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Specific spatio-temporal activities in the cerebral ganglion of *Incilaria fruhstorferi* in response to superior and inferior tentacle nerve stimulation

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ABSTRACT

In terrestrial gastropod mollusks (slugs and snails), olfaction is the dominant sensory modality guiding various kinds of behavior. Anatomical studies indicate that olfactory information is processed in the brain (the cerebral ganglion) in two lobes in particular: the procerebrum (PC) and the metacerebrum (MtC). This implies that olfactory functions emerge from simultaneous and cooperative processing in the PC and the MtC. However, no previous physiological study has investigated the activity in these two lobes simultaneously. In the present study, the activity evoked by electrical stimulation of the olfactory nerves, the superior and inferior tentacle nerves, was recorded optically from the whole cerebral ganglion of the terrestrial slug, *Incilaria fruhstorferi*. The results indicated that the evoked activity in the PC and the MtC showed two specific spatio-temporal patterns. First, when either set of nerves was stimulated, the activity of the medial neuropilar region of the MtC (the mMtC) always preceded the activity in the PC. Second, stimulation of the superior tentacle nerves activated the medial and lateral halves of the mMtC almost evenly, whereas stimulation of the inferior tentacle nerves activated the lateral half of the mMtC more strongly than the medial half. These results suggest that the activated region of the mMtC plays an important role in olfactory processing, especially with respect to the functional differences between the superior and inferior tentacles.

1. Introduction

In terrestrial gastropod mollusks (slugs and snails), olfaction is the dominant sensory modality guiding behavior. These animals can identify odors, orient to them, and alter their interpretation of olfactory stimuli as attractive or repellent based on associated events (Gelperin, 1975; Sahley et al., 1990; Teyke, 1995). Olfactory information is received by the olfactory epithelia of two pairs of tentacles: the superior tentacles (STs) and the inferior tentacles (ITs). The STs and the ITs are connected to the cerebral ganglion (CG) via the superior tentacle nerves (STNs) and the inferior tentacle

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Abbreviations: CG, cerebral ganglion; ST, superior tentacle; IT, inferior tentacle; STN, superior tentacle nerve; ITN, inferior tentacle nerve; PC, procerebrum; MsC, mesocerebrum; MtC, metacerebrum; CM, cell mass; TM, terminal mass; IM, internal mass; LFP, local field potential; mMtC, medial MtC; CC, cerebral commissure; CPlC, cerebropleural connective; CPdC, cerebropedal connective; ILN, internal lip nerve; MLN, medial lip nerve; ELN, external lip nerve

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nerves (ITNs), respectively. The CG is a higher center of the brain that comprises three lobes: the procerebrum (PC), the mesocerebrum (MsC), and the metacerebrum (MtC). Anatomical studies indicate that after entering the body of the CG, afferent fibers of the tentacle nerves segregate into several bundles, and terminate in the PC and the MtC (Chase and Tolloczko, 1989, 1993; Chase, 2000). Ample evidence suggests that the MsC is specialized for the control of mating behaviors (Chase and Li, 1994; Chase, 2000; Koene et al., 2000).

Because parts of the bundles from the STN and the ITN converge in the PC, it is considered to be an olfactory center. In *Achatina*, odor stimulation increases metabolic activity in the PC (Chase, 1985). In *Limax*, *Incilaria*, and *Helix*, the PC shows spontaneous oscillatory activity (Gelperin and Tank, 1990; Kawahara et al., 1997; Nikitin and Balaban, 2000) that is modulated by odor stimulation (Gelperin and Tank, 1990; Kimura et al., 1998a; Schütt et al., 1999; Nikitin and Balaban, 2000; Toda et al., 2000). By contrast, few studies have investigated olfactory information processing in the MtC, probably because it lacks distinctive anatomical features and its cellular population appears heterogeneous compared with the other lobes (Chase, 2000).

The MtC is thought to collect multisensory information and to command motor actions when appropriate signals are received (Chase, 2000). Although it is unclear which area of the MtC deals with olfactory processing, several studies have suggested that it plays an important role in olfaction. Teyke and Gelperin (1999) found that isolated cerebral ganglia could discriminate an appetitively conditioned odor from a novel odor, even when the PC oscillation was abolished pharmacologically. Although the authors only discussed the role of the PC oscillation in olfactory recognition, their results suggested that the MtC might discriminate or evaluate important (learned) odors even without the PC. Although it has not been clearly discussed in papers, the importance of the MtC seems to have been suggested by previously reports concerning the functional differences between the ST and the IT (Chase and Croll, 1981; Friedrich and Teyke, 1998; Kimura et al., 1998a,c, 1999). Physiologically, Kimura et al. (1998a,c) showed that when appetitively conditioned odors were applied to the IT there was an increase in the frequency of PC oscillation, while aversively conditioned odors decreased the oscillation frequency; a similar pattern was not observed in the ST. Furthermore, the PC oscillation frequency increased when any region of digits of the ST ganglion and an apical region of the digits of the IT ganglion were electrically stimulated, and decreased when the middle and basal regions of the digits of the IT ganglion were electrically stimulated (Ito et al., 1999). These differences in PC oscillation might be related to the functional differences in olfactory processing between the ST and the IT reported in behavioral studies — for example, the ST is involved in olfactory orientation (Chase and Croll, 1981), whereas the IT is involved in learning (Friedrich and Teyke, 1998) or retrieving odors (Kimura et al., 1999). However, it remains uncertain whether the modulatory effects on PC oscillations are directly caused by afferent inputs from the ST and the IT to the PC, or are induced indirectly via afferent inputs to other regions, such as the MtC. Indeed, there seem to be no differences in the anatomical projection patterns from the STN and the ITN to the PC (Chase and Tolloczko, 1993). Hence, it is important to determine whether the ST and the IT have different effects on the MtC.

It is therefore of interest to clarify the roles of the PC and the MtC in olfactory functions, and to determine how olfactory processes emerge from these two regions. This will aid our understanding of information processing in the brain, because "parallel processing" in any sensory modality is common and is a principal characteristic of highly developed brains. As the structure of the mollusk brain is relatively simple compared with the vertebrate or insect brain, slugs and snails are good models in which to address the fundamental issue of sensory information processing.

The current study investigated olfactory information-flow mechanisms in the CG, particularly regarding the spatio-temporal characteristics of the MtC activity evoked by the ST and the IT stimulations, and the temporal relationships between the MtC and the PC activities. For clarifying these spatio-temporal characteristics, activity of the whole CG in response to accurately-controlled nerve stimulation should be recorded. To this end, we optically recorded the activity of the whole CG when the STN and the ITN were electrically stimulated, and clarified the spatio-temporal properties of the olfactory regions in the CG of the terrestrial slug, *Incilaria fruhstorferi*.

2. Results

2.1. Spontaneous activity

Fig. 1A shows a fluorescent image of the CG stained by the voltage-sensitive dye, Di-4-ANEPPS. From the gross anatomy, three lobes — the PC, the MsC, and the MtC — could easily be identified. Moreover, the PC could be further divided into three regions: the cell mass (CM), the terminal mass (TM), and the internal mass (IM; Fig. 1). As noted in other species of terrestrial slug (Kawahara et al., 1997), the fluorescent intensity of the Di-4-ANEPPS was relatively strong in the neuropile, especially in the TM and the IM of the PC.

Optically recording the whole CG revealed that the PC was the only region showing spontaneous activity (Fig. 2A). The oscillatory activity of the PC was also confirmed by performing electrophysiological recording with a suction electrode (Fig. 1C). Fig. 2B shows the temporal patterns of six points in the CG: each point of the CM, TM, and IM in the PC, one point in the MsC region, and two points in the MtC region. The three regions of the PC showed oscillatory activity at about 0.5 Hz, whereas the MsC and MtC regions were almost quiescent. In each mass of the PC, the oscillatory waveforms seemed to differ. The amplitudes of the oscillatory signals in the TM and the IM were larger than those in the CM. Comparing the waveforms in each mass, the rapid and intense hyperpolarization seemed to be specific to the IM oscillation. Fast oscillatory cycles were seen in the IM that did not seem to correspond to any oscillatory cycle in the other masses (asterisks in Fig. 2B).

The characteristics of the oscillatory activity in each mass were confirmed using cross-correlation analysis. Two reference waves for the analysis were selected from each anatomical center of the TM and the IM, and a cross-correlation