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Neural responses from the filiform receptor neuron afferents of the wind-sensitive cercal system in three cockroach species

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ABSTRACT

The wind-sensitive insect cercal system is involved in many important behaviors, such as initiating terrestrial escape responses and providing sensory feedback during flight. The occurrence of these behaviors vary in cockroach species *Periplaneta americana* (strong terrestrial response and flight), *Blaberus craniifer* (weak terrestrial response and flight), and *Gromphodorhina portentosa* (no terrestrial response and no flight). A previous study focusing on wind-sensitive interneuron (WSI) responses demonstrated that variations in sensory processing of wind information accompany these behavioral differences. In this study, we recorded extracellularly from the cercal nerve to characterize filiform afferent population responses to different wind velocities to investigate how sensory processing differs across these species at the initial encoding of wind. We compared these results and responses from the WSI population to examine information transfer at the first synapse. Our main results were: (1) *G. portentosa* had the weakest responses of the three species over the stimulus duration and possessed the smallest cerci with the least filiform hair receptors of the three species; (2) *B. craniifer* filiform responses were similar to or greater than *P. americana* responses even though *B. craniifer* possessed smaller cerci with less filiform hair receptors than *P. americana*; (3) the greater filiform afferent responses in *B. craniifer*, including a larger amplitude second positive peak compared to the other two species, suggest more synchronous activity between filiform afferents in this species; (4) the transfer of information at the first synapse appears to be similar in both *P. americana* and *G. portentosa*, but different in *B. craniifer*.

1. Introduction

The insect wind-sensitive cercal system has been the subject of many neurobiological and neuroethological studies to investigate general nervous system function. In particular, the cercal system has been a useful model for studying neural mechanisms underlying sensory processing since sensory processing in insects resembles early sensory processing stages in vertebrates. Sensory processing can vary across animals with different life histories and comparative studies are useful for identifying and studying the neural mechanisms underlying these differences. Compared to many vertebrate sensory systems, the insect cercal system is a more tractable system that is readily accessible for neurophysiological, neuroanatomical, and imaging studies.

The insect cercal system consists of two posterior appendages, known as the cerci, that contain wind-sensitive filiform hairs that detect air currents and is one of the most sensitive sensory systems in biology (Barth, 2000, 2004). A single sensory receptor cell attaches to the base of each filiform hair (Gnatzy, 1976) and its axon projects to the terminal abdominal ganglion (TAG). Within the TAG, the afferents monosynaptically connect to a population of wind-sensitive interneurons (WSIs) that carry wind information to the thoracic ganglia containing premotor and motor neurons (motor regions) as well as to the subesophageal and supraesophageal ganglia (Boyan and Ball, 1990; Farley and Milburn, 1969; Spira et al., 1969).

WSIs activate the thoracic motor regions to generate behavior through indirect (via premotor neurons) synapses to motor neurons (Boyan and Ball, 1989; Casagrand and Ritzmann, 1991; Ritzmann, 1981; Ritzmann and Camhi, 1978; Ritzmann and Pollack, 1981, 1986, 1988, 1990; Westin et al., 1988), though direct synapses could also be present in some systems. The two most common behaviors associated with the cercal system are predator detection to initiate terrestrial escape responses (Camhi and Tom, 1978) and providing sensory feedback during flight (Fraser, 1977; Libersat and Camhi, 1988; Ritzmann et al., 1982). Insects that possess a cercal system may differ in the wind-mediated behaviors they exhibit, even between closely related species. In cockroaches (Blattaria), the cercal system mediates both functions in the

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American cockroach *Periplaneta americana*. However, wind does not elicit terrestrial escape responses in the Madagascan hissing cockroach *Gromphodorhina portentosa* and this species does not fly since it lacks wings. In the Death's Head cockroach *Blaberus craniifer*, wind evokes only weak terrestrial responses that are not effective for evading capture (Simpson et al., 1986). However, *B. craniifer* possesses pink flight muscles capable of supporting sustained flight (Bell et al., 2007; Kramer, 1956; Roth and Willis, 1960).

Variations in sensory processing of wind information accompany the behavioral differences across these three species. In a previous study, McGorry et al. (2014) found differences in the response properties of the population of the WSIs in *P. americana*, *B. craniifer*, and *G. portentosa*. Wind evoked the least number of action potentials with the lowest spiking rates from the WSI population in *G. portentosa*. However, wind elicited a similar number of action potentials from WSIs across wind velocities in *B. craniifer* as *P. americana*. Furthermore, WSI responses had similar high spiking rates in *B. craniifer* and *P. americana*, but the WSI population in *B. craniifer* maintained this high spiking rate for a longer duration after stimulus onset than the other two species. Differences in the intrinsic response properties of the WSIs could contribute to the sensory processing differences in these three species at the level of the WSIs. However, initial encoding of the wind stimulus by the filiform hairs and the input provided by the filiform afferents could also influence WSI responses and contribute to species differences in sensory processing.

Previous experiments on the filiform hairs and their afferent responses have focused on: (1) the mechanical properties of the filiform hairs (Tautz, 1979; Fletcher, 1978; Dechant et al., 2006; Bathellier et al., 2012); (2) the relationship between filiform hair length and sensitivity to the acceleration and velocity components of wind stimuli (longer hairs respond to wind velocity while shorter hairs respond to wind acceleration) (Shimozawa and Kanou, 1984; Kanou et al., 1988; Landolfa and Miller, 1995; Kant and Humphrey, 2009); and (3) directional selectivity of the filiform hairs and the encoding of wind direction by the filiform afferents (Nicklaus, 1965; Gnatzy, 1976; Westin, 1979; Dagan and Camhi, 1979; Tobias and Murphey, 1979; Gnatzy and Tautz, 1980; Miller et al., 1991; Barth et al., 1993). Even fewer of these studies have involved cockroaches and have mainly focused on the encoding of wind direction by the filiform hairs and their afferent responses in *P. americana* (Nicklaus, 1965; Westin, 1979; Dagan and Camhi, 1979). These studies tested only a limited number of wind velocities (50–60, 140, and 260 cm/s, Westin, 1979; <1 cm/s, Dagan and Camhi, 1979) with short stimulus durations (80 ms, Westin, 1979; 100 ms, Dagan and Camhi, 1979).

In the current study, we examined the responses of the filiform afferent population in *P. americana*, *B. craniifer*, and *G. portentosa* across wind velocities to determine how sensory processing differs across these species at the initial encoding of the wind stimulus. Since the recording setup and system to generate wind stimuli was the same as used to measure WSI responses (McGorry et al., 2014), we compared the filiform afferent and WSI responses to examine the transfer of information at the first synapse of the cercal sensory system neural circuit, which may reveal additional information about sensory processing in these three species. We also measured morphological features of the cerci, including the number of filiform hairs, to relate to the physiological responses. Previous cercal system studies have focused primarily on *P. americana* and the cricket *Acheta domesticus*. Here, we investigate and compare closely related species that have different life histories to determine whether differences in sensory processing exist. Once identified, future experiments can target the neural mechanisms underlying these differences, which could reveal novel neural mechanisms involved in sensory processing.

2. Methods

2.1. Animals

This study examined wind-sensitive filiform hair afferent responses in three different cockroach (Blattaria) species: *P. americana* (Linnaeus, 1758) (Blattidae: Blattinae), *G. portentosa* (Schaum, 1853) (Blaberidae: Oxyhaloinae), and *B. craniifer* (Burmeister, 1838) (Blaberidae: Blaberinae). Each species was lab-reared from colonies maintained at the College of Charleston. They were fed on cat chow, provided water, and raised between 24 and 28 °C in 30–60% humidity using a 14:10 day:night cycle.

2.2. Morphology measurements

Body length and cercal lengths for each species were measured using a digital caliper (KD Tools, Cockeysville, MD) under a stereomicroscope (Model SD6 Leica Microsystems SD6, Buffalo Grove, IL). Cercal segments and filiform hair counts were also performed under the same microscope.

2.3. Neural recordings

Animals were anesthetized with CO_2 and pinned dorsal side up on a raised platform with the legs and wings removed. After removing the dorsal abdominal cuticle, the gut was detached from the anus and placed outside of the body cavity. Care was taken to minimize spillage of the gut contents into the body cavity while removing the gut. The body cavity was rinsed several times with saline (Fielden, 1960) to wash out any gut contents that may have entered the body cavity. Care was also taken to keep the cerci clean and free of saline during the surgery and experiments. This included plugging up the anus with Surgident periphery dental wax (Heraeus Kulzer, Armonk, NY) to prevent saline from leaking out of the body cavity and onto the cerci. Trachea and reproductive organs were dissected away to reveal the cercal nerves and the TAG. The cercal nerve was cut where it entered the TAG as were all peripheral nerve branches other than the one entering the cercus itself that contained the filiform sensory cell axons. A suction electrode recorded neural activity from the cut end of the cercal nerve. Data collection began one minute after obtaining the recording to allow the recording to stabilize.

2.4. Experimental setup

Wind puffs were generated using the building's compressed air supply and gated using a solenoid valve (Model 2S025-1/4-1-D, Sitzo Technical Corporation, Palo Alto, CA). These were similar to the wind stimulus used in behavioral studies of cercal-mediated wind-evoked terrestrial escape responses in *P. americana* (Camhi and Tom, 1978) and *B. craniifer* (Simpson et al., 1986), as well as other species, such as crickets (Tauber and Camhi, 1995; Baba and Shimozawa, 1997). To reduce turbulence in the wind puffs, air passed from the solenoid into a baffle and through a PVC pipe (35 cm length and 2.5 cm inner diameter) filled with coffee stirrers (4 mm diameter) in the last 14 cm of the pipe closest to the prep (Fig. 1A). A gap between the solenoid and the baffle decoupled the stimulus presentation setup from the recording setup, which prevented neural responses to vibrations generated by the opening/closing of the solenoid (Fig. 1A). The experimenter controlled stimulus presentation using a push button mechanism, with the stimulus duration precisely controlled by an electronic timer (Model RTE-P1D12, IDEC, Osaka, Japan) in the stimulus presentation circuit. Stimulus magnitude was controlled using a pressure regulator (Model 14R113Fc 1/8″ body ¼″ port, Parker Hannifin