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Gustatory feedback affects feeding related motor pattern generation in starved 3rd instar larvae of *Calliphora vicina*

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ABSTRACT

Gustatory feedback allows animals to distinguish between edible and noxious food and adapts centrally generated feeding motor patterns to environmental demands. In reduced preparations obtained from starved Calliphora larvae, putatively appetitive (ethanol), aversive (sodium acetate) and neutral (glucose) gustatory stimuli were applied to the anterior sense organs. The resulting sensory response was recorded from the maxillary- and antennal nerves. All three stimuli increased the neural activity in both nerves. Recordings obtained from the antennal nerve to monitor the activation pattern of the cibarial dilator muscles, demonstrated an effect of gustatory input on the central pattern generator for feeding. Ethanol consistently enhanced the rhythmic activity of the CDM motor neurons either by speeding up the rhythm or by increasing the burst duration. Ethanol also had an enhancing effect on the motor patterns of a protractor muscle which moves the cephalopharyngeal skeleton relative to the body. Sodium acetate showed a state dependent effect: in preparations without spontaneous CDM activity it initiated rhythmic motor patterns, while an ongoing CDM rhythm was inhibited. Surprisingly glucose had an enhancing effect which was less pronounced than that of ethanol. Gustatory feedback therefore can modify and adapt the motor output of the multifunctional central pattern generator for feeding.

1. Introduction

Feeding is one of the most essential animal behaviors and gustatory cues are utilized to distinguish edible from noxious food. The imagos of various insect groups provide well understood model systems in which the physiology of gustatory organs located on the mouth parts and legs, and the interaction of gustatory feedback with feeding behavior like proboscis extension have been thoroughly analyzed, e.g. Lepidoptera (*Heliothis virescens*: Blaney and Simmonds, 1990; Jorgensen et al., 2007; Kvello et al., 2010; Ramaswamy, 1987), Hymenoptera (*Apis mellifera*: Abisgold and Simpson, 1988; Sanchez et al., 2005; Takeda, 1961) and Diptera (*Calliphora*, *Drosophila*: DeJianne et al., 1985; Dethier, 1976; Duerr and Quinn, 1982; Galindo and Smith, 2001; Singh, 1997; Stocker, 1994).

The gustatory and olfactory systems of *Calliphora* and *Drosophila* larvae comprise various external and internal sense organs. The external gustatory organs, located on the cephalic lobes and around the mouth are the terminal-, ventral- and labial organs (Bolwig, 1946; Chu-Wang and Axtell, 1972a; Chu-Wang and Axtell, 1972b; Honda and Ishikawa, 1987a; Honda and Ishikawa, 1987b; Hückesfeld et al., 2010; Richter, 1962; Singh and Singh, 1984; Szpila et al., 2008; Vosshall and Stocker, 2007). The prominent dorsal organs which are also located on the cephalic lobes primary serve an olfactory function (Fishilevich et al., 2005; Honda and Ishikawa, 1987a; Oppliger et al., 2000). The internal labral organ described for *Calliphora* (Hückesfeld et al., 2010; Ludwig, 1949) possibly correlates to three groups of pharyngeal sensilla in *Drosophila* (Singh and Singh, 1984; Vosshall and Stocker, 2007). The sensory pathways of the gustatory organs are well established: the maxillary nerve (MN) and its side branches innervate the terminal-, ventral- and labial organs while the dorsal- and labral organs are innervated by the antennal nerve (AN, Columb et al., 2007; Gendre et al., 2004; Schoofs et al., 2009; Stocker, 1994; Vosshall and Stocker, 2007).

Feeding behavior is a cycle of repetitive movement components mediated by identified muscle groups: the cephalopharyngeal skeleton (CPS) is rhythmically extended and withdrawn by protractor and retractor muscles accompanied by elevation and depression of the mouth hooks (Schoofs et al., 2009). Food ingestion is imbedded into this cycle and mediated by a suction pump

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powered by rhythmic contractions of the cibarial dilator muscles (CDM) which are innervated by neurons projecting through the AN (Hanslik et al., 2010; Schoofs et al., 2009, 2010). The rhythmic contractions of feeding related muscle groups are driven by a central pattern generator located in the central nervous system (Schoofs et al., 2009, 2010). Although isolated CPGs are *per definition* capable of producing fictive behaviors (Decolmyn, 1980; Marder, 2002; Marder and Bucher, 2001) sensory inputs are required to shape the correct final motor pattern in the intact animal (Reingold and Gelperin, 1980; Shetreat-Klein and Cropper, 2004; Wendler, 1974).

The perception of smell and taste in *Drosophila* larvae has been intensively studied with molecular–biological methods to reveal the neural architecture of the system, the functionality of olfactory and gustatory receptor neurons and their role in the context of chemo-sensation and learning (for reviews see: Cobb, 1999; Gerber and Stocker, 2007; Stocker, 1994, 2001; Vosshall and Stocker, 2007). The genetic toolbox available for *Drosophila* and the revived interest in feeding regulation initiated research into the circuitry of the *Drosophila* gustatory system and its influence on larval behavior (Bader et al., 2007; Balakireva et al., 2000; Gerber and Stocker, 2007; Heimbeck et al., 1999; Hendel et al., 2005; Melcher et al., 2007; Melcher and Pankratz, 2005; Miyakawa, 1982; Schipanski et al., 2008; Vosshall and Stocker, 2007). Surprisingly, to our knowledge the obvious hypothesis that gustatory input regulates the activity of feeding related muscle groups was never approached electrophysiologically.

In this qualitative "proof of concept" investigation *Calliphora* larvae were used to address this issue. Although this species lacks genetic accessibility, the large larvae are ideally suited for electrophysiological experiments. The cephalic sense organs of starved larvae were stimulated in reduced preparations either with a putative appetitive (ethanol), aversive (sodium acetate) or neutral (glucose) gustatory stimulus. The overall afferent neural activity was simultaneously recorded from the MN and AN. All three stimuli caused a sensory response in the form of an increased action potential frequency. Furthermore all three stimuli had a profound effect on the motor patterns recorded from the AN (food ingestion) and PaN (CPS protraction) which implies that gustatory feedback is capable of adopting the centrally generated motor output according to the environmental necessities.

2. Material and methods

2.1. Animals

About 100 flies (*Calliphora vicina*) were kept in a 25 × 40 × 35 cm cage and supplied with water and sugar *ad libitum*. They were offered an oviposition site (50 ml beaker filled with minced meat) each day for 3 h, thus the age of the larvae was roughly synchronized. The hatched larvae were supplied with meat according to necessity. Feeding 3rd instar larvae were collected daily and starved in a refrigerator for 24 h before they were used for the experiments.

2.2. Preparation

The larvae were pinned down, dorsal side up, in a Petri dish coated with clear silicone (Wacker Silicones, Elastosil RT 601) and covered with saline (in mM: NaCl: 140, KCl: 3, $CaCl_2 \cdot 6H_2O$: 2, $MgCl_2 \cdot 6H_2O$: 4, Sucrose: 10, HEPES: 5, pH 7.2). After opening them along the dorsal midline the esophagus, salivary glands, fat body and gut was removed. The pharyngeal complex with the cephalic lobes bearing the terminal- and dorsal organs was dissected free, taking care not to damage the nerves innervating the sense organs (antennal- and maxillary nerve). Except for the set of experiments in which recordings were obtained from the accessory prothoracic nerve (PaN) all other nerves were transected.

2.3. Electrophysiology

To record the neural activity a small petroleum jelly pool based on a piece of Parafilm was constructed around the respective nerve. The saline level was lowered below the rim of the pool and the recording electrode (Ag/AgCl) placed in it. The indifferent electrode was placed in the surrounding saline. Action potentials were amplified (5000 fold) and filtered (high pass 3 kHz, low pass 100 Hz). The recorded signals were digitally stored in real time using a 4-channel AD board (CED 1401 micro) and processed with Spike2 (Cambridge Electronic Design).

2.3.1. Sensory response

The sensory response due to gustatory stimulation was simultaneously recorded from the ipsilateral MN and AN. Both nerves were transected just anterior to the central nervous system (CNS, see Fig. 1, right side). The neural activity was recorded for at least 1 min before and 2 min after stimulus application. The overall neural activity was detected using Spike2 and plotted as "mean frequency" calculated for successive 2 s intervals. Nine recordings were performed for each type of stimulus.

2.3.2. Motor patterns

Both pairs of MN and AN were still connected to the CNS. Fictive food ingestion patterns were recorded *en passant* from the AN (see Fig. 1, left side). In one set of experiments the gustatory effect of ethanol on the motor pattern of the PaN was recorded. The nerve was cut distally to the recording site. In the intact animal this nerve innervates the "dorsal protractor A", a muscle that participates in the protraction of the cephalopharyngal skeleton (CPS) during feeding and locomotion.

The neural activity was recorded for at least 2 min before and 2 min after the gustatory stimulation. At least 10 recordings were performed for each set of experiments.

2.4. Gustatory stimulation

A relatively large petroleum jelly pool (volume approx. 70 μl) was constructed around the pharyngeal complex, with the cephalic lobes exposed to its lumen (see Fig. 1). In preliminary experiments the pool was completely emptied with a micropipette. This exposed the sense organs to air and already produced a sensory response. The procedure was therefore changed: the saline in the pool was only removed so far that its remains were just covering the cephalic organs. A comparative large amount (at least 10 fold) of the respective substance dissolved in saline was then filled into the pool with a micropipette. Care was taken to produce as little turbulences as possible during this process. Any sensory response was therefore exclusively caused by the respective substance. We used:

- 5% ethanol as a putative appetitive stimulus.
- 0.5 M sodium acetate as a putative aversive stimulus.
- 1 M glucose as a putative neutral stimulus.

The stimuli were dissolved in saline because distilled water produced a strong sensory response itself. Additionally the neural activity in the nerves ceased completely after about 1 min, suggesting an osmosis related damage of the sensory structures.

3. Results

3.1. Sensory response due to gustatory stimulation (Fig. 2)

Three types of gustatory stimuli were applied to the cephalic sense organs: ethanol (5%), sodium acetate (0.5 M) and glucose (1 M). The resulting overall neural activity was recorded simultaneously from the MN and AN. It could not be determined from