behavior in *B. mori* males.

Neural mechanisms of odor-source searching behavior

In *B. mori* males specific subsets of descending interneurons (DNs) which link the brain and the thoracic motor system show a characteristic state-dependent activity resembling an electronic 'flip-flop circuit' which has two distinct firing frequencies: high and low (Olberg, 1983). Switching back and forth between the two states occurs upon pheromonal stimulation. The flip-flop is the basic element of 'memory' in electronic circuits. We have characterized two types of DNs (Group IIA,D and Group IIC) which respond with a flip-flopping activity pattern and a brief excitation pattern, respectively, in response to pulsed pheromonal stimulation (Kanzaki *et al.*, 1994; Mishima and Kanzaki, 1999). Both groups of DNs have dendritic arborizations in particular areas of neuropile in the protocerebrum of the brain: the lateral accessory lobe (LAL) and the ventral protocerebrum (VPC).

Our results indicate that the pheromone-mediated preprogrammed orientation behavior is controlled by these two types of DNs; i.e. straight-line walking is controlled by the brief excitation by the Group IIC DNs and the subsequent zigzagging turns and looping are controlled by the flip-flopping activity of Groups IIA and IID DNs.

Neural network

To test our hypothesis for the neural control of pheromone-mediated orientation in *B. mori* males we built a behavioral model for odor-source searching behavior. It seems obvious from the results of our studies of the DNs involved in this behavior that long-lasting excitation and reciprocal inhibition are important for generating the flip-flop responses, and these ideas were incorporated into our model.

Long-lasting excitation

We have characterized serotonin immunoreactive protocerebral bilateral neurons (PBNs) which link both LALs and VPCs (M. Iwano and R. Kanzaki, unpublished observations). Moreover, pressure injection of the serotonin into the LAL drove an enhancement of the pheromone response in some LAL neurons (E.S. Hill and R. Kanzaki, unpublished observations). Therefore, we also incorporated the demonstrated neuromodulatory actions of serotonin in generating long-lasting excitation into our model.

Dynamics of the concentration of neuromodulator is described below. $s_i(t)$ is damped slowly and the time constant τ_{ph} determines this long-lasting response.

$$\tau_{ph}\frac{ds_i(t)}{dt} = -s_i(t) \qquad (1)$$

where *i* represents the side, i.e. right or left LAL–VPC. When the antennae receive a pheromone stimulus, an input signal goes into both LAL–VPC regions. We assumed that a short time lag occurred according to which antenna receives the stimulus. *i* represents the side that received a stimulus, $\bar{i}$ represents the opposite side and the time lag was described as:

$$\begin{aligned} s_i(t_{\text{stim}} + 400\ \text{ms}) &= 0.4 \\ s_{\bar{i}}(t_{\text{stim}} + 720\ \text{ms}) &= 0.4 \end{aligned} \tag{2}$$

where t_{stim} is the moment at which a pheromone stimulus reaches the antenna.

Reciprocal inhibition

We have characterized γ-aminobutyric acid (GABA) immunoreactive protocerebral bilateral neurons (PBNs) which link the left and right LALs (M. Iwano and R. Kanzaki, unpublished observations). GABA is an inhibitory neurotransmitter in the insect brain. It is consistent with what is known from other rhythmically active neural circuits (Calabrese *et al.*, 1989) that alternating activity patterns could be generated by these GABA immunoreactive PBNs which may make reciprocal connections (Mishima and Kanzaki, 1999). It is also known from other reciprocally active systems that fatigue of the cell plays an important role for alternating the activity state. The membrane potential of the LAL–VPC region of our model is shown below.

$$\tau_U \frac{\mathrm{d}U_i(t)}{\mathrm{d}t} = U_i(t) + c_1 X_{\bar{i}}(t) + s_i(t) \tag{3}$$

where

$$X_i(t) = sigm(U_i(t), h_i(t)) \tag{4}$$

$U_i(t)$ is the average membrane potential of the region, $X_i(t)$ is the activity ratio of the region determined by equation (4) and τ_U is time constant of the membrane potential. The output function *sigm* depends on the membrane potential $U_i(t)$ and the threshold level $h_i(t)$ that has the fatigue effect described below.

$$\tau_h(U_i) \frac{\mathrm{d}h_i(t)}{\mathrm{d}t} = -h_i(t) + c_2 X_i(t) + h_{i0} \tag{5}$$

The time constant $\tau_h(U_i)$ is a variable dependent on the membrane potential $U_i(t)$, so that as the membrane potential $U_i(t)$ becomes higher, the cell fatigues more quickly. Since $\tau_h(U_i)$ has a low value under conditions where the $U_i(t)$ is less than the static level of the threshold h_{i0}, the threshold $h_i(t)$ recovers to the initial static level h_{i0} quickly when the region becomes inactive.

Evaluation by an insect-size robot

In order to evaluate the behavioral model under circumstances in which it controls a real body interacting with a real environment, we built an insect-size mobile robot and used our model as the control system for the robot. To make the pheromone field around the robot equivalent to the pheromone field around the moth, our robot was built to the approximate dimensions of the real insect (length: 31 mm, width: 18 mm, height: 30 mm). Antennae excised from live *B. mori* males were used as pheromone sensors, which were attached in front of the robot with an inter-antennal spacing similar to the moth. The electroantennogram, the depolarization of the antennal nerve which appears between the tip and the base of the antenna upon pheromone detection, was used as the odor input signal to the behavioral control system. Sensor values recorded in the pheromone field were transmitted to a host computer every 20 ms using a wire. The host computer calculated the next action according to the behavioral model by solving simultaneous differential equations. System parameters used in this model were determined in the simulation. The robot received action commands from the host computer and then drove the motors. Power was supplied to the robot by thin wires.

The experiments were performed in a wind tunnel so that the behavior of the robot could be compared directly with the behavior of our insects in response to the same sort of pheromone plume. Upon detection of pheromone the robot responded by executing moth-like behavioral sequences, and by repeating these sequences of the behavior, the robot could reach the pheromone source. Thus, our results indicate that even a pre-programmed sequence of the behavior, without the influence of memory and learning, can support a complex task such as oriention towards and location of the odor source by simply repeating the set and reset of the program according to the distribution of odor filaments in the environment. We now know that pheromone tracking in *B. mori* males is modified by sensory modalities other than olfaction, and by experience (Gatellier *et al.*, 2004; Seki *et al.*, 2005). It is the ultimate goal of our ongoing research to understand how these basic neural systems are modified by other sensory modalities and experience for generating adaptive behavior.

For many decades, invertebrate neuroethology has provided insight into how nervous systems organize and generate behavior, due in part to invertebrates being uniquely suited for multidisciplinary studies at different levels of organization using a variety of methodological approaches. For the coming decades, multidisciplinary contributions of biology (analysis) and engineering (synthesis) will be important for expanding our understanding of neuroethology.

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