Investigation of spiking frequency of chemosensitive neurons in scorpion pectines in relation to photoperiod.

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Summary

Pectines are large, comb-like appendages, located on the ventral body surfaces of all scorpions. The primary sensory structures on pectines are the numerous (10^5) pore-tipped structures, called peg sensilla, each of which is innervated by approximately 10-15 dendrites. Previous studies indicate that pectines are chemosensory and are important during mating encounters and perhaps in the chemical identification of food sources. Since scorpions are most active at night, we were interested in whether the spiking frequencies of peg sensilla are altered by photoperiod. Light-dark cycles were chosen to emulate naturally occurring mid-summer photoperiod (14 hours and ten minutes of light and 9 hours and 50 minutes of dark). Ten animals (*Centruroides vittatus*) were placed in a room with the photophase from 6:30 am - 8:40 pm and scotophase from 8:40 pm - 6:30 am. A second group of ten animals was placed in a room with photophase from 8:40 pm - 6:30 am and scotophase from 6:30 am - 8:40 pm. We referred to the first group of animals as the 'normal' group and the second group of animals as the 'reversed' group. All other environmental factors, such as temperature, humidity, moisture, and nutritional supply were kept constant. For each animal, 3-5 minute extracellular recordings were obtained from approximately five peg sensilla on both right and left pectines. For the two treatment groups, half of the animals were recorded during their photophase period, the other half during their scotophase period. Because scorpions are nocturnal animals, a higher frequency of neural firing was predicted after the onset of the scotophase (nighttime). Under the conditions of our experiment, the normal and reverse photophase animals showed no significant difference in the firing frequency of peg sensilla neurons from that of normal and reverse scotophase animals. Subsequent data analysis suggests that there is variation in spiking frequency, which may be more specific than the resolution of our sampling window. Additionally we have found that spiking rhythms and frequencies of peg sensilla vary widely both within and between animals, indicating that peg sensilla may be heterogeneous in terms of their electrical activity. Future recordings running continuously for 24 hours may allow us to better assess possible rhythmic fluctuations in pectinal spiking frequencies.

Introduction

Circadian rhythms, self-sustained daily rhythms that govern behavioral as well as physiological patterns in organisms, have long been a topic of interest among scientists. Circadian studies have been conducted on such organisms as hamsters, rats, sparrows, lizards, marine snails, and fruit flies (Aschoff et al. 1982, Binkley 1990, Palmer 1974). Circadian rhythms have also been observed in scorpions and visual and non-visual photoreceptors supply the necessary information to the circadian pacemaker (Fleissner & Fleissner 1993). However, not many studies have been conducted on whether photoperiod also affects activity of chemosensory neurons in scorpions. Because scorpions are nocturnal animals, we were interested if scorpion chemosensory neurons are more active during natural or simulated nightfall. Due to accessibility of the scorpion chemosensitive pectinal organs to electrophysiological recordings, these organisms pose as ideal test animals.

The primary chemosensory organs of scorpions are the pectines. Pectines are paired, ventral, comb-like appendages that contain numerous (10^5) peg sensilla (Swoveland 1978) arranged among 40-46 teeth per pecten in *Centruroides vittatus*. Scorpions are the only organisms that possess such unique chemosensitive organs. The pegs are minute, pore-tipped structures innervated by approximately 10-15 dendrites (Brownell 1989, Foelix & Muller-Vorholt 1983, Ivanov & Balashov 1979). The pectines appear to transduce pheromonal information during mating encounters (Gaffin & Brownell 1992) and are used in the chemical identification of food sources (Krapf 1986, Gaffin & Brownell 1997).

Previous studies using extracellular recordings from the sensilla have shown a higher frequency of spiking in peg sensilla of *Paruroctonus mesaensis* during night hours (Gaffin & Brownell 1997). However, such studies did not kept variables such as temperature and humidity constant. In this study, neural activity was assessed from individual peg sensilla on scorpions entrained to two different photoperiod regimes, while keeping other environmental factors, such as temperature, humidity, moisture, and nutritional supply constant. Our prediction was that animals recorded during the lights-off period (scotophase) would show an increased frequency of spiking as compared to animals recorded during their the lights-on period (photophase). Similar day-night spiking frequencies between the two groups of animals would suggest pectinal neuronal activity is regulated by photoperiod, whereas other patterns would suggest that neuronal activity is internally regulated.

Methods

Animals

Twenty adult female *C. vittatus* were the subjects of these investigations. All animals were collected during spring and summer 1997 from post-oak forests in Cleveland County, OK. At the laboratory, animals were housed singly in clear, 4150 ml. glass, pickle jars atop natural soil substrates at 22°C and 65% humidity. Animals were maintained with weekly feedings of small gray crickets (Fluker's Cricket Farm, Inc; Baton Rouge, LA). Animals were misted with approximately 12 ml of water biweekly. All animals were maintained in rooms with a light cycle chosen to simulate mid-summer day-length (14 hours and 10 minutes light and 9 hours and 50 minutes dark). The timing of the photophase (lights-on) and scotophase (lights-off) were altered for the two rooms. Half of the animals were placed in a room with the photophase at 6:30 am -8:40 pm and scotophase at 8:40 pm - 6:30 am. The remaining animals were placed in a room with a photophase at 8:40 pm - 6:30 am and scotophase at 6:30 am - 8:40 pm. We referred to the first group of animals as the 'normal' group and the second group of animals as the 'reversed' group. Animals were allowed at least one week acclimation time in their respective rooms before electrophysiological recording.

Electrophysiology

Following anesthetization by cooling (-5°C for 5 min), animals were quickly secured, ventral side up, to a glass slide using modeling clay. A bridge consisting of a glass cover slip, supported by the clay, was erected to span the ventral body surface just posterior to the genital aperture. The pectines were lifted and secured atop a piece of double-sided tape, which was aligned with the anterior edge of the cover slip. The pectinal teeth were then carefully arranged and pressed into the tape using a metal dissecting probe. The indifferent electrode (an 8-cm, presharpened length of silver wire) was inserted superficially through intersegmental membrane between the first and second metasomal segment. The free end of the electrode (with lead exposed) was secured with clay to keep the electrode in place.

Recording electrodes consisted of tungsten wires (0.12 mm diam., approximately 3 cm long), electrolytically etched to a tip diameter of approximately 1 µm Peg sensilla were visualized under epi-illumination and magnified 500-1000x with a compound microscope equipped with long working distance objectives (Olympus BX50WI). Electrodes were maneuvered and inserted through flexible cuticle at the peg base (Fig. 1) using a micromanipulator (Leitz, joystick model). Signals were amplified 10,000 times by a DAM 80-E differential AC amplifier (World Precision Instruments) and bandpassed between 300 Hz and 3 kHz. A SA-10 solid state stereo amplifier (Realistic Inc) audibilized spikes. Electrical contact with hemolymph was noted by a detectable increase in

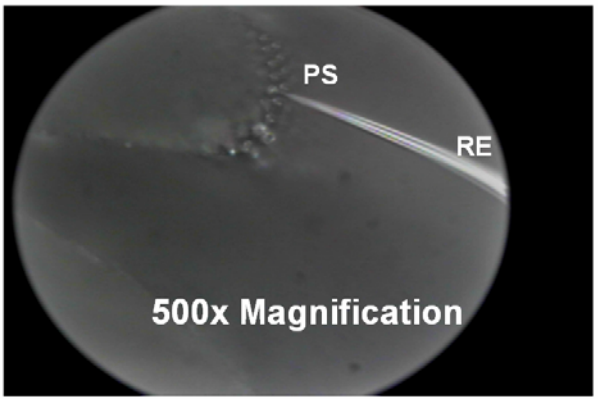

Fig. 1: Electrode placement within the field of peg sensilla. This is a photograph of an extracellular recording electrode (RE) inserted in a peg sensillum (PS) of *C. vittatus* under 500x magnification.

audio frequencies of the amplified signal. Spiking events were typically detected simultaneous with the penetration. Electrode penetration was manipulated until maximum spiking events could be obtained and visualized via the oscilloscope (Tektronix Dual Storage Scope). Signals observed were recorded onto 120-minute videotapes (JVC) using a VHS video tape recorder (Panasonic Omnivision model 420 L).

Extracellular recordings were obtained from 10 animals in the 'normal' group and 10 animals in the 'reversed' group. Within each of the two groups, five animals were recorded during the photoperiod and another five during the scotoperiod. Three to five minutes of spontaneous activity was recorded from centrally located peg sensilla on the central portion of a medially located tooth on the left and right pectines. Although we attempted to record from five pegs from each pecten, we were not always successful and sometimes were only able to record from a minimum of three pegs. Recordings were taken within six hours at the onset of each lights-off and lights-on periods. At the end of each recording session the animal was carefully freed from its clay restraint, cleaned of any adhering clay, and returned to its home container.

Data Processing and Analysis

Spikes were digitally sampled at 20 kHz (CED 1401, Cambridge Electronics) and digitally filtered to further reduce the amplitude of the background noise. Spike analysis software (Spike2, Cambridge Electronics) was used to detect spiking events that exceeded a user-set threshold voltage. Muscle movement and other incongruities were clipped from the records and only stable spiking data were used in determining spiking frequencies. Spiking frequencies were obtained for each sensillum for which there was at least three minutes of clean record. For each animal, the spiking frequencies of all sensilla recorded were averaged to obtain an overall spiking frequency for that animal. Additionally, inter-spike interval histograms (0.01 s bins) were created for each of the pegs recorded.

Results

Extracellular action potentials from *C. vittatus* were easily observed above background noise and ranged between 3-8 in signal-to-noise ratio. Recordings utilizing tungsten electrodes were generally stable for the duration of the recordings. Recordings were sometimes successful shortly following anesthesia and preparation of the animal. However, there were also occasions where an hour or longer would go by before adequate signals were attained.

The four treatment groups tested showed wide variations in their firing frequencies and no pattern with regard to photo regime. For the normal photophase group, the average spiking frequency ranged from 4.13-13.71 Hz. For the normal scotophase group, the average spiking frequency ranged from 5.76-13.71 Hz. For the reverse photophase group, the average spiking frequency ranged from 2.67-12.96 Hz. For the reverse scotophase group, the average spiking frequency ranged from 7.71-11.15 Hz (Fig. 2). There were no significant differences in the mean spiking frequency between the normal and reverse photophase groups as well as the normal and reverse scotophase groups (Fig. 2).

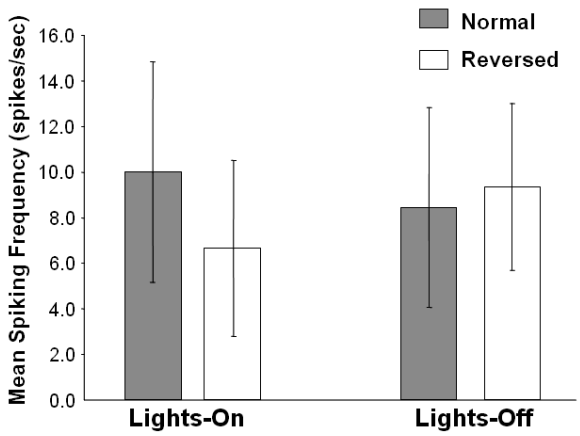

Fig. 2: Relationship between mean spiking frequency within the four treatment groups. The mean spiking frequencies are shown for lights-on and lights-off recording periods for the normal and reversed groups of animals.

Interspike interval histograms (IIH) indicate that large variations exist in spiking patterns within records obtained from individual peg sensilla, even from adjacent pegs on the same animal. Although consistency in firing was often noticed within the same pecten (Fig. 3, records: 112L, 113R, 135L, 74L, 81L, 114R, 121R, 80R), intrapectinal spiking variation at least likely (Fig. 3, records: 104L, 82L, 114R, 115L, 104L, 98R, 123L, 125L and R). For example, in record 104L, the second IIH shows a consistent, high frequency pattern of spiking, while the 3rd, 4th, and 5th are much more eratic. Fig. 3 also demonstrates that there were variations between animals within the same treatment group as well as clear variations in spiking patterns between animals within the same treatment group and between animals in different treatment groups.

Discussion

The aim of this experiment was to find the relationship between photoperiod and chemosensory neural activity. In other words, is there a difference in the activity of pectinal chemosensory neurons of

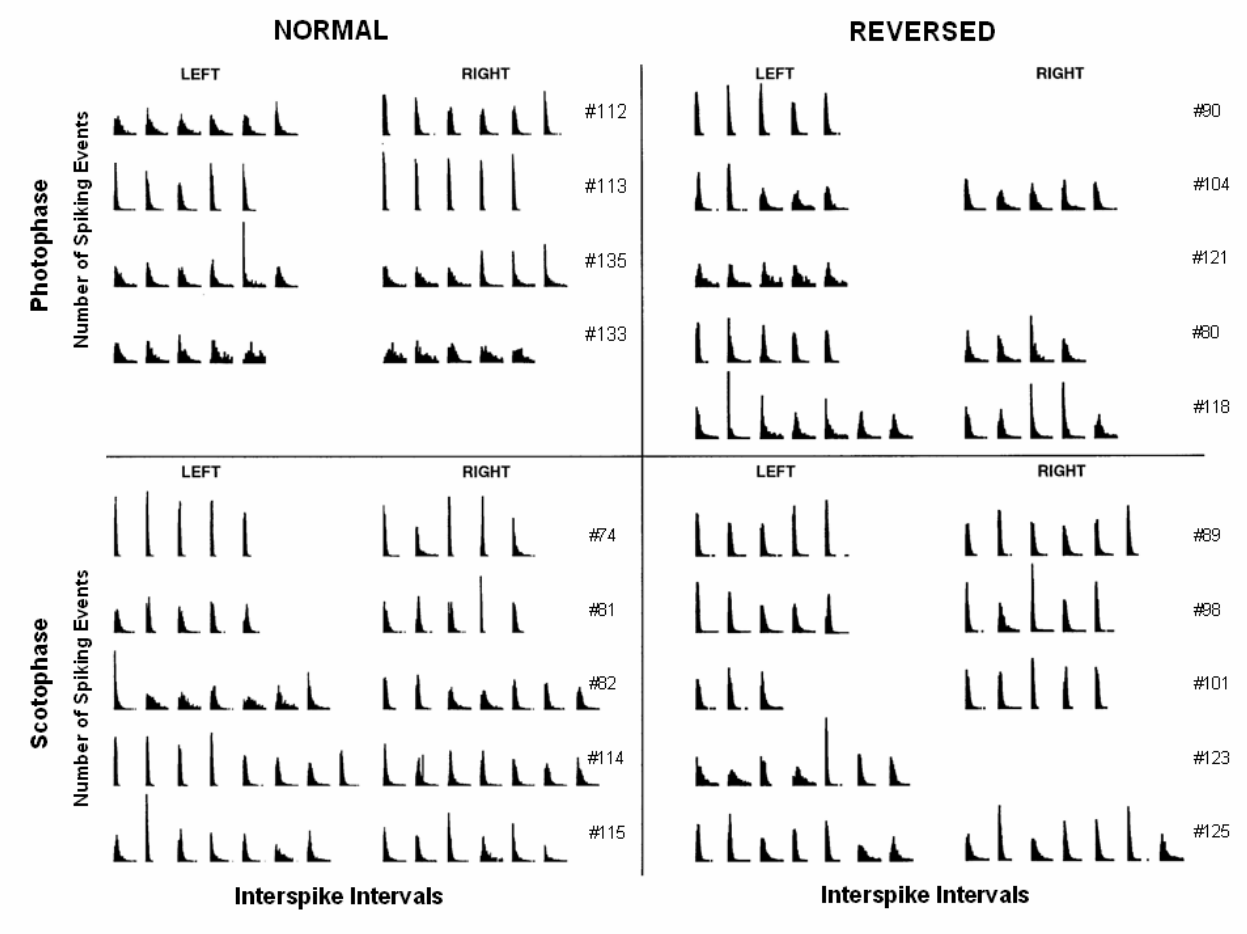

Fig. 3: Variation in spiking patterns of peg sensilla. Interspike interval histograms of all peg sensilla recorded in the experiment are shown grouped by acclimation group, photoperiod recording time (photophase or scotophase), and animal number.

scorpions during photophase and scotophase periods? Because scorpions are nocturnal animals, we predicted they would have a higher level of chemosensory neural activity during "normal" or "reversed" lights-off period than "normal" or "reversed" lights-on period. Under the conditions of our experiment, we found no significant difference in spiking activity between photophase and scotophase recordings for animals entrained to opposite photoperiod regimes.

An interesting finding of this study was that interspike interval histograms showed a wide range of variance in spiking patterns between peg sensilla, both within the same animal and between different animals. Another interesting observation is a lower level of spiking activity seen 250-400 minutes after the onset of the lights-on period (Fig. 4). It is possible that the high variation in peg activity, coupled with the large window of time we chose for this particular study, caused us to miss subtle changes in spiking behavior as suggested by Fig. 4. Also, recordings running continuously for 24 hours from a single peg sensillum may provide a more sensitive assessment of chemosensory spiking patterns in relation to photoperiod.

Additional experimental errors may have been caused by the test animals' exposure to light during the preparation for the recording time. Future electrophysiological studies should keep the animal in the same photo-regime during preparation for recordings as dictated by its classification (i.e. normal photoperiod).

By understanding how photoperiod affects chemosensory neuronal firing activity, we would be able to discover optimal recording times for future electrophysiological studies. We may also gain insight into whether scorpions sense their chemical environment better at night. Ultimately, such neurophysiological behavior in response to photoperiod may shed light on much of scorpion ethology.

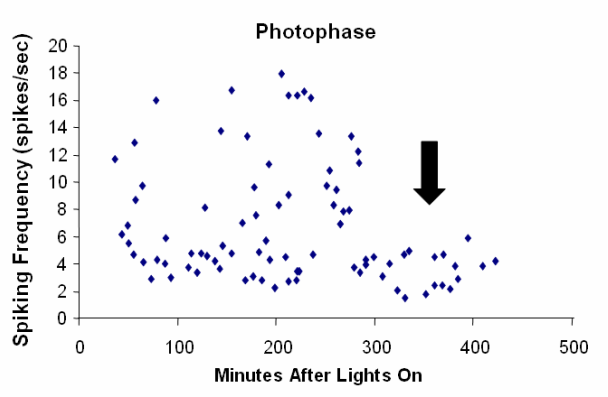

Fig. 4: The spiking frequencies of all recordings (both normal and reversed groups) are shown for the 500 minutes following lights on. The arrow points to a lower spiking frequency occurring 250-400 minutes after the lights-on period.

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