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

Anne C.K. Olsen and Jeffrey D. Triblehorn

Department of Biology and Program in Neuroscience, College of Charleston, 66 George Street, Charleston, SC 29424

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 *Gromphodrhina 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*.

Keywords

Wind; Blattaria; Escape; Sensory; Predator-Prey

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Correspondence with: Jeffrey D. Triblehorn Department of Biology College of Charleston 66 George St. Charleston, SC 29424 USA
triblehornj@cofc.edu phone: 1 843 953 5848 fax: 1 843 953 5453.

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1.0 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 American cockroach *Periplaneta americana*. However, wind does not elicit terrestrial escape responses in the Madagascan hissing cockroach *Gromphodorrhina 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; Landolfi 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.

2.0 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 24-28°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 CO₂ 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 Shimozaawa, 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. 1 A). 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. 1 A). 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 1/4" port, Parker Hannifin

Corporation, Cleveland, OH) with a pressure gauge attached (Model 15.110-100-1/8"-CBM, Noshok, Berea, OH). This system allowed us to present consistent wind stimuli of different velocities as measured by a hot-wire anemometer (Dantec Dynamics, Model 54T30 mini CTA and P16 probe, Denmark) (Fig. 1A). This was the same system used to produce wind puff stimuli as McGorry et al. (2014).

Experiments were conducted within a 90 cm × 110 cm × 100 cm (H × W × D) Faraday cage (Fig. 1A). The animal was placed within 2.5 cm of the PVC pipe opening. The hot-wire anemometer recorded the velocity of each wind stimulus. We recorded cercal afferent responses to seven different wind velocities between 1-250 cm/s as well as to 0 cm/s (solenoid opening and closing without wind). The 0 cm/s presentation was included to measure: 1) the level of spontaneous activity in the cercal nerve and 2) any responses to the solenoid opening/closing (as a control). Each wind velocity was presented consecutively 60 times with a 2 s interstimulus interval, which is enough time for the filiform afferent responses to return to baseline conditions (Dagan and Camhi, 1979). The eight different wind velocities were tested in a random order and there was a 90 s interval between testing each new wind velocity. Figure 1B shows examples of hot-wire anemometer traces (average of three wind stimulus presentations) for five different velocities in the 1-250 cm/s wind velocity range tested.

2.5 Data Collection and Analysis

Neural responses, anemometer traces, and a stimulus marker indicating stimulus onset for each wind presentation were digitized (National Instruments PCI-6221 connected to a BNC 2090A, 40 kHz sample rate) and recorded using WinWCP (John Dempster, University of Strathclyde). Neural signals were amplified by a differential AC amplifier (A-M Systems Model 1800, Sequim, WA USA) and filtered (high pass = 1 Hz; low pass = 20 kHz). Extracellular recordings from the cercal nerve provide a summed response of filiform afferent activity (i.e. “cercogram”; Dagan and Camhi, 1979) (Fig. 2, top trace). The 60 recordings were signal averaged to obtain the filiform afferent response for analysis (Fig. 2., bottom trace) The signal averaged filiform afferent response was rectified and integrated to obtain the magnitude of the afferent response at each wind velocity tested using Data View version 8.3 (William Heitler, University of St. Andrews). Only the first 250 ms of the response was analyzed.

Species stimulus-response (S-R) curves for afferent responses were constructed as follows (Fig. 3). For each animal's data, the best fit curve (either a two phase decay, one phase decay, linear, quadratic, or sigmoidal curve fit function) was calculated using a least squares fit model (Prism 5, GraphPad Software, San Diego, CA) (Fig. 3A). Species S-R curves were generated by entering wind velocity values (0, 5, and 10 up to 250 cm/s in 10 cm/s intervals) into each individual S-R curve equation to obtain predicted filiform afferent responses (Fig. 3B). These filiform afferent responses were then averaged for each wind velocity to generate the S-R curve for the species.

For additional analyses of the first and second positive peaks of the filiform afferent response, the envelope of the signal average response was used. The envelope was generated using a Gaussian smoothing function with a 25 Hz cutoff frequency using Data View version 9.1.3. For the analysis of the first and second peaks, the peak amplitudes (in mV) were measured instead of the integrated neural response.

3.0 Results

3.1 Body and cercal measurements in all three species

P. americana had the smallest body length of the three species, but the longest cerci, which was a sizable percentage of their body length (mean = 18.55 % of body length) compared to the other two, larger species (means = 8.83% in *B. craniifer* and 3.17% in *G. portentosa*; Table 1). Each *P. americana* cercus contained ~ 200 filiform hairs on 18 cercal segments, which is substantially greater than either *B. craniifer* (~ 60 filiform hairs on 15 cercal segments) or *G. portentosa* (~ 20 hairs on 11 cercal segments). Filiform hairs were only located on the ventral surface in all three species.

3.2 Filiform afferent responses to different wind velocities

Extracellular recordings from the cercal nerve provide a summed response of filiform afferent activity. We signal averaged 60 responses to each wind velocity presented, which provides the level of consistent afferent activity for each stimulus presentation. The magnitude and shape of the signal averaged responses was a combination of the magnitude of the afferent response (both number of afferents responding and the number of action potentials they generate) and the level of synchrony in the neural responses across presentations (asynchronous responses will cancel out while synchronous responses will produce peaks or sustained increases in activity). We will use “filiform afferent response” to refer to the signal averaged response throughout.

Filiform afferent responses began with an initial positive peak occurring at the onset of the response across wind velocities (black arrows, Fig. 4). This peak had both a high and low frequency component. The amplitude of this first peak was higher in *P. americana* (Fig. 4, left) and *B. craniifer* (Fig. 4 middle) than in *G. portentosa* (Fig. 4, right). At higher wind velocities, filiform afferent responses contained a second positive peak (open arrows, Fig. 4). In the neural traces shown, this second peak first appears in the 61-74 cm/s neural trace for *B. craniifer*, the 96-100 cm/s neural trace for *G. portentosa*, and the 192-221 neural trace for *P. americana*. However, the wind velocity at which the second peak appeared varied in different individuals (see below). The onset and amplitude of the second peak within the neural trace also varied, occurring earlier in the neural response with increasing amplitude as wind velocity increased (compare 61-74, 96-100 and 192-221 cm/s neural traces for *B. craniifer*; 96-100 and 192-221 cm/s in *G. portentosa*).

From these data, we generated stimulus-response (S-R) curves to: 1) quantify how the filiform afferent responses changed with increasing wind velocity and 2) compare the encoding of wind velocity by the filiform afferent population across species. We quantified the filiform afferent response by rectifying and integrating the signal averaged neural response elicited by different wind velocities (See Methods for more detail). McGorry et al. (2014) found that the WSI responses in these same three species were not consistent throughout the 250 ms duration of the wind stimulus. Compared to the Second 150 ms of the response, the First 100 ms of the response: 1) contained more overall spikes; 2) contained more large amplitude spikes; and 3) had a faster spiking rate for the WSI population (Fig. 4B; dashed line marks First 100 ms). Therefore, the First 100 ms and Second 150 ms of the

responses were analyzed separately. To directly relate the filiform afferent responses to the WSI responses from McGorry et al. (2014), we analyzed the filiform afferent responses in the same manner by analyzing the responses during the First 100 ms and Second 150 ms of the stimulus separately.

3.3 Stimulus-Response (S-R) curves for the afferent population

In all three species, filiform afferent responses increased with wind velocity during the First 100 ms of the stimulus (Fig. 5A). As expected from the neural traces in Fig. 4, filiform afferent responses in both *P. americana* and *B. craniifer* were stronger than *G. portentosa* during the First 100 ms of the stimulus. However, the shapes of the S-R curves for the First 100 ms differed between *B. craniifer* (sigmoidal) and *P. americana* (a gradual curve resembling a one phase decay function). Filiform afferent responses for *B. craniifer* and *P. americana* were similar at wind velocities less than 140 cm/s (mean filiform afferent response = 2.53 ± 0.35 [mean $\pm$ sem] for *B. craniifer* and 2.31 ± 0.39 for *P. americana*), but as wind velocity increased, afferent responses increased more in *B. craniifer* (mean filiform afferent response = 4.72 ± 0.71 at 250 cm/s) than *P. americana* (mean filiform afferent response = 2.99 ± 0.55 at 250 cm/s). In *G. portentosa*, filiform afferent responses increased linearly with wind velocity (0.68 ± 0.1 at 140 cm/s and 0.92 ± 0.16 at 250 cm/s, for comparison with the other two species). In all three species, the variation (the standard error of the mean) in filiform afferent responses increased with stimulus velocity.

The S-R curves in Fig. 5A illustrate the changes in the response magnitude across wind velocities within a species as well as differences in the response magnitude across species. However, comparing the differences in response magnitude cannot determine whether the filiform afferent population in each species actually encodes wind velocity differently. S-R curve shapes, which describe the encoding function of wind velocity by the filiform afferent population, can be compared across species by normalizing the data. To determine whether these species differed in how the filiform afferent population encodes wind velocity, each species S-R curve (Fig. 5A) was normalized to the maximum spike count (Fig. 5B). Previously, we defined the dynamic encoding range for the WSI population as the wind velocity that elicited 0.75 proportion of the maximum response (McGorry et al., 2014). A lower wind velocity indicated a narrower range of encoding wind velocity. We used the same criterion for the filiform afferent responses for comparison with WSI responses (Table 2).

The normalized S-R curves for the First 100 ms of the stimulus were very similar for *P. americana* and *G. portentosa*, though the curve for *P. americana* had a shallower rise at lower wind velocities (Fig. 5B). Both species had similar dynamic encoding ranges (145 for *P. americana* and 141 cm/s for *G. portentosa*) (Table 2). *B. craniifer* had the shallowest rise with its sigmoidal-shaped normalized S-R curve and the highest dynamic encoding range (173 cm/s). Above this wind velocity, the normalized S-R curve for *B. craniifer* matched the other two species.

For the Second 150 ms of the stimulus, the S-R relationship for *G. portentosa* was very similar as for the First 100 ms, both in the magnitude of the response and the shape of the function (Fig. 5C). However, the S-R relationships for *B. craniifer* and *P. americana* were

different from the First 100 ms in three ways. First, *B. craniifer* had stronger filiform afferent responses than *P. americana* at wind velocities over 20 cm/s (Fig. 5C). Second, the S-R curves for both *B. craniifer* and *P. americana* had an inverted U-shape, with both species S-R curves peaking at 170 cm/s (Fig. 5C). At this peak response, the filiform afferent response was almost twice as large in *B. craniifer* as in *P. americana* (filiform afferent response = 5.23 ± 1.05 and 2.87 ± 0.56 , respectively). Third, in *P. americana*, the magnitude of the filiform afferent responses was similar during both periods of the stimulus (as in *G. portentosa*), even though the shape of the S-R curves differed. However, the filiform afferent response in *B. craniifer* was stronger during the Second 150 ms of the stimulus compared to the First 100 ms of the stimulus except at the highest and lowest wind velocities.

All three species encoded wind velocity similarly during the Second 150 ms of the stimulus up to about 200 cm/s based on the normalized S-R curves (Fig. 5D). The dynamic encoding range was slightly higher in *G. portentosa* (101 cm/s) than *P. americana* (95 cm/s) and *B. craniifer* (86 cm/s). The falling phase of the normalized S-R curves (wind velocities over 180 cm/s) for *P. americana* and *B. craniifer* was similar, decreasing to 0.77 of the maximum response in *P. americana* and 0.74 in *B. craniifer* by 250 cm/s.

Filiform afferent response latencies did not vary with stimulus velocity for all species, but *P. americana* had a shorter latency (11.34 ± 0.38 ms; mean $\pm$ s.d.) than *B. craniifer* (14.16 ± 0.46 ms) and *G. portentosa* (14.18 ± 0.93 ms).

3.4 Analysis of the second peak in the filiform afferent responses across species

Differences in the amplitude of second positive peak (Fig. 4, open arrows) relative to the first peak (Fig. 4, black arrows) were the most notable species differences in the filiform afferent responses after overall activity. Therefore, we analyzed the characteristics of the first and second positive peaks across species by removing the high frequency components from the neural traces (see Methods for details) and analyzing the amplitudes (in mV) of the two peaks in the filtered filiform afferent responses. Figure 4C illustrates the results of the filtering process by showing the filtered filiform afferent response trace examples for the highest wind velocity (192-221 cm/s) for each species.

We compared the amplitudes of the first and second positive peaks in the filiform afferent responses that contained both peaks in all three species (Fig. 6A). The dashed line indicates equal amplitudes for the first and second positive peaks; data below the dashed line indicates instances where the amplitude of the first peak was greater while data above the dashed line indicate instances where the amplitude of the second positive peak was greater. For *B. craniifer*, the amplitude of the second positive peak was greater in 98% of the instances (41 out of 42) with the amplitudes of the two peaks being equal in the one exception. The opposite was true in *P. americana*, where the first positive peak larger than or equal to second positive peak in 81% of the instances (34 out of 42). In *G. portentosa*, both positive peaks had similar amplitudes, which were generally smaller than in the other two species.

The relative amplitudes of the first and second positive peaks (expressed as the ratio of the amplitude of the two peaks) varied with wind velocity (Fig. 6B; dashed line represents equal

amplitudes of the first and second positive peaks). In *P. americana*, the second positive peak appeared in the response beginning at wind velocities in the 51-70 cm/s range and did not appear in *B. craniifer* and *G. portentosa* until the 71-90 cm/s velocity range. In *B. craniifer*, the second positive peak was consistently two to four times greater in amplitude than the first positive peak. In *P. americana*, the amplitude of the first positive peak was higher at lower wind velocities (51-170 cm/s) but the amplitudes were similar at higher wind velocities (> 170 cm/s). In *G. portentosa*, the amplitudes of the peaks were similar. In all three species, the time interval between the two peaks decreased similarly with increasing wind velocity (Fig. 6C).

3.5 Comparison of filiform afferent and WSI S-R curves

We compared the filiform afferent S-R curves to WSI S-R curves from McGorry et al. (2014) to examine the input-output relationship at the first synapse in the cercal sensory system neural circuit (Fig. 7).

In both *P. americana* and *G. portentosa*, the S-R curves for the WSIs had similar shapes for the First 100 ms (closed triangles) and Second 150 ms (open triangles) of the stimulus, though more spikes occurred during the First 100 ms in both species. However, the magnitude of filiform afferent responses (closed and open circles) was similar during both periods of the stimulus. The WSI spike counts in these two species appeared proportional to the magnitude of the filiform afferent input since *G. portentosa* WSI spike counts and filiform afferent responses were both less than those in *P. americana*.

The relationship between the filiform afferent and WSI S-R curves in *B. craniifer* appeared to be different from *P. americana* and *G. portentosa*. As with the other two species, the First 100 ms evoked more WSI spikes than the Second 150 ms of the stimulus. However, the situation was reversed for the filiform afferent responses, which were greater during the Second 150 ms of the stimulus. Furthermore, although *B. craniifer* WSI S-R curves for both periods of the stimulus were similar to those in *P. americana* (in terms of both S-R curve shapes and spike counts), the filiform afferent responses in *B. craniifer* were very different from *P. americana* in both S-R curve shape and spike counts.

For all three species, the dynamic encoding ranges were broader for filiform afferent responses than for WSI responses during the First 100 ms and Second 150 ms of the stimulus (Table 2).

4.0 Discussion

In this study, we investigated the neural responses of the wind-sensitive cercal afferent population in three cockroach (Blattaria) species. These species differ in the wind-mediated behaviors they exhibit. Wind evokes terrestrial escape responses in *P. americana*, weak terrestrial responses *B. craniifer* and no responses in *G. portentosa*. Flight has been well-characterized in *P. americana* (Fraser, 1977; Ritzmann, 1982; Libersat and Camhi, 1988; Ganihar et al., 1994) while *G. portentosa* lacks wings and does not fly. *B. craniifer* possesses pink flight muscles capable of supporting sustained flight (Bell et al., 2007; Kramer, 1956; Roth and Willis, 1960) but flight performance has not been well-studied or

characterized in this species. Separate S-R curves for filiform afferent responses were generated for the First 100 ms and Second 150 ms for comparison with previously collected S-R curves for the WSI responses in these three species from McGorry et al. (2014).

Our main results include: 1) *G. portentosa* had the weakest afferent responses of the three species over the stimulus duration and possessed the smallest cerci with the least number of filiform hair receptors of the three species; 2) *B. craniifer* filiform afferent responses were similar to or greater than *P. americana* responses even though *B. craniifer* possessed smaller cerci with less filiform hair receptors compared to *P. americana*; 3) all three species encoded wind velocity similarly during the entire stimulus, but *B. craniifer* did exhibit a larger dynamic range during the First 100 ms of the stimulus and greater encoding sensitivity when wind velocities were below 180 cm/s; 4) *B. craniifer* and *P. americana* filiform afferent responses contained a similar first positive peak response to the wind reaching the cerci that was greater in amplitude compared to *G. portentosa*, but *B. craniifer* filiform afferent responses contained a second positive peak that was larger than the second positive peak in the other two species. We will compare the filiform afferent responses to the WSI responses of these three species (McGorry et al., 2014) and discuss potential differences in sensory processing occurring at the first synapse in the wind-sensitive cercal system circuit in these three species.

4.1 Filiform afferent responses across species

Although wind-sensitive filiform hair receptor neurons are spike producing, extracellular recordings from the cercal nerve lacked distinct individual neural spikes (Fig. 2), but instead recorded the summed neural activity generated by the filiform hair afferent population. The signal averaged response incorporated several characteristics of the filiform afferent activity elicited by wind stimuli, including: 1) the number of filiform afferent neurons activated; 2) the number of action potentials elicited by each filiform afferent neuron; and 3) the level of synchronous filiform afferent activity across stimulus presentations. Since the filiform afferent neurons synapse directly with the WSIs, all three of these characteristics influence activation of the WSIs at the next stage of the cercal system neural circuit

G. portentosa exhibited the weakest filiform afferent responses of the three species tested during both the First 100 ms and Second 150 ms of the stimulus (Fig. 5 A,C). This species also possessed the smallest cerci (both in overall length and relative to body length) and the least filiform hairs (about 20 per cercus, Table 1), so the magnitude of the afferent response appeared related to the number of wind receptors. This was not the case for *P. americana* and *B. craniifer*, however. Despite *B. craniifer* possessing smaller cerci (both in overall length and relative to body length) and less filiform hairs than *P. americana* (~60 per cercus vs. ~200 per cercus, respectively; Table 1), wind velocities up to 140 cm/s evoked similar afferent responses in *B. craniifer* and *P. americana* during the First 100 ms of the stimulus while higher wind velocities evoked stronger responses in *B. craniifer* (Fig. 5A). Furthermore, wind elicited greater filiform afferent activity in *B. craniifer* during the Second 150 ms of the stimulus than *P. americana* across wind velocities (Fig. 5C). These results suggest filiform afferents in *B. craniifer* have one or more properties that allow the filiform afferent population to generate greater activity with less filiform hairs and associated

receptor neurons. Possible ways to enhance the overall activity of the filiform afferent population may include increasing the number of action potentials generated by individual afferents, greater synchrony across afferent activity, or some combination of the two.

The possibility of increased synchrony in *B. craniifer* afferent activity was reflected in the positive peaks appearing in the filiform afferent responses (Fig. 4, black and open arrows). Filiform afferent responses began with an initial positive peak at all wind velocities (Fig. 4, black arrows) generated by synchronous afferent activity responding to the arrival of the wind stimulus. Both *P. americana* and *B. craniifer* had similar first positive peak amplitudes, which were larger than those in *G. portentosa* (Figs. 4, 6A). The second positive peak differed from the first positive peak in three significant ways: 1) it did not occur at all stimulus velocities, but only at wind velocities over 70 cm/s in *B. craniifer* and *G. portentosa* and over 50 cm/s in *P. americana* (Fig. 6B); 2) the latency of the second positive peak after the first positive peak was not consistent, appearing earlier in the filiform afferent neural response as the wind velocity increased (Figs. 4, 6C); and 3) the second positive peak was always larger than the first peak in *B. craniifer* but not the other two species (Figs. 4, 6B). The similarity of the first positive peak amplitude between *B. craniifer* and *P. americana* as well as the larger second positive peak in *B. craniifer* relative to *P. americana* also supports the possibility of increased synchrony among afferent activity in *B. craniifer* despite possessing less filiform hairs than *P. americana*.

The falling phase of the U-shaped function for the Second 150 ms S-R curves in *P. americana* and *B. craniifer* was likely due to the time when the second positive peak appeared in the filiform afferent response. At lower wind velocities, the second positive peak occurred during the Second 150 ms of the stimulus, but within the First 100 ms at higher wind velocities. This would also explain the sigmoidal shape of the S-R curve for the First 100 ms of the stimulus in *B. craniifer*, where the amplitude of the second positive peak was greater in *B. craniifer* than in the other two species.

Differences in the amplitude of the second positive peak across species correlate with the differences in the WSI population spiking rates. McGorry et al. (2014) found that the WSI population in *B. craniifer* and *P. americana* had similar spiking rates that were higher than the WSI population in *G. portentosa*. However, the WSI population in *B. craniifer* maintained this high spiking rate for a longer duration than in *P. americana*. Higher amplitude peaks in the filiform afferent response represent greater afferent activity, providing increased input to the WSIs and could contribute to the sustained high spiking rate of the WSI population in *B. craniifer* compared to the other two species.

While the arrival of the wind stimulus at the cerci evokes the first positive peak, there is no immediate explanation for what elicits the second peak in the filiform afferent response. One possibility is that the second positive peak is a synchronized afferent response to some stimulus property, such as the wind stimulus reaching peak velocity. The time difference between the two positive peaks decreased as wind velocity increased (Fig. 6C), which is consistent with this possibility since wind acceleration increases with wind velocity and reaches peak velocity earlier. Another possibility is that the two peaks represent responses from two different afferent populations. However, if the two peaks are generated by separate

afferent populations, it is unlikely due to separate monosynaptic/polysynaptic pathways or by different sets of afferent conduction velocities since the interval between the peaks is too long (Fig. 6C). The two populations could have different wind velocity thresholds, with one population activated at lower wind velocities and occurring earlier in the response and the other population activated at higher wind velocities and occurring later.

A number of factors influence the S-R properties of individual filiform afferent receptor neurons and, ultimately, the S-R responses of the filiform afferent population. Individual filiform afferent receptor neurons have a limited dynamic range for encoding wind velocity, which overlap with other filiform afferent receptor neurons to provide a wider dynamic range for the entire population of filiform afferent receptor cells (Landolfa and Miller, 1995). Three filiform afferent receptors with overlapping dynamic ranges can have ten times broader range as a single receptor. Landolfa and Miller (1995) conducted their study in crickets (*Acheta domesticus*), which possess filiform hairs of different lengths. Filiform hair length is a mechanical property that contributes to the response characteristics of a single filiform receptor cell, including sensitivity to the velocity (longer hairs) or acceleration (shorter hairs) component of wind. *P. americana* possesses less variation in filiform hairs lengths than *A. domesticus*, with hair lengths in the upper range of intermediate hair lengths in crickets (around 725 μm ; Nicklaus, 1965). This length is close to, but just below, the 800 μm cutoff for a filiform hair to be termed “velocity sensitive” (Shimozawa and Kanou, 1984; Landolfa and Miller, 1995). Membrane biophysics of the filiform afferent receptor neurons also contribute to determining the dynamic range for each receptor neuron (Landolfa and Miller, 1995). Differences in the membrane biophysics of filiform afferent receptor neurons could contribute to differences in the population filiform afferent responses between these three cockroaches, but such investigations are lacking for cockroach species.

Despite the differences in the magnitude of the filiform afferent responses, the normalized responses indicate that the encoding of wind velocity was similar across the three species during both the First 100 ms and Second 150 ms of the stimulus (Figs. 5 B,D). The most notable exceptions were: 1) *B. craniifer* during the First 100 ms of the stimulus for wind velocities below 180 cm/s and this species also had a higher dynamic encoding range and 2) *P. americana* and *B. craniifer* during the Second 150 ms of the stimulus at wind velocities over 200 cm/s due the inverted U-shaped function of their S-R curves

It may not be so surprising that *B. craniifer*, with less filiform hairs than *P. americana*, exhibits such strong afferent and WSI responses since Dagan and Volman (1982) showed that first instar *P. americana* are able to encode wind direction and perform directional running escape responses while only possessing four filiform hairs (two per cerci). However, *G. portentosa* also possesses less filiform hairs but had much weaker afferent and WSI responses than the other two species. It is not clear what mechanism(s) allow first instar *P. americana* to perform as adult *P. americana* or what advantage the larger number of filiform hairs convey to adult *P. americana*.

4.2 Information transfer from the filiform afferent population to the WSI population

Comparing filiform afferent or WSI responses across species provides relevant information about differences in sensory processing at separate levels of the cercal system circuit.

Considering the relationship between filiform afferent and WSI responses and the transfer of information at the first synapse in the cercal system both within and across species can provide additional insight into sensory processing in these species. This information can identify targets for future investigations into general neural mechanisms involved in sensory processing.

The relationship between filiform afferent input and WSI responses, and thus information transfer, appeared to be similar in both *P. americana* and *G. portentosa*. In both species, the WSI S-R curves had similar shapes, with wind evoking more WSI spikes during the First 100 ms than the Second 150 ms of the stimulus even though the magnitude of filiform afferent input was similar during both periods of the stimulus (Fig. 7). However, the WSI responses (output) seem proportional to the filiform afferent input since WSI spike counts and filiform afferent responses in *G. portentosa* were less than those in *P. americana*. The magnitude of filiform afferent input may account for the differences between WSI spike counts between *P. americana* and *G. portentosa*, but cannot account for the WSI spike count differences between the First 100 ms and Second 150 ms of the stimulus in both species. The WSI spike count differences between the two stimulus periods must be due to other factors than afferent input (discussed below).

Information transfer in *B. craniifer* appears to be different from *P. americana* and *G. portentosa*. The shapes of the WSI S-R curves for *B. craniifer* for the First 100 ms and Second 150 ms of the stimulus were similar to both *P. americana* and *G. portentosa* and the spike counts during both the First 100 ms and Second 150 ms were similar to *P. americana* (Fig. 7). However, the input from the filiform afferents in *B. craniifer* was very different in several ways from the other two species. First, the shapes of the *B. craniifer* filiform S-R curves during the First 100 ms and Second 150 ms of the stimulus were very different (sigmoidal vs. inverted U-shaped, respectively). Second, filiform afferent responses were greater in *B. craniifer* than in *P. americana*, but this increased input did not result in a proportionate increase in WSI spike counts (as was the case comparing *P. americana* and *G. portentosa*). Although WSI spike counts were greater during the First 100 ms than the Second 150 ms of the stimulus in *B. craniifer*, the opposite was true for the filiform afferent responses (greater during the Second 150 ms than the First 100 ms of the stimulus). These results suggest that information transfer, and thus sensory processing of wind information, is different in *B. craniifer* compared to the other two species.

The magnitude of afferent input is not the only factor that may influence the output of WSI responses at the first synapse of the cercal sensory system. The pattern of connectivity and synaptic efficacy between individual filiform receptor neurons and the WSIs affect WSI responses (Miller et al., 1991). Filiform receptor neurons project to multiple WSIs of the same characteristics to preserve direction, velocity, and acceleration information and these synapses may have different efficacies (Boyan and Ball, 1990). Furthermore, filiform afferent and WSI synapses can facilitate or depress based on the presynaptic activity controlled by the retrograde signal nitric oxide (Davis and Murphey, 1993). WSI activity patterns are not just generated by the presynaptic input, but WSIs also have intrinsic properties that influence their activity pattern, classified as phasic, tonic, or phasic-tonic (Westin, 1979; Kanou and Shimozaawa, 1984). These intrinsic properties include the

membrane biophysics of the WSIs, possibly by the presence and distribution of ion channels responsible for spike rate adaptation, such as Na^+ - and Ca^{2+} -activated K^+ channels (Sah, 1996; Sah and Faber, 2002; Sanchez-Vives et al., 2000), which has not been extensively studied in insect WSIs. Indirect connections between filiform afferent and WSIs also influence WSI responses. Inhibitory inputs from local interneurons shape directional sensitivity and possibly influence WSI responses to velocity and acceleration (Miller et al., 1991; Bacon and Murphey, 1984; Jacobs and Miller, 1985; Jacobs et al., 1986; Levine and Murphey, 1980). Levine and Murphey (1980) also described the effects of a primary afferent depolarization (PAD) on cercal afferents in *A. domesticus*. The PAD controls afferent response duration and magnitude to prevent presynaptic depression. The time course of the PAD is 20-70 ms in *A. domesticus*, which could contribute to differences in the filiform afferent and WSI responses during the First 100 ms and Second 150 ms of the stimulus if also present in these cockroach species. The filiform afferent and WSI curve comparisons are limited in that they do not provide information about the underlying mechanisms. However, these comparisons do provide some insight into information transfer for future studies to investigate the underlying neural mechanisms responsible for the differences between species.

4.3 B. craniifer as a focus of future investigations

The higher level of neural activity elicited by wind stimuli at both the filiform afferent (current study) and WSI (McGorry et al., 2014) levels of the cercal sensory system is paradoxical given the reduction in cercal length relative to body length and smaller number of filiform hair receptors compared to the *P. americana* (Table 1). This suggests that sensory processing is substantially different in *B. craniifer*. The results from these two studies are particularly intriguing since wind evokes only weak terrestrial escape responses in *B. craniifer* that are ineffective for evading predators (Simpson et al., 1986). This also begs the question of why this level of neural activity does not activate strong motor responses and generate wind-evoked responses similar to *P. americana*. Future comparative investigations involving *B. craniifer* and *P. americana* (and possibly *G. portentosa*) will not only focus on the mechanisms underlying these increased neural responses in *B. craniifer*, but also on mechanisms preventing wind-evoked behaviors in *B. craniifer*. These investigations could reveal new neural mechanisms in sensory processing underlying these differences that may also occur in other invertebrate and vertebrate sensory systems. Such investigations could also provide insight into on how the cercal system has evolved in insects, which can provide a better understanding on how nervous systems evolve in general.

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Highlights

- Cercal system filiform afferent responses were compared in three cockroach species.
- The species differed in their wind-evoked escape responses and flight ability.
- Wind elicited the weakest responses in *Gromphadorhina portentosa*.
- Wind elicited stronger responses in *Blaberus craniifer* than *Periplaneta americana*.
- Sensory processing in *B. craniifer* may be different than in the other two species.

Fig. 1. Schematic of the experimental setup (A) and representative hot-wire anemometer traces for five wind velocities (B)

(A) Schematic illustrates the experimental setup, including the system used to generate wind stimuli, the position of the preparation and the hot-wire anemometer used to measure wind velocities. (B) The average waveform of three anemometer traces used to measure the wind velocity. The dashed line indicates 100 ms after the solenoid opened (indicated by the arrow).

Fig. 2. Neural traces of a single response and the signal averaged response of 60 single responses
Top trace: Neural response of filiform afferent population to a single wind puff (209 cm/s) recorded extracellularly from the cercal nerve with a suction electrode. Bottom trace: Signal averaged neural response of filiform afferent population using 60 stimulus presentations. The signal averaged response was used to analyze the filiform afferent population response for each wind velocity tested. This example comes from *B. craniifer*.

Fig. 3. Construction of species stimulus-response (S-R) curves from individual S-R curves
 (A) Example of eight filiform afferent responses (squares) during the First 100 ms of the stimulus from one *B. craniifer* individual. The line shows the best curve fit for this data set (Boltzmann sigmoidal curve fit: $R^2 = 0.9476$, $y = 186159 + ((1000000 - 186159) / (1 + (\exp((168.2 - x) / 30.26))))$). (B) Filled circles are the predicted filiform afferent responses across wind velocities based on entering wind velocities of 0, 5, and 10 up to 250 cm/s in 10 cm/s intervals based on the best curve fit in (A). These predicted filiform afferent responses were then averaged across individuals for each wind velocity to generate the S-R curve for the species (shown in Fig. 5A,C).

Fig. 4. Neural traces of signal averaged filiform afferent responses at four wind velocity ranges for *P. americana*, *B. craniifer*, and *G. portentosa*

(A) All four traces for each species come from the same individual and are the results of signal averaging 60 responses to the same wind velocity (see Methods). An initial positive peak (black arrows) marked the beginning of the response at all wind velocities for all species while a second positive peak occurred at higher wind velocities (open arrows). (B) Example of WSI responses from all three species to a high velocity wind stimulus taken from McGorry et al. (2014) for comparison with filiform afferent responses in (A). (C) Envelopes of the signal averaged responses for the 192-221 cm/s range for all three species used for analysis of the first and second peaks. Dashed lines indicate 100 ms after the solenoid opened.

Fig. 5. Stimulus-Response (S-R) curves for the filiform afferent response for the three cockroach species

(A) S-R curves for filiform afferent responses during the First 100 ms of the stimulus for *P. americana* (open circles), *B. craniifer* (closed circles), and *G. portentosa* (closed triangles).

(B) Normalized S-R curves for filiform afferent responses during the First 100 ms of the stimulus. Each species' S-R curve was normalized to the maximum filiform afferent response in (A).

(C) S-R curves for filiform afferent responses during the Second 150 ms of the stimulus. (D) Normalized S-R curves for filiform afferent responses during the Second 150 ms of the stimulus. Each species' S-R curve was normalized to the maximum filiform afferent response in (C). *P. americana* n = 13; *B. craniifer* n = 10; *G. portentosa* n = 10.

Error bars represent standard errors of the mean (sem).

Fig. 6. Analysis of the first and second positive peaks in filiform afferent responses for the three cockroach species

(A) Each data point represents the amplitudes (mV) of the first and the second positive peaks in all filiform afferent responses that contained a second positive peak (all filiform afferent responses contained a first peak) for *P. americana* (open circles), *B. craniifer* (closed circles), and *G. portentosa* (closed triangles). The dashed line indicates equal first and second positive peak amplitudes. (B) Ratio of the amplitudes of the first and second positive peaks as a function of wind velocity for all three species. The dashed line indicates equal amplitudes for the first and second positive peaks (ratio = 1). Positive ratios indicate that the amplitude of the second positive peak was greater than the first positive peak. Negative ratios indicate that the amplitude of the first positive peak was greater than the second positive peak. Data were grouped into 20 cm/s wind velocity intervals. (C) Time difference between the first and second positive peaks as a function of wind velocity in all three species. Data were grouped into 20 cm/s wind velocity intervals.

Fig. 7. Comparison of the filiform afferent response and WSI S-R curves for the three cockroach species

Comparison of the S-R curves for the filiform afferent response (circles, left axis) and the WSI responses (triangles, right axis) during the First 100 ms (closed symbols) and Second 150 ms (open symbols) of the wind stimulus for each species. WSI data come from McGorry et al. (2014) (*P. americana* n = 15, *B. craniifer* n = 12; *G. portentosa* n = 11).

Table 1

Cercal system structure morphological characteristics from the three species.

Species	Mean Body Length (mm)	Mean Cercal Length (mm)	% of Cercal Length to Body Length	Number of Cercal Segments	Approximate Number of Filiform Hairs per cercus
<i>P. americana</i>	33.97 ± 3.7	6.26 ± 0.79	18.55 ± 2.59	18.13 ± 2.1	~200
<i>B. craniifer</i>	48.46 ± 2.21	4.28 ± 0.18	8.83 ± 0.29	14.94 ± 0.46	~60
<i>G. portentosa</i>	57.55 ± 3.61	1.83 ± 0.19	3.17 ± 0.23	10.89 ± 0.32	~20

Table 2

Dynamic encoding ranges for filiform afferent and WSI responses.

	P. americana	B. craniifer	G. portentosa
First 100 ms			
Afferents	145 cm/s	173 cm/s	141 cm/s
WSIs	31 cm/s	56 cm/s	55 cm/s
Second 150 ms			
Afferents	95 cm/s *	86 cm/s *	101 cm/s
WSIs	3 cm/s	25 cm/s	66 cm/s

* Both species had inverted U-shaped S-R curves with maximum response at 170 cm/s