# ARTICLE
# Studying Temporal Properties of Stimulus-Evoked Responses in the Ventral Nerve Cord of Insects

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Students in undergraduate laboratory settings learn many of the foundational principles of sensory processing in the comparatively simple, easy to study invertebrate nervous system. In this example preparation, the American cockroach, students record action potentials from the fibers in the ventral nerve cord (VNC) that participate in a well explained escape behavior in response to stimulation of its cerci, a pair of mechanosensitive abdominal appendages. A system that allows good control over the time and amplitude of the air pulse delivered to the cerci is described. This experimental setup enables students to extract and display temporal information from recordings to learn how to interpret those responses in the context of the properties of the stimulus. I offer examples of specific investigations and analyses that work well for this purpose in an undergraduate laboratory.

*Key words: electrophysiology, cerci, giant fibers, interspike interval, autocorrelation, adaptation, phase locking*

Many college level neurobiology courses feature lessons on the importance of temporal information carried by neural networks that mediate behaviors such as the jamming avoidance response in the weakly electric fish, echolocation in insectivorous bats, and localization of the azimuth of a sound source by the owl (Carew, 2000; Zupanc, 2010). However, students often find it challenging to understand the variety of ways that the time-of-occurrence of action potentials can be displayed to reveal information about the behavior of cells as a step towards building models of circuits. This lab module gives students the opportunity to gain practical experience applying these types of analyses using the action potentials they have recorded in the ventral nerve cord (VNC) preparation of the American cockroach. The VNC is a tractable system for students at the undergraduate level to record electrophysiological responses to mechanical stimulation of the cerci (a pair of abdominal appendages receptive to air movements). Axons emanating from the directionally sensitive filiform hairs that cover each cercus contact giant interneurons that travel along the VNC to contact thoracic ganglia that coordinate escape from predators. Evasive responses can be rapidly evoked at a peak air speed as low as 1-5 mm/s accounting for the detection of predators from distances of at least 10 cm away (Camhi et al., 1978; Camhi and Tom, 1978; Camhi, 1980). In some species of insects filiform hairs may be selectively sensitive to periodic displacements of the wingbeats of predators (Tautz, 1977).

The cockroach VNC preparation is commonly employed to study sensory-motor integration in the central nervous system because students can master all of the manual skills necessary to record multiunit activity within the time constraints typical of most college-level lab sections. Rather than investing time trying to overcome technical challenges, students may be asked to conduct experiments that they design and later, when off-line, can analyze by methods traditionally employed by sensory physiologists. However, one challenge to studying mechanosensitive VNC responses in an undergraduate level course with a limited budget is the availability of a system that can deliver pulses of air with a pressure and duration that can be directly controlled (e.g., by a picospritzer). Although more convenient practices like squeezing air from a pipette bulb may well simulate a stimulus that a cockroach might encounter in nature and be effective in promoting discharges, it is not suitable if the pedagogical goal is to learn how to apply analytical techniques that depend upon referring unit activity to the timing of the stimulus (e.g., peri-stimulus time histograms [PSTH]). That limitation becomes particularly apparent when studying how the patterns embedded in the timing of the response contributes to the encoding of the physical attributes of the stimulus. This article gives suggestions for a system that allows control of stimulus properties and works well in evoking robust responses from the VNC. In addition, I describe how students can use those responses to observe and quantify the components of the basic mechanisms underlying temporal performance including phase locking, precision, and adaptation.

## MATERIALS AND METHODS

The results reported in this article were collected by students who were enrolled in a junior level Neurobiology course and who had no previous training with electrophysiological techniques. Despite being relatively untrained, two sessions (each three hours) provided them with ample time to master the dissection, conduct experiments, and make significant progress on analyzing recordings. Preparation of figures was typically completed outside of the scheduled time.

### *Dissection*

A full description of the dissection to expose the ventral nerve cord of the American Cockroach *Periplaneta americana* can be found in Oakley and Schafer (1978). In

order to make the cerci accessible to stimulation the animal was anchored with insect pins near the surface of a glass Petri dish filled with paraffin wax or Sylgard (Dow).

*Recording*

Students routinely required only one or two attempts to perform a clean dissection and obtain multiunit recordings with waveform characteristics, response properties and spontaneous activity that resembled what has been previously reported for giant fibers and the cercal nerve (Dagan and Parnas, 1970; Libersat and Camhi, 1988; Watson and Ritzmann, 1994). Bipolar electrodes were fashioned from a pair of 0.25 mm silver wires (catalog# AGW1030, WPI Instruments), with tips slightly bent, and housed in a glass tube that can be lowered directly on the dorsal surface of the VNC by a micromanipulator (M3301, WPI Instruments). When a micromanipulator was not available, a surgical forceps (#7) was used to carefully position a pair of wires just under the VNC. The signal detected at the electrode was amplified by 0.5-2 K in differential mode, filtered 0.3 – 3 kHz (Dagan EX-1), and monitored audiovisually with an oscilloscope (40 MHz, B&K Precision) and battery-operated speaker (Radio Shack). Raw recordings were digitally sampled at a minimum of 20 kHz (Powerlab w/CHART7 software; ADI Instruments). A stainless steel platform (Kinetic Systems) was used to secure instruments mounted on magnetic stands (e.g., micromanipulator), but vibration isolation was not necessary to obtain recordings that remained stable for over three hours. Shielding of spurious electromagnetic signals with a Faraday cage was not necessary (for advice on minimizing noise in recording setups see Paul et al., 1997).

*Mechanosensory stimulation*

A system that delivered natural-like stimuli to the cerci was able to (1) evoke reliable VNC responses and (2) allowed control of stimulus strength and timing. A robust burst of air pressure was produced by either a mechanical wave driver (Pasco), a tool commonly used in physics lab to study the vibration of strings, or a battery operated audio speaker (Radio Shack). The movement of the piston of the wave driver or the diaphragm of the speaker was powered by a square pulse of variable duration, strength and frequency from either a stimulator (Grass SD-9 or A&M Systems Model 2100) or function generator (Instek or B&K Precision). Either the trigger signal or stimulus itself was recorded on a second channel of the acquisition system as a reference for the construction of histograms. Stimuli presented at low frequencies were, like natural stimuli, effective in the near field up to a distance of several centimeters, a distance of separation of a fraction of the stimulus wavelength. Measurement of the signal pressure produced by a speaker was made by a sound level meter (C weighting, fast integration mode; Radio Shack) positioned at the preparation. A pressure of around 60-70 dB was sufficient to reliably evoke VCN discharges when the speaker was positioned within the near field.

*Analysis*

Students were trained to separate a single giant fiber response among multiple units for subsequent plotting of histograms using either the spike histogram module available for Chart (ADI Instruments) or custom written routines in Matlab (Mathworks). Figures and statistics were prepared in Sigmaplot 12 (Systat).

## RESULTS

*Regularity (Phase locking)*

The regularity of discharges was apparent in the response to audio presentation of a train of square pulses at variable frequencies. In the case of the unit firing the largest action potential in figure 1A, a single spike was evoked by the offset (rarefaction) phase of each pulse delivered at 2 Hz (above), and the second, smaller unit preferred the onset (condensation) phase. At 5 Hz (below), the larger unit continued to phase lock although it did not fire to every cycle of the stimulus waveform. The strict regularity of the discharges apparent to the eye is displayed in the interspike interval histogram (ISIH; figure 1B) and the autocorrelation histogram (ACH, figure 1C).

Simply, the ISIH tallies and displays the intervals between successive action potentials and the ACH does the same for the intervals between each spike and *all* subsequent spikes. Any apparent periodicity, whether stimulus-driven or intrinsic, should be evident in the position of any peaks along the dimension of time. Here, the largest peak of the ISIH centered at the period of the stimulus is accompanied by successively smaller peaks positioned at whole integer intervals of the stimulus periods. The greater number of “harmonics” appearing in the response to 5 Hz stimulus is likely attributable to adaptation. Any apparent suggestion of the presence of phase locking in the raw record can be corroborated with a period histogram (Figure 1B, inset), which plots the cumulative scattering of spikes over the period of the stimulus. In this case, the narrow distribution of spikes reveals robust phase locking. If statistical verification of the strength of neural phase locking is desired refer to the classic article of Goldberg and Brown (1969) for a description of vector strength (which is equivalent to the first Fourier component of the normalized period histogram).

*Precision*

Displacement of the tactile hairs on the cerci produces a pattern of one to few spikes with regular latency in giant fibers. The narrow width of the peaks (5 ms bin width) in the ACH indicates that the giant fiber discharges fairly precisely to the phase of the stimulus. That precision, or temporal jitter, was quantified by delivering a stimulus produced by a mechanical wave driver once every second over 120 trials. In the upper half of figure 2, the time of occurrence of each discharge was plotted as a dot raster. For this particular unit, every stimulus presentation evoked at least a single spike, yet no more than two (17.5% trials), with a mean latency to the initial spike of 22.6 ms. The standard deviation of this giant fiber unit was a remarkable 4.7 ms (coefficient of variation = 0.21) which is clearly evident in the tight clustering of spike counts in the bins (1 ms in width) centered near the mean latency in the PSTH.

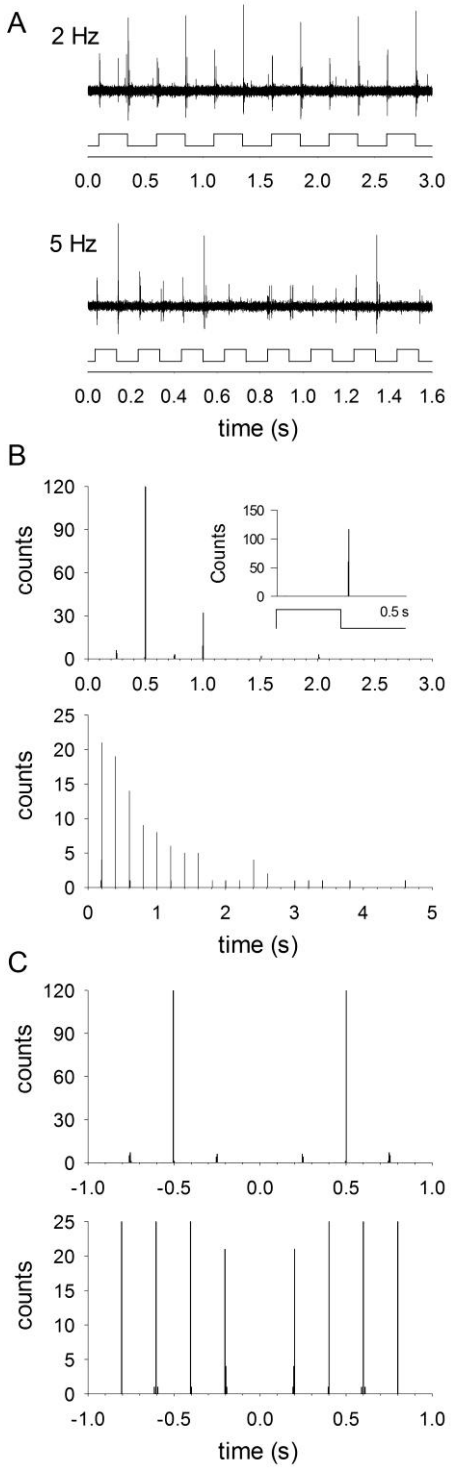


*Figure 1*. Response Periodicity and Phase Locking. *A.* Ventral nerve cord multiunit response displayed above square wave stimulus (top panel 2 Hz, bottom panel 5 Hz; same format is used in *B* and *C*), *B.* ISIHs, period histogram (inset; referred to 0.5 s period of the displayed stimulus) and *C*, ACHs. The histograms display results from only the largest unit observed in *A.* Bin width is 5 ms in ISIH and ACH, and 50 µs in period histogram. In both cases care was taken to adjust signal pressure to 74 dB SPL.

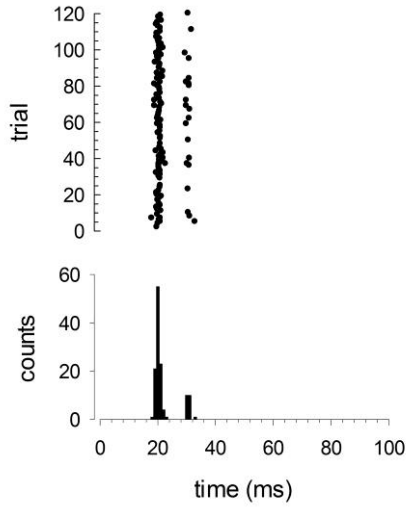


*Figure 2.* Neural Precision. Dot raster (top) and PSTH (bottom) of a VNC unit to a pulse of air displacement (driven by a 70 V pulse to wave driver) presented 120 times, at once every second. Bins are 1 ms wide.

*Adaptation*

The phenomenon of ignoring a persistent stimulus is a common feature of sensory systems that can easily be observed by using the mechanical wave driver to produce a train of air currents delivered at variable rates (Figure 3a). While the extent of the rate of adaptation varies from unit to unit, it tends to correlate directly with the rate of presentation of the stimulus. At 5 Hz, a single fiber fired to almost every stimulus for over 3 seconds. However, as the repetition rate increased the response(s) observed at the beginning of the train were much less reliable to later stimuli (8 Hz), or ceased altogether (21 Hz) (Figure 3b).

## DISCUSSION

A total of 63 groups, or approximately 190 students, have completed this experiment since the autumn semester of 2006. The module complements a laboratory done the previous semester in our Introduction to Neuroscience course when many of the same students observe and quantify cockroach orientation behaviors using ethograms. Students had no direct experience recording electrical activity from *in vivo* preparations. Despite that, almost all major technical issues were worked out in the first session; each group successfully recorded unit activity that was evoked by manipulation of the cerci. With limited technical challenges to overcome, students can devote more time to build skills in experimental design, data analysis and writing.

Each lab group was required to identify questions that they might be able to tackle with the tools available. Before doing so, they were encouraged to consult a set of historically important articles made available on the course Moodle site and to review the results of the behavioral analysis they completed in the introductory course mentioned above. Most groups immediately keyed in on

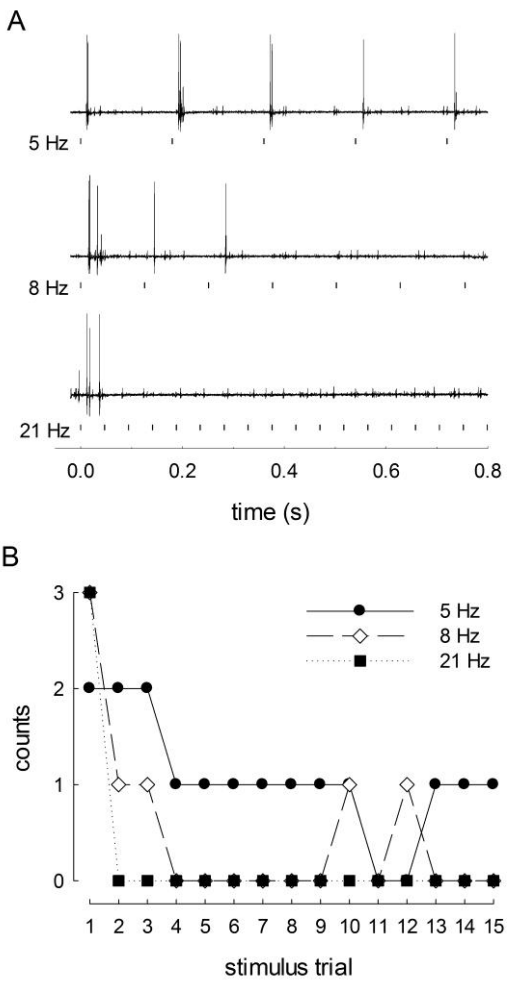


*Figure 3*. Adaptation. *A.* VNC unit response to pulses of air displacement (driven by a 40 V pulse to wave driver) presented at 5, 8 and 21 Hz. Stimulus marker indicated by vertical lines below each recording. *B.* The number of spikes occurring to the first 15 stimulus presentations at each frequency.

questions that were both reasonable in concept and doable.

*How might this simple nervous system…*
*…exploit cues for localizing a stimulus?*
*…ignore an ongoing or repeated stimulus (behavioral habituation)?*
*...extract an important signal out of the background (e.g., from an approaching predator on a windy day)?*
*…represent the intensity or other dynamic attributes of a stimulus?*

Approaching these questions required a controlled stimulus whose temporal properties could serve as a reference for the neural response--the delivery system described above has worked well for my students in this regard.

It is recommended that a laboratory session be dedicated to analyzing data and preparing figures. Since silver wires tend to record the activity from multiple units, for further analysis students had to first learn how to discriminate a single action potential based upon the amplitude and width of its waveform. If the unit activity was stable and could be isolated above the baseline noise, this task was handled after a little practice. Since undergraduate level students lack direct experience working with unit recordings, they often struggle to understand how to interpret the information displayed in the various types of histograms commonly used in the analysis of stimulus-evoked unit responses. One of the more important lessons obtained from the module is that there are several ways to display information embedded in the time-of-occurrence of action potentials. When a stimulus is repeated over many trials, by totaling the number of spikes that occurs at each time point across all trials, the resulting PSTH may reveal temporal response patterns that can be used as an electrophysiological signature to identify its source, the way cutaneous mechanoreceptors in the skin (Johnson, 2001) and cells in the auditory brainstem (Rhode and Greenberg, 1992) are now commonly identified. When plotted in reference to the time period of the frequency of an oscillating stimulus, the resulting period histogram makes it apparent whether the unit can “lock onto” a specific phase angle. Alternatively, measuring the intervals between each discharge and plotting them as an ACH will reveal whether there is an intrinsic or stimulus-driven periodicity present.

There are many ways to bridge the outcomes of this lab module into the material in the classroom. While my course features many classical neuroethological models (Carew, 2000; Zupanc, 2010) the concepts that are studied in animals are relevant to human hearing and balance. Prior to the lab, most students understand that the number of spikes occurring in response to a stimulus is useful in deriving tuning curves or receptive field properties; however, the utility of knowing *when* the firing of action potentials occur within central circuits that encipher the physical senses may be less obvious. In class we typically include a general discussion on how information carried in the timing of each spike is a critical component of the coincident detection model for computing interaural time delays (ITD) the putative mechanism for localizing the source of a sound along the horizon (Jeffress, 1948). Having observed and measured the precision of well-timed neural responses helps students make better sense of how the membrane time constant, the biophysical property that determines the limits of phase locking, contributes to setting the edges of systems-level performance. Within the context of these discussions students are asked to consider how comparative systems that rely on timing to perform everyday activities have adapted anatomic and biophysical specializations at the synaptic and cellular level to convey spikes with high fidelity and precision (Trussell, 1997). In summary, the stimulus generation system as used in this lab module provides a valuable opportunity for students to work with data that is easy to collect under well controlled conditions, placing the focus on how a careful, well designed analysis might lead to revelations about the way the nervous system represents information about a stimulus.

## REFERENCES

Camhi JM (1980) The escape system of the cockroach. Sci Am 243:158-172.

Camhi JM, Tom W (1978) The escape behavior of the cockroach *Periplaneta Americana* I. Turning response to wind puffs. J Comp Physiol A 128:193-201.

Camhi JM, Tom W, Volman S (1978) The escape behavior of the cockroach *Periplaneta Americana* II. Detection of natural predators of air displacement. J Comp Physiol A 128:203-212.

Carew TJ (2000) Behavioral neurobiology: the cellular organization of natural behavior. Sunderland, MA: Sinauer.

Dagan D, Parnas I (1970) Giant fibre and small fibre pathways involved in the evasive response of the cockroach, *Periplaneta Americana*. J Exp Biol 52:313-324.

Goldberg JM, Brown PB (1969) Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: some physiological mechanisms of sound localization. J Neurophysiol 32:613-636.

Jeffress LA (1948) A place theory of sound localization. J Comp Physiol Psychol 41:35-39.

Johnson KO (2001) The roles and functions of cutaneous mechanoreceptors. Curr Opin Neurobiol 11:455-461.

Libersat F, Camhi JM (1988) Control of cercal position during flight in the cockroach: a mechanism for regulating sensory feedback. J Exp Biol 136:483-488.

Oakley B, Schafer R (1978) Experimental neurobiology: a laboratory manual. Ann Arbor, MI: University of Michigan Press.

Paul CA, Beltz B, Berger-Sweeney J (1997) Discovering neurons: the experimental basis of neuroscience. Woodbury, NY: Cold Spring Harbor.

Rhode WS, Greenberg S (1992) Physiology of the cochlear nuclei. In: The mammalian auditory pathway: neurophysiology (Popper AN, Fay RR; eds.) pp 94-152. New York, NY: Springer-Verlag.

Tautz, J (1977) Reception of medium vibration by thoracal hairs of caterpillars of *Barathra brassicae* L. (Lepidoptera, Noctuidae) I. Mechanical properties of the receptor hairs. J Comp Physiol 118:13-31.

Trussell LO (1997) Cellular mechanisms for preservation of timing in central auditory pathways. Curr Opin Neurobiol 7:487-492.

Watson JT, Ritzmann RE (1994) The escape response versus the quiescent response of the American cockroach behavioral choice mediated by physiological state. Anim Behav 48:476-478.

Zupanc GHK (2010) Behavioral neurobiology 2nd ed. New York, NY: Oxford.

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