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Proprioceptors involved in stinging response of the honeybee, *Apis mellifera*

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ABSTRACT

Two types of mechanosensitive proprioceptor organ are present on the stinging apparatus of the honeybee: campaniform sensilla and mechanosensory hairplates. The campaniform sensilla are located on the surface of the tapering sting-shaft, which comprises an unpaired stylet and paired lancets. Each sensillum on the lancet differs from that on the stylet in terms of their topography and external morphology. The sensory afferents of the campaniform sensilla display slow-adapted firing responses to deformation of the cuticle that would be caused by the action of inserting the sting into a substrate, and their afferent signals induce and/or prolong the stinging response. By contrast, the mechanosensory hairplates are located at basal cuticular plates and on the posterior surface of the lancet valves. Two fields of hairplates on the second ramus at the ventral edge of the groove and on the antero-lateral edge of the oblong plate respond synchronously to protraction of the lancet. During the stinging response, these hairplates are likely to detect any sliding movement of the lancet and its position relative to the stylet. Afferent signals produced by them are likely to provide important information to the neuronal circuit for the generation and modulation of the stinging motor pattern.

1. Introduction

The exocuticle of insects and crustaceans contains a large number of mechanosensitive proprioceptors of various morphological types. These provide sensory information about the state and performance of exopodites and have a part in controlling the movement and posture of effectors during locomotion (reviewed by Bässler and Büschges, 1998; Pearson, 1993; Zill et al., 2004). In the honeybee, *Apis mellifera*, two kinds of external proprioceptor have been described on the stinging apparatus: campaniform sensilla and hairplates with trichoid sensilla (Hermann and Douglas, 1976a,b; Shing and Erickson, 1982). The campaniform sensilla located on the sting probably detect the depth of sting insertion by assaying the increasing cuticular deformation that occurs with successively deeper penetrations, whereas the hairplates located on the cuticular plates of the stinging apparatus are likely to be proprioceptors detecting the relative position of movable parts of the sting during the stinging response (Shing and Erickson, 1982). However, there are no published physiological studies available on the response characteristics and functional roles of these proprioceptive organs in the stinging response.

The stinging response is the culminating stage of the defensive behavior of the honeybee, involving penetration of a substrate by the shaft of the sting and the release of venom from the venom sac (Breed et al., 2004; Collins et al., 1980). Recently, the stinging response has been used for a new conditioning protocol in the honeybee, and has received much attention in the framework of learning and memory (Carcaud et al., 2009; Giurfa et al., 2009; Roussel et al., 2010; Vergoz et al., 2007). During the stinging response, in addition to the protraction of the entire shaft out of the abdomen tip, the paired ventral parts of the sting (i.e. the 'lancet' versus the unpaired dorsal component, the 'stylet') exhibit alternating rhythmic sliding (Snodgrass, 1956; Dade, 1962). This movement results from the coordinated action of four pairs of stinging muscle (M196s, M197s, M198s, and M199s) and enables the sting to be inserted deeper into the target. A previous study described that afferent inputs to the terminal abdominal ganglion (TAG), in which the central pattern generator for the stinging movements is located, modulate the frequency of the rhythmic sliding and maintain the relationship between cycle period and burst duration of the stinging muscle activity at various frequencies (Ogawa et al., 1995). The sensory signals from the mechanosensitive proprioceptors in the stinging apparatus also appear to have an effect on the stinging motor patterns, but it is currently unknown how and which proprioceptors are involved in the modulation of the stinging response.

In the present study, we first describe the topography, detailed morphology and central projection of the proprioceptors, from

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which the effective stimulus to each receptor is inferred. We also analyze the electrophysiological responses of these receptors to the inferred effective stimuli, and examine the effects of the afferents on the stinging motor activity.

2. Materials and methods

2.1. Animals

Specimens of honeybees (*A. mellifera* L.) were obtained from outdoor colonies at Okayama University. All the experiments presented were performed with foraging bees.

2.2. Preparation

Bees were anesthetized at 4° C for 20 min. The abdomen was severed from the thorax and pinned on a paraffin platform. Following longitudinal lateral-line incision, the terga of the 3rd to 6th segments and the gut were removed to expose the stinging apparatus. To avoid any venom leaking from the preparation, the acid gland was coated with Vaseline.

2.3. Morphology

The topography and location of proprioceptors mediated by the stinging response were examined in whole sting shafts and basal cuticular plates that were removed from abdomens, under a transmission light microscope (BH-RFL, Olympus, Tokyo, Japan). The exact distribution and the external structure of proprioceptors were examined with a scanning electron microscope (SEM; T-300, JOEL, Tokyo, Japan) in isolated stinging apparatus that had been fixed with acetone (Wako Pure Chemical, Osaka, Japan), dehydrated, CO_2-critical-point dried and then coated with gold.

Sectional planes of the campaniform sensilla were examined using a transmission electron microscopy (TEM). The lancets and stylet dissected from the stinging apparatus were prefixed for 2 h at 4 °C with 4% glutaraldehyde (in 0.1-M sodium phosphate buffer, pH 7.4, Sigma–Aldrich, St. Louis, MO, USA). They were then post-fixed for 2 h at 4 °C in a 2% OsO_4 solution in the same buffer, and finally dehydrated and imbedded in Epon 812 araldite (CY-230, Ciba Geigy, Tokyo, Japan). Ultra-thin serial sections were double-stained with uranyl acetate and lead citrate, and observed under the TEM (H-300, Hitachi, Tokyo, Japan).

To stain the central projection of the sensory neurons innervating the proprioceptors, 10% agar gel consisting of 10% $NiCl_2$ was located either at the cut end of the sting (stylet or lancet) or at the end of hair sensilla on the valve for 8–24 h at 4 °C. After filling, the 6th and terminal abdominal ganglia were isolated into honeybee saline (NaCl 270 mM, KCl 3.2 mM, $MgCl_2$ 10 mM, $CaCl_2$ 1.8 mM, $NaHCO_3$ 7.1 mM, dextrose 50 mM, Tris-buffer 10 mM, pH 7.4, Wako). The nickel ions were then precipitated within the neurons by addition of rubeanic acid (Wako) to the honeybee saline. After fixation with 70% ethanol, the ganglia were dehydrated and cleared with methyl salicylate for whole-mount viewing. The ganglion stained with the nickel ion was intensified, according to the method of Bacon and Altman (1977). The stains were drawn as a whole mount using a camera lucida attached to the microscope (Olympus).

2.4. Electrophysiology

Electrophysiological experiments were performed with the exposed stinging apparatus in the above-mentioned manner. For extracellular recordings of neuronal responses of the proprioceptors, a glass suction electrode filled with honeybee saline was placed on a cut proximal stump of the lateral nerve, A8 or A9, innervating the proprioceptors. A reference electrode was placed in the abdomen. Campaniform sensilla were stimulated by a stainless steel probe attached to a micromanipulator (Narishige, Tokyo, Japan). The tip of the probe was placed on the surface of the sting, and pressed against the cuticle. The bend of the sting shaft or the barbs of the lancet led to excitation of the campaniform sensilla. To stimulate the hair sensilla on the second ramus or on the oblong plate, a lancet was moved forward and backward alternately via a small stainless wire connected to the arm of a vibrator.

For recordings of electromyograms (EMGs), electropolished tungsten wires (ø = 70 μm) were inserted into the stinging muscles (a protractor M198 and a retractor M199; see Ogawa et al., 1995). A reference electrode was placed in the abdomen. Recordings of the neuronal activity of sensory afferents and the EMGs were viewed on an oscilloscope and stored on magnetic tape.

3. Results

Two types of proprioceptive sense organ were observed on the honeybee stinging apparatus (Fig. 1). One is the campaniform sensilla, which are strain-sensitive mechanoreceptors distributed on the long shaft of the sting. The second type is mechanosensory hairplates located on the basal cuticular plates.

3.1. Campaniform sensilla

3.1.1. Topography and external morphologies

The campaniform sensilla on the sting were classified into two groups according to their distributions. The first group was

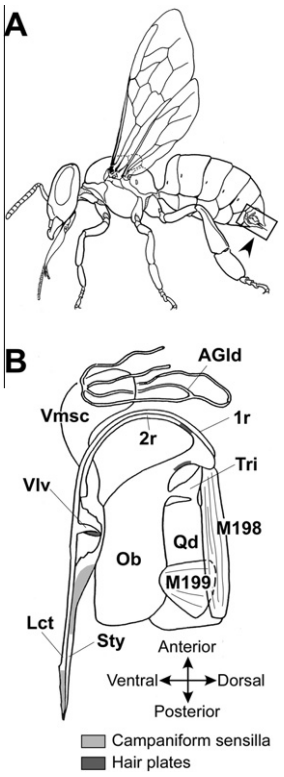

Fig. 1. Overview of the proprioceptors on the stinging apparatus. (A) Drawing indicating location of the stinging apparatus in the honeybee (square enclosed area). (B) Schematic illustration of the distribution of proprioceptors on the stinging apparatus (lateral view). Dark-gray areas indicate a field on which the mechanosensory hairplates are located, and light-gray areas indicate a field on which the campaniform sensilla are located (see also Fig. 8). 1r, first ramus; 2r, second ramus; AGld, acid gland; Lct, lancet; Ob, oblong plate; Qd, quadrate plate; Sty, stylet; Tri, triangular plate; Vlv, valve on lancet; Vmsc, venom sac.