

Behavioral Determination of Odor Preference is Coded by the Oscillation Frequency in a Collective Oscillating Network of a Terrestrial Mollusk

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Introduction

The brain receives sensory inputs, processes them and finally produces outputs in the form of behavior. In olfaction, many excellent studies have clarified the initial step of neural processing. However, the next step, i.e. how the meaning of the odor is added to the recognized quality of the chemical odor, is poorly understood. This is a critical problem in brain science, because the meaning of a stimulus finally determines the behavior of the animal. In this paper, we first review the characteristics of the olfactory CNS of the land slug *Limax marginatus* and related species, focusing on the synchronized network oscillation of membrane potentials of the neurons and then report our recent study on a neural mechanism for determining the aversive meaning of odors in the mollusk.

Odor-aversion conditioning in the *Limax*

The land slug *Limax* has a highly developed ability to discriminate and identify odors and can be conditioned aversively (Sahley *et al.*, 1981). If a slug is subjected to a paired presentation of the odor of a favorite food and a noxious stimulus such as electrical shock to the head, or quinidine to the lips, the slug learns the relationship between the two stimuli and starts to avoid the previously attractive odor.

Oscillatory activity of the neural network in the *Limax* olfactory center

The procerebrum (PC) is an olfactory center of the gastropod mollusks (Chase, 1985). The *Limax* PC is a highly developed neural network consisting of $\sim 10^5$ neurons that are classified into two types, bursting (B) and nonbursting (NB) neurons (Gelperin *et al.*, 1989; Kleinfeld *et al.*, 1994). B neurons have projections within the cell body layer of the PC while NB neurons have projections extending to the neuropil layers (Watanabe *et al.*, 1998). The NB neurons receive direct input from the afferent fibers from the tentacle ganglion, the primary olfactory center (Inoue *et al.*, 2000). The projection pattern of NB neurons is topographically organized, potentially enabling the mapping of information onto a spatial pattern (Kawahara *et al.*, 1997). Furthermore, we have recently studied odor-evoked activity in individual NB neurons using perforated patch recordings (Murakami *et al.*, 2004) and the result suggest that activity of NB neurons may encode odor identity.

The PC shows regular oscillatory activity at ~ 0.7 Hz (Gelperin and Tank, 1990; Kleinfeld *et al.*, 1994; Kawahara *et al.*, 1997; Kimura *et al.*, 1998) with a slight phase difference along the PC, which produces waves propagating from the apical to basal region (Kleinfeld *et al.*, 1994; Kawahara *et al.*, 1997; Inoue *et al.*, 1998). Perforated-patch recording revealed that B neurons have Cl⁻-dependent excitatory and K⁺-dependent inhibitory glutamate receptors (Watanabe *et al.*, 1999). On the other hand, NB neurons only have the latter ones. Our recent study revealed a spatial difference in the Cl⁻-dependent periodic depolarizations in B neurons, which can account for the unidirectional propagation of waves (Watanabe *et al.*, 2003).

Neuronal interactions producing the oscillatory activity are mediated by various putative transmitters including glutamate, acetylcholine (Watanabe *et al.*, 2001), serotonin and NO (Gelperin *et al.*, 1993; Inoue *et al.*, 2001). The activity level of the bursting neurons determines the oscillation frequency, which can be increased by action of acetylcholine on B neurons (Watanabe *et al.*, 2001).

What is the role of the oscillation in the PC in information processing?

One of the major issues with the neural oscillation is what role the oscillation could have in information processing. There are reports that the PC oscillation is involved in odor discrimination (Teyke and Gelperin, 1999). We also proposed a phase-dependent filtering of olfactory information (Inoue *et al.*, 2000). However, one way to answer this question more clearly is to identify the neural pathway from the PC to behavioral output and to analyze the causal relationship between oscillatory dynamics in olfactory processing networks and odor-guided behavior. Since such attempts remain elusive in the mammalian brain, an *in vitro* nervous system of the *Limax* may lead to an answer. An advantage of using *Limax* is that molluscan nervous systems retain their function in the isolated brain, which can survive for a long period of time in physiological saline. However, there have been so far few studies focusing on the regions outside the PC, which may lead to identification of pathways from the PC to a motor output.

In vitro index of odor-guided behavior

We identified the odor-evoked motor output pathway (Inoue *et al.*, 2004). We observed the behavior of the slug in response to odorants very carefully and found that when the animal approached an aversive odorant (innately or conditioned), it showed shortening of the mantle, before turning away from the repellent. In contrast, attractive odorants did not induce mantle shortening. Therefore, contraction of the mantle muscle can be used as an index of an aversive response. We identified a motoneuron that projects to the mantle and innervates the mantle muscle and named it posterior visceral neuron, *p*-VN. Electrical stimulation of the superior tentacle nerve or application of innately aversive odor induced the action potentials in the *p*-VN. On the other hand, the attractive odors hardly induced firing of the *p*-VN.

These results indicate that aversive odors selectively increase the number of *p*-VN spikes, namely, that odor-elicited muscle contraction *in vivo* can be reproduced *in vitro*, in terms of the activity of the *p*-VN.

Neural correlate of the aversive meaning of the odor

The isolated nose–brain preparation was conditioned by simultaneous application of an odor of attractive food to the tentacle and electrical stimulation of the lip nerve. In the test of the learning, the

conditioned (now aversive) and unconditioned (attractive) odors were applied to the nose while monitoring the activity of the *p*-VN and LFP of the PC. Application of the conditioned odor elicited discharges of the motoneuron. However, application of an unconditioned odor did not induce the discharges of the motoneuron. These results were obtained repeatedly, indicating that the isolated brains can acquire the odor memory. Thus an *in vitro* conditioning system has been established.

Simultaneous recording of the LFP oscillation of the PC lobe and *p*-VN activity has revealed strong correlation between discharges of the *p*-VN and the frequency increase in the LFP oscillation, both of which are induced selectively by aversive odors, the frequency increase preceding the *p*-VN discharge.

We examined the effect of the acetylcholine application, which increases LFP oscillation frequency, on the discharges of the *p*-VN. Selective application of acetylcholine to the PC greatly increases the LFP oscillation frequency, but has little effect to the motoneuronal discharges. However, application of odor, not only aversive but also attractive, could elicit discharges of the *p*-VN while the PC oscillation frequency was increased by ACh application. This result suggests that the increase in the oscillation frequency in the PC does not activate the *p*-VN directly, but has a modulatory effect on the odor recognition, which results in the discharges of the *p*-VN and the subsequent contraction of the mantle muscle.

The conclusion of the present study is that the oscillation frequency in the olfactory CNS is a regulatory element for determining the aversive meaning of odors. In other words, the present study revealed how the meaning of the odor is added to the recognized quality of the chemical odor and how it leads to behavior.

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