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Neural responses from the wind-sensitive interneuron population in four cockroach species

Clare A. McGorry¹, Caroline N. Newman², and Jeffrey D. Triblehorn^{1,2}

¹Department of Biology, College of Charleston, 66 George Street, Charleston, SC 29424

²Program in Neuroscience, College of Charleston, 66 George Street, Charleston, SC 29424

Abstract

The wind-sensitive insect cercal sensory system is involved in important behaviors including predator detection and initiating terrestrial escape responses as well as flight maintenance. However, not all insects possessing a cercal system exhibit these behaviors. In cockroaches, wind evokes strong terrestrial escape responses in *Periplaneta americana* and *Blattella germanica*, but only weak escape responses in *Blaberus craniifer* and no escape responses in *Gromphadorhina portentosa*. Both *P. americana* and *Blab. craniifer* possesses pink flight muscles correlated with flight ability while *Blat. germanica* possesses white flight muscles that cannot support flight and *G. portentosa* lacks wings. These different behavioral combinations could correlate with differences in sensory processing of wind information by the cercal system. In this study, we focused on the wind-sensitive interneurons (WSIs) since they provide input to the premotor/motor neurons that influence terrestrial escape and flight behavior. Using extracellular recordings, we characterized the responses from the WSI population by generating stimulus-response (S-R) curves and examining spike firing rates. Using cluster analysis, we also examined the activity of individual units (four per species, though not necessarily homologous) comprising the population response in each species. Our main results were: 1) all four species possessed ascending WSIs in the abdominal connectives; 2) wind elicited the weakest WSI responses (lowest spike counts and spike rates) in *G. portentosa*; 3) wind elicited WSI responses in *Blab. craniifer* that were greater than *P. americana* or *Blat. germanica*; 4) the activity of four individual units comprising the WSI population response in each species was similar across species.

Keywords

Cercal; Wind; Blattaria; Escape; 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

Information gathering and behavioral control are two essential functions of the nervous system. Sensory systems allow organisms to extract information about their environment that the nervous system then uses, or ignores, to guide behavior. Additionally, how a sensory modality is incorporated into behavior can affect sensory processing of that modality in an organism. Therefore, how sensory information is encoded and used can vary across organisms. Comparative studies using a model system that involves a measurable behavior with neural correlates that are tractable and accessible for neurophysiological studies are valuable for understanding the neural mechanisms underlying how nervous systems extract, encode and use sensory information to guide behavior. The insect wind-sensitive cercal system, which has been the subject of many neurobiological and neuroethological studies to better understand general principles of nervous system function, can serve as this model system.

The cerci, two posterior appendages, contain filiform hairs that detect air currents and are one of the most sensitive sensory receptors in biology (Barth, 2000, 2004). A single sensory receptor cell attaches to the base of each filiform hair and its axon projects to the terminal abdominal ganglion (TAG). Within the TAG, the afferents monosynaptically connect to the population of wind-sensitive interneurons (WSIs) that often have large axon diameters and have been termed “giant interneurons” (or GIs). In some insects, the largest nine axons, many of which are WSIs, can comprise as much as 31% (*Periplaneta americana*) of the abdominal connective cross-sectional area (Boyan and Ball, 1986). The WSIs often occupy the dorsal and ventral intermediate tracts (DIT and VIT, respectively), but have also been found in other anatomical tracts, including the lateral and medial dorsal tracts (LDT and MDT, respectively), dorsal lateral tract (DLT), ventral medial tract (VMT), and ventral lateral tract (VLT). The WSI axons project 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). Current evidence indicates 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 cercal system has been mostly studied in Orthoptera (i.e. locusts and crickets) and Dictyoptera (i.e. cockroaches and mantids) (reviewed in Boyan and Ball, 1990; Camhi, 1984; Comer and Robertson, 2001; Ritzmann, 1984) as well as Thysanura (firebrats and silverfish; Edwards and Reddy, 1986). Although the basic elements of the cercal system are present across these insect groups, cercal systems vary greatly across species. These variations include differences in cercal morphology (i.e. cercal length, segmented vs. unsegmented cerci, orientation relative to the body axis), filiform hairs (i.e. number, length, and location of hairs), and WSIs (i.e. total number, anatomical tract location, axon diameters, and response properties). The thoracic connections between the WSIs and premotor/motor neurons likely also differ across species, but this has not been widely studied.

The two most common functions of 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). These behaviors have been mostly studied in the cockroach *P. americana* and several cricket species. In predator detection, wind directed at the cerci initiates a two-component terrestrial escape response consisting of a rapid initial turn away from the wind source followed by more variable running and/or, in the case of crickets, jumping (Baba and Shimozawa, 1997; Camhi and Tom, 1978; Dupuy et al., 2011; Tauber and Camhi, 1995). WSIs located in the VIT initiate the turn response (Westin et al., 1977) while WSIs located in the DIT are thought to be involved in the more variable running/jumping phase (Camhi and Nolen, 1981; Ritzmann and Pollack, 1981). WSIs located in the DIT also form the ascending pathway in a sensory feedback loop providing wind information to the flight central pattern generator (CPG) located in the thoracic ganglia (Libersat et al., 1989). This sensory feedback loop functions in maintaining flight through self-generated wind (i.e. “flight maintenance;” Fraser, 1977; Libersat and Camhi, 1988; Ritzmann et al., 1982) and altering flight behavior based on cercal-directed wind generated by external sources (i.e. bat predators; Ganihar et al., 1994). Flight maintenance appears to be a secondary function of WSIs within the DIT, and the cercal system in general, incorporated during the evolution of insect flight (Edwards, 1997).

In some species, such as *P. americana*, the cercal system mediates both functions and possesses WSIs with large diameter axons in both the DIT and VIT. However, this is not the case for all insects possessing cercal systems. For example, the praying mantis *Parasphendale agrionina* does not exhibit wind-evoked terrestrial responses, but males incorporate wind directed at the cerci into its flight responses (females do not fly) (Triblehorn, 2003). Furthermore, axons within the VIT occupy less connective space in male *Para. agrionina* compared to species that exhibit wind-mediated terrestrial escape responses while axons in the DIT occupy similar connective space as other species that fly and possess a cercal system. In female *Para. agrionina*, however, DIT axons occupy less connective space (Triblehorn et al., 2012). *Para. agrionina* and *P. americana* illustrate that variations in the cercal system may correlate with cercal system function in a species.

Differences in wind-mediated behaviors (terrestrial escape responses and flight maintenance) can also occur in closely related species. In cockroaches (Blatteria), wind puffs presented at room temperature evoke terrestrial escape responses in the German cockroach *Blattella germanica*, but not in the Madagascan hissing cockroach *Gromphadorhina portentosa*. Furthermore, Simpson et al. (1986) found that wind evoked weak terrestrial responses in *Blaberus craniifer* (Death’s head cockroach) compared to *P. americana*. These weaker responses would not be effective for evading capture despite *Blab. craniifer* possessing large diameter axons comparable to *P. americana* in the VIT of the abdominal connectives (Simpson et al., 1986). *G. portentosa* does not fly since the species lacks wings. Although both *Blab. craniifer* and *Blat. germanica* possess fully formed wings, only *Blab. craniifer* possesses pink flight muscle, which is correlated with flight ability, while *Blat. germanica* possesses white flight muscles that cannot support flight (Bell et al., 2007; Kramer, 1956). Compared to white flight muscles, pink flight muscles contain more

muscle fibers, larger muscle fibers, and have higher metabolic respiration rates since they possess more mitochondria (cytochromes contribute to the pink coloration of the muscle) and have higher activity from metabolic enzymes such as isocitrate dehydrogenase and citrate synthase (Zera et al., 1997).

Here, we compare the cercal system sensory processing of wind information in these four species (*P. americana*, *Blat. germanica*, *Blab. craniifer*, and *G. portentosa*) that vary in their wind-mediated behaviors. Our study focused on the WSI population since the WSIs provide input to the premotor/motor neurons that initiate and modify behavior. We used a wind stimulus similar to that used to study the behavioral terrestrial escape responses of *P. americana* (Camhi and Tom, 1978) and *Blab. craniifer* (Simpson et al., 1986), referred to as a “bulk flow” stimulus. Using this stimulus allowed us to make meaningful comparisons between our physiological responses of WSI activity to results from those behavioral studies. We characterized the neural responses by examining how the WSI population encodes wind velocity as well as the spike rates of WSI responses to different wind velocities. We also identified and analyzed individual units comprising the WSI population response.

2.0 Methods

2.1 Animals

This study examined wind-sensitive interneurons (WSIs) in four different cockroach (Blattaria) species: *Periplaneta americana* (Linnaeus, 1758) (Blattidae:Blattinae), *Blattella germanica* (Linnaeus, 1767) (Blattellidae:Blattellinae), *Gromphadorhina portentosa* (Schaum, 1853) (Blaberidae:Oxyhaloinae), and *Blaberus craniifer* (Burmeister, 1838) (Blaberidae:Blaberinae). These species provide examples of the four different combinations of the presence or absence of terrestrial escape response and flight maintenance wind-mediated behaviors (summarized in Table 1). 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 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 kept intact and placed outside the body cavity to expose the ventral nerve cord. Neural activity was recorded between A2 and A3 from one connective and both connectives were cut between A1 and A2 to record only ascending neural activity. For *P. americana*, *G. portentosa*, and *Blab. craniifer*, a silver wire (0.01” A-M Systems, Sequim, WA USA) hook electrode recorded neural activity. A mineral oil-Vaseline mixture was applied to prevent desiccation. Due to the small size of *Blat. germanica*, a well using sticky wax (Kerr’s) was built around the dorsal abdominal cuticle to hold enough saline (Fielden, 1960) to maintain a viable recording for the duration of the experiment. Therefore, a suction electrode recorded neural activity for *Blat. germanica*.

2.3 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 *Blab. 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. 1). 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). 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. 2).

Experiments were conducted within a 90 cm × 110 cm × 100 cm (H × W × D) Faraday cage (Fig. 1). 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 WSI 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 connective and 2) any responses to the solenoid opening/closing (as a control). Each wind velocity was presented consecutively ten times with a 15 s interstimulus interval. The eight different wind velocities were tested in a random order and there was a 90 s interval between testing each new wind velocity.

2.4 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). Spike counts occurring within the first 250 ms of the solenoid opening were counted using DataView version 6.3.2 (William Heitler, St. Andrews University) and averaged over the ten presentations for each wind velocity.

Species stimulus-response (S-R) curves were constructed as follows (Fig. 3). For each animal's data, the best fit curve (typically either a two phase decay, one phase decay, or linear curve fit) 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 of 0, 5, and 10 up to 250 cm/s in 10 cm/s intervals into each individual S-R curve

equation to obtain spike counts predicted by each S-R curve (Fig. 3B). These spike counts were then averaged for each wind velocity to generate the S-R curve for the species.

For each species, individual units were identified using principle components analysis followed by cluster analysis performed using DataView version 8.6.2 (William Heitler, St. Andrews University).

3.0 Results

3.1 Wind-evoked neural responses across species

Wind puffs evoked neural responses in all four cockroach species, indicating that each species possessed WSIs (Fig. 4). Multiple WSIs contributed to the neural responses based on the different spike amplitudes comprising each species' responses. Neural responses generated by the WSI population increased with wind velocity, from low range (< 10 cm/s) to medium range (108–125 cm/s range) to high range (> 185 cm/s) wind velocities across all species. There also was a qualitative difference in the WSI population response earlier in the stimulus compared to later in the stimulus in all four species, particularly at the higher wind velocities (indicated by dashed line placed 100 ms after stimulus onset, Fig. 4).

From these data, we generated stimulus-response (S-R) curves to: 1) quantify how the number of wind-evoked WSI spiking responses changed with increasing wind velocity and 2) compare the encoding of wind velocity by the WSI population across species. Since the neural responses were not consistent for the duration of the wind stimulus, we analyzed the wind-evoked responses occurring during the First 100 ms and Second 150 ms of the stimulus separately.

3.2 S-R relationships for the wind-sensitive interneuron population across species

S-R curves for neural responses occurring during the First 100 ms of the stimulus had similar shapes across species (Fig. 5A). All species S-R curves exhibited a rapid increase in spike counts at lower wind velocities before plateauing at higher velocities. Wind consistently evoked the least number of spikes from WSIs in *G. portentosa*, a species that does not fly and wind does not evoke an escape response (maximum average = 33 spikes). Both *P. americana* and *Blat. germanica* exhibit wind-mediated escape responses, but only *P. americana* is capable of sustained flight. Wind elicited slightly more WSI spikes in *P. americana* than *Blat. germanica* across all wind velocities (maximum average = 47 vs. 41 spikes respectively). However, wind elicited the strongest maximum average WSI responses in *Blab. craniifer* (49 spikes), a species that is capable of sustained flight but exhibits only a weak terrestrial escape response to wind stimuli.

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 between species. However, comparing the differences in response magnitude cannot determine whether the WSI population in each species actually encodes wind velocity differently. S-R curve shapes, which describe the encoding function of wind velocity by the WSI population, can be compared across species by normalizing the data. Normalization also better describes the dynamic encoding range limit of wind velocity of the WSI population, where increases in

wind velocity does not increase the number of WSI responses. To determine whether these species differed in how the WSI population encodes wind velocity, each species S-R curve (Fig. 5A) was normalized to the maximum spike count (Fig. 5B). We defined the dynamic encoding range limit as the wind velocity that elicited 0.75 proportion of the maximum response. A lower velocity indicated that the WSI population encodes wind velocity over a narrower wind velocity range.

The normalized responses to the First 100 ms were very similar across species, but did show some slight differences (Fig. 5B, Table 2). Both *Blab. craniifer* and *G. portentosa* had similar dynamic range limits (56 and 55 cm/s, respectively), but *Blab. craniifer* had a slightly shallower rise in its S-R curve at lower wind velocities compared to *G. portentosa*. Both *P. americana* and *Blat. germanica* had steeper rises in their normalized S-R curves compared to *Blab. craniifer* and *G. portentosa*. Consequently, *P. americana* and *Blat. germanica* had lower dynamic range limits (31 and 24 cm/s, respectively).

Variation in the WSI responses across individuals during the First 100 ms of the stimulus also showed species differences (Fig. 6A). Responses varied most in *P. americana* (> 8 SDs) and the least in *Blab. craniifer* (< 4 SDs over most of the wind velocities). *Blat. germanica* responses were more variable than *G. portentosa* at lower velocities (7 vs. 5 SDs), but were similar to each other at higher wind velocities (6 SDs).

During the Second 150 ms of the stimulus, WSI responses differed from the First 100 ms in three ways. First, wind evoked fewer spikes from the WSI population during the Second 150 ms in all four species, even though the interval was 50 ms longer (Fig. 5C). Based on the maximum spikes elicited, this ranged from a 17% decrease in *Blat. germanica* (from 41 to 34 spikes) to a 49% decrease in *Blab. craniifer* (from 49 to 25 spikes). Second, there were greater differences in wind velocity encoding between species compared to the First 100 ms (Fig. 5D, Table 2). Two species, *P. americana* and *Blab. craniifer*, exhibited less encoding of wind velocity during the Second 150 ms of the stimulus. The S-R relationship for *P. americana* was virtually flat over most of the wind velocity range (dynamic range limit = 3 cm/s) indicating this species is not encoding wind velocity over most of this range (or at least there is no relationship between wind velocity and WSI spike responses in *P. americana* during the Second 150 ms of the stimulus). *Blab. craniifer* was similar, but had a higher dynamic range limit (25 cm/s) than *P. americana*. Conversely, both *Blat. germanica* and *G. portentosa* exhibited encoding of wind velocity over a wider range during the Second 150 ms. *Blat. germanica* showed a linear increase with wind velocity and was the only species where the response did not plateau within the range tested (thus, no dynamic range could be calculated, Table 2). The S-R curve for *G. portentosa* did plateau, but had a shallower rise resulting in a higher dynamic range limit (66 cm/s). Third, the individual variation in WSI responses was greater in three of the species (*Blat. germanica*, *G. portentosa*, and *Blab. craniifer*) during the Second 150 ms of stimulus, especially at higher wind velocities (Fig. 6B). The variation was similar in *P. americana*, which was already high during the First 100 ms of the stimulus (> 8 SDs).

3.3 Firing rates for the wind-sensitive interneuron population across species

The spike rate of the WSI population can be as important as the number of spikes elicited by wind stimuli for controlling behavior. We examined differences in the spike rate of the WSI population across species during the 250 ms stimulus duration across three velocity ranges: Low (< 50 cm/s), Medium (51–140 cm/s), and High (> 141 cm/s) (Fig. 7).

For both *P. americana* (terrestrial escape response and flight) and *Blat. germanica* (terrestrial escape response and no flight), stimulus onset elicited the maximum spike rate (600–700 spikes/s) by 25 ms after stimulus onset for all three wind velocity ranges. At the Low velocity range, the spike rate immediately began decreasing gradually before reaching a steady spike rate that was higher for *P. americana* (100–150 spikes/s) than *Blat. germanica* (< 50 spikes/s). At the Medium velocity range, the spike rate dropped after stimulus onset but remained elevated (500–600 spikes/s) before gradually decreasing to a steady spike rate that was similar to the Low velocity range (50–100 spikes/s for *Blat. germanica*; 90–120 spikes/s for *P. americana*). At the High velocity range, the maximum spike rate for *P. americana* was higher than *Blat. germanica* (> 600 spikes/s compared to 500–600 spikes/s, respectively), but the spike rate remained elevated for a slightly longer period in *Blat. germanica* than *P. americana* before gradually decreasing to a higher steady spike rate (150–200 spikes/s).

For *Blab. craniifer* (flight and weak terrestrial escape response), stimulus onset elicited the maximum spike rate (about 550 spikes/s) by 30 ms at the Low velocity range. The spike rate remained elevated (> 400 spikes/s) longer than *P. americana* or *Blat. germanica* before decreasing to a steady spike rate of 50–100 spikes/s. At both Medium and High velocity ranges, the maximum spike rates (600–650 spikes/s) were similar to *P. americana*, but the spike rates remained elevated for a longer duration than the other species. Furthermore, *Blab. craniifer* spike rates were higher over the entire wind stimulus duration compared to the other species, as the decline in the spike rates was slower and the steady spike rate was higher (200–300 spikes/s).

For *G. portentosa* (no terrestrial escape response and lacks wings), the onset of the stimulus also elicited the highest spiking rates, but were consistently lower than the other three species across wind velocity ranges (480–520 spikes/s). Furthermore, the maximum spiking rate occurred in *G. portentosa* later than the other three species (35 ms after stimulus onset). The decrease in spiking rate followed a similar pattern as *P. americana* and *Blat. germanica*, reaching a steady rate of 50–100 spikes/s across wind velocities.

3.4 Activity of individual units across wind velocity ranges

Species differences in sensory processing of wind stimuli could appear in the response properties of individual units comprising the WSI population response. Therefore, we performed principle components analysis followed by cluster analysis on the spikes comprising the WSI population response to identify, analyze and compare the activity of individual units in all four species. In each species, we were able to classify four units and identified them as Units 1–4 based on increasing amplitude (i.e. Unit 1 = smallest amplitude, Unit 4 = largest amplitude). We analyzed the responses of these four units to four different

velocity regions within the 0–250 cm/s wind velocity range: Lowest (5 ± 3 cm/s; mean $\pm$ SD), Low (37 ± 9 cm/s), Medium (114 ± 12 cm/s), and High (185 ± 11 cm/s). The response properties of the four units differed and their activity varied with wind velocity, both in spike counts (Fig. 8) and spike rates (Fig. 9). However, each unit's response properties were very similar across species. Although we do not claim that these four units are homologous among the four species, we do not completely discount that possibility.

Unit 1 spike counts did not change with wind velocity except in *Blat. germanica*, where spike counts decreased with increasing wind velocity during the First 100 ms (Fig. 8). However, spike counts increased with wind velocity during the Second 150 ms in all species. Unit 2 spike counts exhibited an inverted U-shape relationship with wind velocity during the First 100 ms of the stimulus in all species, as spike counts increased with wind velocity but decreased at the High velocity. During the Second 150 ms, Unit 2 spike counts increased with wind velocity in *Blat. germanica*, plateaued after increasing from the Lowest to Low velocity in *Blab. craniifer* and *G. portentosa*, and did not vary with wind velocity in *P. americana* (Fig. 8).

Both Units 1 and 2 were the most active during the entire stimulus duration (Fig. 9). In most species, Unit 1 spike rate increased gradually and maintained a steady firing rate, especially in response to the High velocity. The exception was *Blab. craniifer* as Unit 1 activity increased rapidly at response onset to all four wind velocities, which began to decrease around 100 ms after stimulus onset. Unit 2 activity increased rapidly at response onset in all species before decreasing to a steady rate around 100 ms. Unit 2's rapid increase was greater for *Blat. germanica* and *Blab. craniifer* than *P. americana* or *G. portentosa*.

Units 3 and 4 (the largest amplitude units) exhibited very similar activity across species. Both Units 3 and 4 spike counts increased with wind velocity during the First 100 ms of the stimulus (Fig. 8). Although Unit 4 was largely inactive during the Second 150 ms of the stimulus, Unit 3 remained active, but with a lower spike count that generally increased with wind velocity (except in *P. americana*). Both Units 3 and 4 were the first to respond in all four species (Fig. 9). Increasing wind velocity not only increased the number of spikes produced by these units, but also prolonged the duration of their high spike rate activity before decreasing (Unit 3) or ceasing (Unit 4).

4.0 Discussion

In this study, we investigated four cockroach (Blattaria) species that possess a wind-sensitive cercal system, but differ in whether they exhibit two cercal-mediated wind behaviors: terrestrial escape responses and flight maintenance. Wind only evokes terrestrial escape responses in *P. americana* and *Blat. germanica*. In *Blab. craniifer*, wind only elicits weak terrestrial escape responses and elicits no terrestrial escape responses in *G. portentosa*. Flight has been well-characterized in *P. americana* (flight speed ~ 200 cm/s; Ganihar et al., 1994), which uses cercal-directed wind to maintain and adjust flight behavior (Fraser, 1977; Ganihar et al. 1994; Libersat and Camhi, 1988; Ritzmann et al., 1982) while *G. portentosa* does not fly since this species lacks wings. *Blab. craniifer* possesses pink flight muscles capable of supporting sustained flight while *Blat. germanica* possesses white flight muscle

that cannot support flight (Bell et al., 2007; Kramer, 1956). The diversity of wind-mediated behaviors exhibited by these four species make them ideal for comparatively investigating differences in the neural components underlying these behaviors, including the processing of wind sensory information. Here, we focused on the WSIs since these neurons provide the “driving force” for behavior by providing the input to premotor/motor neurons that generate behavior. We measured the response properties of the WSI population (both number of spikes and spike rate) as well as individual units comprising the population response.

Our main results were: 1) all four species possessed ascending WSIs regardless of whether the species exhibits a strong wind-mediated terrestrial escape response with (*P. americana*) or without (*Blat. germanica*) flight ability, a weak wind-mediated terrestrial escape response with flight ability (*Blab. craniifer*), or no terrestrial escape response and lacks flight ability (*G. portentosa*); 2) wind elicited the weakest WSI population responses (lowest spike counts and spike rates) in *G. portentosa*; 3) wind elicited WSI population responses (spike counts and spike rates) in *Blab. craniifer* that were equivalent to or greater than *P. americana* or *Blat. germanica*; 4) the activity of four individual units comprising the WSI population response in each species was similar across species. However, we cannot determine from extracellular recordings whether these units include the activity of one or multiple WSIs and do not claim that these four units are homologous among the four species. We will relate our results to the differences in wind-mediated behavioral responses in these four species.

4.1 The First 100 ms of the stimulus: The initiation of terrestrial escape responses

The First 100 ms of our stimulus contained the acceleration component of the wind stimulus, which initiates the rapid turn in terrestrial escape responses (Plummer and Camhi, 1981). Based on previous work in *P. americana* (Westin et al. 1977) and the cricket *Gryllus bimaculatus* (Hirota et al., 1993), WSIs located in the VIT respond phasically at a high firing rate to the acceleration component of wind stimuli (Kanou and Shimozawa, 1984) and activate the thoracic motor regions to initiate terrestrial escape responses (Casagrand and Ritzmann, 1991; Ritzmann, 1981; Ritzmann and Camhi, 1978; Ritzmann and Pollack, 1981, 1986, 1988, 1990; Westin et al., 1988). The WSIs with the largest axon diameters are found in the VIT in *P. americana* (Daley et al. 1981). In all four species, Units 3 and 4 likely represent activity of WSIs located in the VIT since they responded phasically (Figs. 8, 9) and had the largest spike amplitudes (spike amplitude is correlated with axon diameter) (Fig. 9, inset). Species differences in sensory processing of wind information by the WSI population and individual units that may be related to initiating terrestrial escape responses would occur during the First 100 ms of the stimulus.

In the two species that exhibit strong terrestrial escape responses (*P. americana* and *Blat. germanica*), wind elicited a similar number of spikes in the WSI population during the First 100 ms of the stimulus (Fig. 5A). The spike rate of the WSI population in these two species was also similar, though the spike rate during the First 100 ms in *P. americana* was slightly higher at wind velocities > 141 cm/s. There were no notable differences in the spike counts (Fig. 8) or spike rates (Fig. 9) of the individual units between these two species. In *G. portentosa* (no terrestrial escape response), wind elicited less spikes in the WSI population than *P. americana* and *Blat. germanica* (Fig. 5A). Thus, the WSIs in *G. portentosa* provide

less input to the premotor/motor neurons. A fast spike rate could compensate for the fewer spikes produced through temporal summation. However, *G. portentosa* also had a lower spike rate from WSIs at all three wind velocity ranges (Fig. 7). Units 2 and 4 in *G. portentosa* had lower spike counts compared to the other species and contributed most to the decreased activity in the *G. portentosa* S-R curve during the First 100 ms of the stimulus (Fig. 5A). In *Blab. craniifer* (weak terrestrial escape response), both WSI population spike counts and spike rates were equivalent to or stronger than those recorded in *P. americana* and *Blat. germanica* (Figs 5A, 7). In fact, at Medium and High wind velocity ranges, the *Blab. craniifer* WSI population maintained a faster spike rate for a longer duration than the other species (Fig. 7B,C). The activity (spike counts and spike rates) of individual units in *Blab. craniifer* was also similar to either *P. americana* or *Blat. germanica* or both (Figs. 8, 9).

The shapes of the S-R curves for the First 100 ms for all species were similar (Fig. 5A,B), but *P. americana* and *Blat. germanica* dynamic range limits were almost half that of the other two species (Table 2). This lower dynamic range suggests that encoding wind velocity may not be important in species that exhibit strong terrestrial responses. The cercal system may simply monitor the presence/absence of wind based on a threshold velocity (an “on/off switch” instead of a “dimmer switch”) to initiate escape responses. In *Blab. craniifer*, the strong wind-evoked WSI responses with the larger dynamic encoding range seems paradoxical to the weak behavioral responses described by Simpson et al. (1986). These results suggest that it may be worthwhile to reinvestigate wind-evoked terrestrial responses in *Blab. craniifer*. The larger dynamic encoding range in *G. portentosa*, along with the reduced WSI spike counts and spike rates suggest there may be a different role for cercal directed wind in this species, which will be discussed below.

The lack of a wind-mediated terrestrial escape response in *G. portentosa* could be partially explained by the reduced WSI spike counts and spike rates. These reduced WSI responses could fail to activate thoracic motor regions and prevent a cercal-mediated wind-evoked terrestrial response. Although this reduced response could contribute to the lack of a wind-mediated terrestrial response in *G. portentosa*, other neural mechanisms are also likely involved. The potential for other neural mechanisms is illustrated by *Blab. craniifer*, which only exhibits a weak wind-mediated escape response even though the WSI population responses (both spike counts and spike rates) are as great as or greater than those in *P. americana* and *Blat. germanica*. The activity of the individual units is also similar between these three species. These results suggest that these *P. americana*, *Blab. craniifer*, and *G. portentosa* would be useful systems for further investigating the neural mechanisms underlying the lack of a wind-mediated escape response in *Blab. craniifer* and to provide a better understanding of the basic neural mechanisms involved in controlling behavior.

4.2 The Second 150 ms of the stimulus: Continued running and flight maintenance behavior

The Second 150 ms of our stimulus contained the constant velocity component of the wind stimulus. The cercal system uses the velocity component to maintain flight behavior (Fraser, 1977; Libersat and Camhi, 1988; Ritzmann et al., 1982) and may mediate the continued

running phase of the terrestrial response, which is less stereotyped than the initial turn (Camhi and Nolen, 1981; Ritzmann and Pollack, 1981). WSIs located in the DIT that respond tonically to the velocity component of wind stimuli (Kanou and Shimozaawa, 1984) mediate these responses. WSIs in the DIT have smaller diameter axons than those in the VIT in *P. americana* (Daley et al. 1981). In all four species, Units 1 and 2 likely represent activity of WSIs located in the DIT since they responded tonically (Figs. 8, 9) and had the smallest spike amplitudes (Fig. 9, insets). Species differences in sensory processing of wind information by the WSI population and individual units that may be related to flight maintenance and continued running in the terrestrial escape response would occur during the Second 150 ms of the stimulus.

In all four species, the total spikes generated by the WSI population during the Second 150 ms (Fig. 5C) decreased across wind velocities compared to responses during the First 100 ms (Fig. 5A). This drop in the population response was largely due to decreased activity in Units 3 and 4 during the Second 150 ms of the stimulus in all four species (Fig. 8). Unit 4 activity was essentially absent during the Second 150 ms of the stimulus (Figs. 8, 9) while Unit 3 activity continued at a reduced spike rate (Fig. 9). Unit 2 activity also decreased during the Second 150 ms of the stimulus in *Blat. germanica* and *Blab. craniifer*, but was similar as during the First 100 ms in the other two species (Fig. 8). The differences in Unit 2 activity could be due to variation in spike rate adaptation across the species. In all four species, Unit 1 activity during the Second 150 ms either increased or was similar to activity during the First 100 ms.

Only *G. portentosa* and *Blat. germanica* (both non-flyers) exhibited any encoding of wind velocity by the WSI population during the Second 150 ms of the stimulus. In both species, spike counts increased with wind velocity though the S-R relationships differed between species (Fig. 5C,D). *Blat. germanica* had a linear S-R relationship, with greater increases in WSI spikes across the wind velocity range while *G. portentosa* had a two phase decay S-R relationship that plateaued at higher wind velocities. The fairly flat S-R curves of *P. americana* and *Blab. craniifer* suggest that the WSI population in these species monitor the presence of wind, but do not encode velocity and wind velocity may not affect flight maintenance. In these two species, the activity of the individual units reflected the activity of the WSI population represented in the S-R curves. In *G. portentosa* and *Blat. germanica*, most spike counts of the individual units increased with increasing wind velocity. In *Blab. craniifer*, unit spike counts only increased between the Lowest and Low wind velocities. Spike counts did not vary with wind velocity in *P. americana* except for Unit 1 to the High velocity.

The neural basis of continued running during wind-evoked escape responses is not as well-understood as the initial turn. Input provided by the WSIs during the Second 150 ms to the thoracic motor regions could affect whether the wind stimulus evokes continued running in *P. americana* and *Blat. germanica*. For *Blat. germanica*, the increasing linear S-R relationship of the WSI population (reflected also in individual unit activity) suggests that higher wind velocities may either increase the probability of evoking a continued running response or increase the magnitude of the response (i.e. velocity, acceleration, and/or distance), or both. For *P. americana*, the WSI population S-R relationship was flat as the

number of spikes did not vary with wind velocity during the Second 150 ms of the stimulus (also reflected in the activity of the individual units). This suggests only the presence of wind may be important and the continued running response could be independent of wind velocity. The potential influence of wind velocity on both continued running and flight maintenance can be tested behaviorally in these species and is the focus of future experiments.

4.3 Individual differences in WSI responses: An indicator of the importance of wind velocity encoding in a species?

Traits important for a species' fitness, such as anti-predator defenses or mate-attraction signals, may exhibit little variation across individuals. Therefore, the degree of individual variation could suggest the level of importance a trait has in a species. Here, a low degree of variation in WSI responses, indicated by low standard deviations (Fig. 6), suggests that encoding wind velocity is potentially important to the species while higher standard deviations suggests the opposite.

P. americana exhibited the greatest variation (> 8 SDs), which seems counterintuitive since this species exhibits a strong wind-evoked terrestrial escape response and uses wind to maintain flight. However, this supports the previous assertion that the cercal system may simply monitor the presence/absence of wind in this species. *G. portentosa* exhibited a low degree of variation in WSI responses, especially during the First 100 ms of the stimulus.

Blab. craniifer had the lowest degree of variation, which is interesting since wind also evoked the most WSI responses with the highest spike rates during the First 100 ms of the stimulus. The low degree of variation further suggests that the cercal system may underlie some other function where wind velocity may be important, but also that it may be worthwhile to reinvestigate wind-evoked terrestrial escape responses in *Blab. craniifer*.

4.4 The potential role of wind information and the cercal system in *G. portentosa*

Although *G. portentosa* lacks wings and wind does not elicit terrestrial escape responses, there are several potential explanations why *G. portentosa* possesses a cercal system. One possibility is that *G. portentosa* once exhibited a wind-evoked terrestrial escape response, but it is no longer beneficial due to a different lifestyle and the cercal system is vestigial. Similarly, *G. portentosa* may exhibit cercal-mediated terrestrial escape responses only in the nymphal stages and the neural circuit declines as *G. portentosa* become adults. Several studies have shown that both wind-mediated behavioral responses as well as the neural responses from WSIs can be stronger during the nymphal stages compared to the adult stage (Dangles et al., 2007; Matsuura and Kanou, 2003, 2005). A third possibility is that wind could evoke other defensive behaviors. *G. portentosa* is widely known as the "hissing cockroach" due to the sound generated by expelling air from modified spiracles (Guthrie, 1966; Roth and Hartman, 1967). This hissing sound can be used to startle predators. Wind may also evoke responses in *G. portentosa* that make them more cryptic, such as flattening their body against a substrate or move further into a hiding space. In nymphal praying mantids, visual stimuli can evoke "cryptic ducking" responses (Liske et al., 1999) and wind evoked similar responses in adult male *Para. agrionina* mantids (Triblehorn, 2003). A

fourth possibility is that *G. portentosa* use their cercal system in communication. The filiform hairs on the cerci would be the likely receivers for the hissing communication signals. The encoding of wind velocity for both the First 100 ms (55 cm/s dynamic range limit) and Second 150 ms (66 cm/s dynamic range limit) for *G. portentosa* suggests that the magnitude of generated “hissing” (i.e. velocity of expelled air) could elicit differences in spiking responses of nearby individuals and, as a result, different behavioral responses. The low degree of variation in the WSI responses during the First 100 ms of the stimulus (Fig. 6) would be consistent with this potential function of the cercal system. Behaviors elicited through communication signals would potentially be subtle and occur over a longer period of time than terrestrial escape responses. Carefully designed and controlled experiments would be necessary to determine whether *G. portentosa* use wind detected by the cerci for intraspecific communication.

4.5 Comparative studies of the insect wind-sensitive cercal system in neurobiological research

The insect wind-sensitive cercal system is well-suited for neurobiological investigations since it consists of a well-known neural circuit that is accessible to physiological and anatomical techniques and linked to quantifiable behaviors. Insects represent a diverse group of animals that inhabit almost every ecological niche available. Reflecting this diversity, the cercal system’s morphological and neural components vary across species based on the evolutionary history and behavioral needs of the individual species, which includes groups where the cercal system is absent (Hemiptera, Neuroptera, and all holometabolous insects; Edwards and Reddy, 1986). These differences could also extend to the neural mechanisms involved in cercal system function and even lead to different emergent properties of the neural circuit. Comparative studies that take advantage of this diversity and accessibility are valuable, if not essential, for identifying and targeting these mechanisms for further investigations. Such studies could reveal new neural processing mechanisms that may also occur in other invertebrate and vertebrate systems. These investigations could also provide additional insight into cercal system evolution and provide a better understanding of how nervous systems evolve in general. Continued investigations will focus on other levels of the neural circuit (such as the encoding of wind stimuli by the filiform afferent population) and species from other insect groups as well as additional Blattarian species.

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Highlights

- We compared cercal wind-sensitive interneuron responses in four cockroach species.
- Species differed in exhibiting wind-evoked escape responses or flight ability.
- All four species possessed ascending wind-sensitive interneurons.
- The species lacking both wind-evoked behaviors exhibited weakest neural responses.
- The species with a weak escape response but flies exhibited strongest responses.

Fig. 1. Schematic of the experimental setup for recording WSI responses to different wind stimulus velocities

Wind velocity was varied by adjusting the pressure from a « compressed air source using a regulator and a solenoid valve controlled wind presentation. Air passed from the solenoid valve through a tube filled with coffee stirrers to laminarize the wind. A hot-wire anemometer placed between the tube and the insect preparation measured each wind stimulus presentation. The solenoid valve was decoupled from the recording setup to prevent vibration responses to the solenoid opening and closing.

Fig. 2. Representative hot-wire anemometer traces for three wind velocities (64, 126, 232 cm/s) (A) Ten superimposed anemometer traces for the three different velocities, demonstrating the consistency of the wind stimulus with each presentation. (B) The average waveform of the ten anemometer traces from (A), which was used to measure the wind velocity. Dashed line indicates 100 ms after the solenoid opened.

Fig. 3. Construction of species stimulus-response (S-R) curves from individual S-R curves
 (A) Spike counts (squares) were collected for seven different wind velocities spanning the 1–250 cm/s range as well as 0 cm/s (solenoid opening and closing without wind) for each individual. The S-R curve for the individual was generated by using a linear, one phase decay, or two phase decay function to the eight data points using a least squares fit model (curve fit based on highest R^2 value). In this example from an individual *Blab. craniifer*, the two phase decay function (line) fit best ($R^2 = 0.9984$; $y = 51.42 - 21.2738 * e^{(-803.4 * x)} - 28.24621 * e^{(0.03293 * x)}$) and became that animal's S-R curve. (B) Wind velocities of 0, 5,

and 10 up to 250 cm/s in 10 cm/s intervals were entered into each individual's S-R curve ($y = 51.42 - 21.2738 * e^{(-803.4 * x)} - 28.24621 * e^{(0.03293 * x)}$ in this example) to obtain predicted spike counts (indicated by filled circles). These spike counts 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 WSI responses at three wind velocity ranges demonstrating all four species possess WSIs

All three traces come from one individual per species. Dashed line indicates 100 ms after the solenoid opened. In all four species, there is a difference in the WSI neural responses occurring during the First 100 ms and Second 150 ms of the stimulus (marked by the dashed line). More neural responses and larger amplitude spikes characterize the neural response during the First 100 ms of the stimulus. Also, neural responses increase with increasing wind velocity in all three species.

Fig. 5. Stimulus-Response (S-R) curves for the WSI population for four cockroach species
 (A) S-R curves for WSI spike counts during the First 100 ms of the stimulus. In all four species, spike counts increased rapidly at low wind velocities and plateaued at higher wind velocities. (B) Normalized S-R curves for WSI response during the First 100 ms of the stimulus. Each species' S-R curve was normalized to the maximum spike count in (A). All species encoded wind velocity similarly, but had slightly different dynamic encoding range limits (see text and Table 1). (C) S-R curves for WSI spike counts during the Second 150 ms of the stimulus. Less spikes occurred during the Second 150 ms of the stimulus compared to the First 100 ms in all four species and there were greater species differences in the Second 150 ms S-R curves. (D) Normalized S-R curves for WSI response during the Second 150 ms of the stimulus. Each curve was normalized to the maximum spike count in (C). Species

encoded wind velocity differently during the Second 150 ms of the stimulus. *P. americana* n= 15; *Blat. germanica* n = 12; *Blab. craniifer* n = 12; *G. portentosa* n = 11.

Fig. 6. Individual variation in WSI responses across wind velocities as measured by the standard deviation

(A) During First 100 ms of the stimulus. (B) During Second 150 ms of the stimulus. In all species except *P. americana*, there was more individual variation during the Second 150 ms than during the First 100 ms. Same individuals as in Fig. 5.

Fig. 7. Instantaneous spike rates for WSI responses to different wind velocity ranges (A) Low wind velocity range (< 50 cm/s). (B) Medium wind velocity range (51–140 cm/s). (C) High wind velocity range (> 141 cm/s). In all four species, fastest spike rates occurred at stimulus onset and remained elevated for most of the First 100 ms of the stimulus before gradually decreasing to a steady rate (< 200 spikes/s) during the Second 150 ms of the stimulus. In general, instantaneous spike rates were highest in *Blab. craniifer* and remained elevated for a longer duration while spike rates were lowest in *G. portentosa*. *P. americana*

and *Blat. germanica* were similar though *P. americana* had higher spike rates at stimulus onset in the High velocity range. Same individuals as in Fig. 5.

Fig. 8. Spike counts for four identified units in each species to four different wind velocities
 The top row contains all spikes sorted into four units from one experiment using *Blat. germanica*, but is representative of all species. Units were numbered 1–4 in each species based on increasing spike amplitude. Columns contain spike counts for each unit while rows contain data from different species. Spike counts are divided into those occurring during the First 100 ms and Second 150 ms of the stimulus. Data are from four different wind velocities: Lowest (5 ± 3 cm/s; mean $\pm$ S.D.), Low (37 ± 9 cm/s), Medium (114 ± 12 cm/s),

High (185 ± 11 cm/s). Data came from six individuals per species. See text for additional details.

Fig. 9. Spike rates for the four identified units in each species to four different wind velocities
 The top row contains all spikes sorted into four units from one experiment using *Blat. germanica*, but is representative of all species. Units were numbered 1–4 in each species based on increasing spike amplitude. Columns contain spike counts for each unit while rows contain data from different species. Same wind velocities and individuals as in Fig. 8. See text for additional details.

Table 1

Wind-evoked behavioral responses exhibited by species included in study

Species	Terrestrial escape response?	Flight maintenance?
<i>Periplaneta americana</i>	Yes	Yes
<i>Blattella germanica</i>	Yes	No
<i>Blaberus craniifer</i>	No	Yes
<i>Gromphadorhina portentosa</i>	No	No

Table 2

Calculated dynamic encoding velocity range limits

Species	First 100 ms	Second 150 ms
<i>Periplaneta americana</i>	31 cm/s	3 cm/s
<i>Blattella germanica</i>	24 cm/s	Not defined
<i>Blaberus craniifer</i>	56 cm/s	25 cm/s
<i>Gromphadorhina portentosa</i>	55 cm/s	66 cm/s